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PAPER

Synthesis, DNA binding, docking and photocleavage studies of quinolinyl chalcones

P. J. Bindu,*[a] K. M. Mahadevan,[a] T. R. Ravikumar Naik[b] and B.G. Harish[c]

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A series of simple quinoline-chalcone canjugates have been synthesized by Claisen Schmidt condensation reactions of substituted acetophenones with 2-Chloro-3-formyl-quinoline and evaluated for their nucleolytic activity. The structures of the synthesized quinoline-chalcone canjugates were confirmed by IR, ^{1}H NMR, ^{13}C NMR and Mass spectral analyses. Most of the prepared compounds showed significant DNA binding and photocleavage activitites. The incorporation of the electron-donating group into the ring A, caused a moderate increase in the DNA binding and photocleavage activities. The compounds **3c** and **3d** exhibited promising DNA photocleavage studies against pUC 19 DNA with 85% inhibition at 100 μM concentration. A structure–activity relationship analysis of these compounds was performed; the compounds **3c** and **3d** are potential candidates for future drug discovery and development.

Introduction

There is presently interest in low molecular weight ligands that can interact with nucleic acids in a sequence-selective manner. The development of therapies which are selective for tumour tissues is one of the most important goals in anticancer research, within this framework photodynamic therapy (PDT) can be considered as a very promising approach. Photodynamic therapy (PDT) is a minimally invasive treatment that destroys target cells in the presence of oxygen when light irradiates a photosensitizer, generating highly reactive singlet oxygen.[1] Singlet oxygen then attacks cellular targets, causing destruction through direct cellular damage, vascular shutdown, and activation of an immune response against targeted cells. PDT has several advantages over conventional therapies because of its noninvasive nature, its selectivity, the ability to treat patients with repeated doses without initiating resistance or exceeding total dose limitations (as associated with radiotherapy), the fast healing process resulting in little or no scarring, the ability to treat patients in an outpatient setting, and the lack of associated side effects.[2] Current clinical applications of PDT include the treatment of solid tumors in skin (basal cell carcinomas), lung, esophagus, bladder, head and neck, brain, ocular melanoma, ovarian, prostate, renal cell, cervix, pancreas, and bone carcinomas.[3-5]

On the other hand, photodynamic therapy (PDT) and vascular disrupting agents (VDA) each have their advantages in the treatment of solid tumors. Among the different types of photosensitizers being used in PDT, chalcones are the most extensively studied, due to their photophysical and biological properties.[6] Recently, chalcones act as vascular disrupting agents and destroy tumour neovasculature.[7] However, it was noticed that during VDAs the non targeted (unvascularised) cells lead the tumour to regrow at viable rim of tumor vasculature after VDA treatment. During this period the viable cells rapidly proliferates and recover their blood supply within 24 h. It is hence necessary to associate the VDAs with other therapy to block the blood supply to the tumor vasculature.[8]

The functionalized chalcone framework continues to occupy an important place in medicinal chemistry, due to the presence of a reactive ketovinyl moiety in the molecule and associated with flavonoid family. These functionalized chalcones are one of the major classes of natural products with widespread distribution in fruits, vegetables and soy bean based foodstuff have been recently subjects of great interest for their interesting pharmacological activities.[9] Chalcones have been reported to possess many useful properties, including antibacterial,[10] antifungal,[11] anticancer,[12] anti-inflammatory,[13] antitubercular,[14] antihyperglycemic,[15] antimalarial agents,[16] modulation of nitric oxide production[17] and so on. These compounds are important synthons for the preparation of five and six membered ring systems[18a] as well as intermediate in the synthesis of many pharmaceuticals.[18b] Having such a varied pharmacological activity and synthetic utilities, the chalcones have attracted the chemists to develop newer molecule for their biological activity.

We found that, most of the naturally occurring chalcones are hydroxylated in their aryl ring A and therefore such compounds have mainly been object of anticancer studies.[19] Little is known about the position effect of a phenolic group located in the aryl ring B. The electron donating groups are considered to play a significant role in anticancer activity of chalcones.[19] And also the SAR analysis of the cahalcones shows that, the presence and the position of alkoxy groups on both ring A and ring B favours the biological activity. In particular the hydroxy and methoxy substitution at 2′ and 4′ positions of ring A are more favours for the anticancer activity.[20]

Recently, quinoline and their derivatives have been extensively

explored for their biological, antimalarials, antimicrobial and antitumor activities.[21] Quinoline has attracted significant interest, mainly due to its aromaticity, chemical stability, low toxicity and it has been used as the most attractive pharmacophore for drug design and discovery. On the other hand, experimental evidence has proved the ability of quinolines as potential antitumor agents.[22]

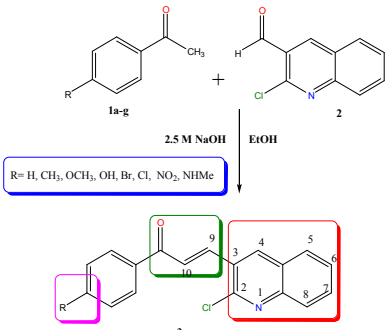
Thus, in continuation of our research on the synthesis of substituted quinoline derivatives,[23] herein we report the synthesis of quinolinyl chalcones with diverse substituents in the aromatic rings, mainly on ring A and probed for the nucleolytic activity. Our objective was to find the DNA cleavers for developing the new anticancer drugs based on the quinoline-chalcone skeleton.

