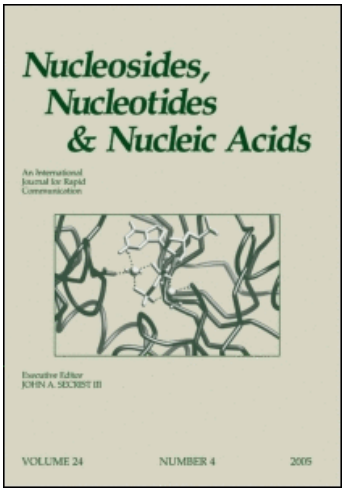

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Interaction of 2-Chloro-N^{10}-Substituted Phenoxazine with DNA and Effect on DNA Melting

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ABSTRACT

Five N^{10}-substituted phenoxazines having different R groups and −Cl substitution at C-2 were found to bind to calf −thymus DNA and plasmid DNA with high affinity as seen from by UV and CD spectroscopy. The effect of phenoxazines on DNA were studied using DNA-ethidium bromide complexes. Upon addition of phenoxazines, the ethidium bromide dissociated from the complex with DNA. The binding of phenoxazines to plasmid $_P$UC18 reduced ethidium bromide binding as seen from the agarose gel electrophoresis. Butyl, and propyl substituted phenoxazines were able to release more ethidium bromide compared with that of acetyl substitution. Addition of phenoxazines also enhanced melting temperature of DNA.

Key Words: Phenoxazine; DNA; Ethidium bromide; Agarose gel electrophoresis; DNA melting temperature.

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INTRODUCTION

DNA-intercalating ligands are a group of compounds of diverse structures, which have the capacity to bind tightly yet reversibly to DNA by intercalating a flat, aromatic chromophore between the base pairs. A majority of intercalators have either no site selectivity or show selectivity to G–C base pairs.[1] However some intercalators have shown selectivity to A–T base pairs.[2] The primary interest in such ligands has been their potential as anti-cancer drugs.[3] Actinomycin D was the first such compound to enter clinical trails in the sixties.[4]

Many of the DNA intercalating agents are potent inhibitors of nucleic acid synthesis, and this was originally considered to be their primary mode of action.[5] Now they have been shown, in addition, to cause sister chromatic exchange[6] and to form micronuclei.[7] Some intercalaters have been demonstrated to act as allosteric effectors of DNA conformation and will convert Z DNA to an intercalated right handed form under solution conditions.[8] Most of the DNA intercalating agents which are potent anticancer drugs. One major drawback, that is, they development of drug resistance. The most common type of such acquired resistance to intercalating agents is development of the 'pleiotropic' or multidrug resistance' phenotype.[9]

In our studies we have synthesized phenoxazine datives with an objective to overcome or circumvent multidrug resistance and to increase the nucleic acid intercalating activity and hence antitumor activity. Our earlier studies have shown that substitution on the phenoxazine ring at position N-10 increased the antiproliferative[10,11] and anti MDR activities of the phenoxazins.[12,13] However these studies did not reveal the mechanisms underlying the increase antiproliferative and anti MDR activities. Hence in this study we have explored the nature of interaction between DNA and phenoxazine derevaties. We have shown that hydrophobic effects and substitution at C-2 of the phenoxazine ring by –Cl or substitution at the N-10 by different alkyl groups have an important role in bringing about structural changes in DNA.

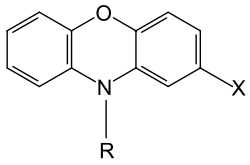
MATERIALS AND METHODS

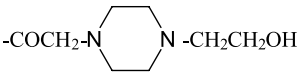
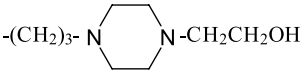
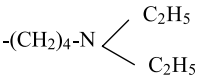
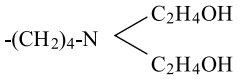
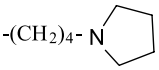
Preparation of DNA

Calf–thymus DNA (CT DNA) was obtained from E. Merck Co., Germany and was purified further by methods[14] involving phenol- chloroform extraction followed by ethanol precipitation. CT DNA thus purified was dissolved in 10 mM Tris-HCl buffer pH 7.4 and was used for spectroscopic binding analysis. The DNA concentrations were determined by using an extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm.

Plasmid DNA used herein were grown in *E. coli* cells and isolated in our laboratory by the method involving phenol-chloroform extraction followed by polyethylene glycol purification.[15] N^{10}-substituted phenoxazines were synthesized by Thimmaiah et al. using methods established in our laboratory[10,11] and they are listed in Table 1.

Table 1. Structural formulae of N^{10}-substituted phenoxazines.



Comp no.	R	X	Name
1		H	10-[[(β-hydroxyethyl) piperazino]acetyl] phenoxazine (PiAP)
2		Cl	10-[3-[(β-hydroxyethyl) piperazino]propyl-2-chloro phenoxazine (PiPCP)
3		Cl	10-[4-(N-diethylamino) butyl]-2-chloro phenoxazine (DBCP)
4		Cl	10-[4-[N-bis(hydroxylethyl) amino]butyl]-2-chloro phenoxazine (BBCP)
5		Cl	10-[4-N-pyrrolidinobutyl)- 2-chlorophenoxazine (PyBCP)

Spectral Absorption Titrations

Absorption titrations were carried out by keeping the concentration of the probe constant, while adding concentrated solution of the CT DNA in progressively increasing amount into both the cuvettes till the saturation in hypochromism was observed.

