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DRUG–DNA INTERACTION STUDIES OF ACRIDONE-BASED DERIVATIVES

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N10-alkylated 2-bromoacridones are a novel series of potent antitumor compounds. DNA binding studies of these compounds were carried out using spectrophotometric titrations, Circular dichroism (CD) measurements using Calf Thymus DNA (CT DNA). The binding constants were identified at a range of $K = (0.3$ to $3.9 \times 10^5$ M$^{-1}$ and the percentage of hypochromism from the spectral titrations at 28–54%. This study has identified a compound 9 with the good binding affinity of $K = 0.39768 \times 10^5$ M$^{-1}$ with CT DNA. Molecular dynamics (MD) simulations have investigated the changes in structural and dynamic features of native DNA on binding to the active compound 9. All the synthesized compounds have increased the uptake of Vinblastine in MDR KBCh R-8-5 cells to an extent of 1.25- to 1.9-fold than standard modulator Verapamil of similar concentration. These findings allowed us to draw preliminary conclusions about the structural features of 2-bromoacridones and further chemical enhancement will improve the binding affinity of the acridone derivatives to CT-DNA for better drug–DNA interaction. The molecular modeling studies have shown mechanism of action and the binding modes of the acridones to DNA.

Keywords Acridone; drug-DNA intercalation; absorption spectral titrations; circular dichroism measurements: molecular dynamics

INTRODUCTION

DNA-intercalating ligands are a group of compounds of diverse structures that have the capacity to bind tightly but reversibly to DNA by insertion (intercalation) of a flat, aromatic chromophore between the base pairs. Although acridone derivatives show a wide variety of biological effects, the
primary medicinal interest in such ligands recently has the potential as anticancer drugs.\cite{1} Following the success of the natural product doxorubicin, which was discovered by random screening and which proved to be the first really broad-spectrum anticancer drug despite its severe cardiotoxicity,\cite{2} an enormous amount of work has been published on the rational design of synthetic DNA intercalating agents as anticancer drugs. While there have been some clinical success (e.g., amsacrine, mitoxantrone), none of these compounds appear superior to doxorubicin and the future direction of this field of drug development is unclear and lot of anticancer drugs are undergoing studies for DNA intercalation.

The first DNA-intercalating agent widely used as an anticancer drug was actinomycin-D, which entered clinical use in the early 1960s\cite{3} followed by the other randomly discovered antitumor antibiotics daunorubicin in 1965,\cite{2} 9-methoxyellipticine in 1970\cite{4} and doxorubicin in 1974.\cite{5} In 1978, the acridine derivative amsacrine became the first synthetic DNA intercalating agent to be clinically successful.\cite{1,6} Since then the majority of DNA intercalating agents entering clinical trial as anticancer drugs have been synthetic and semi-synthetic compounds and their structures have been given in Figure 1, for example, the amsacrine analog CI-921,\cite{7} the ellipticine analog celipticinium acetate,\cite{8} mitoxantrone,\cite{9} amonafide,\cite{10} bisantrene,\cite{11} crisnatol,\cite{12} and oxanthrazole.\cite{13} One DNA-bisintercalating agent, the natural product echinomycin has also received clinical evaluation.\cite{14}

DNA undergoes various conformational changes to B-form, A-form, Z-form, quadruplexes, triplexes, and others when it binds to different compounds. Among them B-form is the most frequently observed conformation with the base pairs perpendicular to double-helix axis and relatively small peak intensities. DNA intercalators interact with the DNA double helix in a conformation, which is close to the B form, characterized by the presence of major and minor helical grooves. The minor groove has a structured layer of water and its width is less in stretches of DNA rich in adenine-thymine base pairs.\cite{15} On the other hand, the major groove has an environment of hydrophilic phosphate groups and in areas of high guanine-cytosine content, the major groove is the region of highest negative charge.\cite{16} Specific contacts are usually made between drugs and atleast one of these grooves, and such interactions are of great importance both for the degree of sequence selectivity and the biological activity of intercalators. It is not easy to determine the exact nature of these interactions, or the sequence of events by which intercalators dock to their respective intercalation sites. However, detailed structure-activity relationship studies with different classes of synthetic DNA-intercalating compounds have repeatedly suggested that DNA intercalation is a necessary condition for antitumor activity among closely related analogs.
Interaction of Acridones with DNA

