NJC

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/c9nj06346c

An unorthodox metal-free synthesis of dihydro-6*H*-quinoline-5-ones in ethanol/water using a non-nucleophilic base and their cytotoxic studies on human cancer cell line†

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Received 23rd December 2019,
Accepted 17th February 2020

DOI: 10.1039/c9nj06346c

rsc.li/njc

A DBU-catalysed metal-free domino reaction strategy has been developed for the facile synthesis of dihydro-6*H*-quinoline-5-ones. This protocol employs a very expedient route to the synthesis of pyridine frameworks using β-chloro-α,β-unsaturated aldehydes, 1,3-diketones, and ammonium acetate in ethanol : water (1 : 1) solvent under eco-friendly conditions. Diverse types of acyclic and cyclic β-chloro-α,β-unsaturated aldehydes were used to obtain a variety of dihydro-6*H*-quinoline-5-ones. The mechanism of the domino reaction was established by isolating the intermediate compound, which was subjected to the next step of the reaction to obtain the target product. The structure of the intermediate was established from spectral and single crystal XRD studies. Most of the synthesized dihydro-6*H*-quinoline-5-one derivatives were found to be cytotoxic to the HeLa cell lines, showing profound cytotoxicity in MTT assays. The DNA fragmentation assay showed no fragmented DNA in the treated sets, which indicated that the compounds did not induce apoptosis of the HeLa cells. In most of the cases, autophagic cell death was evident from fluorescence microscopy studies, though necrosis was also observed in some cases.

Introduction

In the recent eon of research, the discovery of small molecules with the efficiency to work in complex biological processes to prevent diseases has been one of the major challenges facing researchers.[1] This trend has clearly indicated a paradigm shift of the spotlight from natural product chemistry to combinatorial chemistry.[2] Among numerous nitrogen heterocycles, quinoline is a scaffold of crucial importance with respect to biomedical use. Several quinoline derivatives, isolated from natural resources or prepared synthetically, show a wide variety of biological activities.[3] One such type of important quinoline moiety is dihydroquinoline, which exhibits interesting biological and pharmaceutical activities including antitubercular (**1**), anti-HIV (**2**), anticancer (**3**), apical sodium-dependent bile acid transporter (ASBT) inhibition (**4**), (Fig. 1) *etc.* Compounds, streptonigrin (**5**), ascidiathiazones and lavendamycin, (Fig. 1) showed antibiotic, anticancer, and antiproliferative activities, respectively.[3]

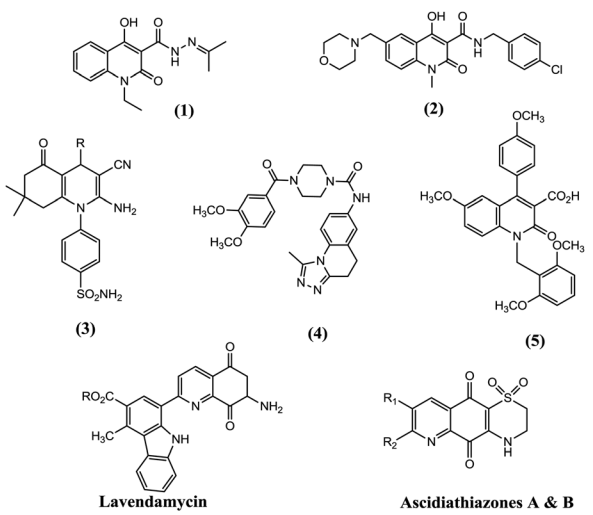

Fig. 1 Some naturally occurring quinoline derivatives.

Numerous skeletal analogues incorporating these crucial moieties have been prepared using synthetic approaches to

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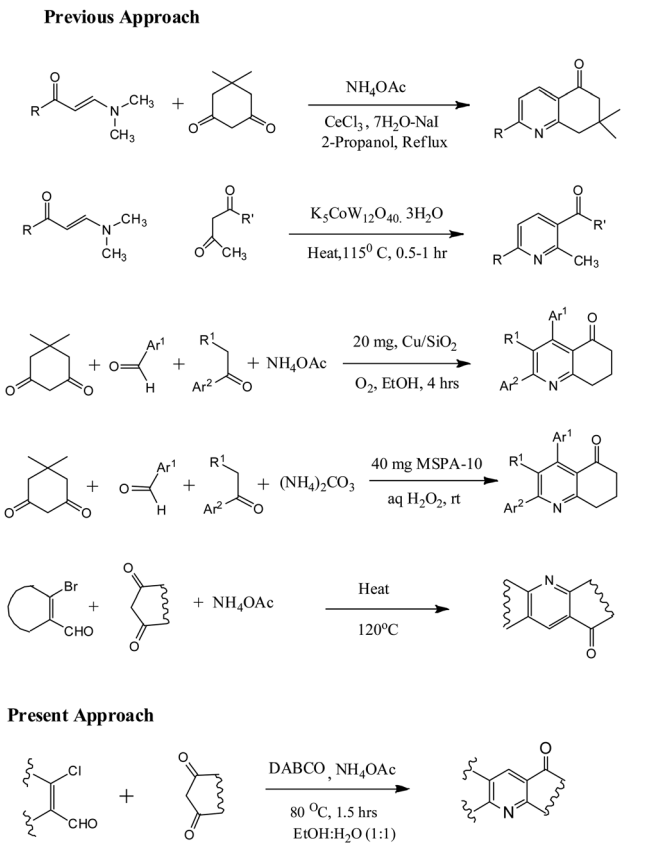
† Electronic supplementary information (ESI) available. CCDC 1542885 and 1542887. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c9nj06346c

