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An ecofriendly synthesis and DNA binding interaction study of some pyrazolo [1,5-*a*]pyrimidines derivatives

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ABSTRACT

The DNA molecule is a target for plethora of anticancer and antiviral drugs that forms covalent and non-covalent adducts with major or minor groove of DNA. In present study we synthesized series of novel Pyrazolo [1,5-*a*]pyrimidine derivatives. 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 measurement and thermal denaturation studies. Further, molecular interactions were revealed for compound **IIIa** and **IVa** by computational methodologies. The preferred mode of ligand binding with double helical DNA as well as preferable DNA groove were explored by molecular docking in different DNA models.

Over the past decades, the design and synthesis of DNA binding compounds have been an area of interest in cancer research.[1–3] DNA acts as a significant intracellular receptor and many chemicals bear their antitumor effects by binding to DNA. Pharmacological actions of these compounds were mediated by means of change in replication of DNA leading to inhibit growth of tumor cells and their effectiveness depends upon the mode and affinity of their DNA binding.[4] A number of synthesized complexes and metal chelates, as agents for mediation of strand scission of duplex DNA and as chemotherapeutic agents, have been used as probes of DNA structure in solution.[5,6] Furthermore, synthesis and kinetic study of transition state analogs have been used as tools for understanding DNA binding for mediation of DNA cleavage or as chemotherapeutic agents.[7]

The fused pyrimidines are an important class of compounds as chemotherapeutic agents in attracting attention of medicinal chemists for their antibacterial[8] antiviral[9] and cytotoxic[10] properties. Pyrazole ring also plays an important role in plethora of biological processes, as many therapeutic agents possess Pyrazole moiety. Certain alkyl pyrazoles have shown significant antiallergic, anti-inflammatory and anti-arthritic properties.[11,12] Many pyrazole containing fused heterocyclic groups have exhibited biological activities and widely studied in pesticide as well as medicine.[13,14] Pyrazolopyrimidines are of considerable chemical and pharmacological importance as purine analogues,[15,16] and have antitumor, antileukemic activities. The role of pyrazolo [1,5-*a*]pyrimidines are well documented as anti-metabolites and shown to have inhibition of purine biochemical reactions.[17,18] Pyrazolopyrimidine and related heterocyclic compounds are found to own a wide important pharmacophore and privileged structure in medicinal chemistry. Pyrazolopyrimidine derivatives exhibit diversified pharmacological activities like CNS depressant[19], neuroleptic[20], tuberculostatic,[10] antihypertensive,[21] analgesic[22] and antimicrobial activity.[23] The Pyrazolo [1,5-*a*]pyrimidine frame work containing a number of fused bicyclic heterocycles has been a versatile scaffold for various pharmacological studies.[23,24] Apart from their utility for several disease targets, pyrazolo [1,5-*a*]pyrimidine derivatives has shown an enormous synthetic value in the preparation of various drugs and bio-active molecules. Prompted by the varied biological activities of pyrazolopyrimidine derivatives, we envisioned our approach towards the synthesis using the alternative green reaction mediums.

The literature survey reveals that the anti-tumor activity of most drugs is due to their intercalations with base pairs of DNA and interferences with normal functioning of enzyme topoisomerase II, which is involved in breaking and releasing of DNA strands.[25–27] The anti-tumor drugs that intercalate DNA are of growing interest in the field of anti-cancer drugs. Particularly, they are characterized by planar chromospheres, which are often constituted by three or four condensed rings, which can intercalate into base pairs.[28] Considering above mentioned fact the

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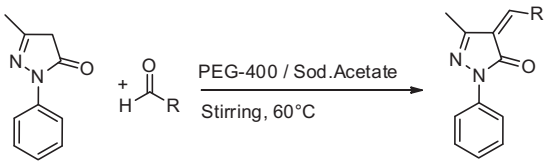
Table 1
Absorption spectral properties of compounds **IIIa** and **IVa** bound to CT-DNA

Sr. No	Compound	λ_{max} (nm)	K_b (M^{-1})	T_m(°C)
1	**IIIa**	320	5.21×10^5	80
2	**IVa**	530	5.7×10^5	82

synthesized pyrazolo [1,5-*a*]pyrimidine derivatives have evaluated for their DNA binding potential. The more efficient and eco-friendly green reaction method for synthesis has been reported in this Letter. The DNA binding ability of designed and synthesized analogues were evaluated by absorption spectroscopy, viscosity measurement, thermal denaturation and molecular docking studies. The DNA-binding studies indicate that compounds **IIIa** and **IVa** have shown more binding with base pairs of Calf Thymes DNA (CT-DNA). The preference of compounds towards major and minor groove has also been supported by molecular docking studies.

