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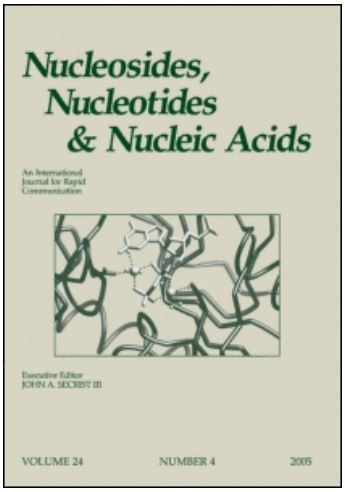

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Synthesis, Antitumor, and DNA Binding Behavior of Novel 4-(2-Hydroxyquinolin-3-yl)-6-Phenyl-5, 6 Dihydropyrimidin Derivatives in Aqueous Medium

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SYNTHESIS, ANTITUMOR, AND DNA BINDING BEHAVIOR OF NOVEL 4-(2-HYDROXYQUINOLIN-3-YL)-6-PHENYL-5, 6 DIHYDROPYRIMIDIN DERIVATIVES IN AQUEOUS MEDIUM

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□ *This article deals with the synthesis of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidin derivatives (**2a–f**), on condensation with various aromatic aldehydes and ketones in aqueous ethanolic NaOH solution yielding the corresponding chalcones (**3**). These chalcones were further reacted with thiourea/urea in the presence of a base, which led to the formation of the titled derivatives (**2a–f**). The newly synthesized heterocyles were characterized by elemental analysis, FTIR, ^{1}HNMR, and electronic and mass spectral data. The compounds (**2a** and **2b**) were evulated for in vitro cyctotoxicity against human breast adenocarcinoma cell (MCF-7). In MTT cytotoxicity studies, both quinolinde derivatives were found most effective. The binding interaction behavior of the compound (**2a**) and (**2d**) with calf thymus-DNA (CT-DNA) was studied by electronic spectra, viscosity measurements, and thermal denaturation studies. On binding to CT-DNA, the absorption spectrum underwent bathochromic and hypochromic shifts. The binding constant (K_b) observed $\mathbf{4.3 \times 10^5\ M^{-1}}$ for (**2a**), and $\mathbf{3.8 \times 10^5\ M^{-1}}$ for (**2d**) suggested that compound (**2a**) binds more strongly with base pairs than (**2d**).*

Keywords Quinoline; Claisen Schmidt condensation; antitumor activity; MTT assay, DNA binding; viscosity measurements; aqueous medium

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INTRODUCTION

Quinoline derivatives are useful heterocyclic aromatic compounds widely used in medicinal chemistry. Additionally, quinoline derivatives have been extensively studied as potential antitumor agents, as they are capable of binding to DNA.[1] Many 4-hydroxy-1,2-dihydro-2-quinolinones have a wide spectrum of pharmacological applications such as anti-herpes simplex virus (anti-HSV),[2] anti-convulsion,[3] and anti-inflammatory[4] activities. Further, the utility of quinoline derivatives in the preparation of some dyes and pigments has been reported.[5] In addition, quinolines are valuable synthons used for the preparation of nano and mesostructures with enhanced electronic and photonic properties.[6] Due to wide range of applicability in the fields of medicine, bioorganics, industry, and synthetic organic chemistry, there has been an increasing interest in the development of efficient methodologies for the synthesis of quinoline derivatives.

On other hand, chalcone are important starting materials for the syntheses of different classes of heterocyclic compounds such as pyrazolines, thiophenes, and pyrimidines. The synthesis of novel pyrimido-quinoline derivatives by a convenient method remains an important synthetic task because of their great importance in pharmacological field.[7–10]

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There is evidence that anticancer activity was due to the intercalation between the drug and the base pairs of DNA and interference with normal functioning of the enzyme topoisomerase II involved in the breaking and releasing of DNA strands.[11] In recent years, various fused systems such as thiophene,[12] furan and pyridine analogues of ellipticine,[13] and benzothiazoloquinoline[14] have been studied for their intercalative properties. Recently, Cao et al. studied DNA affinity properties of Safranine T, which features a planar phenazine ring and have shown that the electrostatic binding plays an important role in the intercalation of safranine T.[12] The results of these various binding studies have been useful in designing new and promising anticancer agents for clinical use.[15]

The recent interest in green chemistry has posed a new challenge for organic synthesis to find new reaction conditions that reduce the emission of volatile organic solvents and the use of hazardous toxic chemicals. Organic reactions in aqueous media have attracted increasing interest currently because of environmental issues and the understanding of biochemical processes. Water as reaction solvent offers many practical and economic advantages including low cost, safe handling, and environmental compatibility. Recently, many organic reactions in aqueous media have been described in the literature.[16] Hence in continuation of our research program, [17–20,31,32,34] we described a highly efficient, cost-effective, and environmentally method for the synthesis of new series of novel quinoline derivatives (**2a–f**). The newly synthesized selected compounds were used for in vitro antitumor and DNA binding studies.