Interaction of DNA with various biologically important organic molecules has been extensively reviewed in the literature.[17–19] Ligand
binding to DNA can be characterized by three modes, that is, interaction–intercalation characterized by a 30–40% change in intensity with a red shift of 20–40 nm because of ligand stacking between base pairs of DNA, electrostatic binding induces smaller change in intensity with no shift in the wave length of absorption maxima and the groove binding shows change in both.[20]

Acridones are the bioisosteres of phenothiazine and phenoxazine that binds reversibly to DNA by intercalation facilitated by cationic ionization and molecular planarity. Study of Drug–DNA interactions is very important for complete understanding of replication and transcription process, which further helps in better design of anticancer and antibiotics.[21] Most of the marketed anticancer drugs acts by DNA binding and their modes of binding will be either DNA intercalation or groove binding or covalent binding. These drugs act by preventing DNA relaxation, blocking gene expression, and inhibiting DNA replication.[22] Intercalation is a process in which planar aromatic constituent is inserted between two base pairs of DNA, causes unwinding and lengthening of DNA helix. Whereas groove binding causes little distortion of DNA structure. Studies proved that DNA intercalating compounds can show bathochromic shifts and hypochromicity in the UV-visible graph.[23] Our previous studies investigated the noncovalent interactions of the novel acridone derivatives with the duplex DNA by ESI-MS technique. And identified that acridones interacts with duplex DNA by intercalation, possesses higher affinity to GC than AT base pairs of the DNA and could not interact with the minor grooves.[24]

The determination of hypochromism is a basic method to interpret the denaturation of DNA. One of the interesting physical methods is the Circular dichroism (CD) ideally suitable to study the drug–DNA interactions. It is a highly sensitive, fast, and simple method which provides important information about the conformation of biomolecules. Other methods like X-ray, NMR, and conformational analysis have many disadvantages because of limited experimental conditions. CD spectra help in identifying the information about the binding modes of complex (Compound+DNA) by monitoring the asymmetric environment. The difference between the absorption of right and left handed circularly polarized light by chiral molecules is called CD. It is expressed in degrees and this phenomenon is called ellipticity. The CD spectrum of free CT DNA shows negative band at 245 nm due to helicity and a positive band at 279 nm due to base stacking which is the characteristic of DNA.[25]

Our investigation and results suggested that $N^{10}$-substituted acridones are good modulators of multidrug resistance in cancer in vitro as well as in bacteria in addition to their anticancer activity. Adequate understanding of the structural and thermodynamic aspects of the interactions between a drug and the target biological macromolecule is one of the important aspects in the design of new therapeutic agents. In this direction,
binding of these acridone molecules with DNA has been undertaken as part of understanding the nature of action of these molecules on cancer and bacterial cells. Ten compounds which showed very good cytotoxicity were selected and studied the interaction of these molecules with calf thymus DNA (CT DNA) by absorption titrimetry and CD techniques. To further probe the structural changes of native DNA on binding to active compound 9, molecular dynamics simulations were performed using Schrödinger Suite 2013.

The study of DNA intercalators has been closely tied to the development of our understanding of DNA itself. In the last few years, DNA intercalators have provided key insights into the function of a DNA-associated enzyme, topoisomerase II. The future may see the application of intercalators, to advance our understanding of other DNA-associated proteins and in the process, uncover new cancer treatment methods.

**MATERIALS AND METHODS**

CT DNA was obtained from E. Merck Co., Germany and was used without further purification. Solutions of CT DNA in 3.3 mM sodium chloride-sodium citrate buffer (SSC buffer) (pH 6.8) gave a UV absorbance ratio of $A_{260}/A_{280} > 1.8$.