Results and Discussion

Synthesis

By far the most popular way of synthesis of chalcones consists in base-catalyzed Claisen-Schmidt condensation of an appropriate acetophenone with benzaldehydes.[24] Number of reports has been published on the comparative studies of chalcone synthesis under both acidic and alkaline conditions.[25] In an ongoing project on the synthesis of quinolines anticancer drug, we have selected 2-chloro-3-formyl-quinoline as the starting material in the present investigation (Scheme 1).[26] The 2-chloro-3-formyl-quinoline thus obtained was confirmed by ^{1}H NMR and ^{13}C NMR spectral data. 2-Chloro-3-formyl-quinoline shows a singlet at δ 10.54 ppm for aldehydic (HC=O) proton, singlet at δ 8.73 ppm for proton C-4 position of quinolines moiety, a set of doublets at δ 8.06-8.04 (d, 1H), δ 7.98-7.96 (d, 1H), δ 7.89-7.85 (t, 1H) and δ 7.66-7.62 (t, 1H) for aromatic protons. The ^{13}C NMR spectra of 2-chloro-3-formyl-quinoline showed signal at δ = 189.07 (C=O), 150.03, 149.51, 140.22, 133.54, 129.66, 128.54, 128.08, 126.47, 126.31 ppm respectively.

In general, we observed that chalcones bearing electron-donating or electronically neutral groups were formed in better yields when compared to the chalcones bearing electron-withdrawing groups (Table 1). The desired products were obtained on an average yield of 84% after purification and their structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. The IR freqency of 3c was observed at,1656 (C=O), 1570, 1511 (C=C) cm^{-1}. The ^{1}H NMR spectrum showed the presence of methoxy functional group at δ = 3.90 and this was also confirmed by ^{13}C NMR at δ = 55.3.2 . The protons, H_α and H_β appeared at δ= 7.75 and δ= 8.15, respectively.



Scheme 1

Table 1. Scope of 3-(2-chloroquinolin-3-yl)-1-phenylprop-2-en-1-one

Products[a]	R	Time (min)	Yield(%)[b]	M.P. (°C)
3a	H	60-120	85	180-182[29]
3b	*p*-CH_3	60-120	87	151-153
3c	*p*-OCH_3	60-120	80	226-228
3d	*p*-OH	60-120	82	178-181
3e	*p*-Cl	60-120	85	170-173
3f	*p*-Br	60-120	88	187-189
3g	*p*-NO_2	60-120	80	165-167
3h	*p*-NHMe	60-120	73	172-175

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

Nucleolytic activity

The choice of rational screening strategy for new chemical entities constitutes a significant challenge for small academic organizations. The presently preferred target-oriented screening as a primary tool for evaluation of new compounds seems to be justified only if applied to dozens of different targets, as testing against a single target could result in a waste of precious potential of new chemical entities with potentially important biological properties.

Drug development has, for the most part, been based on the non-covalent interaction of small organic molecules (agonists, antagonists) with specific protein targets to elicit a desired pharmacological response. In many instances however, functionally orthogonal proteins can share structural features and/or mechanisms of action. This can result in unwanted side-effects if a drug binds indiscriminately to proteins other than the one for which it was intended.[27]

DNA-binding studies of 3-(2-chloroquinolin-3-yl)-1-phenylprop-2-en-1-ones

The DNA-binding modes of these QCs were investigated using absorption spectroscopy, fluorescence and viscosity measurements. Electronic absorption spectroscopy has been widely employed to determine the binding characteristics of small molecules with DNA. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm.[28] Relative binding of the QC to calf-thymus (CT-DNA) was studied by fluorescence and viscosity measurements with CT-DNA solution in tris–HCl/NaCl buffer (pH-7.2) at room temperature.[29]

Absorption spectral studies

Electronic absorption spectroscopy was an effective method for examining the binding mode of DNA with organic molecules.[28] If the binding mode was intercalation, the π* orbital of the intercalated ligand can couple with the π orbital of the base pairs, thus decreasing the π---π* transition energy and resulting in bathochromism. On the other hand, the coupling π orbital was partially filled by electrons, thus decreasing the transition probabilities and concomitantly resulting in hypochromism.[28a] The interaction of **3c** with CT-DNA was monitored by the blue shift in UV-visible spectra. The observed maximum wavelength of **3c** at 390 nm when it was mixed with CT-DNA [Fig**.** 1(a)]. Further, the Fig-1(b) shows the interaction of **3d** with CT-DNA at 410 nm. The intrinsic binding constants K_b of the 2-chloro-3-

quinolinyl-3-phenylpropen-2-ones with CT-DNA were determined and presented in Table 2.

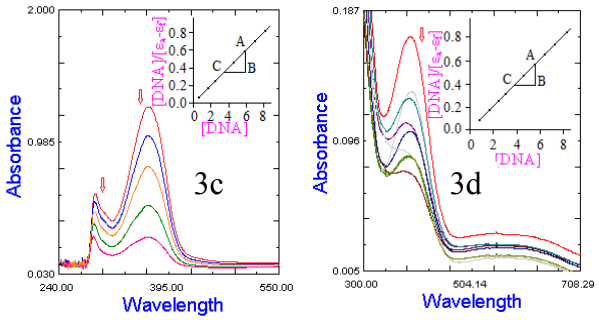


Fig. 1(a). UV absorption spectra of **3c** upon addition of calf-thymus (ds) DNA. **3c**; control [DNA] = 0.5 μM [---], [**3c**] + [DNA] =10 μM [---]; 20 μM [---]; 30 μM [---]; 40 μM [---] DNA respectively. Arrow shows the absorbance changing upon the increase of DNA concentration. The inner plot of [DNA]/(ε_a-ε_f) *vs* [DNA] for the titration of DNA with **3c**.

Fig. 1(b). UV absorption spectra of **3d** upon addition of calf-thymus (ds) DNA. **3d**; control [DNA] = 0.5 μM [---], [**3d**] + [DNA] =10 μM [---]; 20 μM [---]; 30 μM [---]; 40 μM [---]; 50 μM [---] DNA respectively. Arrow shows the absorbance changing upon the increase of DNA concentration.

When CT-DNA was added to the complex solutions, the electronic absorption spectra for QCs exhibited detectable hypochromism in the absorption intensities in all the chalcones studied. The percentage hypochromism of **3c** and **3d** were found to be 20.5, 23.7 % respectively. The intrinsic binding constants of **3c** and **3d** which were found to be 2.8×10^4 M^{-1} and 1.9×10^4 M^{-1}, respectively. The observed K_b values of QCs were compared with classicial intercalators (Figure 1 and Table-2).[29c,30] The resulting values suggested that, the stacking interaction between the QCs and the base pairs of DNA has intercalative binding.

