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Synthesis, DNA binding, photocleavage, molecular docking studies of 2-oxo-3-quinoline-3-oximes

P.J. Bindu,[1] T.R. Ravikumar Naik,*[,2] S. Chandrasekar,[1,2] K.M. Mahadevan,[2,3]

[1]Department of Organic Chemistry, Indian Institute of Science, Bangalore 560 012, India. [2]Centre for Nano Science and Engineering (CeNSE), Indian Institute of Science, Bangalore 560 012, India. [3]Department of Studies and Research in Chemistry, Kuvempu University, Shankaraghatta 577 451, India.

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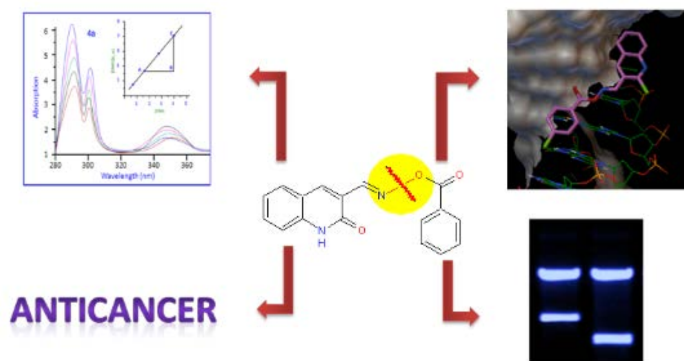
ABSTRACT



A series of 2-oxo-quinoline-3-carbaldoxime esters (OQCE) were designed and synthesized based on the 2-oxo-quinoline structure core as novel anticancer agents. The primary results shows that most of the compounds had moderate cytotoxicities against both tested human cancer cells. The oxygen analogue with alkoxy substituent significantly affected the cancer cells compare to other derivatives. Further, DNA binding, molecular docking and photonuclease studies activities of 2-oxo-quinoline-3-carbaldoxime esters (OQCE) were investigated. The experiment results showed that the prepared compounds have a good DNA binding and photocleavage activity. The molecular simulation results indicate that the presence of oxygen at second position of quinoline core structure improve the binding of designed molecules to DNA protein.

Keywords: 2-Oxo-quinoline-3-carbaldoxime esters, DNA Binding, DNA Photocleavage, Molecular docking, Anticancer activity

INTRODUCTION

Cancer is the cause of one-quarter of all deaths in developed countries. It is now the second leading cause of death in the United States, and is anticipated to surpass heart diseases as the leading cause of death in the futures.[1] Hence, the discovery of novel therapeutic agents for advanced invasive cancers is at the forefront of preclinical and clinical research.[2] The subject of tumor targeted treatment has drawn more and more attention as a result of increasing knowledge of biology and physiology. A tumor specific agent with high therapeutic index, good bioavailability and easy preparation is highly preferred.[3] To reach this goal, rational design and development of synthetic advanced compounds based on active pharmacophore remain as the most successful strategy in cancer chemotherapy.[4–8]

*Corresponding Author: Dr. T.R. Ravikumar Naik
Address: Centre for Nano Science and Engineering, Indian Institute of Science, Bangalore, India
Tel: +91 9900792675 E-mail: naikravi7@gmail.com

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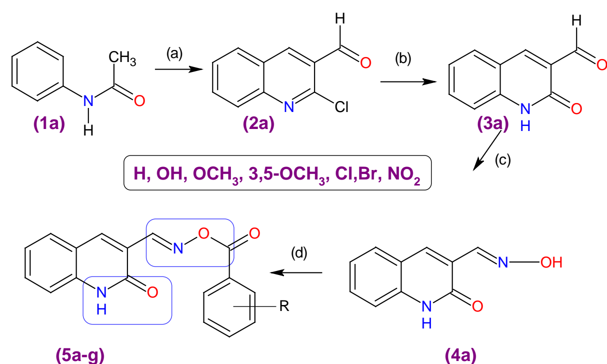
Metal-complexes are the foremost and widely used anticancer drugs for cancer therapy, but these possess inherent side effects, solubility issues and acquired drug resistance. Therefore, considerable attempts are being made to replace these drugs with suitable alternatives, and numerous small molecules have been synthesized and tested for their anticancer activities.[9] In the heterocyclic small molecules related to interest of this study, 2-oxo-quinoline have been reported to show significant biological activities such as anticancer, antiproliferation and anti-inflammation.[10]

In addition, quinoline derivatives have received considerable attention because of their pivotal role in various biological processes and numerous derivatives of quinolines have been reported to have wide biological activities including the anticancer activity.[11–14] Therefore, in a search for novel antitumor agents with an expected nucleolytic activity, we report the synthesis of a new series of 2-oxo-quinoline-3-carbaldoximes bearing an oxime ester moiety. Recently, the chemistry of oximes and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance.[15–17]