**Absorption Spectral Titrations**

Absorption spectra were recorded on SHIMADZU model UV 1601 spectrophotometer. Quartz cells of 1 cm path-length were used and measurements were carried out at 25°C and pH 6.8 in 3.3 mM sodium chloride-sodium citrate buffer, unless otherwise mentioned. Concentration of DNA calculated in base molarities was determined spectrophotometrically with $\varepsilon_{260} = 6600 \text{ dm}^{-3}\text{mol}^{-1}\text{cm}^{-1}$. Absorption titration was performed at a fixed concentration of acridones (15 $\mu$M) in a sodium phosphate buffer (20 mM sodium phosphate, 150 mM NaCl, pH 6.5). 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 upon these absorption titrations may be made by the half-reciprocal plot method using the literature procedure. The intrinsic binding constant ($K$) for a given complex with CT DNA was obtained from the
plot of $D/\Delta \varepsilon_{\text{app}}$ versus $D$ according to the equation.

$$\frac{D}{\Delta \varepsilon_{\text{app}}} = \frac{D}{\Delta \varepsilon} + \frac{1}{\Delta \varepsilon \times K}$$

Where “$D$” is the concentration of DNA in base molarity, $\Delta \varepsilon_{\text{app}} = |\varepsilon_a - \varepsilon_f|$ 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, “$\varepsilon_a$” was obtained by calculating $A_{\text{obs}}/[\text{Acridone}]$. The data were fitted to the equation, with a slope equal to $1/\Delta \varepsilon$ and y-intercept equal to $1/(\Delta \varepsilon \times K)$. The intrinsic binding constant ($K$) was determined from the ratio of the slope to y-intercept.

**CD Measurements**

CD spectra were recorded on a JASCO J-715 spectro polarimeter. All measurements were done in 3.3 mM SSC buffer in quartz cell of 0.2 cm path-length at pH 6.8 and 25°C. Each spectrum is the average of three independent scans. Titration’s in the DNA region (UV CD) and in induced CD region (ICD) was carried out by adding progressively increasing amounts of ligand to a solution of DNA of constant concentration.

**Molecular Modeling**

A double stranded B-DNA was built using the Build Module in Schrödinger 2013 Suite programs. Two DNA models were built using only cytosine (C) and guanine (G) base pairs based on the fact that acridones are known to intercalate between the C and the G base pairs. The first DNA model (DNA Model 1) a hexamer was built with alternating C and G base pairs. The second DNA (DNA Model 2) model also a hexamer was constructed with C on the primary strand and G on its complementary strand. The acridone derivatives were individually placed in an intercalating fashion between the third and the fourth nucleotides of the DNA models.

The structures of the 10 acridone derivatives were built with the Schrödinger Suite 2013. These acridones were prepared using the Ligprep module of Schrödinger Suite 2013. As the molecules were assayed at pH 7.3, the states of ionizable residues were set to conform to this pH.

The acridones have a bromine atom at the third position on the acridone ring; this creates two distinct modes of intercalation of the acridones to DNA Model 1. Thus for each acridone derivative three complexes were formulated – one with DNA Model 1 and two structures with DNA Model 2 as shown in Figure 2. Each DNA-acridone complex was solvated with TIP3P waters and the complex neutralized by addition of Na$^+$ and Cl$^-$ ions. Subsequently, an MD simulation of the DNA-acridone assembly was carried out...
FIGURE 2 Ring with the bromine atom positioned between the two C’s (A) and between the two G’s (B).

using Desmond v3.1 under semi-isotropic conditions at temperature 300 K and 1 bar pressure. The temperature and pressure were maintained by coupling to a Langevin thermostat and barostat. The equilibrated systems were simulated for 1.2 ns under NPT ensemble.