Table 2. The Data of Binding, Docking Energy, Inhibition Constants and DNA Binding Constant by Docking and Absorption Spectral Study

Entry	Docking Energy (Kcal/mol)	Inhibition constant (M)	Nucleotide residues involved in H-bond	Bond Length (Å)	RMSD	DNA binding constant K_b
3a	-9.28	5.72 x 10^{-7}	DT7 DT19	2.77 2.56	1.1	5.2 x 10^4 M^{-1}
3b	-9.53	5.4 x 10^{-7}	DT18	2.69	1.0	4.9 x 10^4 M^{-1}
3c	-22.71	1.49 x 10^{-16}	CBR9	2.05	0.4	2.8 x 10^4 M^{-1}
3d	-22.72	1.42 x 10^{-16}	DA5	2.22	0.3	1.9 x 10^4 M^{-1}
3e	-9.16	5.62 x 10^{-7}	DT7 DT19	2.48 2.69	1.3	5.13 x 10^4 M^{-1}
3f	-9.2	4.68 x 10^{-7}	DT20	2.05	1.5	4.2 x 10^4 M^{-1}
3g	-9.44	5.31 x 10^{-7}	DT18	2.45	1.2	4.91 x 10^4 M^{-1}
3h	-8.45	1.45 x10^{-16}	DA5	2.02	0.2	3.5 x 10^4 M^{-1}

Fluorescence studies

To further clarify the binding of quinolinyl chalcone **3d** with DNA, the emission spectra were recorded in the absence and presence of CT-DNA, as shown in Figure 2. The emission intensity increased apparently when CT-DNA was added to the solution (Figure 2), indicating that the emission of this ligand was 'switched-on' by the DNA solution. The quenching constants K of QCs (3a-h) were calculated according to the classical Stern-Volmer equation (*cf*.ESI†).[31] The fluorescence quenching curves of QCs was shown in Figure 3 (*cf*.ESI†). At a ratio of [DNA]/[QC] = 4.0, the emission intensity is saturated and the relatively emission intensity (r = I/ I0) is ca. 1.8. This behavior may be explained by the fact that, the bound cations of **3d** are protected from the anionic water-bound quencher by the array of negative charges along the DNA phosphate backbone.[31] The fluorescence indicated that QCs binds to DNA by intercalation.

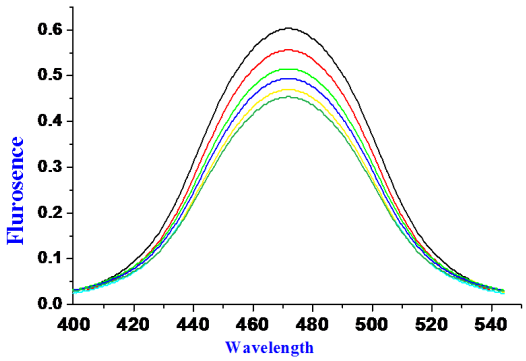


Fig. 2. The emission spectra of quinolinyl chalcone (**3d**) in the presence and absence of CT-DNA.

Viscosity Measurements

The results of the Electronic absorption spectroscopy and Fluorescence studies clearly indicate the strong binding of the QCs to the DNA. The viscosity experiments, being sensitive to the change of length of double helix DNA, were considered as one of the most unambiguous methods to determine the binding mode of complex to DNA in absence of crystal data.[32,33]

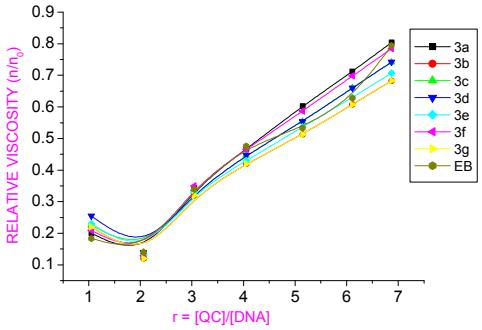


Fig. 4. Relative specific viscosity of ct DNA in the presence of QCs (**3a-g**) and ethidium bromide [---] as a function of the ligand-to-DNA ratio, *c*DNA = 1 mM bp in phosphate buffer.

In general, the relative viscosity of DNA in presence of ligand in an intercalation mode will be increased, because it separate the base pairs of DNA, and thus lengthen the DNA helix. The experiments on relative viscosity of rod-like CT-DNA in the presence of QC (3a-g), were analyzed as a plot of the cubic root of the relative viscosity of the solution *versus* the ligand-to-DNA ratio r and compared with the results obtained with ethidium bromide, i.e. a typical intercalator, under identical conditions were carried out and the results are shown in Figure 4. Thus, the viscosity mesurements supported to the above results that, the stacking interaction of these QCs with the base pairs of DNA and lengthens the DNA helix, indicating these QCs may bind to calf thymus DNA in a nonclassical intercalation mode.

DNA docking studies

Molecular docking techniques are an attractive scaffold to understand the drug-DNA interactions in rational drug design, as well as in the mechanistic study by placing a small molecule into the binding site of the target specific region of the DNA mainly in a non-covalent fashion.[28a]

In the present study, the quinolinyl chalcones were screened for targeted ds-DNA base pairs d(CGCGAATTCGCG)$_2$ dodecamer (PDB ID:1BNA) and provide an energetically favorable docked pose that is shown in Fig. 5 and Table 2. The result shows that, quinolinyl chalcone 3c and 3d which were showed highest affinity -22.71 and -22.72 kcal/mol docking energy, 1.49x10^{-16} and 1.42x10^{-16} estimated inhibition constants with an RMSD of 0.4 and 0.3 compare to the other derivatives. The Fig. 5a and 5b shows that, the quinolinyl chalcone **3c** and **3d** were completely enfolded in the entire binding pocket of ds-DNA. In this model, it is clearly indicated that the compound 3d formed hydrogen bonded between the -OH and N1 of adenine, which is DA5 with the bond length of 2.22 Å (Fig. 6a).[34]The molecular docking studies of QC 3h shows two hydrogens bonds at N1 and N9 of adenine respectively (Fig. 6b) (*cf*.ESI†).[31]