In our attempts to search for novel antitumor agents,[18] we extended the interest to 2-oxo-quinolines and synthesized 2-

oxoquinoline-3-carbaldoxime esters with the aim of evaluating their DNA binding, molecular docking and photonuclease studies (Scheme 1). From our previous report, the quinoline oxime was retained and attention was focused predominantly on the introduction of 2-oxo-substituent on the quinoline aromatic ring. The **5a** 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 anticancer drugs particularly by radical species. After the successful substitution of oxygen to quinoline ring, more attention was focused on alkoxy substituents of the phenyl ring of the oxime ester to improve anticancer activity.



Scheme 1: Synthetic route of 2-oxo-quinoline-3-carbaldoxime esters. Reagents and conditions: (a) DMF/$POCl_3$, 90°C; (b) 70% acetic acid, reflux, 7-8 h; (c) $NH_2OH.HCl$, Et_3N, EtOH, rt; (c) RCOCl, Et_3N, CH_2Cl_2, 0°C.[19]

Table 1. Scope of 2-oxo-quinoline-3-carbaldoxime esters

Products [a]	R	Time (hr)	Yield(%)[b]	M.P. (°C)
5a	H	7-8	84	208-210
5b	4-OH	7-8	81	258-260
5c	4-OCH_3	7-8	83	232-234
5d	3,5-OCH_3	7-8	80	240-242
5e	4-Cl	7-8	85	265-268
5f	4-Br	7-8	80	272-274
5g	4-NO_2	7-8	78	310-312

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

The white shiny colored quinoline oxime **4a** was prepared from 2-oxo-quinoline with hydroxylamine hydrochloride in the presence of Et_3N in ethanol.[19] The OQOE were prepared according to the published procedure.[19] Reaction of the mixture of quinoline oxime **4a** with substituted benzoyl chlorides followed by Et_3N in the presence of dichloromethane gave **5a–g** shown in Scheme 1, Table 1. The desired products were obtained on an average yield of 80-85% after purification. The target compounds OQCE were successfully prepared by the reaction of acetic acid with ethanol in excellent yield (80%). The product was then recrystallized from dichloromethane or ethanol, affording the product as white solid. The structures and yields of these compounds were shown in Table 1. All newly synthesized compounds were well identified by their structures were established with IR, ^{1}H NMR, ^{13}C NMR and mass spectra. The target compounds were prepared using a simple three-step method (Scheme 1).

DNA-binding studies of 2-oxo-quinoline-3-carbaldoxime esters.

The DNA-binding modes of these OQCEs were investigated using absorption spectroscopy 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.[20,21] Relative binding of the OQCEs to CT DNA was studied by fluorescence and viscosity measurements with CT DNA solution in tris–HCl/NaCl buffer (pH 7.2) at room temperature.[20,21]

Absorption spectral studies

Electronic absorption spectroscopy was an effective method for examining the binding mode of DNA with organic molecules.[20,21] 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.[20,21] The interaction of **5a** with CT-DNA was monitored by the blue shift in UV-visible spectra. The observed maximum wavelength of **5a** at 320 nm when it was mixed with CT-DNA [Fig. 1(a)]. The intrinsic binding constants K_b of the 2-oxo-quinoline-3-carbaldoxime esters with CT-DNA were determined and presented in Table 2.

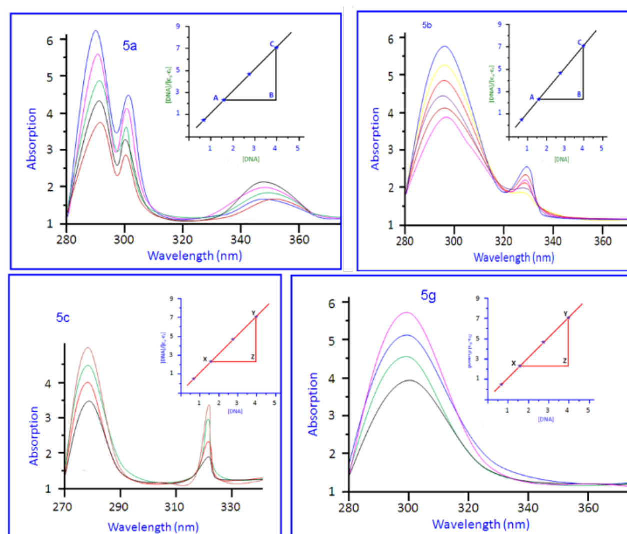


Figure 1. UV absorption spectra of 4b upon addition of calf thymus (ds) DNA. 5a; control [DNA] = 0.5 μM [---], [5a] + [DNA] =10 μM [---]; 20 μM [---]; 30 μM [---]; 40 μM [---] DNA respectively. Arrow shows the absorbance changing upon the increase of DNA concentration. The inner plot of [DNA]/(εa-εf) vs [DNA] for the titration of DNA with 5a.