RESULTS AND DISCUSSION

Absorption Titrations

Under the present experimental conditions, the presence of aromatic nucleobases with stacking ability and outside negative charges (phosphodiester anions of the backbone) in duplex DNA offers scope for interactive as well as electrostatic modes of binding for the acridone derivatives that contain planar aromatic rings. To examine this aspect, 10 acridone derivatives (Table 1) were selected which showed good anticancer, anti-MDR and antibacterial activity and studied the interaction 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 various acridone compounds upon addition of CT DNA. The interaction of these compounds with CT DNA was studied by monitoring the changes in the UV-visible absorption spectra of the various acridone compounds upon addition of CT DNA. Absorption spectra of the compounds (1–10) in the absence and presence of varying amount of CT DNA are shown respectively in Figures 3(a–j). In the range from 325 to 550 nm, all N10-substituted acridone derivatives exhibit two strong absorption peaks with maxima near 390 and 410 nm. Wavelength of maximum absorbance and extinction coefficients at that wavelength are given in Table 2. The binding of acridone derivatives to CT DNA led to strong decrease in the absorption intensities in the absorption maxima of the acridones. Also widely accepted that acridone compounds intercalate with DNA and UV-visible curve of their
complex shows bathochromic shifts and hypochromicity.\(^{[29–31]}\) By comparing the values of binding affinity and percentage of hypochromism with our previous work on fluoro acridones was comparatively more significant, we suggest that fluorine enhances the binding affinity because of its lipophilicity than to bromine.\(^{[32]}\) It is clear that the progressive addition of DNA leads to strong hypochromism in the absorption intensity in all the compounds studied. The percentage hypochromism calculated by the below formula and values are tabulated in Table 2.

\[
\% \text{ Hypochromism} = \frac{\varepsilon_{\text{free}} - \varepsilon_{\text{bound}}}{\varepsilon_{\text{free}}} \times 100
\]
FIGURE 3 (a–j) Absorption titrations of compound 1–4 and 6–10 with CT DNA. traces 1 in all the panels show the UV-visible spectra due to the acridone alone. Subsequent traces were obtained upon incremental addition of CT DNA as shown. Inset shows the plots of the relative optical density ($A/A_0$) versus (DNA).
Among the two maxima of the $N^{10}$-substituted acridones one at 395–399 nm range showed maxima hypochromism (30–54%) than the maximum at 410–419 nm having hypochromism 28–49% and therefore, the change in absorption of the peak at 395–399 nm were used for the calculation of intrinsic binding constant “$K$” (Table 2). The percentage, hypochromism of $N^{10}$-substituted 2-bromopropyl acridone compounds varies from 30 to 54 (Table 2) and hypochromism of $N^{10}$-substituted 2-bromobutyl acridone compounds is 41–53% (Table 2). The degree of hypochromism generally correlates well also with overall binding strength. The extent of hypochromicity as a function of DNA binding, plotted reciprocally as $A_o/A$ versus [DNA], is found to provide good measure of relative binding affinity. Inset in Figure 3(a–j) shows the plots of the relative optical density $A_o/A$ versus [DNA]. Because hypochromism is a manifestation of stacking interactions, there is enough reason to believe that there is atleast partial stacking with all the acridones studied here which show substantial hypochromism (Table 2).
The red shift in the wavelength is characteristic of stacking intercalation of aromatic chromophore with base pairs of DNA by overlap of $\Pi$ electron cloud of acridone compounds. It has been identified that mobility of drug is restricted at the DNA binding site due to hydrophobic environment inside the DNA and causes increase in emission intensity to three times. This phenomenon is observed on binding of antiviral drug Valacyclovir to DNA.\cite{33}

Experimental observations revealed that acridone derivatives with propyl spacer have shown intrinsic binding constant $K$ at a range of $0.37-1.86 \times 10^5$ M$^{-1}$ and butyl spacer with $1.06-3.97 \times 10^5$ M$^{-1}$ indicating that butyl spacer compounds are having good binding affinity with DNA because of hydrophobicity. Increase in the alkyl chain length has shown appreciable binding affinity toward DNA. We also observed that nature of surrounding tertiary amine have shown profound impact on the binding affinity. The basicity of pyrrolidine, piperidine, and morpholine has shown good binding affinity whereas weakly basic piperazine with poor binding affinity. However, further structural modifications to be done to identify the structural features to improve DNA binding interactions.