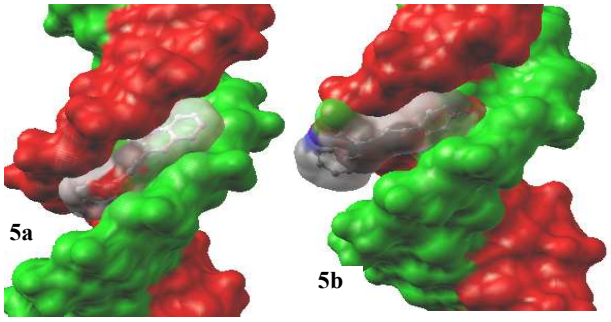


Fig. 5. View of the energy minimized docked poses of QC **3c** and **3d** with DNA d(CGCGAATTCGCG)$_2$ (PDB ID: 1BNA).

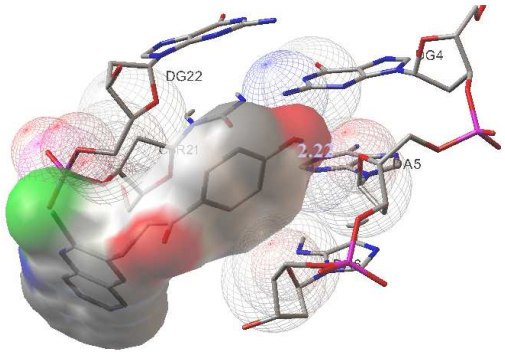


Fig. 6(a). A molecular docked model of QC **3d** showing chemically significant hydrogen-bonding interactions with DNA d (CGCGAATTCGCG)$_2$ (PDB ID: 1BNA).

Moreover, the other derivatives of QC formed less H-bond interaction with the DNA due to the orientation of aromatic ring involved in Van-der Waals interactions (Wireframe model) and flat hydrophobic regions of the binding sites of DNA (Table 2). Thus, we can conclude that there is a mutual complement between spectroscopic techniques and molecular docking, which can provide valuable information about the mode of interaction of the QCs with DNA and the conformation constraints for adduct formation.[34]

DNA Photocleavage studies

The H abstraction from C-4' is the most important process in DNA cleavage. The gel Electrophoresis was an effective method for examining the DNA cleavage studies.[35a,b] When circular plasmid DNA is subject to electrophoresis, relatively fast migration will be observed for the intact supercoil form (Form I). If scission occurs on one strand (nicking), the supercoil will relax to generate a slower-moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) that migrates at rates between Form I and Form II will be generated.[35b,c] The Fig. 7, shows gel electrophoresis separation of pUC19 DNA after incubation with different concentration of quinolinyl chalcones and irradiated for 2h, in 1:9 DMSO/trisbuffer (20 µM, pH- 7.2) at 365 nm. No DNA cleavage was observed for the control in which quinolinyl chalcones were absent (lane 1) (Fig. 7**)**. With increasing concentration of these quinolinyl chalcones (lanes 2-9), the amount of Form I of pUC 19 DNA diminished gradually, whereas Form II increased (Fig. 7**)**.

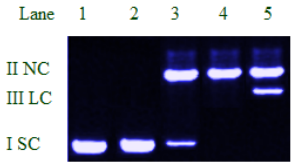


Fig. 7. Light-induced DNA cleavage by quinolinyl chalcones at different concentration. The 2-chloro-3-quinolinyl-3-phenylpropen-2-ones were irradiated with UV light at 365 nm. Lane; 1: control DNA (without compound), Lane; 2: 20µM (**3d**), Lane; 3: 40µM (**3d**), Lane; 4: 60µM (**3d**), Lane; 5: 80µM (**3d**).

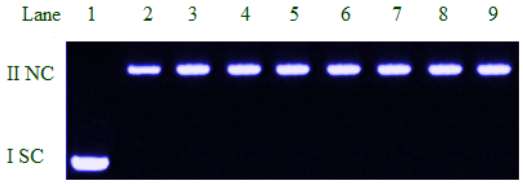


Fig. 8. Light-induced cleavage of DNA by quinolinyl chalcones at 365 nm. Lane; 1: control DNA (without compound), Lane; 2: 60µM (**3a**), Lane; 3: 60µM (**3b**), Lane; 4: 60µM (**3c**), Lane; 5: 60µM (**3d**), Lane; 6: 60µM (**3e**), Lane; 7: 60µM (**3f**), Lane; 8: 60µM (**3g**), 9; 60µM (**3h**).

On the other hand, the QC (**3a-g**) exhibited different cleaving efficiency for the plasmid DNA. At 40 µM concentration, the Compound (**3d**) can promote only 24% conversion of DNA from Form I to II (Fig. 7**)** . At the concentration of 80 µM, compound (**3d**) can almost promote the about 70% conversion of DNA from Form I to II (Fig. 7**)**. The Fig. 8, shows the DNA cleavage of pUC19 DNA at 60 µM concentration. However, other derivatives exhibits much lower cleaving efficiency for pUC 19 DNA. Compound **3h** shows moderate binding constant compare to other derivatives,but it fails in DNA cleavage studies. Even at the

concentration of 80 μM, it can promote only 40% conversion of DNA from form I to II (Fig. 9**)**. The compound **3c** also shows similar DNA cleavage activity compare to the other derivatives. But at higher concentrations around 140 μM, the compounds gets precipitated and there is no moment in the DNA. This reveals that, quinolinyl chalcones nuclease is capable to accelerate the cleavage of plasmid DNA is purely concentration dependent.The percentage of DNA cleavage with different concentrations of QCs (20-100 μM) was shown in Fig. 10. At the concentration of 20 μM and 40 μM, QCs can almost promote 8 and 24% conversion of supercoiled DNA to nicked form DNA. Even at 60 μM concentration, the QCs can exhibit about 50% conversion of form I to II. However, at the 80 μM concentration, the QCs promoted almost 70% conversion supercoiled DNA to nicked and 24 % linear DNA. Also, electrophoresis experiment showed that, the plasmid DNA cleavage depends on the substituent's of the molecule. Excited states of QCs are known to photocleave DNA by H-abstraction mechanisms.[35c,d] The alkoxy groups are highly reactive radicals, which abstracts hydrogen atoms efficiently at C-4' of 2-deoxyribose. It is of interest to note that hydroxyl group has been reported to bring about oxygen radical mediated DNA damage in the presence of photoirradiation.

