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Full Length Research Paper

An efficient synthesis and DNA binding interaction study of some novel heterocyclic fused-pyarzolequinolines: A potent antimicrobial agent

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In the present paper, the synthesis of 3-(5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)quinolin-2-ol (2a)/(3a) and 3-(2-hydroxyquinolin-3-yl)-5-phenyl-4,5-dihydro-1*H*-pyrazole-1 carbothioamide(4a) were prepared according to Claisen-Schmidt condensation, *via* condensation of 2- hydroxyquinoline-3-carbaldehyde with ketones. Then finally, *α*, *β*-unsaturated ketone (3) reacts with hydrazine hydrate, phenylhydrazine and thiosemicarbazide obtained from the corresponding substituted fused-pyarzolequinolines. The newly synthesized compounds were characterized by elemental analysis, IR, ^{1}H-NMR, and Mass spectral data. The selected compounds were studied for interaction with calf thymus-DNA(CT-DNA) using electronic spectra, viscosity measurements as well as thermal denaturation studies. On binding to CT-DNA, the absorption spectrum underwent bathochromic and hypochromic shifts. The binding constant (K_b) gave value of 5.3×10^4 M^{-1} for (2a) and 6.5×10^5 M^{-1} for (4a). The viscosity measurements indicated that the viscosities of sonicated rod like DNA fragments were increased. The newly synthesized dihydro-pyarzolequinolines derivatives were screened for antimicrobial activities.

Key words: Quinoline, chalcone, pyrazole-1-carbothioamide, DNA binding, viscosity measurement, antimicrobial activity.

INTRODUCTION

In recent times, problems of multi-drug resistant microorganisms have reached an alarming level in many countries around the world. A numbers of recent clinical reports described the increasing occurrence of meticillin-resistant *Staphylococcus aureus* and other antibiotic-resistant human pathogenic microorganisms in United State and European countries. Infections caused by those microorganisms pose a serious challenge to the medical community and the need for an effective therapy has led to a search for novel antimicrobial agents (Ferlini et al., 2000). Hence, Quinoline derivatives are useful heterocyclic aromatic compounds and are widely used in medicinal chemistry. Many 4-hydroxy-1, 2-dihydro-2-quinilinones have a wide spectrum of pharmacological applications such as anti-tumor (Kishor et al., 2008) anti HSV (Hasegawa et al., 1991) anti-convulsion (Afonso et al., 1995) and anti-inflammatory (Rowely et al., 1993) activities.

Nowadays, pyrazole have attracted considerable attention in agrochemical and medicinal research, since these compounds have been found to pose good bioactivities such as anti-tumor, anti-senile dementia, antibacterial, antiviral and anticonvulsive activities (Moussaoui et al., 2002; Baraldi et al., 2004). A variety of methods have been reported for the preparation of this class of compounds. After the pioneering work of Fischer and Knoevenagel in the 19th century, the reaction of α,β-unsaturated aldehydes and ketones with phenylhydrazine in acetic acid by refluxing became one of the most popular methods for the preparation of pyrazolines (Levai et al., 2005). 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 that was involved in the breaking and releasing of DNA strands (Kidwai et al.,

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2006). In recent years, various fused systems such as thiophene (Zhang et al., 2004), furan and pyridine analogues of ellipticine (Xinhua et al., 2007) and benzothiazoloquinoline, (Gatto et al., 1999), have been studied for their intercalative properties (Cao et al., 1998; Singh et al., 1992; Baez et al., 1983). Recently, Cao and He 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 (Kidwai et al., 2006). In our previous work, we reported synthesis of substituted quinoline derivatives and their DNA binding interaction studies (Nandeshwarappa et al., 2005, 2006; Lamani et al., 2008; Prakash et al., 2009).

In continuation of our research program (Nandeshwarappa et al., 2005, 2006; Lamani et al., 2008; Prakash et al., 2009; Prabhakara et al., 2007) toward the synthesis of potentially bioactive anticancer agent via a simple and practical approach, herein, we reported a rapid and efficient method for the synthesis of novel fused-pyarzolequinolines derivatives in acetic acid aqueous media with quantitative yield. The synthesized compounds (2a) and (4a) interact with CT-DNA.

EXPERIMENTAL SECTION

All organic solvents used for the synthesis were of analytical grade. The TLC was performed on Baker-Flex silica gel 1B-F (1.55) plates using ethyl acetate and petroleum ether. Melting points were 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 was 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 the Cochin University, Sophisticated Test and Instrumentation Center, Kochi, Kerala, India. Ammonium hexaflurophosphate (NH_4PF_6) was 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. Calf thymus DNA (CT-DNA) was purchased from Bangalore Gene, Bangalore, India. Ultraviolet-visible absorption spectra were determined in a Perkin–Elmer model 554, UV-Vis recording spectrophotometer using quartz cuvettes of 10 mm path-length.

