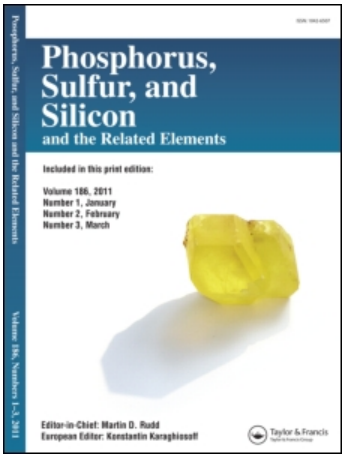

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2-Thieno/Selenopyrano[2,3-*b*]quinolines: Microwave-Induced One-Pot Synthesis, DNA Binding, and Photocleavage Studies

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*We have characterized a new class of 2-thieno/2-selenopyrano[2,3-b]quinolin-2-ol (**2c/3c**) intercalators exhibiting a novel type of DNA binding and photocleavage activity. The dynamic behavior of these compounds with DNA was investigated by absorption spectra (obtained K_b constant for **2c** is 3.6×10^6 and for **3c** is 2.8×10^5), viscosity, and thermal denaturation studies. Intrinsic binding constants (K_b) have been estimated under a similar set of experimental conditions. An absorption spectral study shows the strong interaction between synthesized **2c**, **3c**, and base pairs CT-DNA. In addition, the effects of these compounds on DNA cleavage were investigated by photoirradiation at 360 nm and show that the hybrids effectively cleaved double-stranded DNA with UV light of a long wavelength and without any additive.*

Keywords CT-DNA-binding-photocleavege; thieno-seleno-quinoline

INTRODUCTION

Quinoline and its derivatives have been reported to be associated with interesting pharmacological properties.[1–5] They are found in numerous commercial products, including pharmaceuticals, fragrances, and dyes. Quinoline alkaloids, such as quinine, chloroquine, mefloquine, and amodiaquine, are used as efficient drugs for the treatment of malaria.[6–13] A literature survey shows that there is evidence that

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antitumor activity is due to the intercalation between the base pairs of DNA and interferences with the normal functioning of enzyme topoisomerase II, which is involved in the breaking and releasing of DNA strands.[14] The antitumor drugs that intercalate DNA are of growing interest in the field of anticancer derivatives. Generally, they are characterized by planar chromospheres, which are often constituted of three or four condensed rings that can intercalate into base pairs. Results of these various binding studies have been useful in designing new and promising anticancer agents for clinical use.[15] DNA binding studies of pyrimidothienoquinolines have been recently reported in the literature.[16–17]

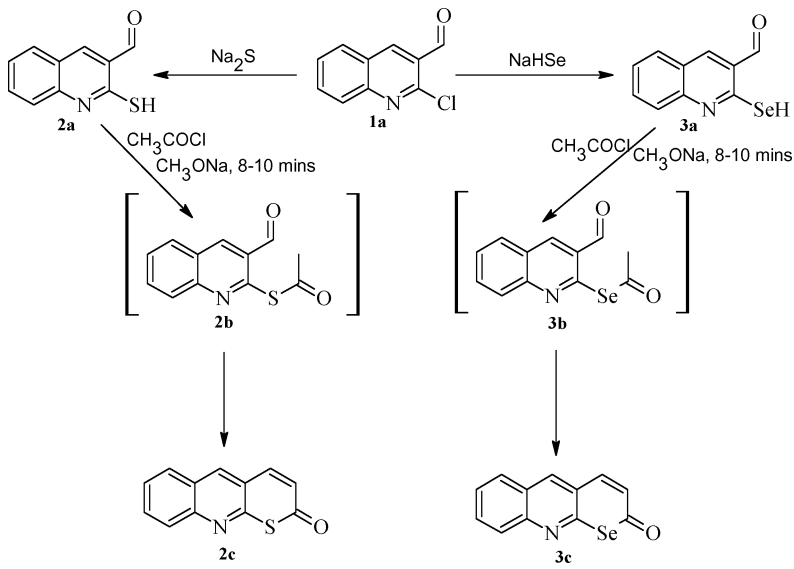
In addition, the biological and pharmaceutical activities of different selenium/sulfur compounds are of special interest because they have been associated with carcinogenicity,[18] toxicity,[19] modification of metal toxicity,[20] and prevention of cancer.[21] In the same field of research, the results of many studies[22–25] have related Se-deficient bioavailability and intake to human cancer mortality. Organoselenium compounds have substantially greater bioavailability than that of inorganic selenium.[26] More importantly, organic selenium is usually found to be less toxic than inorganic forms of the element.[27–30]