### CD Measurements

CD is defined as the differential absorbance of the left and the right circularly polarized light was used to obtain additional insight concerning

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**TABLE 2** Results of DNA titration with acridone derivatives as revealed from the UV-Vis, CD measurements

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>Molar extinction coefficient ($\varepsilon$) Lit M$^{-1}$cm$^{-1}$</th>
<th>% Hypochromism</th>
<th>$K \times 10^5$ M$^{-1}$</th>
<th>ICD (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>395</td>
<td>3796</td>
<td>43</td>
<td>0.3783</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>410</td>
<td>3574</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>397</td>
<td>540</td>
<td>30</td>
<td>1.8602</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>412</td>
<td>516</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>396</td>
<td>2290</td>
<td>33</td>
<td>0.9362 (+)</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>414</td>
<td>2173</td>
<td>29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>396</td>
<td>4424</td>
<td>54</td>
<td>0.9435 (+)</td>
<td>318</td>
</tr>
<tr>
<td></td>
<td>415</td>
<td>4240</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>398</td>
<td>4274</td>
<td>43</td>
<td>1.7219 (+)</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>417</td>
<td>4074</td>
<td>37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>399</td>
<td>3796</td>
<td>43</td>
<td>2.0011 (+)</td>
<td>320</td>
</tr>
<tr>
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<td>419</td>
<td>2996</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>398</td>
<td>2313</td>
<td>46</td>
<td>2.4717</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>418</td>
<td>2139</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>398</td>
<td>2921</td>
<td>53</td>
<td>2.7066</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>418</td>
<td>2772</td>
<td>49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>393</td>
<td>5398</td>
<td>46</td>
<td>3.9768 (+)</td>
<td>319</td>
</tr>
<tr>
<td></td>
<td>410</td>
<td>5103</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>398</td>
<td>6489</td>
<td>41</td>
<td>1.0623 (+)</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>418</td>
<td>6170</td>
<td>38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
the DNA recognition of the acridone derivatives used in this study. It is a reliable tool which monitors the asymmetric environment of the compound when bound to DNA, as the free compounds do not have CD spectra. The free CT DNA gives a negative band at 245 nm due to helicity and positive band at 279 nm because of base stacking which is characteristic of B form of DNA.\(^{[34–36]}\)

Four compounds 4, 6, 9, and 10 were used for this study. The effects of addition of increasing concentration of 4, 6, 9, and 10 into constant concentration of DNA are shown respectively in Figures 4(a–d). All the compounds enhance the ultraviolet CD (UV CD) of B-DNA with 2-bromo substituted acridones (6, 9, and 10) having a greater effect compared to propyl derivatives at given ‘r’ values (r is defined as the ratio of the total concentration of acridone to that of nucleic acid in base molarity) as evident from the plot of observed ellipticity values of the resultant complexes as a function of added ligands [Figures 4(a-d) inset]. Both positive and negative bands of the DNA doublet are affected almost equally. At the maximum ‘r’ values as indicated in figures the positive band is blue-shifted by about 4 nm in all the cases except in 4, there is 2.7-nm blue-shift of the crossover point of
the DNA band, whereas in all the other complexes, there is approximately 2.4-nm red-shift of the crossover point of the DNA band.