UV-visible absorption studies

The concentration of CT-DNA per nucleotide [C(p)] was measured using its known extinction coefficient at 260 nm ($6600 M^{-1}cm^{-1}$) (Reichmann et al., 1954). The absorbance at 260 nm (A_{260}) and 280 nm (A_{280}) for CT-DNA were measured in order to check the purity level. The ratio A_{260}/A_{280} was found to be 1.8 to 1.9, indicating that CT-DNA was satisfactorily free from protein. Buffer (5 mM tris (hydroxymethyl) aminomethane, pH 7.2, 50 mM NaCl) was used for the absorption, viscosity and thermal denaturation experiments.

Absorption titration experiments were carried out by varying DNA concentration (0 to 100 µM) and maintaining the compound concentration constant (30 µM). Absorption spectra were recorded after each successive addition of DNA and equilibration (approximately 10 min.). For both the compound (2a) and (4a), observed data were then fit into (1) in order to obtain the intrinsic binding constant, K_b:

$$[DNA] / (\varepsilon_a - \varepsilon_f) = [DNA] / (\varepsilon_b - \varepsilon_f) + 1/ K_b (\varepsilon_b - \varepsilon_f) \text{---------- --(1)}$$

where ε_a,ε_f, and ε_b are the apparent, free and bound compound extinction coefficients at 332 nm, (2a) and 294 nm (4a), respectively. A plot of [DNA]/ (ε_a-ε_f) versus [DNA] gave a slope of $1/(\varepsilon_b-\varepsilon_f)$ and an intercept y equal to $1/K_b(\varepsilon_b - \varepsilon_f)$, where K_b is the ratio of the slope to the intercept *y* (Wolfe et al., 1987).

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 (Raja et al., 2005).

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 to 10 µM) and absence of each complex. As such, the melting temperature (T_m), at which 50% of double-stranded DNA becomes single-stranded (absorption increase was noticed in the curve width (σT) and temperature range between 10% and 90%) occurred and was calculated as reported (Zhang et al., 2005).

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

(Method-A) - Synthesis of 3-(5-phenyl-4,5-dihydro-1H- pyrazol-3-yl)quinoline-2-ol (2a-c)

A mixture of chalcone (2.75 g 0.01 mol), and hydrazine hydrate (0.52 ml 0.01 mol) dissolved in acetic acid (20 ml) and potassium hydroxide (1 g) in water (5 ml) were placed in a flask and heated under reflux for 6 to 8 h. The completion of reaction was monitored by thin layer chromatography (TLC). The reaction mixture was neutralized by dil hydrochloric acid. The dark brown precipitates were filtered and washed with cold water. The resulting product was recrystallized from suitable solvent system and purified by silica gel column chromatography with pet -ether/ chloroform (2:1). Similarly, the same procedure was used for the synthesis of (2b-c) and (3a-c).

(Method-B) - Synthesis of 3-(2-hydroxyquinolin-3-yl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide derivatives(4a)

To a two-necked 100 ml flask, a mixture of *α*, *β*-unsaturated ketone

(2.75 g 0.01 mol) and (0.91 g 0.01 mol) thiosemicarbazide were dissolved in 30 ml acetic acid and sodium acetate (0.8 g) in water (5 ml) was heated under reflux for 6-8 h. The reaction was monitored by thin layer chromatography (TLC). The reaction mixture was poured in ice cold water to give pale yellowish solid. The solid was washed with petroleum ether and then recrystallized from ethanol, similarly, some procedure followed for 4b-c. The compounds was purified by silica gel column chromatography with *V*(petroleum ether/ ethyl acetate = 3:1). The spectral data of all newly synthesized compounds were summarized in Table 1.

RESULTS AND DISCUSSION

The data of physical constants, elemental analysis, ^{1}H-NMR, mass and IR of synthesized compounds are shown in Tables 1 and 2, respectively. We prepared the 3-(5-phenyl-4,5-dihydro-1*H*- pyrazol-3-yl)quinoline(2a)/(3a) and 3-(2-hydroxyquinolin-3-yl)-5- phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide(4a) compound from the reaction of *α*, *β*-unsaturated ketone with hydrazine hydrate, phenylhydrazine and thiosemicarbazid for the cyclization of some reported pyrazole derivatives. Here, different bases, such as sodium carbonate, potassium carbonate and pyridine were tried. It was found that dilute potassium hydroxide and sodium acetate were the most effective one. This method suggests that the condensation of hydrazine with carbonyl occurred first to form hydrazone, then, the imino hydrazone was added to the carbon-carbon double bond to afford the pyrazole derivative. Due to the electron withdrawing effect of the carbon-nitrogen double bond, α-carbon was more electron deficient compared to *β* carbon, and thus, the nitrogen of the imino wad attacked at the α-carbon as shown in Scheme 1 to form the five-membered pyrazole derivatives (Xinhua et al., 2007; Kalouli et al., 1989).