Determination of intrinsic binding constant (K) for a given complex with DNA, based on these absorption titrations were made by the half -reciprocal plot.[16] The intrinsic binding constant (K) for a given complex with CT DNA was obtained form the plot of $D/\Delta\varepsilon_{app}$ vs. D according to the equation.

$$\frac{D}{\Delta\varepsilon_{app}} = \frac{D}{\Delta\varepsilon} + \frac{1}{\Delta\varepsilon xK}$$

Where 'D' is the concentration of DNA in base molarity, $\Delta\varepsilon_{app} = |\varepsilon_a - \varepsilon_f|$ and $\Delta\varepsilon = |\varepsilon_b - \varepsilon_f|$. Where, '$\varepsilon_b$' and '$\varepsilon_f$' are respective extinction coefficient of the complex in the presence and absence of DNA. The apparent extinction coefficient, 'ε_a' was obtained by calculating A_{obs}/[phenoxazine]. The data were fitted to the equation, with a

slope equal to $1/\Delta\varepsilon$ and y-intercept equal to $1/(\Delta\varepsilon xK)$. The intrinsic binding constant (K) was determined from the ratio of the slope to y-intercept. The percentage hypochromism was calculated as

$$\% \text{ hypochromism} = \frac{(\varepsilon_{free} - \varepsilon_{bound}) \times 100}{\varepsilon_{free}}$$

CD Measurements

CD spectra were recorded on a JASCO J-715 spectropolarimeter. All measurements were made in Tris-HCL buffer pH 7.4 in a quartz cell of 0.2 cm path-length at pH 6.8 and 25°C. In case of UV CD measurments, the CD values were expressed as molar ellipticity, [θ] by following the equation, [θ] = [100 observed ellipticity (deg)/l × c] degcm^2 dmol^{-1}, where [θ] is the observed ellipticity in degrees, l the path length of the cell in centimeters and c is the concentration of the DNA in base molarity. Each spectrum is the average of three independent scans. Titration's in the DNA region (UV CD) and in induced CD region (ICD) were carried out by adding progressively increasing amounts of ligand to a solution of DNA of constant concentration.

Effects of Phenoxazines on the Fluorescence Properties of Ethidium Bromide-Plasmid DNA ($_P$UC 18) Complex

The effects of addition of various concentration of phenoxazines on the fluorescence emission spectra of intercalated bound ethidium bromide–DNA complex were examined. Known concentrations of different phenoxazines were separately added in small increments to solution cantaining Plasmid DNA (5 mM Tris-HCL buffer, pH 7.4). After each addition, the mixtures were carefully stirred followed by recording the corresponding fluorescence emission spectrum (550–700 nm) upon excitation at 266 nm. Each spectrum is the average of three independent scans.

The changes in the fluorescence emission peak (590 nm) due to DNA complexed ethdium bromide were plotted against the concentration of each phenoxazine. The concentration of phenoxazines required to obtain 50% quenching of the maximal fluorescence were compared to find out relative efficiencies of different phenoxazines.

Agarose Gel Electrophorosis

The phenoxazines–DNA association complexes using various concentrations of different phenoxazines were made by mixing the same amount of plasmid, ($_P$UC 18), (1 μg/reaction) at pH 7.4 Tris HCl (20 μM) followed by incubation at 37°C for 5 min in 20 μl. Solution was directly loaded onto 1% agarose gel with loading dye (bromophenol blue and xylene cyanol FF). All the DNA samples as well as dye from the respective lanes were electrophoresed under constant electric field of 80 V for

90 min and then stained with ethidium bromide (0.5 μg/ml) for 2 h. Bands of DNA were detected and photogragraphed under UV light.

Melting Temperature Measurements

Melting temperatures(T_m) for pure CT-DNA or Phenoxazine–CT DNA complexes were measured by following the changes in absorption at 260 nm (A_{260}) as a function of temperature in a Shimadzu Model UV-2100 UV-Vis spectrophotometer. Temperature of the cuvette chamber was maintained using a Jalabo Model F-10 water circulating bath and the temperature inside the cuvette were measured before and after recording each spectrum. All the experiments were carried out with 0.531×10^{-5} M CT DNA at pH 7.4, 5 mM Tris-HCL buffer. The absorption intensity at 260 nm were plotted against individual temperatures and mid points of inflection region in the temperatire-A_{260} curves were taken as the corresponding T_m values.[17]

RESULTS AND DISCUSSION

Absorption Titrations

To examine intercalative and electostatic binding of phenoxazines to DNA, five compounds **1–5** (listed in Table 1) were selected. These compound had anti-cancer, anti-MDR and antibacterial activity.[10–13] We studied the interaction of these compound with duplex DNA. The interaction of these compounds with CT DNA was studied by monitoring the changes in the UV-visible absorption spectra of the phenoxazines upon addition of CT DNA. Absorption spectra of the compounds (**1–5**) in the absence and presence of varying amount of CT DNA are shown in Fig. 1. In the range from 200 to 220 nm all N^{10}-substituted phenoxazines exhibit strong absorption peak with maxima near 208–214 nm. The binding of phenoxazine derivatives to CT DNA led to strong decrease in the absorption intensities in the absorption maxima of the phenoxazines. Progressive addition of DNA led to strong hypochromism in the absorption intensity in all the compounds studied. The percentage hypochromism were found to be 8,48, 22, 74, and 47% for compounds **1–5** respectively. Spectra obtained upon complex formation with CT DNA by each of the phenoxazines exhibit clear isosbestic points around 215 nm. The presence of isosbestic point in each of these titrations suggests a chemical equilibrium between the bound and free ligand with no spectrophotometrically detectable intermediates.

The peaks at 208–214 nm represent the absorption due to free ligand in solution (**Trace 1** in Fig. 1), while corresponding peaks at 208 –220 represent the red shifted intercalation complex between ligand and CT-DNA. Compounds **3** and **5** caused red shift of 5 nm (Fig. 1. Panel C and E) and compound **4** caused red shift of 6 nm (Fig. 1 Panel D). However compound **1** did not show any red shift (Fig. 1 Panel A).