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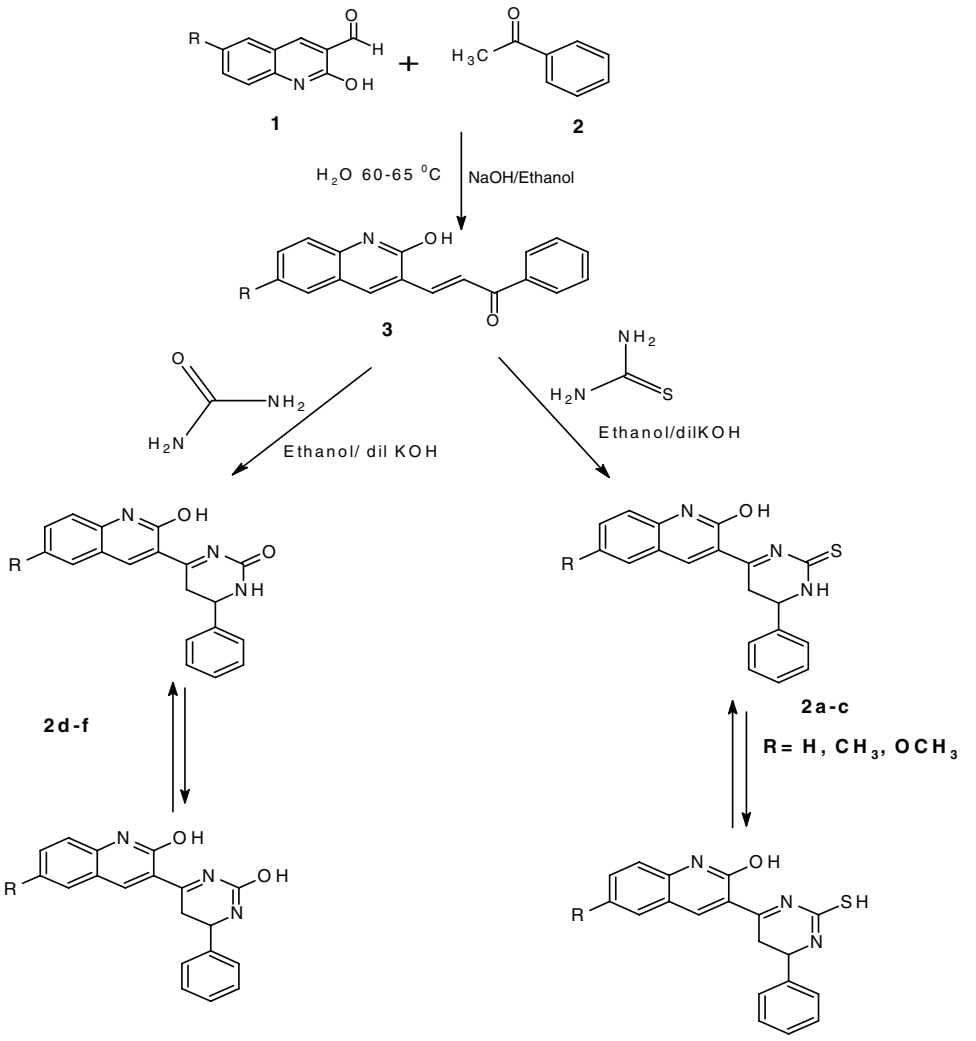
RESULTS AND DISCUSSION

The chemistry of title derivatives (**2a–f**) performed following steps as per the (Scheme 1). The structural elucidation of the newly synthesized compounds was established on the basis of their elemental analysis, infrared (IR), ^{1}H NMR, and mass spectral data.

The IR spectra of the compound (**2a–f**) showed the absence of aldehyde carbonyl stretching frequency, instead it gave a band at 1657–1720 cm^{-1} and two broad peaks observed in the region of 3401 cm^{-1} 3268 cm^{-1} due to –OH- and –NH- groups, respectively. The generation of new sharp absorption band at 1290–1293 cm^{-1}, which attributed to tautomeric form of (C=S)



SCHEME 1 Synthesis of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidin derivatives.

stretching frequency.[17,18] Further structure (**2a**) was confirmed by ^{1}H-NMR spectra show a broad peak δ: 11.72–11.70 due to (Ar-OH-) second position of quinoline (**2a**) and resonate peak δ: 11.32–11.23 ppm, corresponds to the cyclized (-NH-pyrimidene). The signal exhibits multiplets at δ: 7.18–8.00 pp-m for aromatic protons,[19,20] and its mass spectra having molecular ion peak at m/z = 333[M + H]. The obtained elemental analysis values are in good agreement with theoretical data. Hence, we synthesized six more title compounds, which exhibited similar spectral data, were summarized in experimental section.