IR spectra of the compound (2a-c)/(3a-c) and (4a-c) showed broad absorption bands observed in the region of 3539 to 3285 cm^{-1} due to -OH- quinoline (–NH-pyrazole ring) and S=C-NH_2 groups. This confirms the occurrence of subsequent cyclization. Further structure was confirmed by ^{1}H-NMR spectra, which show a broad peak at 12.02 to 12.08 ppm due to–OH- present at second position of quinoline. The resonate triplet at 3.11 to 3.16 ppm corresponds to -CH-CH_2- adjacent protons of fused pyrazole ring. The signal exhibits multiplets at 7.27 to 8.24 ppm for aromatic protons, and mass spectra having molecular ion peak at m/z = 289 [$M^{\pm}$] for (2a) and m/z = 364 [$M^{\pm}$] for (3a) (Nandeshwarappa et al., 2005, 2006; Lamani et al., 2008). Similarly, for compounds (4a), ^{1}H-NMR spectra show triplet at 3.19 to 3.26 ppm due to -CH-CH_2. The resonate multiplets at 7.20 to 7.77 ppm (m, 5H, Ar-H Quinoline). In addition to this, a broad singlet was observed at 4.23 to 4.27 due to the S=C-NH_2 (carbothioamide) and a molecular ion peak at m/z = 348[$M^{\pm}$] (Prakash et al., 2009). The obtained elemental analysis values stands are in good agreement with theoretical data (Table 2). Hence, we synthesized nine more title compounds, which exhibited similar spectral data summarized in Table 1. All the established derivatives have been screened for antimicrobial activity. The sulfur containing carbothioamide (4a-c) compounds exhibited significant antibacterial as well as antifungal activities compared to (2a-c) and (3a-c), the obtained protocols were summarized in Table 4.

DNA binding studies (Electronic absorption spectroscopy)

The application of electronic absorption spectroscopy in CT-DNA binding studies is one of the most important techniques (Raja et al., 2005). The DNA binding studies were characterized by absorbance maximum at 332 for (2a) and 294 nm for (6tyh). 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 the formation of more stable complexes (Figures 1 and 2). The interaction of (2a) and (4a) with DNA resulted in the decrease of absorption intensity accompanied by a shift towards higher wavelengths (~3 and 5 nm). About 7% to 10% reduction (Hypochromism) of absorption was observed at maximum peak of 332 and 294 nm in the presence of an excess calf thymus DNA. The spectral changes (including red shift, and hypochromicity) were used to evaluate the intrinsic binding constant (K_b), it observed 5.3×10^4 M^{-1} for (2a) and 6.5×10^5 M^{-1} for (4a), which are intercalated with (2a) and (4a) into DNA base pairs (Lamani et al., 2008; Prakash et al., 2009; Prabhakara et al., 2007) (Figures 1 and 2 and Table 3).

Viscosity measurements

The binding modes of (2a) and (4a) with CT-DNA, were further confirmed by viscosity measurements. Optical phatophysical probes provide the necessary, but not sufficient clues to support a binding mode. Viscosity experiment is considered as one of the least ambiguous and the most critical tests of a binding mode in solution in the absence of crystallographic structure data. An increase in viscosity of native DNA is regarded as a diagnostic feature of an intercalation process (Prabhakara et al., 2007). A classical intercalative mode causes a significant increase in viscosity of DNA solution due to an increase in separation of base pairs at intercalation sites hence, an increase in overall DNA length. In contrast, the compounds that bind exclusively in the DNA grooves by partial and / nonclassical intercalation under the same conditions, typically cause negative or no change in DNA solution viscosity. [33, 34] 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 are shown in Figure 3. The presence of

Table 1. IR, ^{1}H-NMR and Mass characterization of the synthesized compounds.