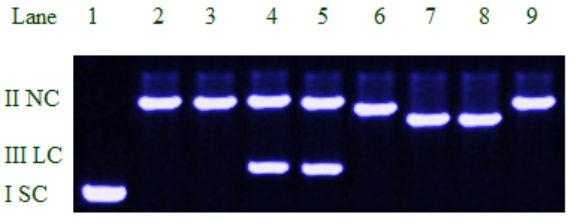


Fig. 9. Light-induced cleavage of DNA by quinolinyl chalcones at 365 nm. Supercoiled DNA runs at position I (SC), linear DNA at position III (LC) and nicked DNA at position II (NC). Lane; 1: control DNA (without compound), Lane; 2: 80μM (**3a**), Lane; 3: 80μM (**3b**), Lane; 4: 80μM (**3c**), Lane; 5: 80μM (**3d**), Lane; 6: 80μM (**3e**), Lane; 7: 80μM (**3f**), Lane; 8: 80μM (**3g**), 9; 60μM (**3h**).

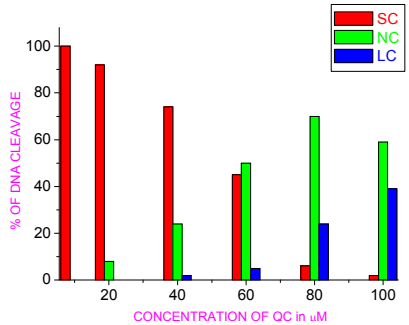


Fig. 10. Plot representation the percentage of pUC 19 DNA cleavage with different concentrations of quinolinyl chalcones (20-100 μM) at 37 °C.

Hence, the mechanism of cleavage of DNA by these QCs is most probably due to oxygen radical intermediate (hydroxy radical) abstracting hydrogen from the C-4' position of the deoxyribose sugar and subsequent cleavage of phosphodiester backbone as showed in Fig. 11. Calculations show that the (4') C-H bond is the weakest in 2-deoxyribose and the most sterically exposed in B-DNA. The molecules having halogen and nitro groups are less active compare to others.

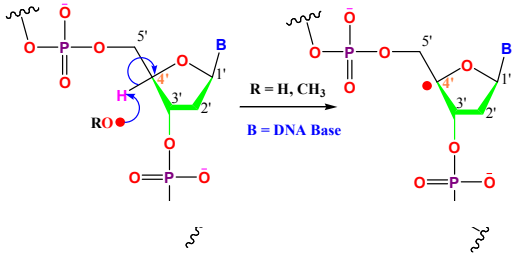


Fig. 11. Hydrogen abstraction from C-4' of DNA sugar

Structure–activity relationship using *in silico* analysis

Structure–activity relationship is the relationship of chemical structure and biological activity of drug molecule. The study involving the structural modification of the drugs with the systematic fashion and determination of undesired effects or low bioactivity. Osiris Property Explorer is one such knowledge based activity prediction tool which predicts drug likeliness, drug score and undesired properties such as mutagenic, tumorigenic, irritant and reproductive effect of novel compounds based on chemical fragment data of available drugs and non-drugs as reported.[36]

Table 3. Drug likeliness properties of synthesized (E)-3-(2-Chloroquinolin-3-yl)-1-phenylprop-2-en-1-ones (3a-3h).[36]

Entry	Mol. wt	*C* log *P*	Drug-likeness	Drug-score	Toxicity risks[a]			
					M[b]	T[c]	I[d]	R[e]
3a	293	4.32	0.58	0.49	(+)	(+)	(+)	(+)
3b	307	4.67	0.34	0.42	(+)	(+)	(+)	(+)
3c	323	4.25	1.32	0.53	(+)	(+)	(+)	(+)
3d	309	3.98	1.66	0.60	(+)	(+)	(+)	(+)
3e	327	4.93	3.26	0.46	(+)	(+)	(+)	(+)
3f	371	5.05	-1.82	0.25	(+)	(+)	(+)	(+)
3g	340	3.72	1.28	0.18	(-)	(+)	(+)	(-)
3h	322	3.92	0.64	0.30	(+)	(-)	(+)	(+)

Ranking as (+) no bad effect, (+/−) medium bad effect, (−) bad effect.

b; M (mutagenic effect).

c; T (tumorigenic effect).

d; I (irritant effect).

e; R (reproductive effect).

Quinolinyl chalcones with electron-donating substituent like hydroxy and methoxy groups on the ring A increases DNA binding and DNA cleavage activity. A lone pair of electrons on oxygen atom of methoxy group delocalizes into the π space of benzene ring, thereby increasing the activity. Similarly, electron-withdrawing substituents, such as halogens, lower the activity. The quinoline chalcones with the 4-hydroxy and 4-methoxy A ring show the highest cytotoxicities, as expected for DNA photocleavage studies. Replacement of a hydrogen or hydroxyl group by a halogen atom is a strategy widely used in drug development to alter biological function.[37] However the result shows that, the activity of halogen substituted QC (3e) was significantly lower. Therefore, designing chalcone derivatives with electron-withdrawing substituents on the ring A increases

DAN binding and cleavage activity. Observed and predicted analysis of quinolinyl chalcone derivatives are presented in Table 3.