accomplish a vast and diverse chemical library.[4] Development of a green technique requires fulfillment of certain criteria such as, use of nontoxic reagents and absence of additives, ambient reaction conditions, atom economy and procedural simplicity. Coupled with this concern, one-pot multicomponent reactions are equally significant as they give rise to complex structures by simultaneous formation of two or more bonds in time-saving procedures.[5] A number of synthetic strategies have been utilized for the preparation of dihydro-6*H*-quinoline-5-one skeletons either by the reaction of β-enaminones with β-dicarbonyls in the presence of $CeCl_3$/NaI in 2-propanol,[6] DBU in butanol,[7] ammonium acetate using microwave,[8] $K_5CoW_{12}O_{40}{\cdot}3H_2O$[9] *etc.* or by the reaction between a ketone, 1,3-diketone, and an aldehyde in the presence of a copper incorporated nanorod like mesoporous silica catalyst using molecular O_2[10] or MCM-41 supported HPF_6.[11] One crucial feature of multicomponent reactions is cost efficiency. Besides many other factors, cost efficiency depends on the number of starting materials and reagents used or steps required for the formation of the product. In this regard, development of reaction protocols involving inexpensive starting materials or minimal number of steps to reach the target molecule is more acceptable.

A literature review on the synthetic methodologies for 7,8-dihydroquinolin-5-ones and similar derivatives reveal that most of the synthetic techniques engage either more than one step with costly metal catalysts or a three to four component-strategy. The majority of groups have reported the preparation of dihydro-6*H*-quinoline-5-one derivatives using a two-step procedure entailing β-enaminone intermediates, which could be obtained from the reaction between dimethylformamide, dimethyl acetals and aryl methyl ketones.[6–9] Mukhopadhyay *et al.*[10,11] demonstrated a four-component-reaction strategy for the preparation of dihydro-6*H*-quinoline-5-one derivatives (Scheme 1).

To enrich this library, we focused on the synthesis of different dihydroquinoline derivatives. Here, the synthesis of dihydro-6*H*-quinoline-5-one derivatives was developed by taking the advantages of the skeletal motif of β-chloro-α,β-unsaturated aldehydes. Goroi *et al.*[12] had reported the synthesis of dihydro-6*H*-quinolin-5-ones from similar motifs of β-bromo-α,β-unsaturated aldehydes by using a three-component methodology between 1,3-diketones, and ammonium acetate without any additional catalyst or metal salt at 120 °C, where the methodology involves the elimination of a more labile bromide group. Moreover, flexibility of the methodology with an acyclic analog of β-bromo-α,β-unsaturated aldehydes was not reported. Additionally proof of mechanistic pathway was not established in the approach. In the pursuit of an easier and more green technique at a lower temperature we have adopted a metal-free protocol in an ethanol/water solvent starting from different β-chloro-α,β-unsaturated aldehydes as a source of carbonyl functionalities. To the best of our knowledge this is the first report of the formation of dihydro-6*H*-quinoline-5-ones from β-chloro-α,β-unsaturated aldehydes as a source of carbonyl functionality.

Quinolines and their derivatives are known for their diverse (antimicrobial, anticancer, anti-HIV, anti-inflammatory, antidepressant *etc.*) biological activities and have been studied for centuries. Anticancer drugs with quinoline moieties can induce



Scheme 1 A comparative study of previous and present work.

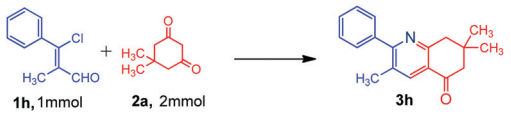
apoptosis, dysregulate cell cycles and inhibit angiogenesis (Afzal *et al.*, 2015).[13] Koprulu and coworkers (2019)[14] reported the synthesis of methoxy and hydroxy substituted quinoline compounds and their antiproliferative properties against human adenocarcinoma (HT29), human cervical cancer cell line-HeLa and rat glioblastoma (C6). In the present study, the anticancer activity of the synthesized dihydro-6*H*-quinoline-5-one derivatives on cervical cancer cells *in vitro* (HeLa, HPV 18 positive cell line) was explored. As the compounds have a planar structure, they tend to intercalate within the DNA bases, causing DNA damage and inhibiting replicative processes (Okten *et al.*, 2017),[15] leading to cell death. To evaluate the mode of cell death, DNA fragmentation assays were undertaken and the presence of autophagic vacuoles was studied.

Results and discussion

In order to find out the suitable reaction conditions associated with the synthesis of dihydro-6*H*-quinoline-5-one, we optimized the reaction by carrying out extensive screening tests utilizing a representative reaction between 3-chloro-2-methyl-3-phenyl-acrylaldehyde (**1h**) (1 mmol) and dimedone (**2a**) (2.0 mmol) in the presence of NH_4OAc (5 mmol) and different bases by varying solvents to obtain **3h**.