Compound	IR cm^{-1} (KBr)	^{1}H-NMR, ($CDCl_3$) δ :(ppm) (400 MHz,),	Mass spectra m/z %
2a	3536 (-OH-); 3233; (-NH); 3035 (Ar-CH); 1543;(C=N); 1023 (-N-N-)	12.04 (s, 1H, -OH Quinoline), 11.06 (s, 1H, -NH- pyrazol ring); 5.55 (d, 1H, -NH-CH- pyrazol); 3.11 (t, 2H, -CH_2 pyrazol), 7.12-7.77 (m, 5H, Ar-H, Quinoline), 7.88-8.21(m, 5H, Ar-H, Phenyle).	m/z =304 [M+H]
2b	3539 (-OH-); 3236 (NH); 3033 (Ar-CH); 1020 (N-N); 1553 (C=N)	12.02 (s, 1H, -OH, Quinoline), 11.08 (s, 1H, -NH- pyrazol ring); 5.57 (d, 1H, - NH-CH- pyrazol); 3.16 (t, 2H, -CH_2- pyrazol), 7.12-7.79 (m, 4H,Ar-H, Quinoline), 7.87-8.21(m, 5H, Ar-H, Phenyle), 2.58 (s, 3H, CH_3).	m/z =290[M+H]
2c	3539 (-OH-); 3236 (NH); 3034 (Ar-CH); 1022 (-N-N-); 1653 (C=N)	12.02 (s, 1H, -OH Quinoline), 11.06 (d, 1H, - NH- pyrazol ring); 5.56 (d, 1H, -NH- CH- pyrazol); 3.16 (t, 2H, -CH_2- pyrazol), 7.12-7.79 (m, 4H, Ar-H, Quinoline), 7.87-8.22(m, 5H, Ar-H, Phenyle), 2.19 (s, 3H, OCH_3).	m/z =319[M$^+$]
3a	3535 (-OH-); 3035 (Ar-CH); 1026 (-N-N-); 1653 (C=N)	12.07 (s, 1H, OH Quinoline), 5.58 (d, 1H, NH-CH- pyrazol); 3.16 (t, 2H, -CH_2- pyrazol), 7.20-7.70 (m, 5H, Ar-H, Quinoline); 7.84-8.33(m, 10H, Ar-H, Phenyle).	m/z =364[M$^+$]
3b	3537 (-OH-); 1650 (C=N); 3036 (Ar-CH); 1023 (-N-N-)	12.08 (s, 1H, OH Quinoline), 5.56 (d, 1H, NH-CH- pyrazol); 2.58 (s, 3H, CH_3); 3.17 (t, 2H, -CH_2- pyrazol), 7.22-7.73 (m, 4H, Ar-H, Quinoline), 7.84-8.32 (m, 10 H, Ar-H Phenyle).	m/z =379 [M$^+$]
3c	3534 (-OH-); 3038 (Ar-CH); 1026 (-N-N-); 1643 (C=N)	12.08 (s, 1H, OH Quinoline), 5.56 (d, 1H, NH-CH- pyrazol); 3.17 (t, 2H, -CH_2- pyrazol), 2.19 (s, 3H, OCH_3)7.20-7.72 (m, 4H, Ar-H Quinoline), 7.84-8.32 (m, 10 H, Ar-H Phenyle).	m/z =396[M+H]
4a	3534 (-OH-); 3262 (NH_2) 1296 (C=S); 3035 (Ar-CH); 1023 (-N-N-); 1643 (C=N)	12.08 (s, 1H, OH Quinoline), 4.23 (s, br,2H, -NH_2- carbothioamide); 5.53 (d, 1H, -NH- CH- pyrazol); 3.20 (t, 2H, -CH_2- pyrazol), 7.20-7.85 (m, 5H, Ar-H Quinoline), 8.02-8.24 (m, 5H, Ar-H Phenyle).	m/z =348[M$^+$]
4b	3537 (-OH-); 3264 (NH_2) 1294 (C=S); 3031 (Ar-CH); 1024 (-N-N-); 1644 (C=N)	12.08 (s, 1H, OH Quinoline), 4.24 (s, br,2H, -NH_2- carbothioamide); 5.52 (d, 1H, -NH- CH- pyrazol); 3.20 (t, 2H, -CH_2- pyrazol), 2.56 2.25 (s, 3H, CH_3); 7.20-7.84 (m, 4H, Ar-H Quinoline), 8.02-8.26 (m, 5H, Ar-H Phenyle).	m/z =363[M+H]
4c	3537 (-OH-); 3265 (NH_2) 1294 (C=S); 3033 (Ar-CH); 1022 (-N-N-); 1645 (C=N)	12.08 (s, 1H, OH Quinoline), 4.24 (s, br,2H, -NH_2- carbothioamide); 5.52 (d, 1H, -NH- CH- pyrazol); 3.20 (t, 2H, -CH_2- pyrazol), 2.56 2.25 (s, 3H, OCH_3); 7.21-7.84 (m, 4H, Ar-H Quinoline), 8.02-8.25 (m, 5H, Ar-H Phenyle).	m/z =351[M+H]

compound had an obvious effect on relative viscosity of CT-DNA with an increase in concentration of the added compounds (Figure 3).

Thermal denaturing studies

Thermal denaturation studies of CT-DNA are useful in determining the ability of the present compound to stabilize the double stranded DNA. The intercalation of small molecules into the double helix was known to increase the DNA melting temperature (T_m). The melting temperature (T_m) of DNA characterizes the transition from double-stranded to single standard nucleic acid (Haq et al., 1995). The DNA melting studies were carried out with CT-DNA in absence and presence of compound. The melting profiles (T_m) for CT-DNA in absence of compound had the value 60 ± 5℃. Under the same experimental conditions, the presence of compounds increased the melting temperature (T_m) of about 8℃ for (2a) and 10℃ for (4a). These variations in DNA melting temperature strongly supported the intercalation of (2a) and (4a) into the double helix DNA (Figure 4). Likewise, the various DNA melting experiments strongly supported

Table 2. Physical and analytical data of synthesized quinoline derivatives.