The degree of hypochromism generally correlated well with overall binding strength. The extent of hypochromicity as a function of DNA binding, plotted reciprocally as A_o/A vs. (DNA), was found to provide a good measure of relative

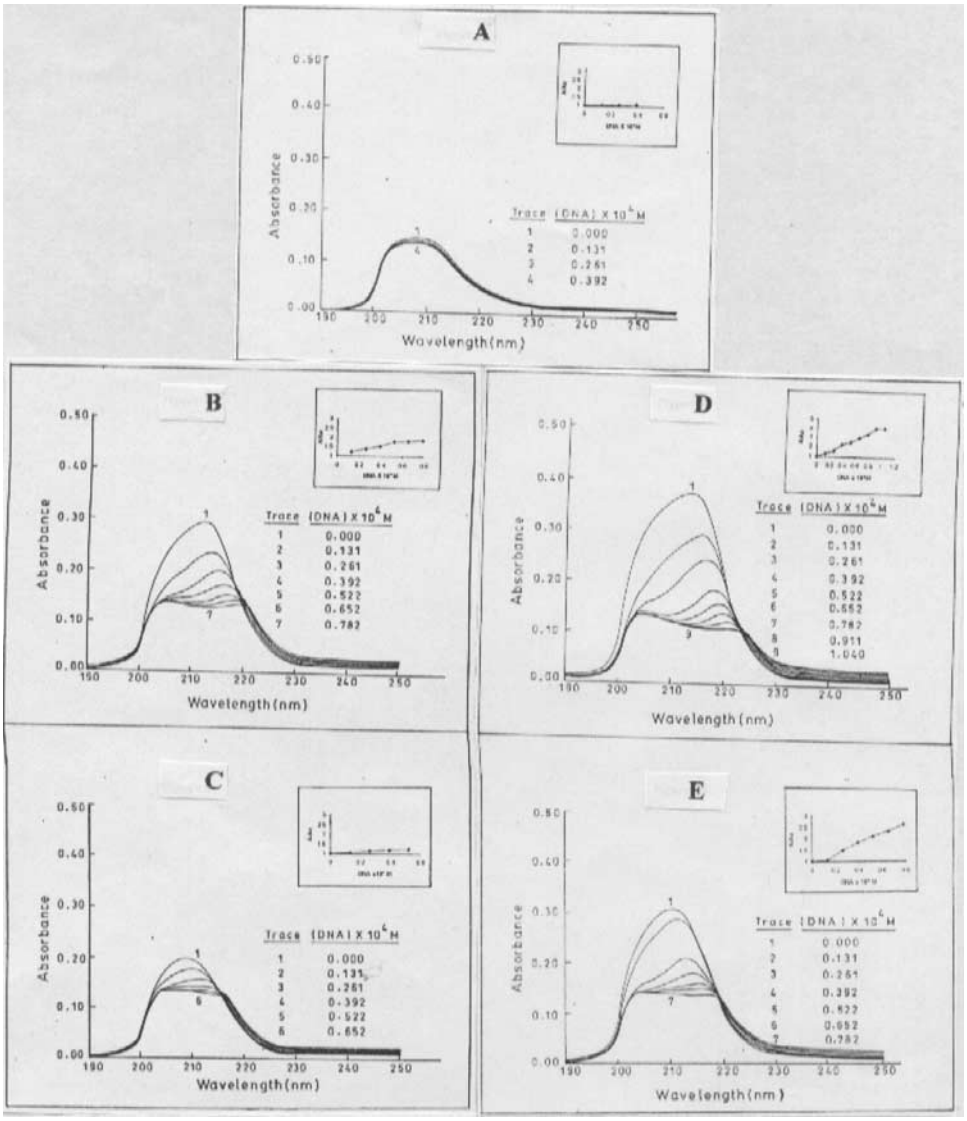


Figure 1. Absorption titrations of different phenoxazines with CT DNA. Trace 1 in all the panels shows the uv-vis spectra due to the phenoxazines alone. Subsequent traces were obtained upon incremental addition of CT DNA as shown. Panels A–E correspond to plots due to the DNA binding of compounds (**1–5**) respectively. Insets shows the plots of the relative optical density (A_o/A) vs. [DNA].

binding affinity (Inset in Fig. 1). Since hypochromism is a manifestation of stacking interactions, there is reason to believe that there is atleast a partial stacking with all the phenoxazines studied here which show substantial hypochromism. Interestingly compound **1** (Fig. 1 Panel A) failed to promote hypochromism. This could be due to

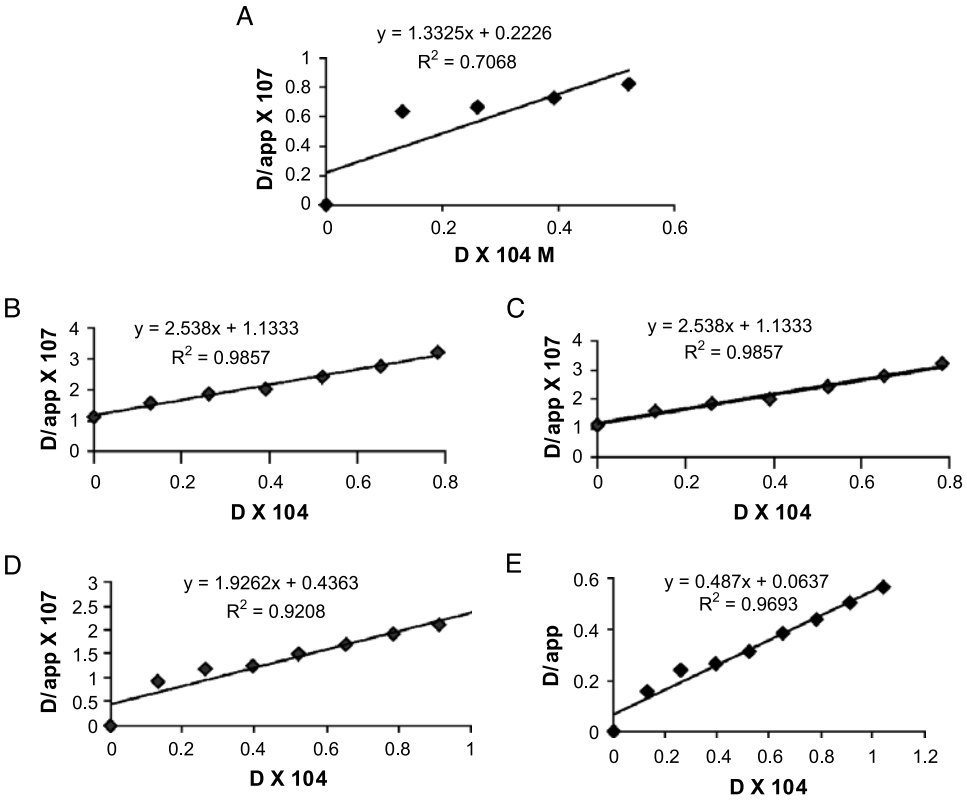


Figure 2. Half reciprocal plots for binding of different phenoxazines with CT DNA. Panels A–E correspond to plots due to the DNA binding of compounds (**1–5**) respectively.