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. Because base pairs must separate vertically to allow for intercalation, the sugar phosphate backbone is disturbed and the regular helical structure is modified. There could be additional interactions between the ligand transition dipoles with that of DNA. From the previous studies a number of acridine molecules have been reported to enhance the UV CD of DNA without perturbing the regular doublet form. In view of the similarity of the UV CD proflavine and 9-aminoacridine complexes which have different substituents on the acridine ring and thus having different binding modes, Dalgleish et al.\textsuperscript{[37]} had proposed that the observed enhancement of the CD bands with increasing concentration of the acridines could be due to shielding of DNA base pairs from one another. Johnson and Tinoco\textsuperscript{[38]} have shown that the magnitude of CD spectrum of DNA is anomalously low due to the cancellation of the rotational strengths of the many CD bands. Based on these studies on acridone derivatives, to complicate the situation further the change in CD in the short wavelength region could also be due to an ICD of the bound ligand. To examine this, CD were recorded in visible region for six compounds 3–6, 9, and 10.

All the ligands investigated in this study are achiral and therefore cannot exhibit any CD signal on their own. Upon binding to DNA by partial or complete intercalation, the ligands experience a chiral environment, which results in the manifestation of the induced CD (ICD) signals in the ligand absorption region of the CD spectrum. ICD were recorded by adding increasing concentration of 3–6, 9, and 10 to fixed concentration of DNA and spectrum are shown respectively in Figures 5(a–f). As the ligand concentrations increase, the resultant CD signals increase in intensity. In all the cases, the CD bands are found to be positive in sign. Compounds 5, 6, and 10 showed CD band at 320 nm, whereas the other substituted acridone derivatives 3 and 9 showed at 319 nm and compound 4 showed CD band at slightly lower wavelength of 318 nm.

The intercalation of a ligand into DNA double helix generally induces

a. red-shift in the absorption maxima of the ligand
b. hypochromism of the absorption band
c. induced CD in the ligand absorption region
d. An altered ellipticity in the UV-region.\textsuperscript{[39]}

Even though the binding of the acridone derivatives investigated in the present study is marked by considerable hypochromism of the ligand absorption band, there is practically no red shift of the absorption maxima.
Absorption titration and UV CD data in the present study do not suggest any considerable difference regarding the binding modes of these compounds. In all the five cases of acridone-DNA complexes, the magnitude of ICD increases as the amount of acridone increases. From the CD studies of aminoacridines bound to DNA, Dalgleish et al.\textsuperscript{[40]} proposed that two possible explanations could be advanced for the observed variation of ICD as a function of acridine concentration. Based on this an acridone derivatives can be postulated as:

**FIGURE 5** (a–f) ICD spectra obtained in binding of compound 3, 4, 5, 6, 9, and 10 with CT DNA.
1. The variation is the result of interaction between bound ligands, which naturally increases at 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 alters 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 the other ligand.

Positive induced CD arises from the perpendicular alignment of the transition moment of an intercalating molecule with the transition moment of the base pairs of DNA into which it is inserted. In contrast, acridones are usually parallel. The basic structural unit in these compounds is same. The difference in their structure arise because of the substituents attached to the N₁₀-position are of diverse functionality. From this study, it is clear that these acridone compounds behave in different manner with DNA due to the structural change. However, more experiments are underway to understand the exact nature of interaction of these molecules with DNA.
**TABLE 3** Definition of the torsion angles in the backbone of DNA

<table>
<thead>
<tr>
<th>Torsion angles</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha ((\alpha))</td>
<td>(O^3'(i-1)-P-O5'-C5')</td>
</tr>
<tr>
<td>Beta ((\beta))</td>
<td>(P-O5'-C5'-C4')</td>
</tr>
<tr>
<td>Gamma ((\gamma))</td>
<td>(O5'-C5'-C4'-C3')</td>
</tr>
<tr>
<td>Delta ((\delta))</td>
<td>(C5'-C4'-C3'-O3')</td>
</tr>
<tr>
<td>Epsilon ((\epsilon))</td>
<td>(C4'-C3'-O3'-P(i+1))</td>
</tr>
<tr>
<td>Zeta ((\zeta))</td>
<td>(C3'-O5'-P(i+1)-O5'(i+1))</td>
</tr>
</tbody>
</table>