Compound	Color	Yield %	m.p ˚C	Cryst solvent	Molecular formula mol/wt	Analysis Calculated (Found) % C	H	N	S
2a	Dark brown	73	178-180	Ethanol	$C_{18}H_{15}N_3O$ (289.33)	74.72 (74.79)	5.23 (5.20)	14.50 (14.56)	--
2b	Brownish	75	198-200	Ethanol /Chloroform	$C_{19}H_{17}N_3O$ (303.35)	75.23 (75.18)	5.65 (5.71)	13.85 (13.88)	--
2c	Brownish	80	190-192	Ethanol/ Chloroform	$C_{19}H_{17}N_3O_2$ (319.35)	71.46 (71.52)	5.37 (5.31)	13.16 (13.22)	--
3a	Yellowish	93	136-138	Ethanol	$C_{24}H_{19}N_3O$ (364.42)	78.88 (78.81)	5.24 (5.28)	11.50 (11.61)	--
3b	Yellowish Orange	83	168-170	Ethanol	$C_{25}H_{21}N_3O$ (379.45)	79.13(79.09)	5.58 (5.56)	11.07 (11.03)	--
3c	Yellowish	78	156-158	Ethanol	$C_{25}H_{21}N_4O_2$ (395.4)	75.93 (75.97)	5.35 (5.41)	10.63 (10.58)	--
4a	Yellowish white	68	178-180	Ethanol	$C_{19}H_{16}N_4OS$ (348.42)	65.5 (65.58)	4.63 (4.57)	16.08 (11.61)	9.20 (9.27)
4b	Pale yellowish	75	228-230	Methanol/Chloroform	$C_{20}H_{18}N_4OS$ (362.44)	66.28 (66.24)	5.01 (5.06)	15.46 (15.51)	8.85 (8.91)
4c	Pale yellowish	72	215-217	Methanol /Chloroform	$C_{20}H_{18}N_4O_2$ (378.44)	63.47(63.41)	4.79 (4.83)	14.80 (14.76)	8.47 (8.42)

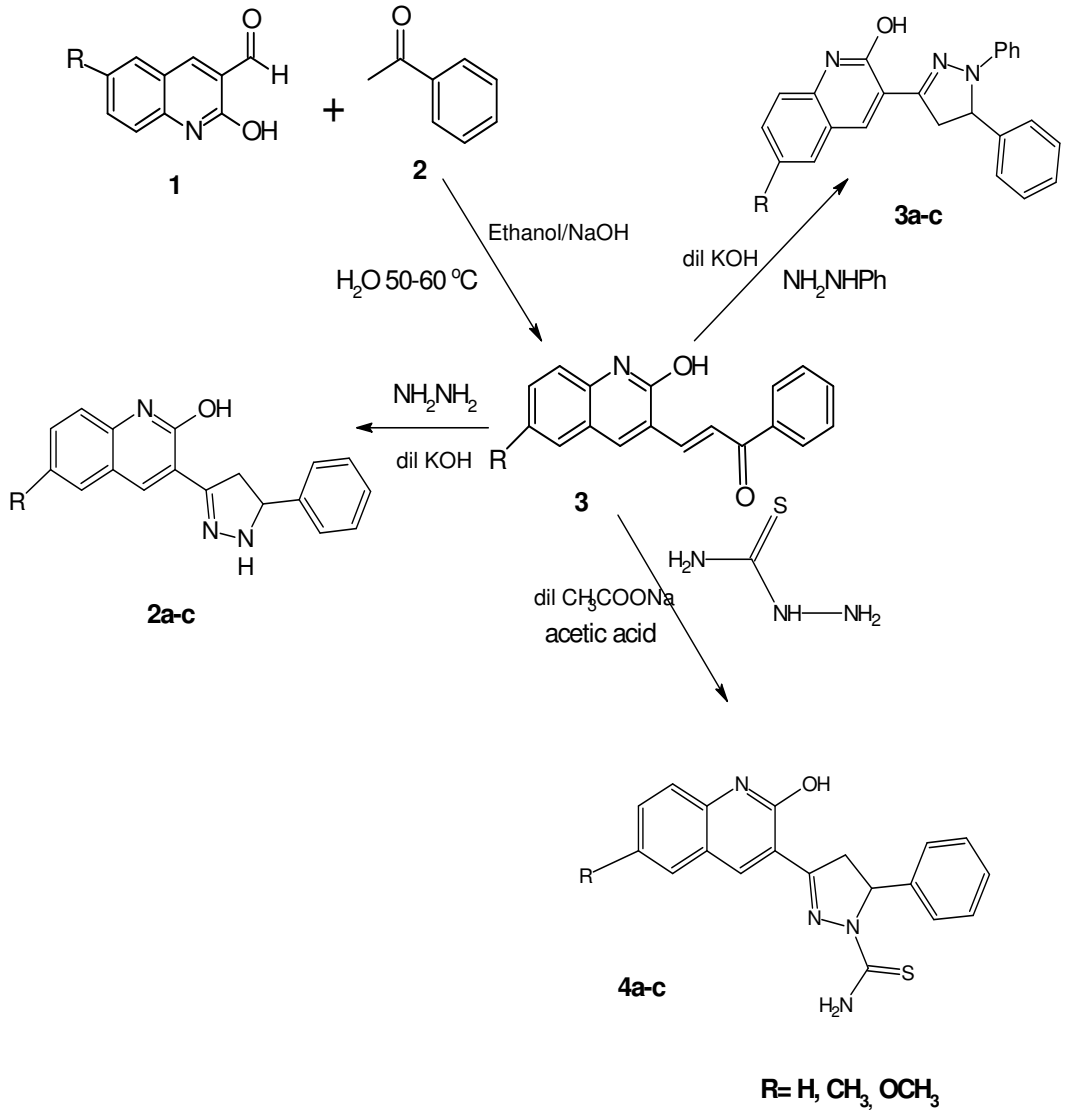
the stabilized double strand calf thymus DNA (Kikuta et al., 1999; Prabhakara et al., 2007) (Figure 4).