With the above facts and in continuation of our interest on DNA interaction studies,[31] we selected 2-thiopyrano/selenopyrano[2,3-*b*]quinolin-2-ol. This study explores the binding mode between the 2-thieno/2-selenopyrano[2,3-*b*]quinolin-2-ol to DNA and the influence ability of sulfur and selenium on the binding of quinolones and DNA.

RESULTS AND DISCUSSION

Chemistry

Accession of **2c**, **3c** via cyclization of intermediate **3a**, **3b** was followed by the reaction between **2a**, **2b** with acetyl chloride in the presence of sodium methoxide as catalyst. These required starting compounds **2a** and **3a**, which were synthesized according to our reported methods in the literature.[31c,e,f,g] The disappearance of a singlet in ^{1}H NMR at δ 2.5 and 2.7 belonging to CH_3 in both **2c** and **3c** confirms the cyclization of intermediate **2b** and **3b**. Similarly, the shift of absorption frequencies in IR spectra at 1635 cm^{-1}, 1647 cm^{-1} of C=O in both **2c** and **3c** shows the formation of **2c**, **3c**, and the appearance of a singlet in ^{1}H NMR at δ 13.2 and 11.3 of OH in **2c** and **3c** shows formation of final products. Finally, the structure was confirmed by its mass spectrum through



SCHEME 1 The synthesis of targeted ligands **2c** and **3c**.

the appearance of a molecular ion peak at m/z 216[M^+] and 263 [M^+] (Scheme 1).

Absorption Spectral Studies

The UV-Vis absorption spectrum of **2c** and **3c** were characterized by two maxima at 293 and 312 nm, respectively. The addition of increasingly higher concentrations of DNA led to bathochromic and hypochromic changes, as seen in Figure 1, i.e., the interaction of **2c** and **3c** with DNA resulted in a strong decrease of the absorption intensity at both peaks, accompanied by a shift towards higher wavelengths. A 19.3% and 16.2% of reduction in absorption were observed at 293 and 312 nm peak maximum respectively in the presence of an excess of DNA at a molar ratio of DNA nucleotide. Two isosbestic points were observed at 302 for **2c** and 325.6 nm for **3c**, respectively. Hypochromism was suggested to be due to strong interactions between the electronic states of the intercalating chromophore and that of the DNA base pairs.[32] The spectral changes that we observed (hypochromicity, red shift, and isosbestic points) were consistent with the intercalation of the chromophore into the stack DNA base pairs.