All the results are summarized in Table 1. Initially the reaction was carried out by using potassium carbonate as the

Table 1 Optimization study using **1h** and aldehyde **2a**



S. no.	Base[a]	Solvent[b]	Time (h)	Temp. (°C)	Yield[c,d] (%)
1	K_2CO_3	Water	12	100	10
2	Triethyl amine	Water	8	100	10
3	Triethyl amine	Ethanol/water (1 : 1)	8	100	20
4	Morpholine	Water	8	100	25
5	Morpholine	Ethanol/water (1 : 1)	8	100	45
6	Pyridine	water	6	100	55
7	Pyridine	Ethanol/water (1 : 1)	6	100	75
8	Piperidine	Ethanol/water (1 : 1)	6	100	60
9	α-Picoline	Ethanol/water (1 : 1)	5	100	75
10	β-Picoline	Ethanol/water (1 : 1)	5	100	80
11	γ-Picoline	Ethanol/water (1 : 1)	4.5	100	85
12	Lutidine	Ethanol/water (1 : 1)	4	100	85
13	Bipyridine	Ethanol/water (1 : 1)	3	100	85
14	DABCO	Ethanol/water (1 : 1)	1.5	100	90
15	DBU	Ethanol/water (1 : 1)	2	100	85
16	**DABCO**	**Ethanol/water (1 : 1)**	**1**	**80**	**96**
17	DABCO	Ethanol/water (1 : 1)	2.5	60	90
18	DABCO	Ethanol/water (1 : 1)	2	70	92

[a] All the reactions were carried out with 3 mmol base. [b] 10 ml of solvent was used in each case. [c] All the reactions were carried out with 3-chloro-2-methyl-3-phenyl-acrylaldehyde (1.0 mmol) and dimedone (2.0 mmol). [d] Yield of isolated product.

base in water, but it resulted in low yield of the product even after a long reaction time at 100 °C (Table 1, entry 1). In the pursuit of more fruitful reaction conditions, we performed the same reaction in water and ethanol–water mixture in the presence of triethyl amine as the base (Table 1, entries 2 and 3) and it was observed that there was a slight increase in the yield of the product in the ethanol–water (1 : 1) solvent after a long reaction time. Consequently, we studied the reaction in water and ethanol–water (1 : 1) in the presence of morpholine and pyridine as the bases (Table 1, entries 4–7), where a marginal enhancement in the product yield was noticed when pyridine was used as the base in an ethanol–water (1 : 1) solvent. Henceforth screening tests were done in ethanol–water (1 : 1) solvent to obtain the best reaction conditions by studying with different non-nucleophilic bases and various reaction times (Table 1, entries 8–15). Ultimately the optimum conditions were realized by using DABCO as the base at 80 °C, which afforded 96% yield of the desired product in ethanol–water (1 : 1) solvent (Table 1, entry 16). A study to observe the effect of temperature on the yield of the product was also performed (Table 1, entries 17 and 18), which indicated that any further alteration of the reaction temperature decreased the yield of the product. To show the scope of this protocol, we utilized the new methodology using different 1,3-dicarbonyl systems with various acyclic β-chloro-α,β-unsaturated aldehydes (Table 2) and cyclic β-chloro-α,β-unsaturated aldehydes (Table 3). In all the cases, the reaction proceeded efficiently with good to excellent yields.

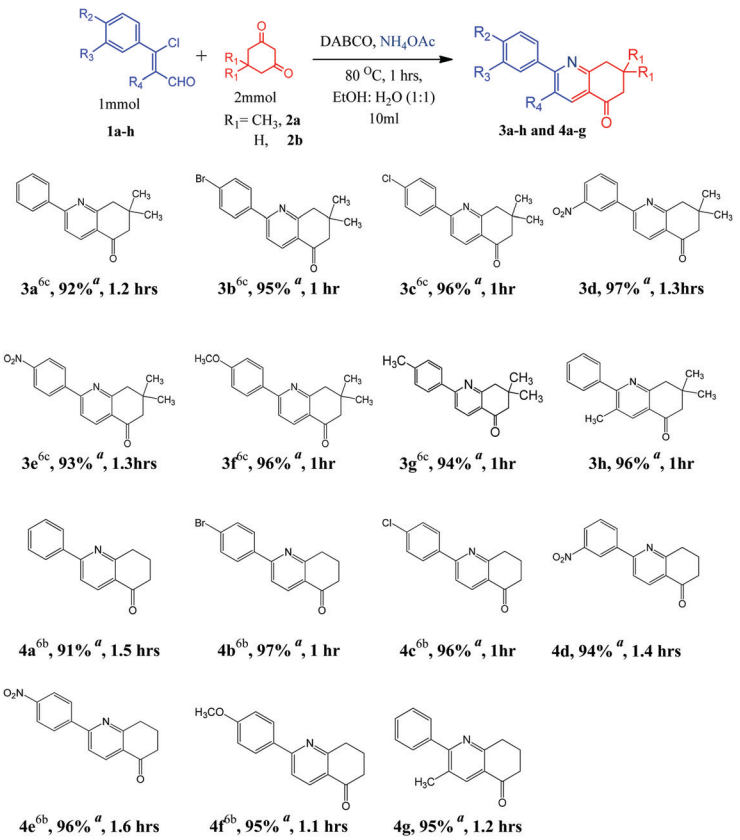
The reactions were simultaneously carried out at the 1 mmol scale and no change in the yield of the product was observed when scaled up to the 10 mmol scale. From the point of view of green chemistry, it was interesting to note that the solvent used herein is a mixture of ethanol and water and the base DABCO is completely benign for the environment.