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
5a	-13.40	5.23 x 10^{-7}	DT7 DT19	2.50 2.52	2.4	5.5 x 10^{4} M^{-1}
5b	-19.64	2.56 x 10^{-16}	DT18	2.63	2.0	2.8 x 10^{4} M^{-1}
5c	-18.29	2.39 x 10^{-16}	CBR9	2.50	2.1	2.9 x 10^{4} M^{-1}
5d	-18.20	2.75 x 10^{-16}	DA5	2.53	2.0	3.1 x 10^{4} M^{-1}
5e	-12.55	5.40 x 10^{-7}	DT7 DT19	2.52 2.54	2.5	5.6 x 10^{4} M^{-1}
5f	-12.88	5.43 x 10^{-7}	DT20	2.56	2.5	5.2 x 10^{4} M^{-1}
5g	-18.75	2.31 x 10^{-16}	DT18	2.58	2.2	3.2 x 10^{4} M^{-1}

In vitro cytotoxicity in MCF-7 and Hela (MTT assay)

Aromatic oxygen compounds offer interesting possibilities for study, but very few have been synthesized and none are known to occur naturally. The in vitro antitumor activities of the target compounds were evaluated by examining their cytotoxic effects using MTT tetrazolium dye assay against a panel of two human tumor cell lines: breast adenocarcinoma (MCF-7) and Hela cells as described by Mosmann.[22] Percentage (%) growth inhibition of cancer cell lines was determined at a concentration of 10 µM, the results are summarized in the **Table 2**. In Table 2, it can be seen that most compounds showed moderate cytotoxicities against both tested cancer cells. The oxygen analogue with methoxy substituent significantly affected the cancer cells. The oxygen analogue of **5d**, showed the good bioactivity against both cell lines.

Table 2: Effect of OQCE on proliferation of human cancer cell lines assessed by MTT assay. A fixed number of cells (1 × 10^4) grown in a 96-well culture plate were exposed to different concentrations of OQCE for 24 h followed by MTT addition and absorbance was recorded at 540 nm by ELISA reader. IC_{50} value of after 24 h drug incubation with MCF-7 and HeLa cell lines by MTT assay. All values are expressed as mean ± SEM.

Drugs	Conc.(µg/mL)	% growth inhibition after 24 h incubation	
		MCF-7 cells	HeLa cells
5a	10	24.45 ± 0.80	21.02 ± 0.62
5b	10	22.26 ± 0.15	22.45 ± 0.35
5c	10	22.50 ± 0.58	20.43 ± 0.92
5d	10	18.61 ± 0.33	18.55 ± 1.20
5e	10	20.17 ± 0.96	22.96 ± 0.44
5f	10	23.12 ± 0.15	23.75 ± 0.15
5g	10	11.45 ± 0.22	10.26 ± 0.88

DNA Photocleavage studies

The DNA cleavage behaviour with OQCE under physiological conditions (pH 7, 37 °C) has been observed by the transformation of the supercoiled form to the nicked and linear forms of plasmid DNA. The H abstraction from C-4' is the most important process in DNA cleavage and the gel-electrophoresis was an effective method for examining the DNA cleavage studies.[19,21] 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) will be generated and that migrates at rates between Form I and Form II.[19,21]

Figure 2. Time-dependence of pUC19 DNA photo cleavage by OQCE (**5a**) at 80µM concentration. The OQCE (**5a**) was irradiated with UV light at 365 nm. Lane; 1: control DNA (without compound), Lane; 2, 3, 4, 5 and 6 irradiated for about 10, 20, 30, 60, 120 minutes respectively.

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Figure 2 shows the cleavage reactions of pUC19 DNA mediated by a variety of scission systems containing oxoquinoline-oximes. The gel electrophoresis separation of pUC19 DNA after incubation with 80µM concentration of OQCE **(5a)** and irradiated for 10, 20, 30, 60, 120 minutes respectively in 1:9 DMSO/trisbuffer (20 µM, pH- 7.2) at 365 nm. DNA cleavages were negligible for controls in which the hybrid compound was absent (Figure 2, lane 1). It is clear from lanes 2-6 that the light induced DNA photocleavage of oxoquinoline oximes was purely depend on the irradiation time. We have already showed the significance of the quinoline oximes as DNA cleavers.