Evaluation of antimicrobial activity

The *in vitro* antimicrobial activity was carried out against 24 h old cultures of two bacteria and two fungi by cup-plate method (Sandan et al., 1998). Compounds have been tested for their antibacterial activity against *Pseudomonas aerugenosa* and *S. aureus* and antifungal activity against *A. niger* and *Candida albicans.* Nutrient agar and potatodextrose agars were used to culture the bacteria and fungus, respectively. The compounds were tested at a concentration of 0.005 mol/mL in DMSO solution. The solution of Chloramphenicol (2 mg/ml) and Flucanazole (2 mg/ml) were prepared in sterilized water and used as standards for comparison of antibacterial and antifungal activities, respectively. The compounds were tested at varied concentration. The minimum inhibition concentration was found to be 0.001 mol/ml in DMSO against all organisms. Inhibition was recorded by measuring the diameter of the zone of inhibition at the end of 24 h for bacteria at 28˚C and 48 h for fungus at 35°C. Moreover, each experiment was repeated thrice and the averages of the three independent determinations were recorded. The newly synthesized derivatives exhibit significant biological activity. The resulted protocols indicates that compounds 2b, 2c 3a and 3c show high activity against *A. niger* and *C. albicans.* Where as compounds 4a, 4b 4c show very good activity against *A. niger* and *C. albicans.* Finally, the result indicates that the presence of 4a, 4b and 4c sulfur compounds exhibits very good antibacterial activity, and compounds 2b, 2c 3a and 3c shows more activity towards antifungal than that of antibacterial activity. Chloramphenicol and fluconazole were used as standards for antibacterial and antifungal activity, respectively (Table 3).

Conclusions

In conclusions, the synthetic pathways adopted for the synthesis of quinoline derivatives was very simple and yielded good yield. The DNA binding studies demon-strates the hypochromicity and bathochromic shifts of the compounds (2a) and (4a) when they binds with base pairs of calf



Scheme 1. Synthetic pathways for the preparation of 3-(5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl) quinoline and carbothioamide derivatives.

Table 3. Evaluation of antimicrobial activity of dihydro-pyarzolequinolines.

Compound	Antibacterial activity zone of inhibition (mm)		Antifungal activity zone of inhibition (mm)	
	P. aerugenosa	***S. aureus***	***A. niger***	***C. albicans***
2a	17	16	13	12
2b	14	12	15	17
2c	18	10	12	13
3a	14	16	16	15
3b	14	15	17	12
3c	15	18	16	16
4a	20	18	22	19
4b	19	21	20	19
4c	19	20	23	20
Chloramphenicol	---	---	25	25
Flucanazole	22	24	----	----

