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An efficient one-pot synthesis and photoinduced DNA cleavage studies of 2-chloro-3-(5-aryl-4,5-dihydroisoxazol-3-yl)quinolines

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ABSTRACT

4,5-Dihydroisoxazoles continue to attract considerable interest due to their wide spread biological activities. Here, we identify an efficient protocol for the preparation of 4,5-dihydroisoxazoles (2-isaxazolines) (**4a**–**g**) from quinolinyl chalcones. The nucleolytic activities of synthesized compounds were investigated by agarose gel electrophoresis. All these compounds were showed the remarkable DNA cleavage activity (concentration dependent) with pUC19 DNA at 365 nm UV light. The DNA cleavage activity was significantly enhanced by the presence of iminyl and carboxy radicals of DIQ.

The interaction of small molecules with nucleic acids has received considerable attention during the past decade.[1] Organic molecules are attractive reagents for the cleavage of nucleic acids due to their inherently diverse structure and reactivity. Metal-based photosensitizers are the foremost and widely used anticancer drugs for cancer therapy, but these possess inherent side effects, solubility and acquired drug resistance. Therefore, considerable attempts are being made to replace these drugs with suitable alternatives, and numerous small molecules have been synthesized and tested for their anticancer activities.[2] Quinoline compounds are regarded as the most promising alternatives to metal complexes as anticancer drugs.[2]

2-Isoxazolines are unique in their chemical behavior not only among heterocyclic compounds in general, but also among related azoles. This is because 2-isoxazoline possesses the typical properties of the aromatic system, which are in fact rather pronounced in these derivatives, together with the high liability of the ring under certain conditions, particularly at the nitrogen–oxygen bond.[3a,3b] 2-Isoxazolines may show interesting medicinal or crop protection properties or have some other industrial utility. It has been well established that isoxazole derivatives possess a wide spectrum of chemotherapeutic activities including antifungal,[4a] antibacterial,[4b] anticonvulsant,[4c] anti-inflammatory,[4d] anti-viral,[4e] analgesic,[4f] antitumar,[4g] chemotherapy activity.[4h] Isoxazoline derivatives also show a good potency in animal models of thrombosis.[4i] Drugs such as Isocarboxazid, Valdecoxib, Oxacillin, Leflunomide, and Micafungin are the examples[5] to substantiate the pharmaceutical acceptance of 2-isoxazoline heterocyclic systems.

On the other hand, the quinoline moieties are reported to act as anticancer and antiviral agents, protein kinase inhibiters for oncolytic drugs, PDE4 inhibitors for antiasthmatic and anti-inflammatory agents,[6a] anticonvulsant agents,[6b] non-opioid analgesics[6c] and antimalarial agents.[6d,6e]

A considerable number of methods have been reported for the synthesis of substituted isoxazoles.[7,8] The most convenient synthesis of isoxazoline and isoxazole ring systems has been executed in the literature via 1,3-dipolar cycloaddition[7] (1,3-DC) reactions of alkenes or alkynes with nitrile oxides generated in situ from aldoximes.[8] Although this three-step procedure is quite general, we found that the parallel synthesis of isoxazoline due to the number of extraction, filtration, and solvent evaporation steps, we sought a one-pot procedure to overcome these limitations. Thus, the development of facile and regioselective methods for the synthesis of DIQ is desirable. We envisioned that, the condensation between hydroxylamine and α,β-unsaturated aldehydes/ketones[9] would be a suitable strategy to achieve this goal (Scheme 1).

Furthermore, there is a real need for discovery of new compounds endowed with anticancer activity, possibly acting through mechanisms of actions, which are distinct from those of well known classes of anticancer agents.[10] Thus, a single molecule containing more than one pharmacophore, each with different mechanism of action could be beneficial for the treatment of cancer. We have been interested in developing the DNA cleavage methods,