a lack of binding ability to DNA. Figure 2 shows the half reciprocal plots for binding of five phenoxazines (**1–5**) with CT DNA.

CD Measurements

CD, defined as the differential absorbance of the left and the right circularly polarized light was used to obtain additional insight concerning the DNA binding of the phenoxazines derivatives used in this study. Figure 3 shows the effects of addition of increasing amounts of phenoxazines to a constant concentrations of DNA. All penoxazines enhance the ultraviolet CD (UV CD) of B-DNA with compound **1** having the least effect compared with compounds **2–5** at a given DNA/Phenoxazine ratio (Fig. 3A–3E inset). The strong enhancement in the magnitude of the DNA doublet in the UV CD could be the result of interaction through gradual alteration of DNA structure. Since base pairs must separate vertically to allow for interaction, the sugar phosphate backbone is disturbed and the regular helical structure is modified. There could be an additional interactions between the ligand transition dipoles with that of DNA.

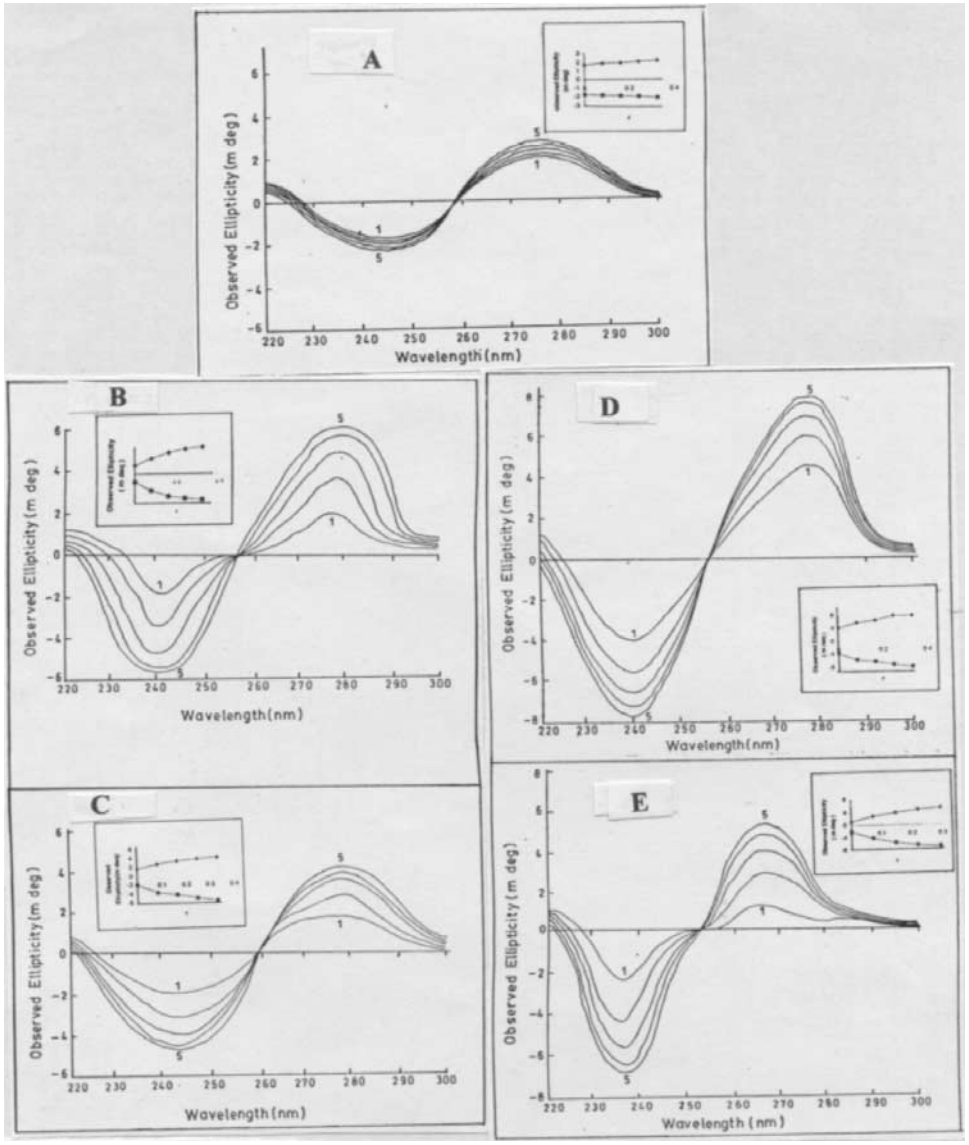


Figure 3. UV-CD studies. Effect of addition of different phenoxazines on the UV-CD of CT DNA. **Trace 1** in all the panels show the CD spectra due to the CT DNA alone. Subsequent traces were obtained upon incremental addition of phenoxazines as shown. Panels A–E corresponds to plot due to the DNA binding of compounds (**1–5**) respectively. Plots of molar ellipticity vs r are given in the inset of each panel.