IN VITRO ANTITUMOR ACTIVITY

In vitro cytotoxicity of synthesized compounds was assessed by standard MTT bioassay in MCF-7 cancer cells at 24 hours of test compounds exposure. The IC_{50} (concentration required to inhibit 50% of cell growth) values for derivatives (**2a** and **2d**) were 50.1 and 76.36 μg/ml at 24 hours, respectively. The percentage of DMSO (0.1%) used in the experiment did not affect the growth of the cells. In the MTT time course study the compounds showed dose-dependent and time dependent activities. The IC_{50} values for derivatives (**2a**) and (**2d**) were 43.35, 59.56 μg/ml and 23.08, 26.82 μg/ml at 48 and 78 hours, respectively. In this study, MTT assays revealed substantial cytotoxicity in MCF-7 cell with increasing exposure to test compounds, the IC_{50} values of compounds at 48 and 72 hours were significantly reduced as compared with 24 hours values. The reduction in the cytotoxicity activity of test compound with increased exposure could be related to degradation of the same in aqueous media. The resulting protocol shows that the in vitro antitumor activity of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidin derivatives exhibited significant cytotoxicity against breast adenocarcinoma cells (MCF-7). It is clear from these data the compound (**2a**) has the highest levels of cytotoxicity activity than that of the compound (**2d**).[21] Many anticancer drugs are effective against MCF-7 by causing apoptosis through the expression of caspase-3, generacting reactive oxygen species (ROS) and damaging DNA.[22] (See Tables 1–3.)

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TABLE 1 In vitro cytotoxic activity of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidine against MCF-7 cells (human breast adenocarcinoma cells) by MTT assay at 24 hours of exposure

	% Cytotoxicity					
Treatment	20 μg/ml	40 μg/ml	60 μg/ml	80 μg/ml	100 μg/ml	IC_{50}
2a	38.69 ± 0.57^a	44.71 ± 0.13^a	55.76 ± 0.37^a	56.94 ± 1.49^a	63.15 ± 1.29^a	50.10
2d	45.61 ± 1.84^a	31.38 ± 1.38^a	33.57 ± 1.2^a	53.57 ± 2.71^a	60.50 ± 1.15^a	76.36

[a]All the values are mean ± SEM of three samples.

TABLE 2 In vitro cytotoxic activity of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidine against MCF-7 cells (human breast adenocarcinoma cells) by MTT assay at 48 hours of exposure

	% Cytotoxicity					
Treatment	20 μg/ml	40 μg/ml	60 μg/ml	80 μg/ml	100 μg/ml	IC_{50}
2a	20.32 ± 0.65^a	28.95 ± 0.44^a	84.33 ± 0.49^a	89.26 ± 0.30^a	90.11 ± 0.55^a	43.35
2d	35.50 ± 0.21^a	31.66 ± 1.24^a	49.77 ± 0.49^a	57.77 ± 2.37^a	60.19 ± 0.27^a	59.56

[a]All the values are mean ± SEM of three samples.

DNA BINDING STUDIES (ABSORPTION SPECTRAL STUDIES)

The application of electronic absorption spectroscopy in calf-thymus DNA (CT-DNA) binding studies is one of the most important techniques.[23] The binding of the molecules to DNA has been well characterized by the large hypochromism. After intercalation the π^* orbital of compounds could couple with π orbital of base pairs, thus decreasing the π^*—π transition of energy, and resulting bathochromism. Hence, the decrease the absorption intensity and significant red shift due to stacking interaction between drug and CT-DNA.[24,25] The DNA binding studies were characterized by absorbance maximum at 329 nm for (**2a**) and 259 nm for (**2d**). The addition of increasing higher concentration of DNA led to hypochromic and bathochromic (red shift) changes in its visible absorption spectra as a result of formation of more stable complexes (Figures 1 and 2). The interaction of (**2a**) and (**2d**) with CT-DNA resulted in the decrease of absorption intensity accompanied by a shift toward higher wavelengths (~3 and 5 nm). Around 9–12% reduction intensity of absorption was observed at 329 and 259 nm peak maximum in the presence of an excess of calf thymus DNA.[26] The lowest observation value observed in spectral changes (including red shift, hypochromicity) were used to evaluate intrinsic binding constant (K_b), it observed value $4.3 \times 10^5\ M^{-1}$ for (**2a**) and $3.8 \times 10^5\ M^{-1}$ for (**2d**) from the spectral result suggested that compound (**2a**) bind more strongly with base pairs than that (**2d**).[27,28]

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TABLE 3 In vitro cytotoxic activity of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidine against MCF-7 cells (human breast adenocarcinoma cells) by MTT assay at 72 hours of exposure>

	% Cytotoxicity					
Treatment	20 μg/ml	40 μg/ml	60 μg/ml	80 μg/ml	100 μg/ml	IC_{50}
2a	40.09 ± 0.80^a	$56..6 \pm 0.14^a$	72.61 ± 0.27^a	77.27 ± 2.33^a	81.23 ± 0.59^a	23.08
2d	43.80 ± 1.62^a	54.05 ± 2.32^a	61.41 ± 1.91^a	66.53 ± 0.42^a	79.27 ± 0.62^a	26.82

[a]All the values are mean ± SEM of three samples.