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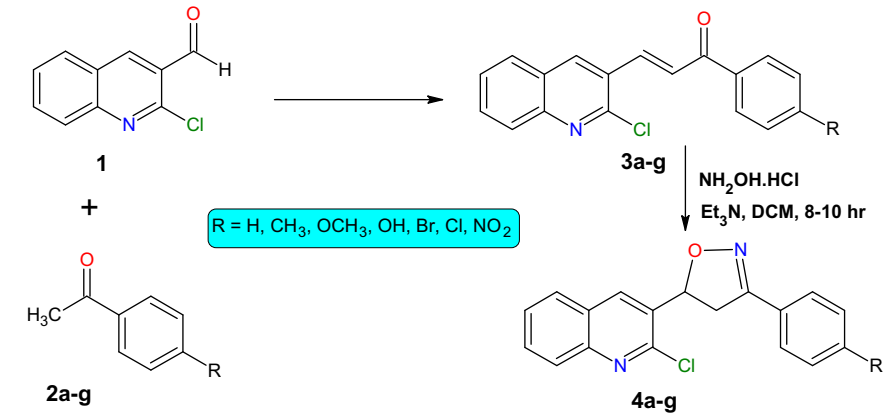
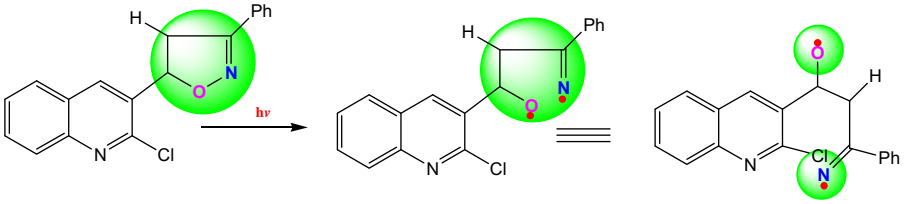


Scheme 1. Synthesis of 2-chloro-3-(5-aryl-4,5-dihydroisoxazol-3-yl)quinolines.



Scheme 2. Photo-irradiation of DIQs.

particularly by radical species. Taking inspiration from the above and as a part of our continuing research on the synthesis of biologically active substituted quinolines.[11] In similar manner, we have synthesized DIQs to cleave the pUC 19 DNA upon UV irradiation (Scheme 2). The classical synthesis of the title compounds involves the Claisen–Schmidt condensation of 2-chloroquinoline-3-carbaldehyde with substituted acetophenones to give quinolinyl chalcones (QC) **(3a–g)**,[12] which on cyclisation with hydroxylamine hydrochloride in presence of Et_3N base furnished 2-chloro-3-(5-aryl-4,5-dihydroisoxazol-3-yl)quinolines **(4a–g)** in good yields (Scheme 1). The results showed that, the presence of Et_3N in dichloromethane increases the yield of isoxazolines.

We have successfully obtained DIQ under the treatment of hydroxylamine with QC. The most efficient set of conditions were to employ 1.8 mmol of quinolinyl chalcones (QC), 64.0 mmol of hydroxylamine and 64.0 mmol of Et_3N in DCM at room temperature (Table 1 and Scheme 1 and 2). However, by using of triethyl amine in DCM found to be more efficient catalyst in terms of high yield and easy isolation of the products. As shown in Table 1, a series of DIQs were readily obtained in good to excellent yields from QC and hydroxylamine under the optimal conditions. In general, substrates containing an electron-withdrawing group were more active to offer higher yields (entries 4, 6, and 7). This result implied that electron density at the aromatic ring played an important role in this process. The synthesized DIQs (**4a–g**) were characterized by IR, ^1H NMR, ^{13}C NMR and mass spectral data.

It has been reported that most DIQ derivatives upon irradiation undergo N–O bond fission as the primary process and afford a variety of products depending on the structure of the starting materials.[13] On photo-irradiation, the weak N–O bond of the DIQ can be selectively cleaved to generate the iminyl and carboxy radicals which can then cause the cleavage of the DNA as shown in Scheme 2.

Recently, some small organic molecules were known to cleave DNA under UV–vis light.[11,14,15] When circular plasmid DNA is subject to electrophoresis, relatively fast migration is generally observed for the intact supercoiled Form I.[11,14] When scission occurs on one strand (nicking), the supercoil relaxes to generate a slower-moving, open-circular Form II. If both strands are cleaved, a linear Form III is generated, migrating between Form I and Form II.[11,14] The 2-chloro-3-(5-aryl-4,5-dihydroisoxazol-3-yl)quinolines (DIQs)[16] were photolysed at 365 nm, at different concentrations in the presence of pUC-19 DNA. Solutions were irradiated for 2 h, in 1:9 DMSO: Trisbuffer (20 μM, pH-7.2) at 365 nm. Figures 1–3 shows the Photo cleavage results of pUC 19 DNA by DIQ, it can be seen that no DNA cleavage was observed for control experiment in which the DIQ was absent (Lane 1).