All the ligands investigated in this study are achiral and cannot exhibit any signal on their own. Upon binding to DNA by partial or complete intercalation, the ligand experiences a chiral environment, which results in the ligand manifestation of the induced CD (ICD) signal in the ligand absorption region of the CD spectrum. Figure 4 shows the effects of addition of increasing amounts of ligands to a fixed concentration

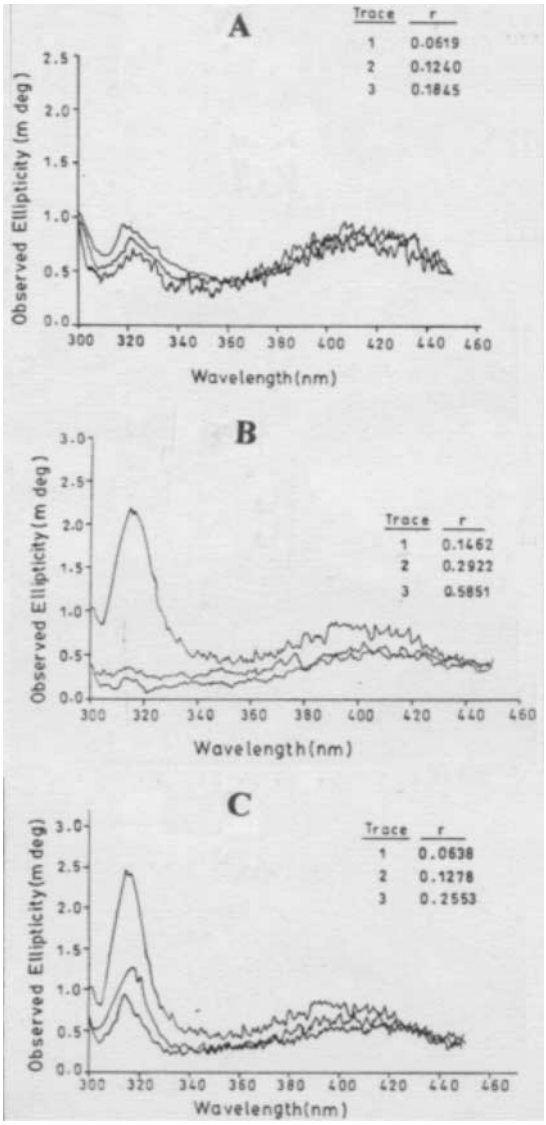
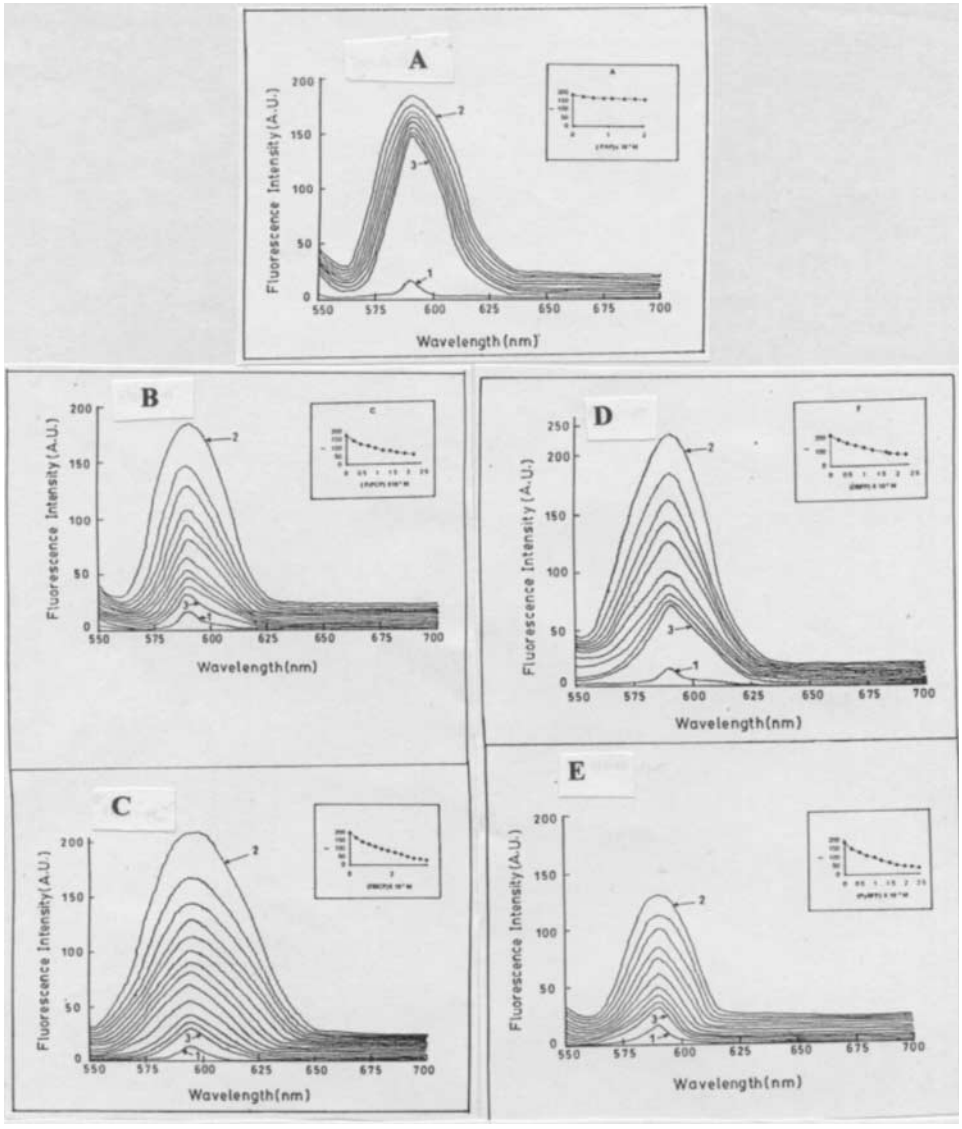


Figure 4. ICD spectra obtained on binding of phenoxazine depravities with CT DNA. Panel (A–C) correspond to spectra due to binding of compounds **1**, **2** and **3** respectively. These spectra were obtained by adding increasing amounts pf phenoxazine depravities to a concentration of CT DNA.

of DNA. As the ligand concentration increases, the resulting CD signals increase in intensity. In all the cases where the phenoxazine nucleus with R group containing butyl side chain, the CD band with the higher wave length was found to be positive in sign. Additional supports for intercalation stems from agarose gel electrophoresis experiments. This implies that the intercalated ligand chromophores undergoes orientation, most probably greater stacking in to the base pairs of DNA.