Conclusion

In summary, we have synthesized substituted quinolinyl chalcones and evaluated their nucleolytic activity. The binding behavior of QCs with DNA was studied by UV spectra, viscosity, fluorescence and gel retardation assay under physiological conditions. Moreover, the DNA docking studies suggested that, the sample 3c, and 3d were completely enfolded in the entire binding pocket of ds-DNA (Fig. 5a and 5b respectively). Thus, an available proton donor in B ring, which is involved in intramolecular hydrogen bonding with N1 of adenine, plays a crucial role in the DNA binding studies of QCs. All the experimental evidences indicate that these QCs can strongly bind to CT DNA via an intercalation mechanism. Furthermore, we clearly demonstrated that an efficient DNA damage may be induced on irradiation of QCs of 3c, and 3d with pUC19 DNA. Upon photoirradiation, the compounds 3c, and 3d are highly reactive radicals which abstracts hydrogen atoms efficiently at C-4' of 2-deoxyribose of B DNA. Results obtained from our present work would be very useful to understand the mechanism of interactions of the small molecules binding to DNA and helpful in the development of their potential applications in biological, pharmaceutical and physiological fields in future.

Experimental

Materials and equipment

All the chemicals used in the present study are of AR grade. Whenever analytical grade chemicals were not available, laboratory grade chemicals were purified and used. Calf thymus DNA (CT DNA) and supercoiled pUC19 DNA (cesium chloride purified) was obtained from Bangalore Genei (India). Agarose (low melt, 65°C, molecular biology grade for DNA gels), ethidium bromide, bromophenol blue, Tris(hydroxymethyl)aminomethane (Tris), sodium chloride, ethylene diamine tetraacetic acid disodium salt (EDTA-Na_2), sodium azide, were of molecular biology grade, obtained from Himedia (India).

Melting points were recorded on an open capillary tube with a Buchi melting point apparatus and are uncorrected. Elemental analyses were carried out using Perkin-Elmer 240C CHN-analyzer. IR spectra were recorded on a FT-IR infrared spectrophotometer. ^{1}H- NMR spectra were obtained using a 300 MHz and 400 MHz on a Bruker spectrometer (chemical shifts in δ ppm). Mass spectra were recorded using a micro spray Q-TOF MS ES Mass spectrometer.

General procedure for the preparation 3-(2-chloroquinolin-3-yl)-1-phenylprop-2-en-1-ones

To a stirred solution of acetophenone (0.6 g, 5.2 mmol) and (1.0 g, 5.2 mmol) 2-chloro-3-formylquinoline dissolved in 10 mL 95 % ethanol containing 2 mL 2.5 M NaOH (aq). The resulting solution was stirred at room temperature and within five minutes the mixture was precipitated. After one hour, the reaction mixture was kept over night at 0-5 °C and then it was poured into cold water, filtered, and the solid rinsed with water and cold ethanol. The crude product thus obtained was recrystallized from MeOH to obtain desired product (Scheme 1, Table 1).

(E)-3-(2-chloroquinolin-3-yl)-1-phenylprop-2-en-1-one (3a)[38]: M.p: 180-182 °C; IR (Neat): 1680 (C=O), 1559 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.45-7.56 (d, 2H, *J* = 8.0, Ar-H), 7.61-7.64 (t, 1H, *J* = 8.0), 7.76-7.77, (d, 1H, *J* = 8.0), 7.78-7.79 (d, 1H, *J* = 8.0, Ar-H), 7.86-7.90 (d, 1H, H_α, *J* = 16.0), 7.96-7.98 (d, 1H, *J* = 8.0), 8.07-8.17 (d, 1H, *J* = 8.0), 8.19-8.23 (d, 1H, H_β, *J* = 16.0), 8.53 (s, 1H) ppm; MS (m/z) 316 (M+23), Calcd (293), Found (316); Anal. Calcd (%) for $C_{18}H_{12}ClNO$: C; 73.60, H; 4.12, N; 4.77. Found: C; 73.58, H; 4.10, N; 4.75.

(E)-3-(2-chloroquinolin-3-yl)-1-(4-methylphenyl)prop-2-en-1-one (3b): M.p: 151-153 °C; IR (Neat): 1662 (C=O), 1487 (C=C) cm^{-1}; cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.43 (s, 3H, CH_3), 7.30-7.32 (d, 2H, *J* = 8.0, Ar-H), 7.35 (d, 1H, *J* = 8.0, Ar-H), 7.57-7.58 (d, 1H, *J* = 8.0), 7.59-7.64 (d, 1H, H_α, *J* = 16.0), 7.73-7.77 (t, 1H, *J* = 8.0), 7.85-7.88 (t, 1H, *J* = 8.0), 7.95-7.97 (d, 1H, *J* = 8.0), 7.99-8.01 (d, 1H, *J* = 8.0), 8.14-8.18 (d, 1H, H_β, *J* = 16.0), 8.50 (s, 1H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 42.3 (CH_3), 126.2, 126.9, 127.2, 127.9, 128.5, 128.6(Cq), 131.5, 133.2, 134.8(Cq), 137.4(Cq), 139.2(Cq), 146.2(Cq), 147.7(Cq), 150.3(Cq), 150.5(Cq), 189.7 ppm; MS (m/z) 330 (M+23), Calcd (307), Found (330); Anal. Calcd (%) for $C_{19}H_{14}ClNO$: C; 74.15, H; 4.58, N; 4.55. Found: C; 74.13, H; 4.56, N; 4.53.

(E)-3-(2-chloroquinolin-3-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (3c): M.p: 226-228 °C; IR (Neat): 1656 (C=O), 1570, 1511 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.90 (s, 3H, OCH_3), 6.99-7.01 (d, 2H, *J* = 8.0, Ar-H), 7.60-7.64 (2d, 2H, *J* = 8.0, Ar-H), 7.75-7.79 (t, 1H, H_α, *J* = 16.0), 7.87-7.89 (d, 1H, *J* = 8.0), 8.01-8.03 (d, 1H, *J* = 8.0), 8.06-8.08 (d, 1H, *J* = 8.0), 8.15-8.19 (d, 1H, H_β, *J* = 16.0), 8.50 (s, 1H), ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 52.32 (OCH_3), 110.7, 123.0, 123.7, 124.4, 124.7, 125.0, 125.2(Cq), 127.2(Cq), 127.8(Cq), 128.2(Cq), 132.8, 135.2(Cq), 144.5(Cq), 147.1(Cq), 160.5(Ar-O), 184.7 (C=O) ppm; MS (m/z) 346 (M+23), Calcd (323), Found (346); Anal. Calcd (%) for $C_{19}H_{14}ClNO_2$: C; 70.48, H; 4.36, N; 4.33. Found: C; 70.46, H; 4.34, N; 4.31.