However, the gel separation of pUC19 DNA was observed after incubation with different concentration of DIQ in the dark (Lane 2–8), while after irradiation at 365 nm for 2 h, in 1:9 DMSO/Trisbuffer (20 μM, pH 7.2). The Micromolar concentrations of the DIQ showed DNA cleavage as evidenced by the disappearance of Form I and appearance of Form II and Form III (Lane 2–8), demonstrating that the DNA cleavage occurred by photoinduced rather than the hydrolytic reaction pathway. Figure 1 indicates that, there is no moment of supercoiled Form I to the nicked Form II and it clears that, at lower concentrations (40 μM) DNA cleavage was not effective. However, with increasing the micromolar concentration of DIQ

Table 1
Synthesis of 2-chloro-3-(5-aryl-4,5-dihydroisoxazol-3-yl)quinolines

Entry[a]	R	Time (hrs)	Yield[b] (%)	M.P(°C)
4a	H	8–10	80	132–134
4b	*p*-CH_3	8–10	83	138–140
4c	*p*-OCH_3	8–10	85	141–143
4d	*p*-NO_2	8–10	90	158–160
4e	*p*-OH	8–10	85	162–164
4f	*p*-Cl	8–10	91	152–154
4g	*p*-Br	8–10	90	169–171

[a] All the products were characterized by elemental analysis, ^1H NMR, ^{13}C NMR and mass spectral data.
[b] Yields of isolated products.

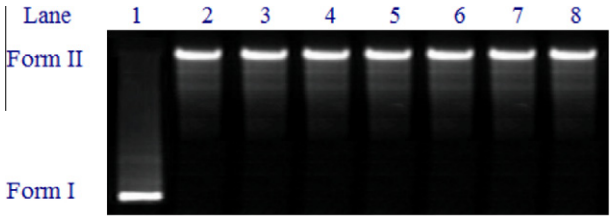


Figure 1. Photo cleavage of DNA by 2-chloro-3-(5-aryl-4,5-dihydroisoxazol-3-yl)quinolines were irradiated with UV light at 365 nm. Supercoiled DNA runs at position I (SC) and nicked DNA at position II (NC). Lane; 1: control DNA (without compound), Lane; 2: 40 μM (**2a**), Lane; 3: 40 μM (**2b**), Lane; 4: 40 μM (**2c**), Lane; 5: 40 μM (**2d**), Lane; 6: 40 μM (**2e**), Lane; 7: 40 μM (**2f**), Lane; 8: 40 μM (**2g**).

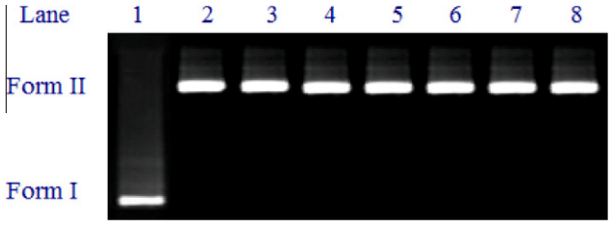


Figure 2. Light-induced cleavage of DNA by 2-chloro-3-(5-aryl-4,5-dihydroisoxazol-3-yl)quinolines with UV light at 365 nm. Supercoiled DNA runs at position I (SC) and nicked DNA at position II (NC). Lane; 1: control DNA (with out compound), Lane; 2: 60 μM (**2a**), Lane; 3: 60 μM (**2b**), Lane; 4: 60 μM (**2c**), Lane; 5: 60 μM (**2d**), Lane; 6: 60 μM (**2e**), Lane; 7: 60 μM (**2f**), Lane; 8: 60 μM (**2g**).

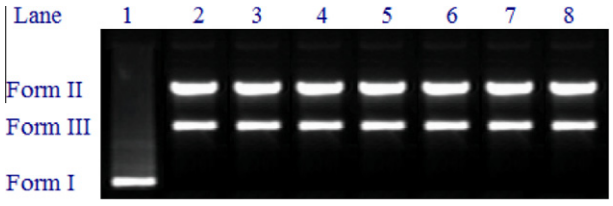


Figure 3. DNA Photo cleavage of 2-chloro-3-(5-aryl-4,5-dihydroisoxazol-3-yl)quinolines at 365 nm with UV light irradiation. Supercoiled DNA runs at position I (SC) and nicked DNA at position II (NC). Lane; 1: control DNA (without compound), Lane; 2: 80 μM (**2a**), Lane; 3: 80 μM (**2b**), Lane; 4:80 μM (**2c**), Lane; 5: 80 μM (**2d**), Lane; 6: 80 μM (**2e**), Lane; 7: 80 μM (**2f**), Lane; 8: 80 μM (**2g**).

form 40 μM to 60 μM (lanes 2–8), the amount of Form I diminished gradually with increasing in Form II of pUC 19 DNA as shown in Figure 2.