In all the five cases of phenoxazine-DNA complexes the magnitude of ICD increased as the amount of phenoxazines increased. Based on the circular dichroism studies of aminoacridines, compounds structurally similar to phenoxazines bound to DNA, Dalgleish et al.[18] proposed that two possible explanations could be advanced for the observed variation of ICD as a function of ligand concentration.

1. The variation is the result of interaction between bound ligands, which naturally increase as the number of molecules in a given interacting group increases. This kind of interaction between bound ligand will result in the formation of exciton bands.



2. The progressive binding of ligand molecules continuously alter the shape of the macromolecule, so that the number of bound ligand molecules in its vicinity determines the environment of any particular bound ligand. On this basis, the cause of the induced optical activity is the interaction of the ligands with the DNA bases surrounding them, rather than with other ligand.

Pasternack et al.,[19] investigated the interactions of tetrakis (4-N-methylpyridyl)porphine (H_2TMPy P-4) and its copper(II), nickel(II), zinc(II), cobalt(III), iron(III), and manganese(III) derivatives with CT DNA and with oligonucleotides. All the metalloporphyrins which do not have axial ligands like Cu(II) and Ni(II) and also the nonmetalated porphyrin show negative ICD with poly(dG-dC) in the ligand absorption region. Thus the metalloporphyrins with axial ligands must be partially interacted in the complex with poly(dA-dT). Thus, in combination with absorption spectral measurements, a negative ICD has been taken as an indication of intercalative GC binding and a positive ICD as that of partial intercalation or external binding to AT regions.

In the present case the intensity of ICD increased in all the cases with increasing concentration of ligand without producing any negative ICD spectrum which is the characteristic of AT specificity. The basic structural unit in these compounds is the same. The difference in their structures arise because the substituents attached to the N^{10}-position are of diverse functionality. From this study it is clear that these compounds behave in an analogous manner with DNA irrespective of their structural difference. However, the exact nature of interaction of these molecules with DNA is not known.

Effect of Phenoxazine Addition on Plasmid DNA ($_P$UC 18)-Ethidium Bromide Complex Using Fluorescence Spectroscopy

The strong intercalative ability of ethidium bromide is well documented in literature. The effects of addition of 2-chloro-N^{10}-phenoxazines on the ethidium bromide-supercoiled DNA complex were examined by adding progressively increasing amounts of phenoxazines to solutions containing DNA-bound ethidium bromide. To verify our results about phenoxazine-DNA interaction,we choose five phenoxazines (**1–5**) for fluorescence studies.

Figure 5. Effects of addition of different phenoxazines on plasmid DNA-bound ethidium bromide. This experiment was carried out by adding increasing amounts of phenoxazines into the plasmid DNA-ethidium bromide complex followed by recording the fluorescence emission spectra after each addition (excitation at 266 nm and emission in the range 550–700 nm). Panels A, B, C, D and E in this figure show the effect of addition of compounds (**1–5**) respectively into the intercalated ethidium bromide-plasmid DNA complex. In all the figures (A, B, C, D, and E), **trace 1** represents the fluorescence emission spectra due to ethidium bromide in 5 mM Tris-HCL buffer (pH 7.4). **Trace 2** represents the absorption spectra of fully plasmid DNA-bound ethium bromide and **trace 3** represents the final spectra (after the attainment of saturation in fluorescence quenching obtained upon addition of phenoxazines of a saturating concentration of a phenoxazines into the plasmid DNA-ethidium bromide complex). The addition of progressive increasing amount of phenoxazines into the plasmid DNA-ethidium bromide complex resulted in spectra from 2 towards 3. The corresponding insets were obtained by plotting the fluorescence intensity of (I) againts phenoxazine concentrations.

Figure 5 shows the changes in the fluorescence emission spectra upon the addition of increasing amounts of phenoxazines to plasmid DNA-bound ethidium bromide. **Trace 1** represents the spectrum due to ethidium bromide alone (2.2×10^{-6} M in 5 mM Tris-HCL pH 7.4). Addition of plasmid DNA into this solution led to enhancement of the fluorescence emission intensity of the resulting mixture (**Trace 2**). The progressive addition of phenoxazines to this DNA-ethidium bromide complex led to gradual fluorescence quenching, finally reaching saturation. **Trace 3** in Fig. 5 (**all panels**) represent the maximally quenched fluorescence.

However addition of several aliquots of N^{10}-substituted acetyl phenoxazine into the DNA-ethidiun bromide complex, the fluoresce spectra of the DNA-bound ethidinm bromide did not alter significantly. This is in marked contract to what was observed in the experiments involving addition of phenoxazines containing alkyl side chains (Fig. 5B–E). The changes in the fluorescence emission intensity (at 590 nm) due to DNA bound ethidium bromide were plotted against the concentration of phenoxazines (shown as insets in Fig. 5). This plot gave relative estimates about the efficiencies of different phenoxazines in inducing destabilization of the ethidium bromide-DNA complex. Amount of phenoxazines required to effect the same extent (50%) of fluorescence quenching varied with the hydrophobic and electrostatic character nature of phenoxazines.

The apparent quenching in the fluorescence emission intensity upon the addition of increasing amounts of phenaxazines could be due to a gradual release of the free ethidum bromide out of the ethidium bromide- DNA complex. This may be due to phenoxazines induced perturbation of DNA organization leading to dissociation of the ethidium bromide from the ethidium bromide- DNA complex. All phenoxazines could influence the ethidium bromide- DNA complex stability although with wide variability.