Besides cost efficiency, the methodology involved procedural simplicity, easy work-up and minimization of chemical impurities. The structures of the products were well characterized by using spectral (IR, ^{1}H and ^{13}C NMR) and elemental analysis data. The structural pattern of the products was fully established by X-ray crystallographic analysis of compound **6a** (CCDC 1542885)† (Fig. 2). We herein synthesized twenty-three compounds and of them, seven are new additions to the literature.

A plausible mechanistic pathway was proposed to explain the base-catalyzed formation of dihydro-6*H*-quinoline-5-ones from β-chloro-α,β-unsaturated aldehydes (Fig. 3). The β-chloro-α,β-unsaturated aldehyde initially went through a Knoevenagel condensation with the 1,3-diketone in the presence of a non-nucleophilic base. Subsequently the presence of a vinylic chlorine atom facilitated the incorporation of the second molecule of 1,3-diketone to produce the xanthene-dione intermediate (**8**). The *in situ* product **8** immediately reacted with ammonia, generated from ammonium acetate under the reaction conditions, to form the β-enaminone through ring opening. This β-enaminone ultimately formed the dihydro-6*H*-quinoline-5-one through internal rearrangement involving N–C bond formation and C–C bond cleavage losing one molecule of the 1,3-diketone.

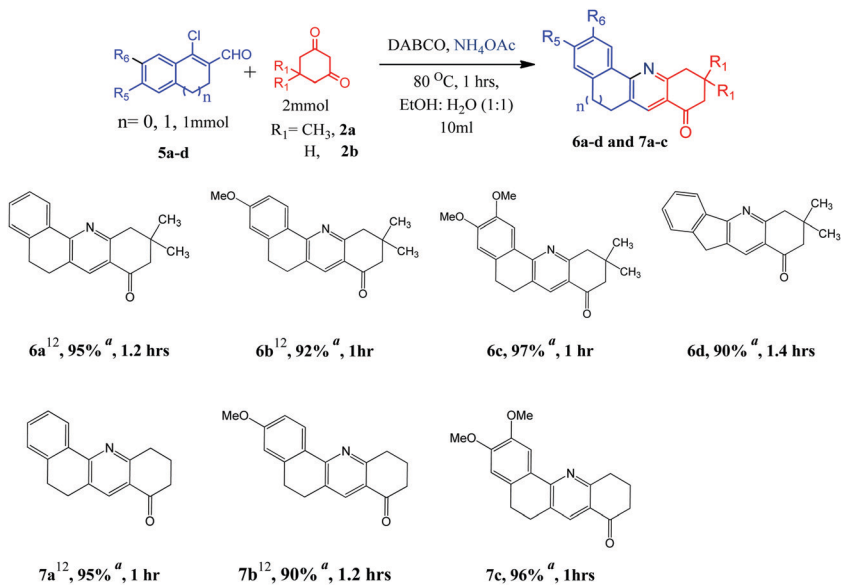
The xanthene-dione intermediate (**8**), proposed in the above-mentioned reaction pathway for the formation of dihydro-6*H*-quinoline-5-one derivatives, had been isolated in the following way. Initially, the reaction between/with (*Z*)-3-chloro-3-phenylacrylaldehyde (**1a**) and dimedone (**2a**) was carried out in the presence of base DABCO in water at room temperature for ten minutes. The intermediate **8** was immediately precipitated, which was

Table 2 Base catalysed dihydro-6*H*-quinoline-5-one derivatives from acyclic β-chloro-α,β-unsaturated aldehydes



[a] % yield was calculated from the isolated product.

Table 3 Base catalysed dihydro-6*H*-quinoline-5-one derivatives from cyclic β-chloro-α,β-unsaturated aldehydes



[a] % yield was calculated from the isolated product.

filtered and further reacted in the presence of ammonium acetate in ethanol–water (1 : 1) solvent at 80 °C when **3a** was obtained after 1.2 hours. A schematic diagram of the aforesaid procedure is shown in Fig. 4. The structure of the xanthene-dione intermediate (**8**) was confirmed by spectroscopic and X-ray crystallographic studies (CCDC 1542887)† (Fig. 5).

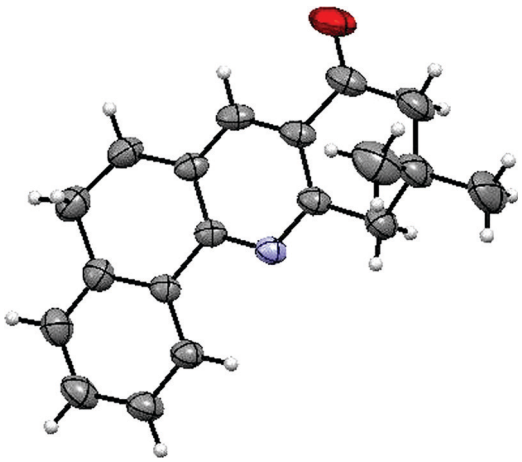


Fig. 2 X-ray crystallographic structure of compound **6a**.

Isolation of intermediate **8** confirmed the proposed mechanistic pathway as shown in the scheme shown in Fig. 3. The role of vinylic chlorine present in the β-chloro-α,β-unsaturated aldehyde for the formation of **8** was thus established.