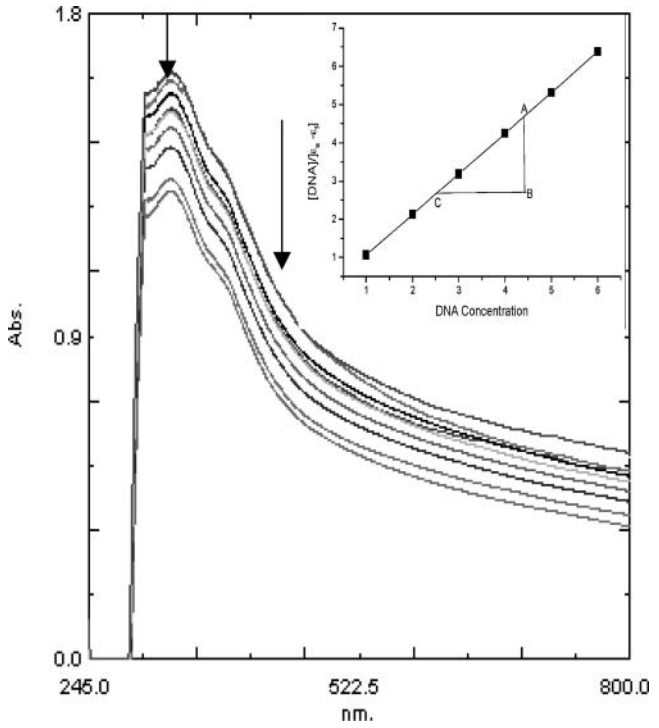

FIGURE 1 Ultraviolet-absorption spectra in Tris-HCl buffer on addition CT-DNA (**2a**) [DNA] = 0.5 μm, = 10 μm, drug, 20 μm; 30 μm; 40 μm; 50 μm; Arrow indicates the absorbance changing on the increase of DNA concentration.

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Viscosity Measurements

To further clarify the interaction modes of (**2a)** and (**2d**) with DNA were investigated by viscosity measurements. An increase in viscosity of native DNA is regarded as a diagnostic feature of an intercalation process.[29,30] We have measured the viscosity changes in short, rod-like DNA fragments. The relative length increase (L/L_0) of the complex formed between (**2a**), (**2d**), and DNA is shown in Figure 3. It is evident that binding of (**2a**) and (**2d**) increased the viscosity of DNA corresponding to an increase in the contour length of the DNA fragments. In order to elucidate the binding mode of the present compound, the viscosity measurements were carried out on CT-DNA by varying the concentration of added compound. The effects of the compounds on the viscosity of rod-like DNA were shown (Figure 3). The presence of compound had an obvious effect on relative viscosity of CT-DNA with an increase in concentration of the added compounds.[31]

Thermal Denaturation

Other strong evidence for the intercalative binding of (**2a**) and (**2d**) into the double helix DNA was obtained from DNA melting studies. The

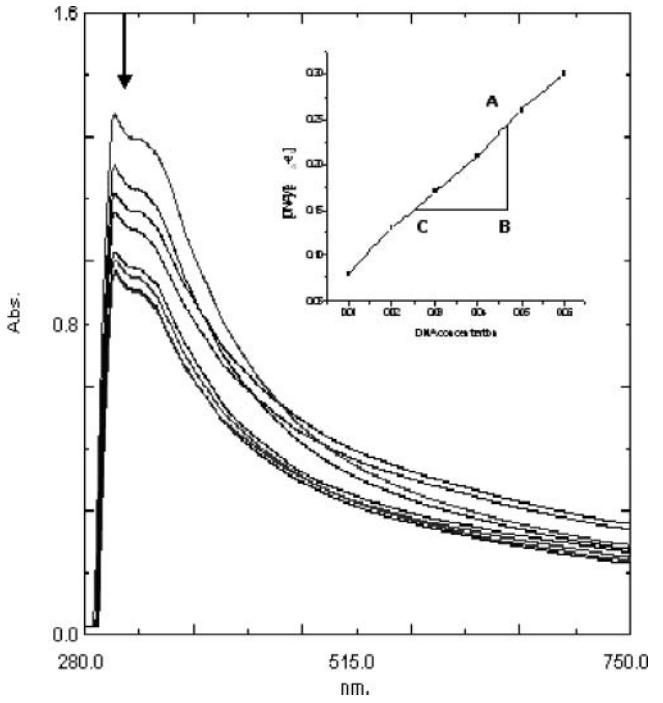

FIGURE 2 Ultraviolet-absorption spectra in Tris-HCl buffer on addition CT-DNA (**2d**) [DNA] = 0.5 μm, = 10 μm, drug, 20 μm; 30 μm; 40 μm; 50 μm; Arrow indicates the absorbance changing on the increase of DNA concentration.