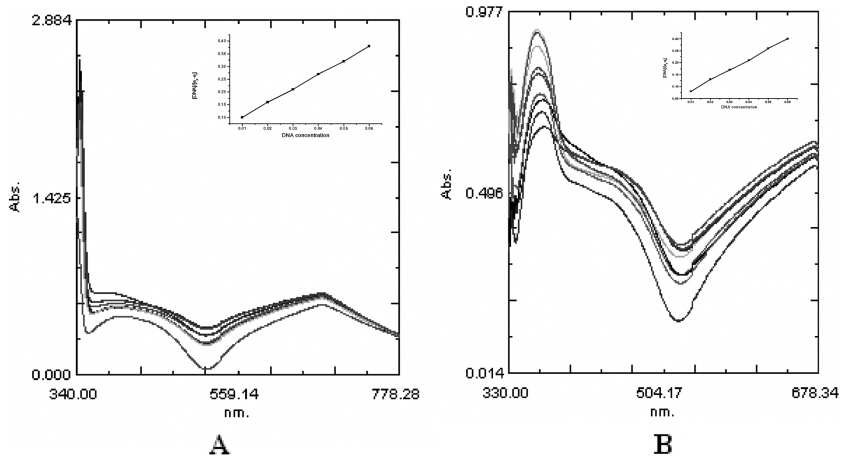


FIGURE 1 Absorption spectra for **A** (**2c**) and **B** (**3c**) in Tris-HCl buffer upon addition of DNA. **2c/3c** = 0.5 μM, [DNA] = 0–100 μM. Showing variation in absorption with increase in conc. of DNA. Inner graph of [DNA]/(ε_a-ε_f) v/s [DNA] for titration of DNA with **2c/3c**.

Viscosity Measurements

To further clarify the interaction modes of the **2c** and **3c** with DNA, they were investigated by viscosity measurements. An increase in the viscosity of native DNA is regarded as a diagnostic feature of an intercalation process.[33,34] We have measured the viscosity changes in short, rod-like DNA fragments. The relative length increase (L/L_0) of the complex formed between **2c/3c** with DNA is shown in Figure 2. It is evident that binding of MPTQ increased the viscosity of DNA, corresponding to an increase in the contour length of the DNA fragments. The measured slope of the plot 1.23 ± 0.03 falls within 63% for **2c**, and 1.05 ± 0.025 falls within 53% for **3c** of the slope of a theoretical curve for an idealized intercalation process (1 + 2r).[35,36] On this basis, we calculate that intercalation of one **3c** molecule provoked an increase of 1.9 Å in the contour length of DNA. Since the size of these sonicated fragments was significantly greater than the persistence length, the estimated 1.9 Å lengthening is probably best regarded as a lower limit.

Thermal Denaturation

Other strong evidence for the intercalative binding of **2c/3c** into the double helix DNA was obtained from DNA melting studies (Table I).

TABLE I Intrinsic Binding Constants (K_b) and DNA Melting Temperature (T_m)

Compounds	K_b (M^{-1})	T_m (°C)
2c	3.6×10^6	60
3c	2.9×10^5	58

The 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.[37] 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.[38] Thus the helix to coil transition temperature can be determined by monitoring the absorbance of DNA at 260 nm as a function of temperature. The DNA melting studies were carried out with calf thymus DNA in the absence and presence of **2c/3c** [1:5 ratio of **2c** and **3c** to DNA-c(P)] (Figure 3). The T_m for calf thymus DNA was 55 ± 1°C in the absence of **2c/3c**, but in the