Being a class of substituted pyridine scaffolds, dihydro-6*H*-quinoline-5-one moieties show high potential towards biological systems. In the present study, we have evaluated the cytotoxic potential of the synthesized dihydro-6*H*-quinoline-5-one scaffolds using MTT cell viability assays on a cervical cancer cell line (HeLa) followed by a DNA fragmentation assay. Morphology of acidic vesicles (vacuoles and lysosomes) and the presence of autophagosomes were observed by fluorescence microscopy after staining with a vital metachromatic dye acridine orange (AO).

Biological study

Cytotoxicity assay. Many of the synthesized quinoline compounds bearing different functional groups exhibit antimetastatic effects. The nature and position of the substituent on the

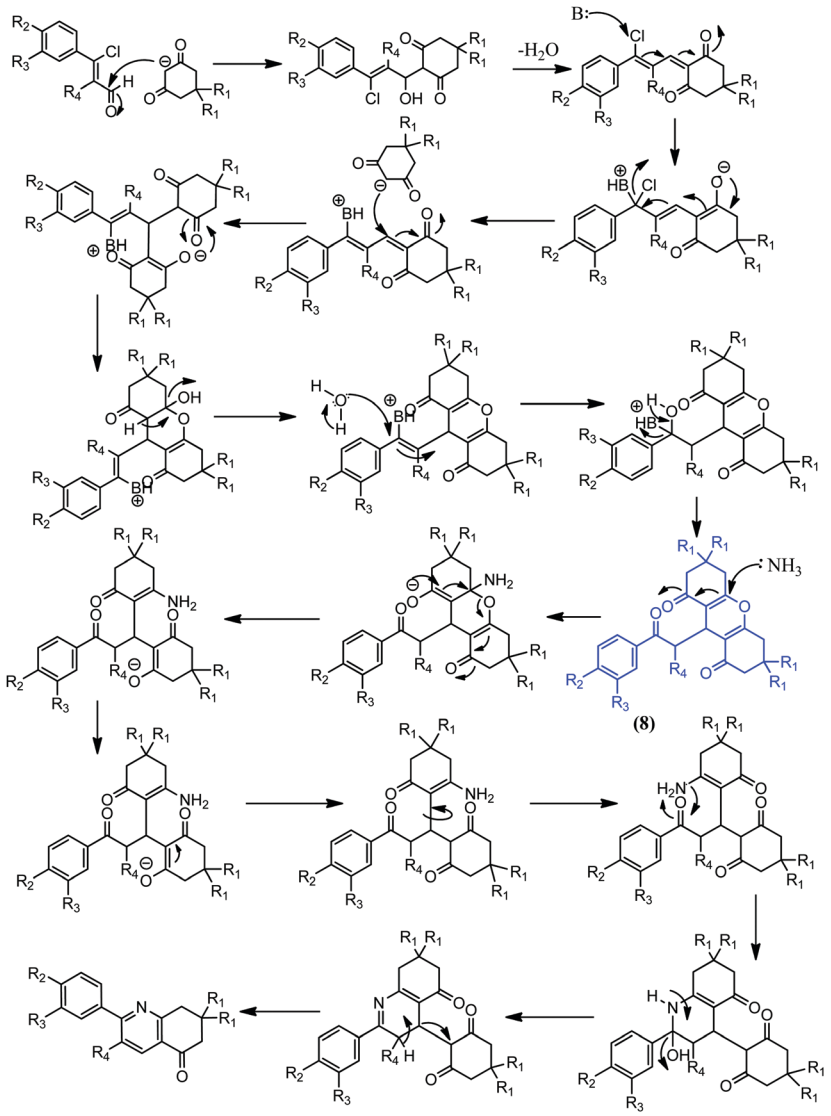


Fig. 3 Plausible mechanistic pathway for the synthesis of dihydro-6*H*-quinoline-5-one derivatives.

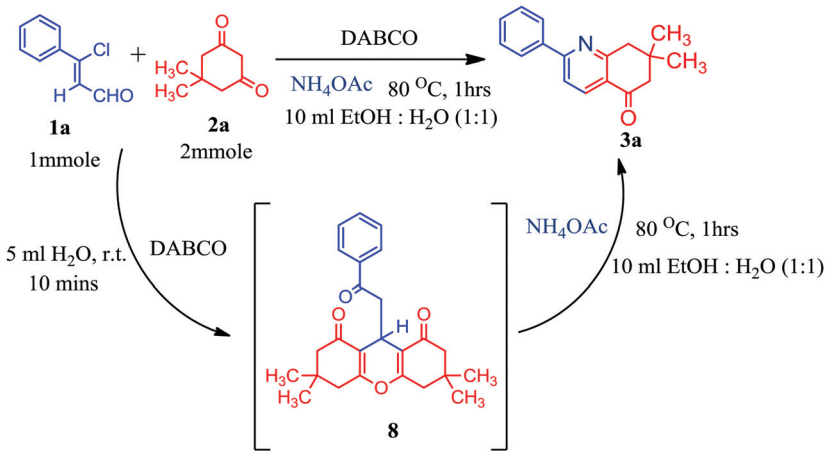


Fig. 4 Schematic diagram of the formation of dihydro-6*H*-quinoline-5-one derivative through xanthene-dione intermediate.