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intercalation of small molecules into the double helix is known to increase the DNA melting temperature (T_m), at which the double helix denatures into single stranded DNA, owing to the increased stability of the helix in the presence of an intercalator.[32] The molar extinction coefficient of DNA bases at 260 nm in the double helical form is much less than the single stranded form; hence, melting of the helix leads to an increase in the absorbance at 260 nm. The DNA melting studies were carried out with CT-DNA in the absence and presence of (**2a**) and (**2d**). T_m (melting temperature) for CT-DNA was $60 \pm 5°C$ in the absence of compounds, but in the presence of (**2a**) and (**2d**) the T_m of CT-DNA increased by 7–10°C. These variations in DNA melting temperature strongly supported the intercalation of compounds into the double helix DNA (Figure 4).[33]

EXPERIMENTAL SECTION

Materials and Methods

All organic solvents used for the synthesis were of analytical grade. The thin layer chromatography (TLC) was performed on Baker-Flex silica gel 1B-F (1.55) plates using ethyl acetate and petroleum ether (1:8).

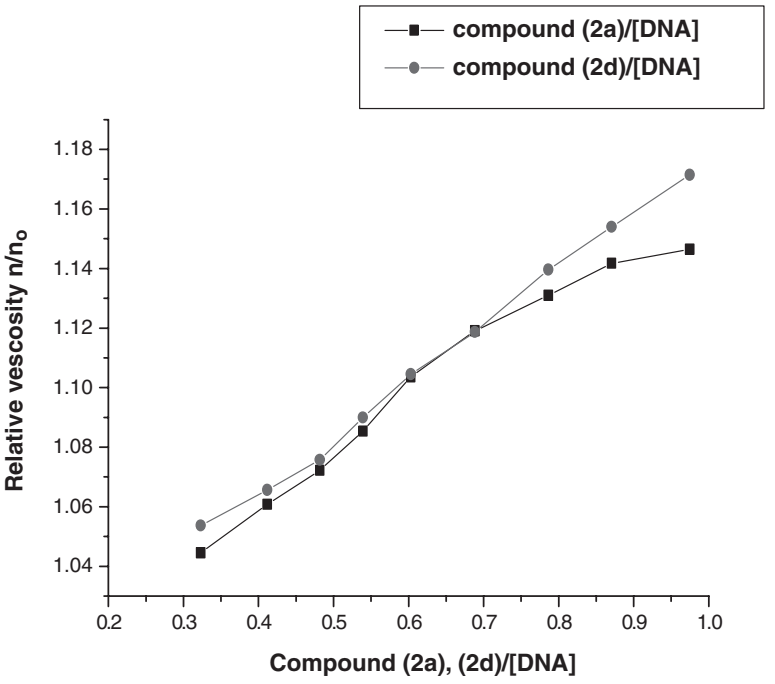

FIGURE 3 Effect of increasing amount of the (**2a**) and (**2d**) on the relative viscosities of CT-DNA, at 25°C.

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Melting point was determined on a Mel-Temp apparatus and were uncorrected. IR spectra were recorded in the matrix of KBr with Perkin-Elmer 1430 spectrometer. ^{1}H NMR spectra were recorded on Jeol spectrometer (400 MHz), and chemical shifts (δ) given in ppm relative to the TMS in $CDCl_3$ solvent. Mass spectra were recorded by electron ionization (EI) on a finnigan MAT 312 spectrometer. C, H, and N analysis were performed at Cochin University, Sophisticated Test & Instrumentation Center, Kochi, Kerala, India. Ammonium hexaflurophosphate (NH_4PF_6) purchased from Qualigens (India). Tris-HCl buffer (5 mM Tris-HCl, 50 mM NaCl, pH = 7.2, Tris = Tris(hydroxymethyl) amino methane) solution was prepared using deionized double distilled water. CT-DNA was purchased from Bangalore Gene, Bangalore, India. Ultraviolet absorption spectra were determined in a Perkin-Elmer (Germany) model 554, UV-Vis recording spectrophotometer using quartz cuvettes of 10 mm path -length.

MTT Assay in Cultured Human Cancer Cells

Cell line: Human breast adenocarcinoma cells (MCF-7) were received from NCCS Pune, India. MEM (minimum essential medium) media was supplemented with 10% fetal bovine serum (FBS), 1% l glutamine, and 50 ug/ml