However, the addition of N^{10}-substituted acetyl phenoxazines could not affect the stability of DNA-bound ethidium bromide complex to any significant extent. This could be due to lack of interaction between acetyl phenoxazines and the super coiled DNA.

While the results obtained with absorption titration experiments involving phenoxazine–DNA complex, and results obtained with circular dichroism experiments, are qualitatively similar to those obtained with fluorescence titration employing ethidium bromide- DNA complex.

Agarose Gel Assay

Ethidium bromide displacement assay has also been used by a number of researchers to estimate the DNA binding efficiency of different classes of DNA binding molecules.[20]

Agarose gel electrophoresis allows one to compare the relative location of the DNA fragments under a constant electric field and gel electrophoresis is routinely used to separate linear double stranded DNA fragmants of different sizes. Each band could be detected visually by ethidium bromide staining under UV light. Since ethidium bromide is probably well characterized intercalator of DNA, and hence intercalation between plasmid DNA and different phenoxazines could be additionally proved by using agarose electrophoresis of the complexes formed between different phenoxazines and DNA.

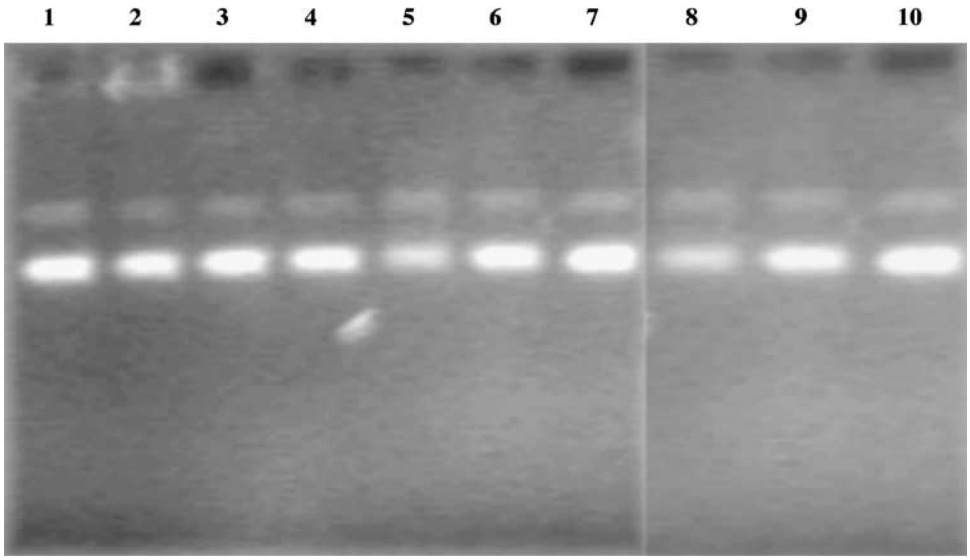


Figure 6. Effect of phenoxazines on the electrophoretic mobility of plasmid DNA in agarose (0.8%) gel. Lane 1 shows plasmid DNA alone, **Lane 2, 3** and **4** shows plasmid DNA in the presence of 1×10^{-3} M, 1×10^{-4} M and 1×10^{-5} M of compound **1** respectively. **Lane 5, 6** and **7** shows in the presence of 1×10^{-3} M, 1×10^{-4} M and 1×10^{-5} M of compound **2** respectively. **Lanes 8, 9** and **10** shows plasmid DNA in the presence of 1×10^{-3} M, 1×10^{-4} M and 1×10^{-5} M of compound **3** respectively.

Figure 6 summerises the salient features of the intercalation between the plasmid DNA and different phenoxazines. **Lane 1** shows plasmid DNA alone, while **lanes 2 to 10** show plasmid DNA in the presence of decreasing concentration of compounds **1–5**.

Our experiments reveal that at high concentration (1×10^{-3} M) of the phenoxazine (compound **2** and **5**), the DNA phenoxazine complex bands in agarose gel showed very faint staining which could be visualized under UV exposure even after 1 hr. At lower concentration (1×10^{-5} M) of the phenoxazines, ethidium bromide staining after the gel electrophoresis was considerably improved. Interestingly, phenoxazines containing acetyl group (compound **1**) have no effect on ethidium bromide staining even at a high concentration. The faint visibility of the DNA bands in the presence of larger concentration of phenoxazines having propyl or butyl side chain (compound **2** and **5**) in the agarose gel even after long ethidium bromide staining suggest that plasmid DNA-phenoxazines complexes lost the ability to intercalate ethidium bromide. This is possibily due to the formation of compaction between DNA-phenoxazine molecule and concomitant alteration in the native DNA structure in water. Although the DNA confrontation under this situation is changed to a compact structure, this form of DNA can still retain the double stranded organization.[21] Faint staining of the DNA could be also be due to condensation of DNA structure.[22] This would leave insufficient space for ethidium bromide to intercalate and hence stain the DNA. Such condensed DNA makes itself inaccessible to small intercalotors such as ethidium bromide.