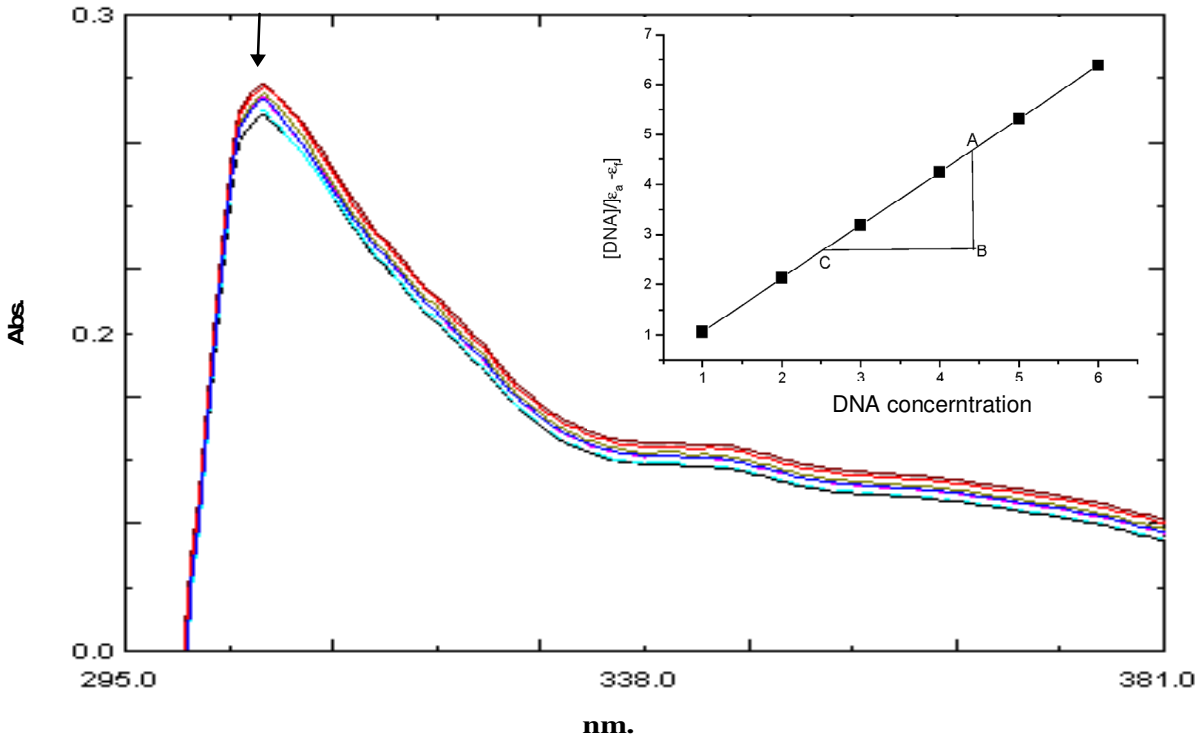


Figure 1. UV- absorption spectra in Tris-HCl Buffer upon addition CT- DNA (**2a**) [DNA]=0.5 µm, = 10 µm, drug, 20 µm; 30 µm; 40 µm; 50 µm; Arrow shows the absorbance changing upon the increase of DNA concentration.