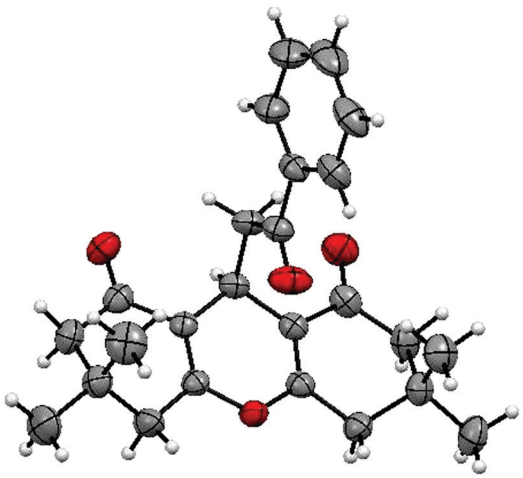


Fig. 5 X-ray crystallographic structure of compound **8**.

quinoline framework show variety in their efficacy in terms of anticancer activity, thus generating structure–activity relationship studies.[16] In our case, the presence of methoxyl groups at different positions with respect to quinoline nitrogen has shown relatively higher cytotoxicity than that of other substituents. We thereby undertook a detailed study with the compounds containing methoxyl groups only. From the MTT assay (Table 4), it was evident that less cytotoxicity was observed in HeLa cells treated with **3f**, **7c**, and **6b** (IC_{50} 120 μm, 100 μm, and 90 μm respectively), whereas cells treated with **4f**, **6c**, and **7b** showed significant cytotoxicity. Among these compounds, it was found that compound **6c** is the most cytotoxic when compared with the others ($IC_{50} \sim 0.95$ μM) (Fig. 6).

DNA fragmentation assay. DNA fragmentation is a hallmark of apoptotic cell death. However, in the treated cells no sign of laddering of DNA (fragments with 180 kb or its multiples) was observed (Fig. 7). Intact genomic DNA was found in all the treated sets, which indicate that apoptosis might not be the mechanism for inducing cell death in the treated ones.

Fluorescence microscopy with acridine orange dye. From the fluorescence microscopy study, it was observed that in the sets treated with compounds **3f**, **4f**, **6b** and **7b** formation of acidic vacuoles (autophagosomes) was evident. Increased amounts of diffused red specks can be easily observed indicating that the cell death pathway was mediated by autophagy, which was induced after 24 hours of chemical treatments, whereas a necrotic cellular morphology was observed in the sets treated with compounds **6c** and **7c** (Fig. 8).

Table 4 Results obtained from an MTT assay of HeLa cells with different dihydro-6*H*-quinoline-5-one derivatives

		Dose								
Compound	% cell viability	0.05 μM	0.1 μM	0.5 μM	0.75 μM	1 μM	25 μM	50 μM	100 μM	250 μM
3f	Average	92	89.67	87	83.67	81.77	75.33	71.67	69.33	3.67
	SD	0.65	0.73	0.5	0.72	0.05	1.6	1.63	1.05	0.4
6b	Average	99.33	92	95.33	95	88.33	88	72.33	45.67	—
	SD	1.25	1.15	0.65	1.15	0.45	0.75	2.08	1.13	—
6c	Average	90.33	90.67	93.33	65.66	47	35.33	20.33	15	—
	SD	2.51	0.23	0.6	0.65	0.43	1.3	0.05	1.5	—
4f	Average	104.69	97.66	101.17	97.26	90.23	82.03	48.44	31.05	—
	SD	3.5	3.1	4.6	4.2	3.5	3.7	0.8	1.8	—
7b	Average	101.7	102.54	100.2	97.07	95.31	26.75	22.07	19.94	—
	SD	7.8	8.14	1.25	2.98	0.8	10.44	0.265	1.01	—
7c	Average	101.37	99.61	100.2	98.44	90.43	81.84	67.77	51.37	—
	SD	1.82	1.2	1.5	0.41	2.12	0.11	2.6	2.62	—
Doxorubicin	IC_{50}	1 μM[17]								

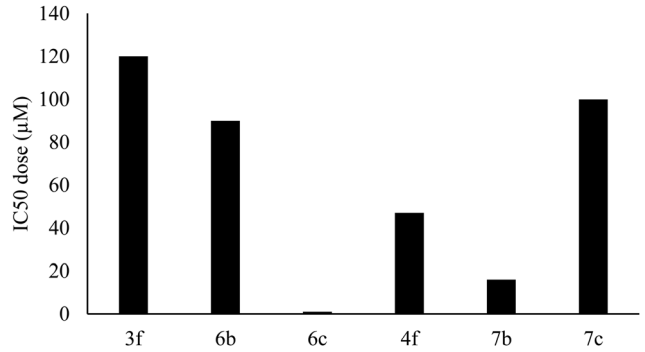


Fig. 6 Bar graph showing IC_{50} doses of the compounds in HeLa cells.