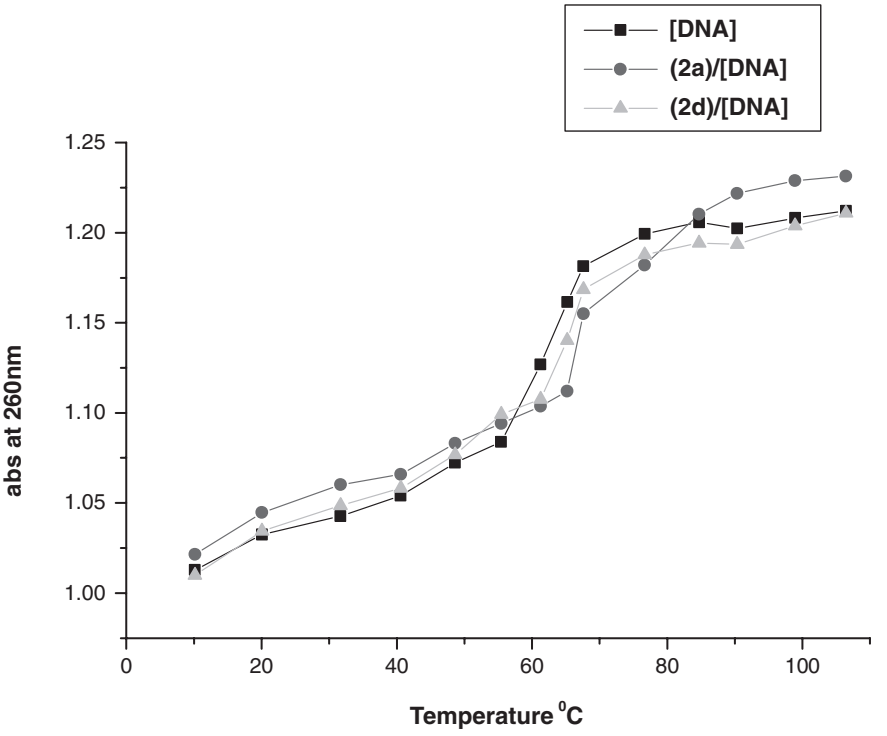

FIGURE 4 Melting cures of CT-DNA in the presence and absences of (**2a**) and (**2d**).

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gentamicin sulphate in a CO_2 incubator (NuAire, dPlymouth, USA) in a humidified atmosphere of 5% CO_2 and 95% air. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent was received from Sigma Aldrich (USA). Tissue culture flasks, 96-well microculture plates were from Tarson and Nunc (USA). Gentamycin was obtained from KMC Hospital, Manipal, India.

In Vitro Cytotoxic Activities (MTT Assay)

In vitro cytotoxicity was determined using a standard MTT assay[34,35] with protocol appropriate for the individual test system. In brief, exponentially growing cells were plated in 96-well plated (1×10^4 cells/well in 100 μl of medium) and incubated for 24-hour attachment. Test compounds were prepared prior to the experiment by dissolving 0.1% DMSO and diluted with medium. The cells were then exposed to different concentrations of test compounds (20–100 μg/ml) in the volume of 100 μl/well. Cells in the control wells received the same volume of medium containing 0.1% DMSO After 24 hours, the medium was removed and cell cultures were incubated with 100 μl MTT reagent (1mg/ml) for 4 hours at 37°C. The formation of the viable cells was solubilized by the addition of 100 μl DMSO. The suspension was placed on micro-vibrator for 5 minutes and absorbance was recorded at 540 nm

by the ELISA reader (BIOTEK-ELx800). The experiment was performed in triplicate. The percentage of growth inhibition was calculated with respect to vehicle control using the formula,% Inhibition = [{(Control absorbance − Blank absorbance) − (Test absorbance − Blank absorbance)}/(Control absorbance − Blank absorbance)] × 100. For MTT time course study, MCF-7 cells (1×10^4 cells/well) seeded in 96-well plates were exposed to different concentrations of test compounds (20–100 μg/ml). The percentage cytotoxicity and IC_{50} values were determined at 48 and 72 hours of test compound incubation. Data obtained were expressed as mean ± SEM.

UV-Visible Absorption Studies

The concentration of CT-DNA per nucleotide [C(p)] was measured using its known extinction coefficient at 260 nm (6600 $M^{-1}cm^{-1}$).[36] The absorbance at 260 nm (A_{260}) and at 280 nm (A_{280}) for CT-DNA was measured to check purity. The ratio A_{260}/A_{280} was found to be 1.8–1.9, indicating that CT-DNA was satisfactorily free from protein. Buffer [5mM tris (hydroxymethyl) aminomethane, pH 7.2, 50mM NaCl] was used for the absorption, viscosity, and thermal denaturation studies.

Absorption titration experiments were carried out by varying DNA concentration (0–100 μM) and maintaining the compound concentration constant (30 μM). Absorption spectra were recorded after each successive addition of DNA and equilibration (approximately 10 minutes). For both the compound (**2a**) and (**2d**) observed data were then fit into (1) in order to obtain the intrinsic binding constant K_b.