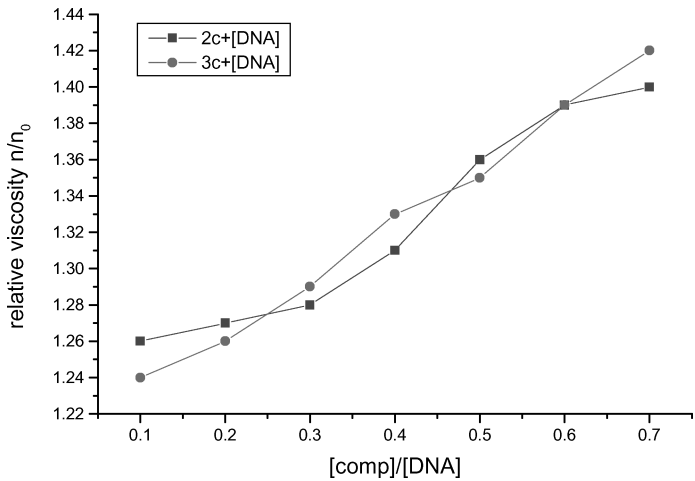


FIGURE 2 Plot of relative viscosity versus [2c,3c]/[DNA] effect of 2-thiopyrano[2,3-*b*]quinolin-2-ol (**2c**) and 2-selenopyrano[2,3-*b*]quinolin-2-ol (**3c**) on the viscosity of CT-DNA at 25 (±0.1°C). 2-thiopyrano[2,3-*b*]quinolin-2-ol (**2c**) = 0–100 μM, 2-selenopyrano[2,3-*b*]quinolin-2-ol (**3c**) = 0–100 μM, and [DNA] = 50 μM.

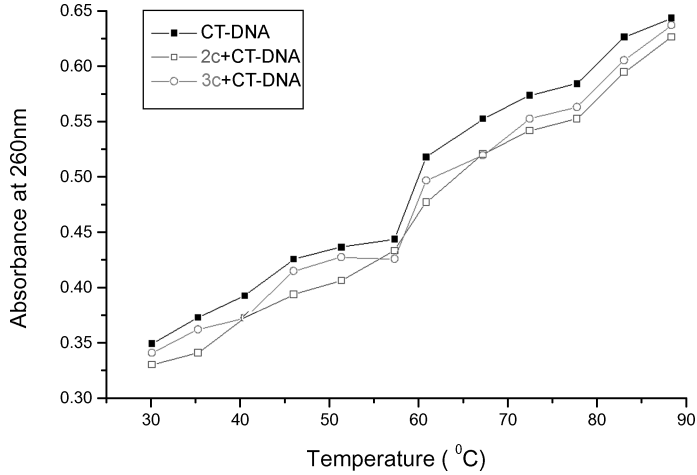


FIGURE 3 Melting curves of CT-DNA in absence and presence of both **2c** and **3c**.

presence of **2c/3c** the T_m of CT DNA was increased by 5°C and 3°C, respectively. The advantage of this method is that it is much easier to identify when more than one transition occurs.[39] These various DNA melting experiments strongly supportd the intercalation of **2c/3c** into the double helix DNA.

The pUC 19 DNA Cleavage Studies

It is now recognized that the extremely reactive $^{\bullet}OH$ radical derived from O_2^- and H_2O_2 is a cause of DNA strand scission in cellular damage.[40] Figure 4 shows the electrophoretic pattern of DNA after UV-photolysis of H_2O_2 (2.5 mmol/L) in the absence or presence of **2c/3c**.

The faster-moving band corresponds to the native form of supercoiled circular pUC19 DNA (SC DNA), i.e., DNA control in the absence of hydrogen peroxide (H_2O_2), and the slower-moving band is the open circular form (NC DNA). The UV irradiation of DNA in the presence of H_2O_2 (lane 2) caused the cleavage of scDNA to give open coiled DNA (NC DNA) and the linear form (linDNA), indicating that $^{\bullet}OH$ generated by UV-photolysis of H_2O_2 produced DNA strand scission.

The results may indicate the important role of sulfur and selenium in these photo-induced DNA cleavage reactions. It is likely that the photocleavage at 360 nm involves photoexcitation of the charge-transfer band, leading to the formation of an excited singlet state that, through the triplet state, activates molecular oxygen to form reactive singlet