Further, the nucleolytic efficiencies of DIQs were investigated at the 80 μM concentration, the pUC19 DNA was completely converted in to form II and form III DNA (Fig. 3). But at higher concentrations (120 μM) was not examined because of the precipitation of DIQs will occurs in the reaction mixture. It was found that, DIQs are the most potent nuclease mimic in terms of molecular structure. Upon photo-irradiation, all the products from reactions may be attributed to the primary cleavage of the N–O bond of DIQs resulting in the formation of radicals, which affect the nucleolytic efficiency of 2-chloro-3-(5-aryl-4,5-dihydroisoxazol-3-yl)quinolines.

Under the similar experimental conditions, the QOE showed photoactivated DNA cleavage.[11]The DNA cleavage was due to H-abstraction by the nitrogen and oxygen radical resulting from N–O bond homolysis, which enhanced DNA Photo cleavage capacity of DIQ is similar to that of QOE.[11] All the substituted DIQs leading to spontaneous DNA cleavage (Fig. 3). The details of the mechanism of its DNA photoinduced cleavage remain to be studied in the future. The percentage of DNA cleavage with different concentrations of DIQs (40–80 μM) was shown in Figure 4. At the

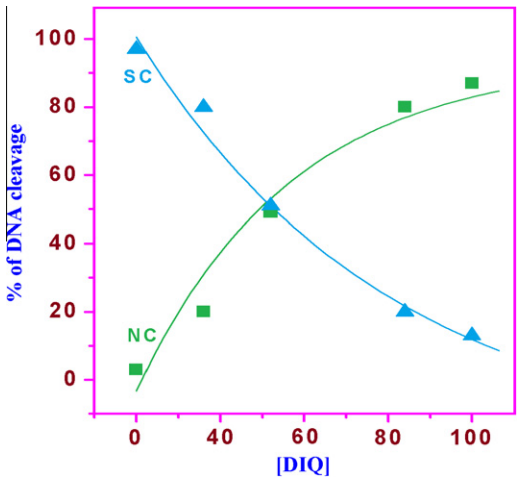


Figure 4. Plot representation the percentage of pUC 19 DNA cleavage with different concentrations of DIQs (40–80 μM) at 37 °C.

concentration of 40 μM, DIQs can almost promote 20% conversion of supercoiled DNA to nicked form DNA. Even at 60 μM concentration, the DIQs can exhibit about 50% conversion of form I to II. However, at the 80 μM concentration, the DIQs promoted almost 85% conversion supercoiled DNA to nicked and linear DNA.

In conclusion, an efficient approach for the highly regioselective synthesis of a diverse array of DIQ was developed. The synthesized DIQs were demonstrated as versatile synthons for the DNA Photo cleavage studies. Study showed that, the iminyl and carboxy radicals resulting from N–O bond homolysis of DIQs are highly reactive radicals, which abstracts hydrogen atoms efficiently at C-4′ of 2-deoxyribose of B DNA. The presences of aryl and quinoline core in DIQs were also responsible for spontaneous DNA photocleavage activity. The details of the mechanism of its DNA photoinduced cleavage remain to be studied in the future.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.034.

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16. General procedure for the preparation of 2-Chloro-3-(5-aryl-4,5-dihydroisoxazol-3-yl)quinolines: To a solution of 2-chloro-3-quinolinyl-3-phenylpropen-2-ones (**3a–g**) (500 mg, 1.8 mmol) in CH_2Cl_2 (30 mL) was added hydroxylamine hydrochloride (220 mg, 64.0 mmol) and Et_3N (5.0 mL, 64.0 mmol) at room temperature. After being stirred for 8–10 h, the reaction mixture was quenched with water and extracted with CH_2Cl_2, which was washed repeatedly with water and dried over anhydrous $NaSO_4$. Purification by flash column chromatography on silica gel *n*-hexane:EtOAc (1:1) gave (480 mg, 92%) as yellow needles (**4a–g**).