(E)-3-(2-chloroquinolin-3-yl)-1-(4-hydroxyphenyl)prop-2-en-1-one (3d): M.p: 178-181°C; IR (Neat): 1653 (C=O), 1459 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.92-6.94 (d, 2H, *J* = 8.0, Ar-H), 6.96-7.00 (d, 1H, H_α, *J* = 16.0), 7.05-7.07 (d, 1H, *J* = 8.0, Ar-H), 7.49-7.51 (d, 1H, *J* = 8.0), 7.51-7.53 (d, 1H, *J* = 8.0), 7.71-7.88 (t, 1H, *J* = 8.0), 7.92-7.96 (d, 1H, H_α, *J* = 16.0), 8.00-8.17 (t, 1H, *J* = 8.0), 8.31-8.35 (d, 1H, H_β, *J* = 16.0), 8.79 (s, 1H), 10.45 (OH, 1H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 113.8, 126.1, 126.8, 127.5, 127.8, 128.1, 128.3(Cq), 130.3(Cq), 130.9(Cq), 131.3(Cq), 135.9, 138.3(Cq), 147.6(Cq), 150.2(Cq), 163.6(Ar-O), 187.8 (C=O) ppm; MS (m/z) 332 (M+23), Calcd (309), Found (332); Anal. Calcd (%) for $C_{18}H_{12}ClNO_2$: C; 69.80, H; 3.90, N; 4.52. Found: C; 69.78, H; 3.88, N; 4.53.

(E)-3-(2-chloroquinolin-3-yl)-1-(4-chlorophenyl)prop-2-en-1-one (3e): M.p: 170-173 °C; IR (Neat): 1604 (C=O), 1459 (C=C)

cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.24-7.28 (d, 1H, *J* = 8.0), 7.40-7.42 (d, 2H, *J* = 8.0), 7.59-7.63 (t, 1H, *J* = 8.0), 7.78-7.82 (d, 1H, H_α, *J* = 16.0), 7.94-7.97 (d, 2H, *J* = 8.0, Ar-H), 8.04-8.06 (d, 1H, *J* = 8.0), 8.17-8.19 (d, 2H, *J* = 8.0, Ar-H), 8.32-8.36 (d, 1H, H_β, *J* = 16.0), 8.62 (s, 1H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 119.6, 119.7, 127.0, 127.1, 128.0, 128.9, 128.9, 129.9(Cq), 130.1(Cq), 134.4(Cq), 136.2(Cq), 137.0, 139.8(Cq), 146.3(Cq), 150.3(Cq-Cl), 196.5 (C=O) ppm; MS (m/z) 350 (M+23), Calcd (327), Found (350); Anal. Calcd (%) for $C_{18}H_{11}Cl_2NO$: C; 65.87, H; 3.38, N; 4.27. Found: C; 65.85, H; 3.36, N; 4.25.

(E)-3-(2-chloroquinolin-3-yl)-1-(4-bromophenyl)prop-2-en-1-one (3f): M.p: 187-189 °C; IR (Neat): 1607 (C=O), 1511 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37-7.39 (d, 1H, *J* = 8.0, Ar-H), 7.57-7.60 (t, 1H, *J* = 8.0), 7.74-7.76 (d, 1H, *J* = 8.0), 7.79-7.81 (d, 2H, *J* = 8.0, Ar-H), 7.82-7.86 (d, 1H, H_α, *J* = 16.0), 7.98-7.80 (d, 1H, *J* = 8.0), 8.14-8.16 (d, 1H, *J* = 8.0), 8.29-8.33 (d, 1H, H_β, *J* = 16.0), 8.51 (s, 1H) ppm; MS (m/z) 395 (M+23), Calcd (372), Found (395); Anal. Calcd (%) for $C_{18}H_{11}BrClNO$: C; 58.02, H; 2.98, N; 3.76. Found: C; 58.00, H; 2.96, N; 3.74.

(E)-3-(2-chloroquinolin-3-yl)-1-(4-nitrophenyl)prop-2-en-1-one (3g): M.p: 165-167 °C; IR (Neat): 1658 (C=O), 1523 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.25-7.28 (t, 1H, *J* = 8.0), 7.35-7.37 (d, 1H, *J* = 8.0), 7.58-7.61 (t, 1H, *J* = 8.0), 7.73-7.75 (d, 1H, *J* = 8.0, Ar-H), 7.81-7.83 (d, 1H, *J* = 8.0, Ar-H), 7.85-7.89 (d, 1H, H_α, *J* = 16.0), 8.03-8.05 (d, 1H, *J* = 8.0), 8.19-8.23 (d, 1H, H_β, *J* = 16.0), 8.60 (s, 1H) ppm; MS (m/z) 361 (M+23), Calcd (338), Found (361); Anal. Calcd (%) for $C_{18}H_{11}ClN_2O_3$: C; 63.82, H; 3.27, N; 8.27. Found: C; 63.80, H; 3.25, N; 8.25.

(E)-3-(2-chloroquinolin-3-yl)-1-(4-(methylamino)phenyl)prop-2-en-1-one (3h): M.p: 165-167 °C; IR (Neat): 1647 (C=O), 1590, 1552 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.875 (S, $NHCH_3$), 7.33-7.35 (d, 1H, *J* = 8.0), 7.38-7.40 (d, 1H, *J* = 8.0), 7.41-7.42 (d, 2H, *J* = 8.0, Ar-H), 7.58-7.63 (t, 1H, *J* = 8.0), 7.75-7.79 (t, 1H, *J* = 8.0), 7.95 (d, 1H, H_α, *J* = 16.0), 8.03-8.05 (d, 1H, *J* = 8.0, Ar-H), 8.06-8.10 (d, 1H, H_β, *J* = 16.0), 8.61 (s, 1H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): ^{13}C NMR (400 MHz, $CDCl_3$): δ = 33.1 (CH_3), 117, 120.0, 123.4, 124.5, 127.0, 127.4, 127.6, 128.2(Cq), 128.3, 130.0(Cq), 131.2(Cq), 136.8(Cq), 138.1(Cq), 147.1(Cq), 149.9(Cq), 186.1 ppm.