In extension of our work on synthesis of bioactive heterocyclic compounds,[29–31] herein we report a novel series of pyrazolo [1,5-*a*]pyrimidines by condensation of 4-substituted benzylidene-3-methyl-1*H*-pyrazol-5(4*H*)-one with 5-aminopyrazole in PEG-400 (Table 1). The starting compound 5-aminopyrazole was prepared in two steps from corresponding amine by diazotization and then treated with malononitrile followed by reaction with hydrazine hydrate (Scheme 1). The novel 4-substituted benzylidene-3-methyl-1*H*-pyrazol-5(4*H*)-one compounds **III**(**a**–**d**) were prepared in presence of sodium acetate and PEG-400 as a green reaction solvent and hetero carbaldehyde (Scheme 2 and Fig. 1). Finally, the synthesis of pyrazolo [1,5-*a*]pyrimidines **IV**(**a**–**d**) were attempted by reacting 5-aminopyrazole with 4-substituted benzylidene-3-methyl-1*H*-pyrazol-5(4*H*)-one in PEG-400 as reaction solvent (Scheme 3 and Fig. 2). Formation of products were assumed to proceed through Michael-type addition of ring nitrogen in 5-amino-pyrazole to activated double bond, followed by intra-molecular cyclisation with elimination of water and then dehydrogenation. Structures of compounds were appropriately established by spectroscopic and analytical methods.

Application of electronic absorption spectroscopy is considered as one of the most important technique in CT-DNA binding studies.[31] Binding of molecules to DNA has been well characterized by the large hypochromism in UV spectrum. After intercalation, the $\pi*$ orbital of compounds could couple with π-orbital of base pairs, thus decreasing the $\pi*-\pi$ transition of energy and resulting in bathochromism. Hence, decrease in absorption intensity and significant red shift were observed due to stacking interaction between compound and CT-DNA.[32] The DNA-binding studies were characterized by absorbance maximum at 320 nm for compound **IIIa** and 530 nm for compound **IVa**. Additionally, increasing concentration of DNA led to hypochromic and bathochromic (red shift) changes in its visible absorption spectra as a result of formation of more stable complex as shown in Figure 3A and B. The interactions of compounds **IIIa** and **IVa** with CT-DNA resulted in decrease of absorption intensity accompanied by a shift towards higher wavelength. Around 12–9% reduction intensity of absorption was



Scheme 2. Synthesis of 4-substituted benzylidene-3-methyl-1*H*-pyrazol-5(4*H*)-one.

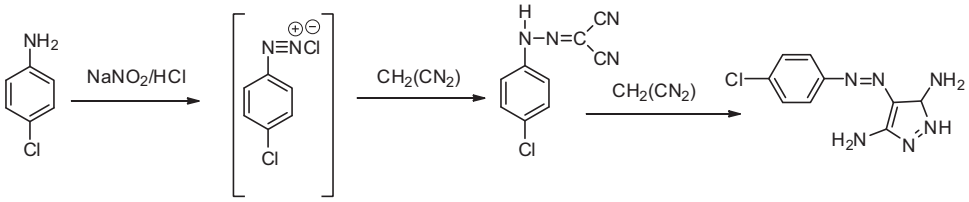
observed at 320 nm and 530 nm peak maximum in presence of an excess CT-DNA. The lowest value observed in spectral changes (including red shift and hypochromicity) were used to evaluate intrinsic binding constant (K_b). The spectra results suggested that, the compound **IIIa** binds strongly than the compound **IVa** with DNA, as the calculated intrinsic binding constant for compound **IIIa** and **IVa** was found to have 5.21×10^{-5} and 5.7×10^{-5} respectively. The difference in binding constant was observed due to difference in mode of binding with DNA. (Table 2).