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f), \quad (1)$$

where $\varepsilon_{a,}\varepsilon_{,}$ and ε_b are the apparent, free, and bound compound extinction coefficients at 329 nm for (**2a**) and 256 nm for (**2d**). A plot of [DNA]/($\varepsilon_a - \varepsilon_{)}$ versus [DNA] gave a slope of $1/(\varepsilon_b - \varepsilon_{)}$ and an intercept y equal to $1/K_b(\varepsilon_b\text{-}\varepsilon_{)}$, where K_b is the ratio of the slope to the intercept y.[37]

Viscosity Measurements

Viscosity measurements were carried out using a semimicro dilution capillary viscometer at room temperature. Each experiment was performed three times and an average flow time was calculated. Data were presented as (η/η_o) versus binding ratio, where η is the viscosity of DNA in the presence of complex and η_o is the viscosity of DNA alone.[38]

Thermal Denaturation

Melting studies were carried out by monitoring the absorption of CT-DNA (50 μM) at 260 nm at various temperatures in the presence (5–10 μM) and the absence of each complex. The melting temperature (T_m), at which

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50% of double-stranded DNA becomes single-stranded and the curve width (σT), the temperature range between 10% and 90% noticed absorption increases occurred and calculated as reported.[39]

GENERAL SYNTHESIS

Synthesis of (2)-1-(2-hydroxyquinolin-3-yl)-3-phenylprop-2-en-1-one (3)

To a mixture of ketone (**2**) (1.63 mL 0.014 mol) and the appropriate aldehyde (**1**) (2.55g 0.014 mol) in oxygen-free ethanol (25 mL) was added a solution of sodium hydroxide in distilled water (5 mL) with constant stirring at 0°C. The reaction mixture was stirred at 60 ± 5°C for and additional 2 hours on a magnetic stirrer at room temperature. The completion of reaction was checked by TLC and poured in ice-cold water; the solid mass that separated out was filtered, washed with water, and crystallized from ethyl acetate to furnish the desired product as yellowish crystals. Yield 79% 180–182°C

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Synthesis of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidine-2(1*H*)-thione(2a)

A mixture of (1.55 g 0.005 mol) required chalcone (**3**), (0.42 g 0.0056 mol) thiourea and KOH (0.66 g) in 20 mL ethanol was heated under reflux for 6–8 hours. The progress of the reaction was monitored by TLC. The reaction mixture was poured onto crushed ice; the greenish yellow solid product was separated by neutralizing diluted HCl and recrystalized from ethanol. Yield 83%, m.p. 215–217°C, IR (KBr): 1290 (C=S), 3401 (OH-quinoline), 3256, (-NH pyrimidine), 2988 (Ar-CH stretching), 1568 (C=N), 1415 (C=C ring skeleton pyrimidine moiety), ^{1}H NMR (400 MHz, $CDCl_3$) 11.72 (s, 1H, OH quinoline), 11.32 (s, 1H, NH pyrimidine), 7.18–7.53 (m, 5H, 4H, 5H, 6H, 7H, and 8H, Ar-H quinoline). 3.67 (d, 1H, -CH-), 4.23 (d, 2H, CH_2), 7.85–8.00 (m, 5H, Ar-H, phenyle). Mass spectra m/z = 333[M^++H]. Anal. (%) for $C_{19}H_{15}N_3OS$, Calcd. C, 68.45; H, 4.53; N, 12.60; S, 9.62; Found: C, 68.47; H, 4.44; N, 12.56; S, 9.68.

Synthesis of 4-(2-hydroxy-6-methylquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidine-2(1*H*)-thione(2b)

Yellowish solid with yield 78%, m.p. 250–252°C, IR (KBr): 1290 (C=S), 3403 (OH- quinoline), 3255(-NH pyrimidine), 2982, (Ar-CH stretching), 1566 (C=N), 1417 (C=C ring skeleton pyrimidine moiety), ^{1}H NMR (400 MHz, $CDCl_3$) 11.72 (s, 1H, OH Quinoline), 11.32 (s, 1H, NH pyrimidine), 3.22, (s, 3H, CH_3), 3.64 (d, 1H, -CH-), 4.26 (d, 2H, CH_2), 7.15–7.52 (m, 4H, 4H, 5H, 7H, and 8H, Ar-H quinoline). 7.85–8.05 (m, 5H, Ar-H, phenyle).

Mass spectra m/z = 347[M+H]. Anal. (%) for $C_{20}H_{17}N_3OS$, Calcd. C, 69.14; H, 4.93; N, 12.09; S, 9.23; Found: C, 69.17; H, 4.94; N, 12.02; S, 9.28.

Synthesis of 4-(2-hydroxy-6-methoxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidine-2(1*H*)-thione (2c)

Yellowish solid with yield 93%, m.p. 238–240°C, IR (KBr): 1290 (C=S), 3400 (OH- quinoline), 3255(-NH pyrimidine), 2987, (Ar-CH stretching), 1566 (C=N), 1415 (C=C ring skeleton pyrimidine moiety), ^{1}H NMR (400 MHz, $CDCl_3$) 11.72 (s, 1H, OH quinoline), 11.33 (s, 1H, NH pyrimidine), 3.86 (s, 3H, -OCH_3), 3.64 (d, 1H, -CH-), 4.25 (d, 2H, CH_2), 7.15–7.53 (m, 4H, 4H, 5H, 7H, and 8H, Ar-H quinoline). 7.85–8.05 (m, 5H, Ar-H, phenyle). Mass spectra m/z = 363 [M+H].Anal. (%) for $C_{20}H_{17}N_3OS$, Calcd. C, 66.10; H, 4.71; N, 11.56; S, 8.82; Found: C, 66.17; H, 4.74; N, 11.51; S, 8.88.