**Molecular Dynamics**

The last frame of the MD trajectory was selected and analyzed for the modulations that the acridones make to the structure and other parameters of the DNA model. Based on the calculation of strain energy of the various complexes with the two DNA models, it was observed that the complex of acridones with DNA Model 1 are in general more stable, and consequently more attention was paid to these complexes. Among the acridone derivatives, biological studies reveal that molecule 9 is the most active and therefore this complex was scrutinized in great detail. In the native B-DNA structure the distance between two adjacent base pair is about 2.3 Å, molecule 9 on intercalation is seen to open up the DNA structure enlarging the distance.
TABLE 4 Polar plot of the torsion angles ($\alpha$, $\beta$, $\gamma$, $\delta$, $\epsilon$, and $\zeta$) in both the primary (A) and complementary (B) strands of DNA Model 1 over the course of the MD trajectory. Angles are measured in an anticlockwise direction from the right which denotes an angle of $0^\circ$. The radial component corresponds to time with the initial angle at $t = 0$ ns displayed at the center of the circle and the final angle at $t = 1.2$ ns displayed on the bounding circle.
TABLE 4  Polar plot of the torsion angles ($\alpha$, $\beta$, $\gamma$, $\delta$, $\epsilon$, and $\zeta$) in both the primary (A) and complementary (B) strands of DNA Model 1 over the course of the MD trajectory. Angles are measured in an anticlockwise direction from the right which denotes an angle of $0^\circ$. The radial component corresponds to time with the initial angle at $t = 0$ ns displayed at the center of the circle and the final angle at $t = 1.2$ ns displayed on the bounding circle (Continued)
between the base pairs to as much as 5.7 Å and bringing about a complete
disruption in the native structure of the B-DNA. This is in tune with the
reported mechanism of the working of acridones as anticancer agents as
shown in Figure 6 and thus the acridones are expected to have a similar
mechanism of action.

The changes caused by the acridones to the native B-DNA structure can
be conveniently explained in terms of the perturbation to the backbone
torsion angles of DNA namely the \( \alpha \), \( \beta \), \( \gamma \), \( \delta \), \( \epsilon \), and \( \zeta \) angles, which are
defined along the DNA backbone. The definitions of these angles are given
in Table 3 and graphically shown in Figure 7.

The polar plots of the 6 torsion angles \( \alpha \), \( \beta \), \( \gamma \), \( \delta \), \( \epsilon \), and \( \zeta \), in the native
B-DNA and in the B-DNA-acridone complexes have been compared in
Table 4. For every torsion angle, it can be observed that there is a great
degree of variation in the two polar plots at every nucleotide, which implies

FIGURE 8 Torsion angles and nucleotide units as defined in Table 2. (N = nitrogen base; S = sugar).
that the acridones are able to bring about a major disruption in the B-DNA. This disturbance to the structure propagates several nucleotides away from the main intercalation site.

The perturbations caused by the acridones to the native structure of B-DNA alter the dihedral angles in the complex such that the new values cannot be categorized as belonging to any of the three forms A, B, or Z-DNA. The greatest impact on the structure of B-DNA is seen in the region bounded by the second and third set of nucleotides in both the primary (A) and the complementary (B) strands of the DNA as defined in Figure 8. Incidentally, this is the region in which the acridone derivatives were intercalated.

CONCLUSIONS

From the studies, novel acridone derivatives have shown good binding affinity with DNA \( (K = 0.3–3.9 \times 10^5 \text{ M}^{-1}) \). Compounds with \( N^{10} \) substituted butyl side chain have shown better affinity of DNA binding than of propyl side chain. Molecular modeling studies have thrown light on the mechanism of action and the binding modes of the acridones to DNA. The acridones cause a large disruption to the structure of DNA that propagates several nucleotides away from the primary intercalation site. A similarity in the mechanism of action to the acridones has also been noted in this study. Further improvement in the chemical structure will enhance the ability of acridones as good DNA intercalators, as it is crucial to exhibit biological activity. With these observations, can conclude that these novel acridone derivatives can be developed as potential DNA intercalators.

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