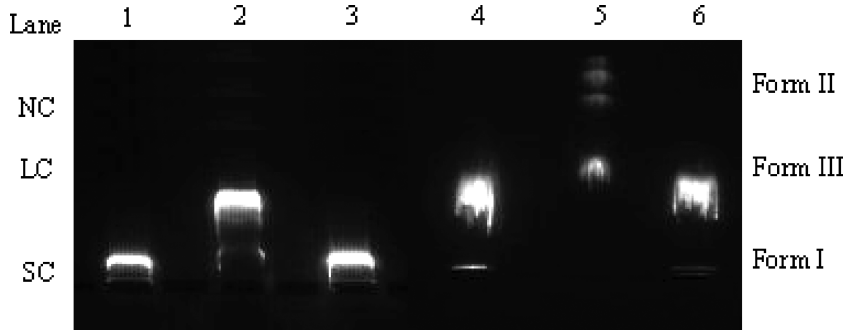


FIGURE 4 Cleavage pattern of supercoiled pUC19 DNA against OH, generated by the photolysis of H_2O_2 at different concentrations of **2c/3c** (200–400 mmol/L). Lane 1, untreated DNA (control); lane 2, 2.5 mmol/L H_2O_2 + DNA; lane 3, **2c** (200 mmol/L) + 2.5 mmol/L H_2O_2 + DNA; lane 4, **2c** (400 mmol/L) + 2.5 mmol/L H_2O_2 + DNA; lane 5, **3c** (200 mmol/L) + 2.5 mmol/L H_2O_2 + DNA; lane 6, **3c** (400 mmol/L) + 2.5 mmol/L H_2O_2 + DNA.

oxygen species. Significant cleavage was observed by the selenium-containing **3c** (70%) compared to that of sulfur containing **2c** (64%). From the above results, we conclude that in the photocleavage activity of **2c** and **3c** at 360 nm, **3c** shows significantly higher cleavage activity than **2c** based on its DNA binding propensity.

CONCLUSION

We demonstrated not only the molecular design and chemical synthesis of novel 2-thiopyrano[2,3-*b*]quinolin-2-ol (**2c**) and 2-selenopyrano[2,3-*b*]quinolin-2-ol (**3c**) hybrids, but also their DNA binding and photocleavage profiles. The described chemistry and biological evaluation provided significant information about the importance of novel and selective DNA binding and photocleaving agents.

EXPERIMENTAL

The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plates using petroleum ether/ethyl acetate solvent. Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded in KBr pellets on a Perkin-Elmer 157 IR Spectrophotometer. ^{1}H NMR spectra were recorded in DMSO-d_6 on an EM-390 (400 MHz) NMR spectrometer, mass spectra were recorded on a MASPEC low resolution instrument operating at 70eV, and UV-visible absorption spectra were recorded using a Shimadzu UV-1650

PC model. All reagents and solvents were AR grade, purchased commercially, and tris-HCl buffer was purchased from Qualigens (Mumbai, India). Calf thymus DNA (CT-DNA) and pUC 19 DNA were purchased from Bangalore Gene, Bangalore, India. Tris-HCl buffer (5 mM tris HCl, 50 M NaCl, pH 7.2, Tris = Tris (Hydroxymethyl) amino methane) solution was prepared using deionized, double-distilled water.

General Procedure for the Synthesis of 2-Mercapto/2-selenopyrano[2,3-*b*]quinolin-2-ol (2c/3c)

A mixture of 2-mercapto/2-seleno quinoline-3-carbaldehyde (**2a/3a**) (0.945/1.18 g, 0.005 mol), acetyl chloride (0.39 g, 0.005 mol), and sodium methoxide (1.08 g, 0.02 mol) was taken in a beaker and irradiated in a microwave oven for about 4–6 min in an interval of 20 sec at 160 W. The progress of the reaction was monitored by TLC; the obtained product was poured into ice-cold water, stirred well, and acidified with dil HCl. The solid that separated out was filtered, washed with water, and dried and recrystallized from methanol.