Synthesis of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidin-2(1*H*)-one (2d)

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Brown solid with yield 93%, m.p. 225–226°C, IR (KBr): 1690 (C=O), 3401 (OH- quinoline), 3258 (-NH pyrimidine), 2982, (Ar-CH stretching), 1569 (C=N), 1414 (C=C ring skeleton pyrimidine moiety), ^{1}H NMR (400 MHz, $CDCl_3$) 11.70 (s, 1H, OH quinoline), 11.33 (s, 1H, NH pyrimidine), 7.18–7.52 (m, 5H, 4H, 5H, 6H, 7H, and 8H, Ar-H quinoline). 3.63 (d, 1H, -CH-), 4.26 (d, 2H, CH_2), 7.87–8.02 (m, 5H, Ar-H, phenyle). Mass spectra m/z = 318 [M+H]. Anal. (%) for $C_{19}H_{15}N_3O_2$, Calcd. C, 71.91; H, 4.76; N, 13.24; Found: C, 71.96; H, 4.69; N, 13.29.

Synthesis of 4-(2-hydroxy-6-methylquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidin-2(1*H*)-one(2e)

Dark brown solid with yield 93%, m.p. 246–248°C, IR (KBr): 1690 (C=O), 3405 (OH- quinoline), 3253 (-NH pyrimidine), 2989 (Ar-CH stretching), 1568 (C=N), 1416 (C=C ring skeleton pyrimidine moiety), ^{1}H NMR (400 MHz, $CDCl_3$) 11.72 (s, 1H, OH quinoline), 11.30 (s, 1H, NH pyrimidine), 3.20 (s, 3H, CH_3), 3.66 (d, 1H, -CH-), 4.24 (d, 2H, CH_2), 7.18–7.52 (m, 5H, 4H, 5H, 7H, and 8H, Ar-H quinoline). 7.87–8.02 (m, 5H, Ar-H, phenyle). Mass spectra m/z = 331[M+].Anal. (%) for $C_{20}H_{17}N_3O_2$, Calcd. C, 72.49; H, 5.17; N, 12.68; Found: C, 72.37; H, 5.20; N, 12.66.0

Synthesis of 4-(2-hydroxy-6-methoxyquinolin-3-yl)-6-phenyl-5,6-dihydropyrimidin-2(1*H*)-one (2f)

Dark brown solid with yield 93%, m.p. 240–242°C, IR (KBr): 1690 (C=O), 3401 (OH- quinoline), 3253 (-NH pyrimidine), 2989, (Ar-CH stretching), 1569 (C=N), 1416 (C=C ring skeleton pyrimidine moiety), ^{1}H

NMR (400 MHz, $CDCl_3$) 11.70 (s, 1H, OH quinoline), 11.30 (s, 1H, NH pyrimidine), 3.82 (s, 3H, -OCH_3), 3.66 (d, 1H, -CH-), 4.26 (d, 2H, CH_2), 7.18–7.53 (m, 5H, 4H, 5H, 7H, and 8H, Ar-H quinoline). 7.87–8.02 (m, 5H, Ar-H, phenyle). Mass spectra m/z = 347 [M^+]. Anal. (%) for $C_{20}H_{17}N_3O_3$, Calcd. C, 69.15; H, 4.93; N, 12.10; Found: C, 69.20; H, 4.91; N, 12.06.

CONCLUSIONS

The synthetic route adopted for synthesis of quinoline derivatives (**2a–f**) was very simple and yielded good yield. The preliminary antitumor studies of 4-(2-hydroxyquinolin-3-yl)-6-phenyl-5, 6-dihydropyrimidin derivatives exhibited significant cytotoxicity against breast adenocarcinoma cells (MCF-7) growth in vitro. The obtained protocol shows that compound (**2a**) exhibits more prominent antitumor activity than that of compound (**2d**). In DNA binding studies, indicate hypochromicity and bathochromic shifts of the compounds (**2a**) and (**2d**) when it binds with base pairs of calf thymus-DNA. The binding constant values of **4**.3 $\times$ 10^5 M^{-1} for (**2a**) and 3.8 $\times$ 10^5 M^{-1} for (**2d**) suggested that the compound (**2a**) binds more avidly to CT-DNA than (**2d**). In addition, increasing viscosity of sonicated rod-like DNA fragments and the melting temperature of CT-DNA, in the presence of compound solutions supports the binding mode.

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