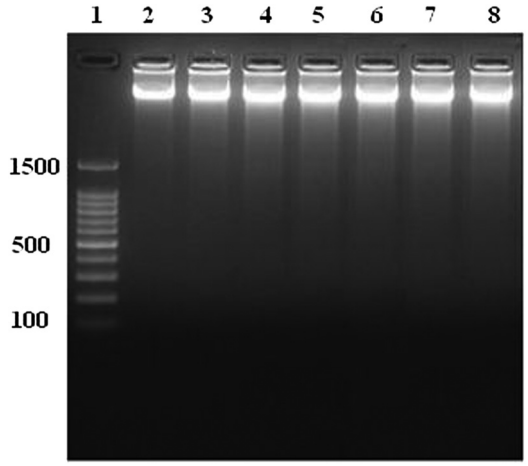


Fig. 7 HeLa cells grown in T25 flask were treated with IC_{50} doses of corresponding treatments for 24 hours. Lane 1: DNA marker (1.5 kb), lane 2: control set, lane 3: compound **3f**, lane 4: compound **6b**, lane 5: compound **6c**, lane 6: compound **4f**, lane 7: compound **7b**, and lane 8: compound **7c**.

Experimental

Procedure for the synthesis of dihydro-6*H*-quinoline-5-one derivatives

2 mmol of a 1,3-diketone compound and 1.2 mmol of β-chloro-α,β-unsaturated aldehydes were stirred in the presence of 5 mmol ammonium acetate in 10 ml ethanol/water (1 : 1) solvent using DABCO as a base at 80 °C for 1–1.6 hours. The reaction was monitored by TLC. After completion of the reaction the whole mixture was extracted with ethyl acetate and the ethyl acetate part was washed with brine three times and evaporated under vacuum. The reaction mixture obtained was subjected to column chromatographic separation using different proportions of a petroleum ether:ethyl acetate mixture. The product was obtained from a 20% ethyl acetate petroleum ether mixture. The products were well characterized by IR, ^{1}H NMR, ^{13}C NMR, elemental analysis and an X-ray crystallographic study.

Synthesis of 3,3,6,6-tetramethyl-9-(2-oxo-2-phenylethyl)-3,4,5,6,7,9-hexahydro-1*H*-xanthene-1,8(2*H*)-dione (8) (proposed intermediate) and its conversion to the 7,7-dimethyl-2-phenyl-7,8-dihydroquinolin-5(6*H*)-one (3a)

A mixture of 3-chloro-3-phenylacrylaldehyde (**1a**) (1 mmol) and dimedone (**2a**) (2 mmol) was stirred in the presence of DABCO in 5 ml water at room temperature for 10 min. The compound 3,3,6,6-tetramethyl-9-(2-oxo-2-phenylethyl)-3,4,5,6,7,9-hexahydro-1*H*-xanthene-1,8(2*H*)-dione was precipitated as a white solid, which was isolated by filtration and washed with water. The isolated xanthene dione was further reacted in the presence of 5 mmol ammonium acetate in 10 ml ethanol/water (1 : 1) solvent at 80 °C for 1.2 hour and the expected 7,7-dimethyl-2-phenyl-7,8-dihydroquinolin-5(6*H*)-one was obtained.

General procedure for *in vitro* evaluation of anticancer activity

Maintenance of cell line. Human cervical cancer cell line, HeLa, a HPV 18 positive cell line was obtained from NCCS, Pune. Cells were maintained in EMEM (Eagle's minimum essential medium), supplemented with 10% FBS, non-essential amino acid and glutamic acid, in a humidified CO_2 (5%) at 37 °C.

Cytotoxicity assay. Cytotoxicity of the chemicals towards HeLa cells was investigated using standard MTT reduction assays.[18] Positively charged tetrazolium dye MTT can penetrate viable cell membranes easily. After reduction by viable cell mitochondrial dehydrogenases and oxidoreductases, the yellow colored MTT dye turns to purple colored crystals. These water insoluble formazan crystals can be solubilized and quantified spectrophotometrically to determine the viability percentage. 1×10^3 cells were seeded in a 96-well plate and were incubated overnight at 37 °C. Cells were then treated with the synthesized compounds in a concentration range of 0.05 μM to 100 μM for 24 hours. Then 100 μg of MTT (Sigma) (5 mg MTT per ml PBS) was added to each well, mixed and then incubated in a 5% CO_2 incubator for another 3–4 hours. Then the media was removed from the wells and the formazan crystals were dissolved with 80 μl of DMSO (Merck). Absorption spectra were recorded using an ELISA microplate reader (BioRad) at 595 nm. The extent of cytotoxicity was determined using the following formula:

$$\%\ \text{inhibition} = [(\text{absorbance of control set} - \text{absorbance of treated set})/\text{absorbance of control set}] \times 100$$

From the absorption spectral data, cell viabilities (in percentage) in treated and control sets were calculated along with standard deviations and bar graphs were made using the standard software (Microsoft Excel).