Oxime esters were the first oxime derivatives to be used for radical generation and they remain the most popular. They have been put to use in two ways; either as sources of *C*-centred radicals or for generating iminyl radicals. On homolysis of their weak N–O bonds, an iminyl radical is accompanied by an acyloxyl type radical (Scheme 2).[19] Most acyloxyls decarboxylate and release *C*-centred radicals very rapidly making these precursors very effective sources of the biological studies.

The cleavage experiments of pUC19 DNA of OQCE under different conditions have been carried out. The time dependence of DNA cleavage reveals that the supercoiled form completely disappears after 30 min, as shown in Figure. 3. The observed distribution of supercoiled and linear DNA in the agarose gels provides the H abstraction from C-4' of DNA strand and this cleavage due to the oxyl and immyl radicals. The OQCE promotes up to 88% conversion of DNA from form I to II (Figure 3). However, other derivative also exhibits good cleaving efficiency for pUC 19 DNA. The oxygen functional group in the quinolines moiety was enhanced the DNA cleavage activity. Interestingly OQCE nuclease is capable to accelerate the cleavage of plasmid DNA is purely on time and concentration dependent.

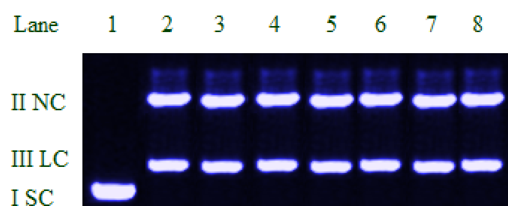
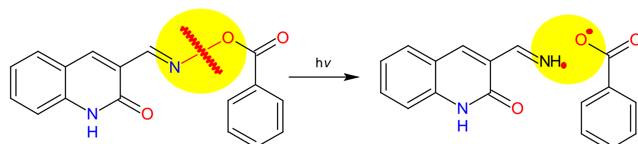


Figure 3. Light-induced cleavage of DNA by OQCE at 365 nm. Supercoiled DNA runs at position I (SC), linear DNA at position III (LC) and nicked DNA at position II (NC). Lane; 1: control DNA (without compound), Lane; 2: 80µM (**5a**), Lane; 3: 80µM (**5b**), Lane; 4: 80µM (**5c**), Lane; 5: 80µM (**5d**),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



Scheme 2: Generating iminyl radicals and acyloxyl type radical on photo irradiation.

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.[20]

In the present study, the OQCE 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 Figure 4. The result shows that, OQCE **5b**, **5c,** **5d** and **5g** which were showed highest affinity -19.64, -18.29, -18.20 and 18.75 kcal/mol docking energy, 2.56×10^{-16}, 2.39×10^{-16}, 2.75×10^{-16} and 2.31×10^{-16} estimated inhibition constants with an RMSD of 2.0. In this model, it is clearly indicated that the compound **5b** formed hydrogen bonded between the -OH and N1 of adenine, which is DT18 with the bond length of 2.63 Å.[22] Moreover, the other derivatives of oxime esters 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. 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 oxime ester with DNA and the conformation constraints for adduct formation.[20]

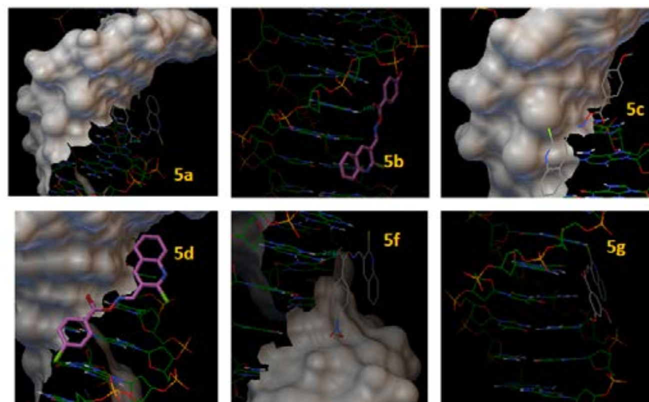


Figure 4. View of the energy minimized docked poses of OQCE 5a, 5b, 5c, 5d, 5f, and 5g with DNA d(CGCGAATTCGCG)$_2$ (PDB ID: 1BNA).

Conclusion

In summary, we described the synthesis, DNA binding, DNA photocleavage, molecular docking and anticancer activity of 2-oxo-quinoline-3-carbaldoxime esters. The primary results showed that, most compounds had moderate anticancer activities with IC$_{50}$ values. Oxime ester **5d** showed more selective cytotoxicity towards MCF-7 and Hela cancer cells and greater potency than the other oxime esters. Also, we clearly demonstrated the DNA binding and DNA photocleavage studies of oxime esters. The above results demonstrate that the rational design of oxime esters as novel anticancer agents. Further studies on the relevant action mechanisms and problematic of the potential toxicity of these compounds are in progress, and will be published in the future.

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