UV-visible absorbance spectral studies[28]

All the experiments involving interaction of the quinolinyl chalcones with CT-DNA were carried out in doubly distilled H_2O buffer containing 5 mM Tris and 50 mM NaCl and adjusted to pH-7.2 with Tris-HCl buffer. A solution of CT-DNA gave a ratio of UV absorbance at 260 and 280 nm of about 1.8-1.9, indicating that the CT-DNA was sufficiently free of protein. The CT-DNA concentration per nucleotide was determined spectrophotometrically by employing an extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm. The quinolinyl chalcones were dissolved in a solvent mixture of 10% DMSO and 90% Tris-HCl buffer (5 mM Tris–HCl, 50 mM NaCl, pH- 7.2) at concentration 10.0 x 10^{-6} M. An absorption titration experiment was performed by maintaining the 10 μM compounds and varying the concentration of nucleic acid. While measuring the absorption spectra, an equal amount of CT-DNA was added to both the compound solution and the reference solution to eliminate the absorbance of CT-DNA itself. The absorption data were analyzed for an evaluation of the intrinsic binding constant K_b. The observed values for the quinolinyl chalcones were then calculated by the equation 1 to obtain the intrinsic binding constant K_b.

Fluorescence spectra[31]

Fluorescence spectra were recorded on a Varian Cary Eclipse Fluorescence Spectrophotometer. Spectrophotometric measurements were performed in thermostated quartz sample cells of 10 mm pathlength at 20 °C. The CT-DNA concentration per nucleotide was determined spectrophotometrically by employing an extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm. The quinolinyl chalcones were dissolved in a solvent mixture of 10% DMSO and 90% Tris-HCl buffer (5 mM Tris–HCl, 50 mM NaCl, pH-7.2) at concentration 10.0 x 10^{-6} M. The prepared solutions were placed into quartz cells and titrated with the titrant solutions in intervals of 0.5-2 equivalent, and absorption spectra were recorded. All spectrophotometric titrations were performed at least 2-3 times to ensure the reproducibility.

Viscometric titration

Viscosity measurements were carried out using a semimicro dilution capillary viscometer at room temperature. Aliquots of the solution of the ligand in Tris-HCl buffer were added to the ct DNA solution (1 mM base pair in the Tris-HCl buffer). Flow times were measured after a thermal equilibration period of 5 min. Each flow time was measured three times and an average flow time value was calculated. As a reference ethidium bromide was employed under identical conditions. The relative viscosity was presented as $(\eta/\eta_0)^{1/3}$.

Molecular docking[28]

The quinolinyl chalcones were designed and the structures were analyzed by using Chem-Draw Ultra 6.0 and it was subjected for geometrical optimization using MM2 and energy minimized by steepest gradient method in Chem3D ultra 6.0. The final selected conformation of quinolinyl chalcones was tested for Lipinski's rule, drug toxicity and other properties through pre ADMET server. The small-molecule topology generator prodrug server automatically generates the coordinates for all the quinolinyl chalcones. Automated docking was used to determine the orientation of inhibitors bind to the ds-DNA. A genetic algorithm method, implemented in the program Auto-Dock 3.0, was employed. The crystal structure of the B-DNA dodecamer, $d(CGCGAATTCGCG)_2$ (NBD code GDLB05) was obtained from the protein data bank. The coordinates for the heteroatom including water and other small molecules were removed. This structure was later added with polar hydrogens and kollmann charges to remove nonintergral chargers. For docking calculations, Gasteigere Marsili partial charges were assigned to the quinolinyl chalcones and nonpolar hydrogen atoms were merged. All torsions were allowed to rotate during docking.

Lennard- Jones parameters 12-10 and 12-6, supplied with the program, were used for modelling H-bonds and van der Waals interactions, respectively. The distance-dependent dielectric permittivity of Mehler and Solmajer was used to calculate the electrostatic grid maps. Random starting points, random orientation, and torsions were used for all ligands. The translation, quaternion, and torsion steps were taken from default values in Auto-Dock. The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were applied for minimization, using default parameters. The number of docking runs was 50, the population in the genetic algorithm was 250, the number of energy evaluations was 100,000, and the maximum number of iterations 10,000.

DNA Photocleavage by Gel Electrophoresis[21a,28]

For the gel electrophoresis experiments, supercoiled pUC19DNA (0.5μg) in Tris–HCl buffer (50mM) with 50mM NaCl (pH 7.2) was treated with quinolinyl chalcones (40 and 80μM) and the solution was irradiated for 2h, in 1:9 DMSO: trisbuffer (20 μM, pH- 7.2) at 365 nm (10 W). After irradiation, the solution was incubated at 37 °C for 1 h. A loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol. Electrophoresis was carried out for 3 h at 50V on a 0.8% agarose gel in Tris–boracic–EDTA buffer. The gel was stained with 1.0μg/ml ethidium bromide. Bands were visualized using UV light and photographed. The cleavage efficiency was measured by determining the ability of the quinolinyl chalcones to convert the supercoiled DNA (SC) to nicked circular form (NC) and linear form (LC).

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Notes and references

[a]*Department of Studies and Research in Chemistry, Kuvempu University, Shankaraghatta- 577 451, INDIA. Tel: +91 9900792675; E-mail:bindu12_naik@rediffmail.com*
[b]*Department of Canter of Nano Science and Engineering, Indian Institute of Science, Bangalore 560 012, INDIA.*
[c]*Department of Biotechnology, M.S. Ramaiah Institute of Technology, MSR Nagar, Bengaluru, Karnataka, INDIA.*

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