DNA fragmentation

Apoptotic cell death can be established by DNA fragmentation assays. DNA ladder assays have previously shown fragmented DNA of 180 base pairs and its multiples (which is an apoptotic phenomenon) in Hep-2 cells treated with only AMK OX-12, a 1,3,4-oxadiazole derivative. To assess the cell cytotoxic potential of the synthesized chemicals on the HeLa cell line, cells were seeded in surface-treated 60 mm culture dishes for 24 hours. Treatments were added according to the IC_{50} doses from MTT cell viability assays and incubated for 24 hours. Total DNA was isolated according to Herrmann and his coworkers (1994) after slight modification.[19] 1×10^6 HeLa cells were harvested using a policeman (cell scrapper) and washed with chilled PBS. Then the cells were dissolved in Tris–EDTA (TE) buffer. Equal amounts of

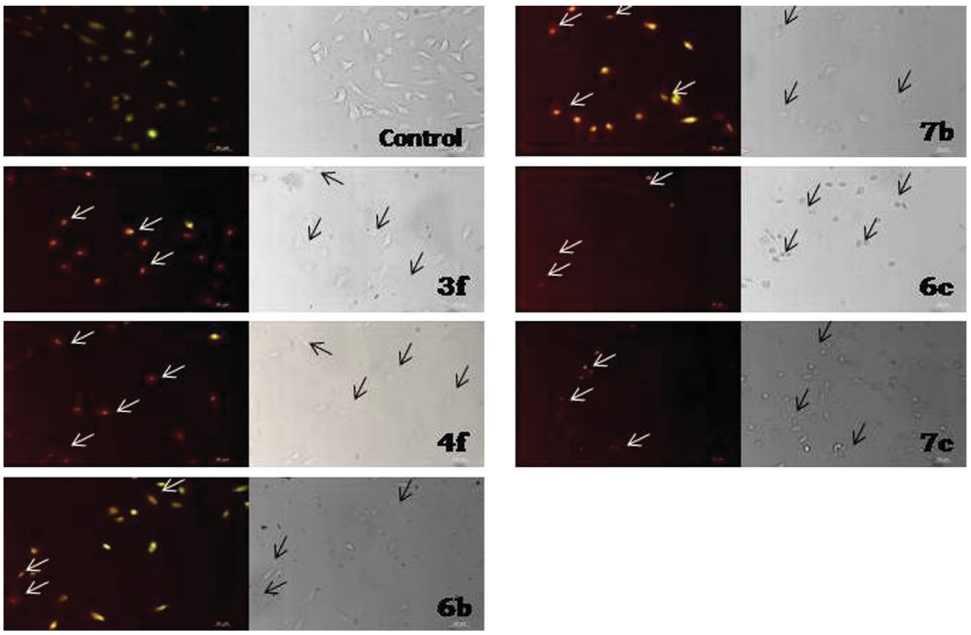


Fig. 8 Fluorescence microscopic study with acridine orange; fluorescence (right) and phase contrast (left) images of treated HeLa cells. Cells were treated with IC_{50} doses of the respective compounds.

2× lysis buffer (200 mM NaCl, 20 mM Tris, 50 mM EDTA, 1% SDS and 2% NP-40) was added, tapped and incubated in ice for 30 minutes. Then a phenol–chloroform–isoamyl alcohol (25 : 24 : 1) mixture was used to eliminate fats and proteins. DNA was collected from the aqueous phase and precipitated with isopropanol and sodium acetate after two rounds of centrifugation. The collected pellet was redissolved in TE buffer and incubated with RNaseA to remove RNA. DNA was finally precipitated with isopropanol and sodium acetate. DNA pellets were washed in 70% ethanol and air dried to evaporate alcohol fully. Then the pellets were dissolved in TE buffer and run on a 1.5% agarose gel and visualized under a UV documentation system.

Fluorescence microscopic study of acidic cellular compartments with acridine orange dye

Acridine orange (AO) is a metachromatic dye. Cell cytoplasm and nucleus are stained as green and the acidic compartments of a cell *viz.*, lysosome and vacuoles are stained orange. Formation of these kinds of acidic compartments in a higher degree represents an increased degree of autophagosome formation, a hallmark of autophagic cell death. In most of the autophagy pathway, the lysosome after fusion with an autophagosome forms an autophagolysosome that can engulf other cellular components leading to completion of the autophagic cell death cascade. Some key proteins are involved for conducting this pathway. Beclin-1, Atg group of proteins are the positive regulatory upstream proteins and LC3B proteins are the key proteins, which act as downstream regulators leading to the formation of an autophagosome.

In this experiment 1×10^4 HeLa cells were seeded on poly-lysine coated 60 mm Petri dishes. On addition of treatments with their respective IC_{50} doses, cells were incubated overnight at 37 °C. Cells were stained with 10 μg ml^{-1} AO and visualized under a fluorescence microscope (Leica).

Conclusion

A green, atom economic metal-free methodology has been developed using a non-nucleophilic base for the syntheses of dihydro-6*H*-quinoline-5-one derivatives from β-chloro-α,β-unsaturated aldehydes with good to excellent yield of the products. Molecular structures of two representative molecules were investigated by means of X-ray diffraction analysis. A mechanistic pathway was established by isolating the xanthenedione intermediate, which was further subjected to the second step of the domino reaction to yield the corresponding dihydro-6*H*-quinoline-5-one derivative. The biological study revealed that dihydro-6*H*-quinoline-5-one derivatives were cytotoxic to HeLa cells and among them, the compound **6c** showed profound cytotoxicity. As the DNA fragmentation assay showed no fragmented DNA in the treated sets, it could be concluded that the dihydro-6*H*-quinoline-5-one compounds did not induce apoptosis to the HeLa cells. In most of the cases autophagic cell death was evident from fluorescence microscopy studies, though necrosis was also observed in a few cases.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors gratefully acknowledge the Department of Chemistry, University of Calcutta for Crystallographic and NMR studies and

the Department of Botany, University of Calcutta for the biological studies. The authors also thank Maulana Azad College, Kolkata for use of the departmental facilities.

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