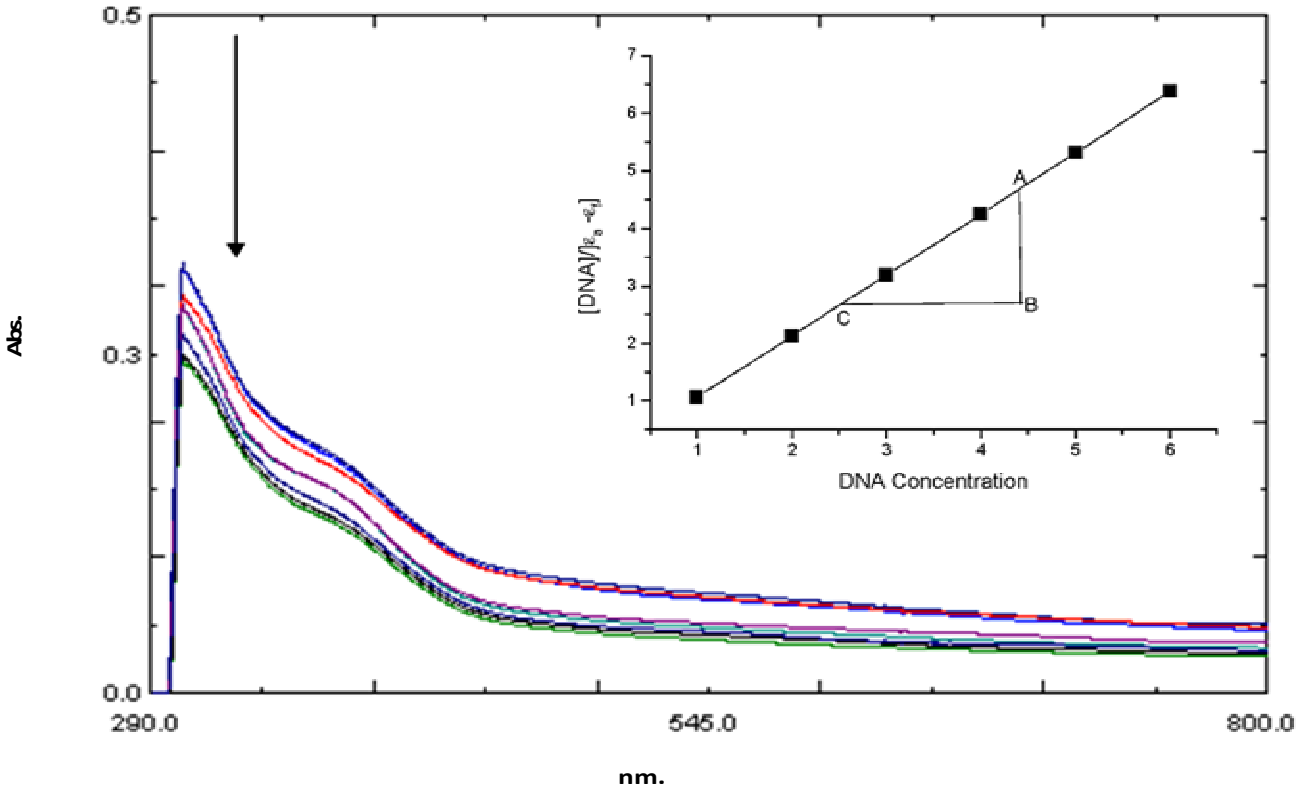


Figure 2. UV- absorption spectra in Tris-HCl Buffer upon addition CT- DNA (**4a**) [DNA]=0.5 µm, =10 µm, drug, 20 µm; 30 µm; 40 µm; 50 µm; Arrow shows the absorbance changing upon the increase of DNA concentration.

Table 4. Absorption spectral properties of compounds (2a) and (**3a**) bound to CT-DNA.

Compound	λ_{max} (nm)	K_b (M^{-1})	T_m (°C)
2a	332	5.3×10^4 M^{-1}	69
4a	294	6.5×10^5 M^{-1}	64

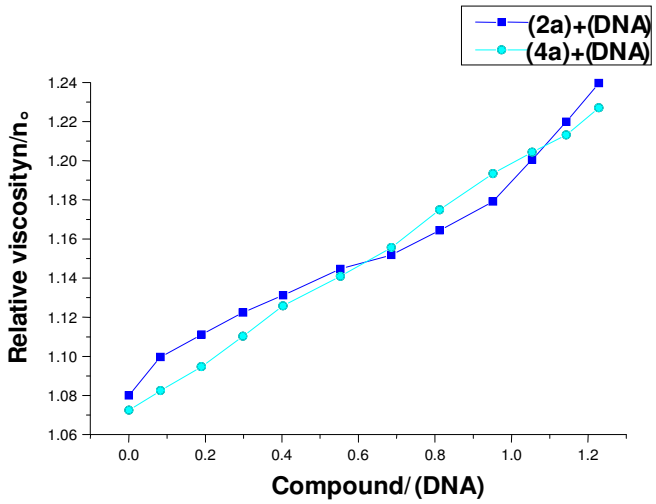


Figure 3. Effect of increasing amount of the (2a) and (**4a**) on the relative viscosities of CT-DNA, at 25°C.

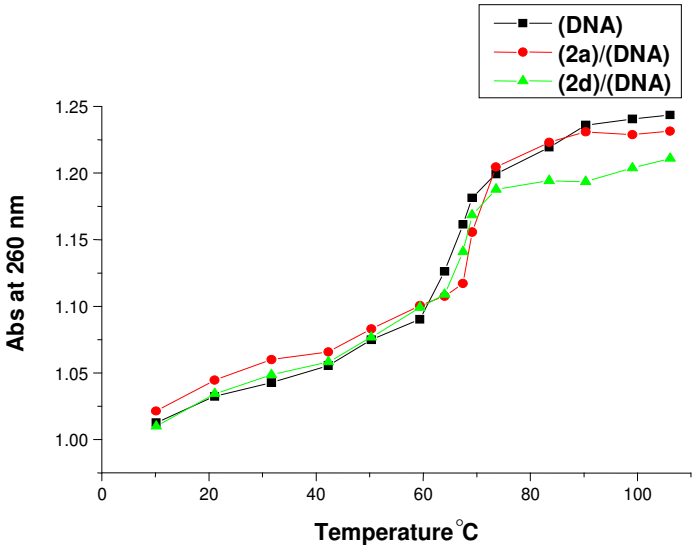


Figure 4. Melting cures of CT-DNA in the presence and absences of (2a) and (4a).

thymus-DNA. The binding constant values of 5.3×10^4 M^{-1} for (2a) and 6.5×10^5 M^{-1} for (4a) suggested that the compound (4a) bind more avidly to CT-DNA than the (2a).

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. The newly synthesized compounds tested for antimicrobial activity from the obtained protocol shows that sulfur containing carbothioamide compounds (4a) exhibited significant inhibitory activity and also bind more strongly with calf thymus-DNA compared to that of (2a).

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