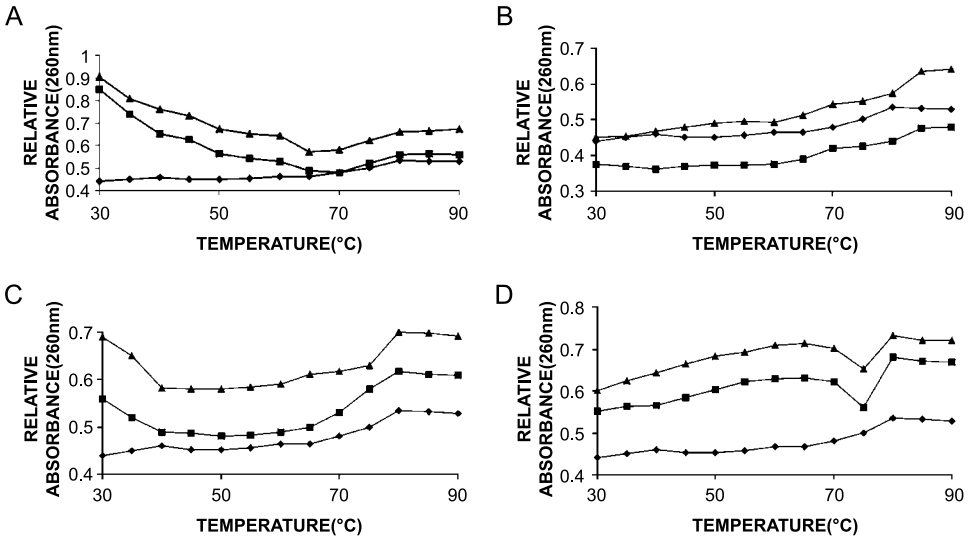


Figure 7. Melting profiles of CT DNA in the presence and absence of different phenoxazines at varying concentrations. In both panel A and B the curve with (▲) represents the melting profile of free CT DNA (0.513×10^{-5} M) and the curve with (●) represents the melting profile of CT DNA (0.513×10^{-5} M) in the presence of 1×10^{-3} M phenoxazines (**a**, **b**, **c** and **d**) respectively. The curve with (■) represents the melting profile of CT DNA (0.513×10^{-5} M) in the presence of 1×10^{-4} M phenoxazines (**a**, **b**, **c** and **d**) respectively.

However, no sighificant differences in the mobilities of different forms of plasmid DNA was observed in the presence of varying concentration of phenoxazines under the condition of gel electrophoresis. Under comparable conditions, the phenoxazine did not affect the band mobilities of the resulting phenaxazines complexes with DNA.

Effects on DNA Melting

We also examined the effects of different phenoxazines on the melting properties of CT DNA. The thermal denaturation on CT DNA were conducted at varying

Table 2. Uv-Visible and CD measurements of titration of DNA with phenoxazines.

Comp. no.	λ_{max} (nm)	Molar extinction coefficient (ε) Lit $m^{-1}cm^{-1}$	% Hypochromism	$K \times 10^4$ M
1	208	3107	8	5.98
2	213	6038	48	2.23
3	210	3204	22	2.23
4	214	5813	74	4.41
5	214	5813	74	7.64

Table 3. The denaturation of calf thymus DNA in the presence of added phenoxazines.

Compound[a]	10^5 (Phenoxazine), M[b]	Tm[c]	DT[d]
None	–	73	14
1	10	77	9
	100	79	10
2	10	77	11
	100	78	12
3	10	69(82)	7(14)
	100	70(82)	7(15)
4	10	76	4
	100	78	4

[a]See Table 1 for structures and notations of the compounds.
[b]The concentration of DNA in base molarities (0.513×10^{-5} M) was kept constant forall the experiments.
[c]See text for condition. The Tm values are accurate within + 1°C.
[d]Widths of thermal denaturation: these values are accurate within + 1°C.

(phenoxazines)/(DNA) ratios in the presence of 5 mM Tris-HCL buffer (pH 7,4) as shown in Fig. 7 panel A–D. The relevant data are summarized in Table 2.

As is evident from Table 3, the DNA melting behavior in the presence of phenoxazines are approximately the same. Modest rises in Tm values with concomitant broadening of the melting profile as a function of phenoxazine concentration were seen.

CONCLUSIONS

Anticancer drugs have been shown to influence DNA organization in several different ways. These include B to A transition,[8] helical destabilization,[6] melting DNA aggregation and condensation of DNA in to compact structure.[5]

The present investigation clearly brings out several points of similarity and differences between effects of phenoxazine on DNA and shows how the hydrophobic effects and specific charges play an important role in bringing about profound changes in DNA structure. We have examined a chromogenic probe (ethidium bromide) that interacts strongly with DNA to form an intercalated complex. The stability of the ethidium bromide- DNA complex is influenced by the addition of phenoxazines leading to the dissociation of probe from the complex. The effect is more pronounced when phenoxazines have propyl or butyl side chain. Comparison of the derivatives for their ability to bind with DNA revealed that they largely follow the order N^{10}-alkyl side chain >N^{10}-acetyl side chain. This trend may be because the attachment of a polar group, -$COCH_2$ to the N^{10} position gives increased hydrophilicity to the molecule. In general, substitution of hydrogen by –Cl increased the ability to bind DNA. This may be due to enhanced lipophilicity of the compounds after–Cl substitution. Careful examination of the results obtained revealed that activity largely

increased as the chain length increased and drastically decreased for $-COCH_2$ group suggesting that hydrophilic group is less effective than hydrophobic groups. The efficiency of the propyl or butyl phenoxazines in destabilizing the probe-DNA complex is nearly three to four orders of magnitude greater than the corresponding capacity of acetyl phenoxazines. Because of the presence of positive charges on nitrogen atom of the phenoxazine nucleus, electrostatic binding of these molecules to the anionic DNA phosphates is facilitated. This results in charge neutralization of the DNA backbone. This in turn reduces the inter and intra strand electrostatic repulsions present in native DNA phosphate backbone. As a result, under these circumstances, the DNA duplexes pack in more compact fashion, leaving insufficient space available for the accommodation of the incoming intercalator molecule or of the once pre-existing with in double strands. Thus, this results in the destabilization of probe-DNA complex. This conclusion is further supported by the lack of ethidium bromide staining of DNA bands in the presence of high concentration of phenoxazines in agarose gel electrophoresis experiment. The phenoxazines also affect the DNA melting behavior as evidenced by modest increases in T_m double to single strand transition. Although, at this level of knowledge, it is difficult to assign the exact mode of interaction, several possibilities exist. The complexation of phenaxazines with DNA might also lead to important changes in the 'structure' of water molecules around the DNA backbone. Our results suggests that alkyl side chain and hydrophobicity increase the interaction of phenoxazines with DNA. It remains to be experimentally verified whether such phenoxazines with increased hydrophobicity are also better than antiMDR agents.

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