*2-Mercaptopyrano[2,3-*b*]quinolin-2-ol (2c)*

Yield 92%, mp 167°C; IR (KBr) cm^{-1}; 3020 (C-H, Ar-H); 1663 (C=O); ^{1}H NMR (DMSO d_6), δ 8.47 (d, 1H, Ar-H, $J = 8.00$), 7.65 (d, 1H, Ar-H, $J = 7.68$), 7.33 (d, 1H, Ar-H, $J = 7.43$), 7.58 (d, 1H, Ar-H, $J = 7.61$), 7.92 (d, 1H, Ar-H, $J = 8.05$), 7.11 (d, 1H, Ar-H, $J = 5.26$), 6.02 (d, 1H, Ar-H, $J = 5.25$), m/z: 213 $[M]^+$Anal. Calcd. For $(C_{12}H_7NOS)$%: C, 67.58; H, 3.31; N, 6.57. Found: C, 67.57; H, 3.32; N, 6.58.

*2-Selenopyrano[2,3-*b*]quinolin-2-ol (3c)*

Yield 85%, mp 192°C; IR (KBr) cm^{-1}; 3015 (C-H, Ar-H); 1678 (C=O); ^{1}H NMR (DMSO d_6), δ 8.44 (d, 1H, Ar-H, $J = 8.03$), 7.68 (d, 1H, Ar-H, $J = 7.65$), 7.35 (d, 1H, Ar-H, $J = 7.41$), 7.61 (d, 1H, Ar-H, $J = 7.62$), 7.95 (d, 1H, Ar-H, $J = 8.05$), 7.16 (d, 1H, Ar-H, $J = 5.25$), 6.11 (d, 1H, Ar-H, $J = 5.25$), m/z: 260 $[M]^+$Anal. Calcd. For $(C_{12}H_7NOSe)$%: C, 55.40; H, 2.71; N, 5.38. Found: C, 55.41; H, 2.70; N, 5.37.

DNA Interaction Experiments

Absorption Spectroscopy

The UV-Vis spectra were recorded on SHIMADZU, UV-1650 PC model spectrophotometer with a slit of 2 nm and scan speed of 400 nm min^{-1}. Quartz cuvettes of 1 cm were used. The absorbance assessments were performed at pH 7.3 by keeping the concentration of DNA or tRNA constant (0.25 mM), while varying the concentration

of flavonoids (0.005–0.3 mM). The values of the binding constants K_b were obtained according to the methods reported.[41,42]

Viscosity Measurements

Viscosity measurements were made according to published procedures[31] using a semimicro dilution capillary viscometer (Viscomatic Fica MgW) with a thermostated bath D40S at 20°C. The flow time for water was 71.1 sec. For the viscosity experiments, samples of calf thymus DNA were sonicated[43] to fragments having an estimated molecular weight of approximately 500,000.[44,45]

Thermal Denaturation Experiments

The DNA melting studies were done by controlling the temperature of the sample cell with a Shimadzu (SHIMADZU, UV-1650 PC) circulating bath while monitoring the absorbance at 260 nm. The temperature of the solution was continuously monitored with a thermo-couple attached to the sample holder.

DNA Cleavage by Photoirradiation

The experiments were performed in a volume of 20 mL containing pUC19 DNA in 5 mmol/L phosphate buffer containing 10 mmol/L NaCl, pH 7.4, in the presence of different concentrations (200–400 mmol/L) of **2c/3c**. Immediately prior to irradiating the samples with UV light, H_2O_2 was added to a final concentration of 2.5 mmol/L.

The reaction volumes were held in caps of polyethylene microcentrifuge tubes, which were placed directly on the surface of a transilluminator (8000 mW/cm) at 360 nm. The samples were irradiated for 5 min at room temperature. After irradiation, 4.5 mL of a mixture containing 0.25% bromophenol blue, 0.25% xylene cyanol FF, and 30% glycerol was added to the irradiated solution. The samples were then analyzed by electrophoresis on a 1% agarose horizontal slab gel in ris-borate buffer (45 mmol/L Tris-borate, 1 mmol/L EDTA). Untreated pUC19 DNA was included as a control in each run of gel electrophoresis, which was carried out at 1.5 V/cm for 15 h. Gel was stained in ethidium bromide (1 mg/mL) and photographed under UV light.

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