Concentration of CT-DNA per nucleotide [$C_{(P)}$] was measured using its known extinction coefficient at 260 nm [6600 M^{-1} cm^{-1}][33] and absorbance at 260 nm (A_{260}) and 280 nm (A_{280}) for CT-DNA was measure to check the purity. The ratio A_{260}/A_{280} was found to be 1.8:1.9, indicating that CT-DNA was satisfactorily free from protein. A phosphate buffer (5 Mm, pH 7.2), was used for the absorption, viscosity and thermal denaturation experiments. Absorption titration experiments were carried out by varying the DNA concentration from 0 to 100 μM and maintaining the compound concentration constant at 30 μM. Absorption spectra were recorded after each successive addition of DNA and equilibrium (approx. 10 min). For both compounds **IIIa** and **IVa**, the observed data were fit into Eq. (1) in order to obtain the intrinsic binding constant, K_b.[29]

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

Where ε_a, ε_f and ε_b are the apparent, free and bound compound extinction coefficient for compound **IIIa** at 290 nm and for compound **IVa** at 305 nm respectively. A plot of $[DNA]/(\varepsilon_a-\varepsilon_f)$ versus [DNA] gave a slope of $1/(\varepsilon_a-\varepsilon_f)$ and an intercept y equal to $1/K_b$ $(\varepsilon_a-\varepsilon_f)$, where K_b is the ratio of slope to y-intercept.

For further clarification, the interaction modes of compound **IIIa** and **IVa** with DNA were investigated by viscosity measurement technique. An increase in viscosity of native DNA is regarded as a diagnostic feature of an intercalation process. During drug-DNA intercalation, base pairs were separated to accommodate the intercalating ligand leads to increase in length of DNA helix usually increasing viscosity of DNA.[34,35] In order to elucidate the binding mode of present compounds, we have measured the viscosity changes in short, rod-like DNA fragments by varying the concentrations of added compounds. The effects of compounds on viscosity of rod-like DNA were shown in Figure 4. The relative length increase (L/L_0) of the complex formed between **IIIa** and **IVa** with DNA as shown in Figure 4. It is evident that the binding of compounds **IIIa** and **IVa** with DNA leads to increase in viscosity of DNA corresponding to an increase in contour length of DNA frag-



Scheme 1. Synthesis of 5-aminopyrazole.

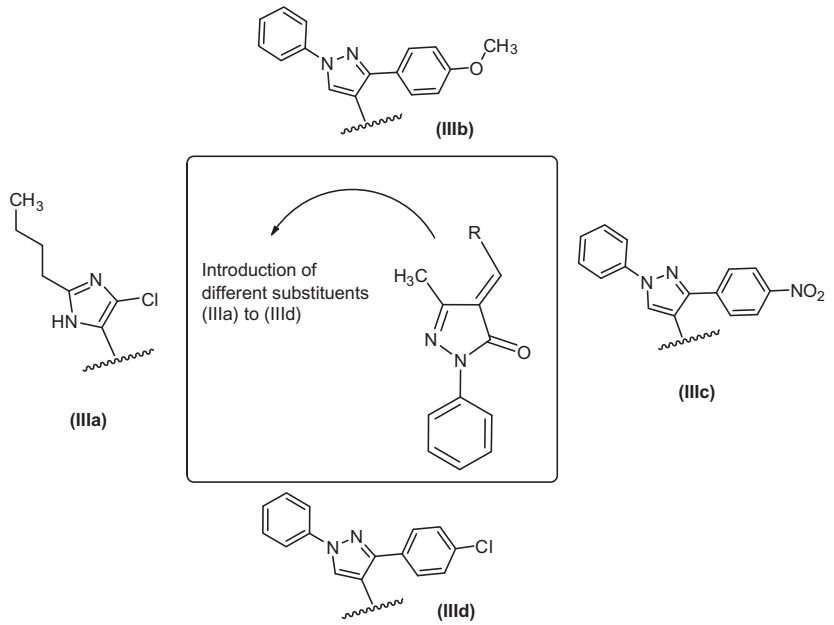
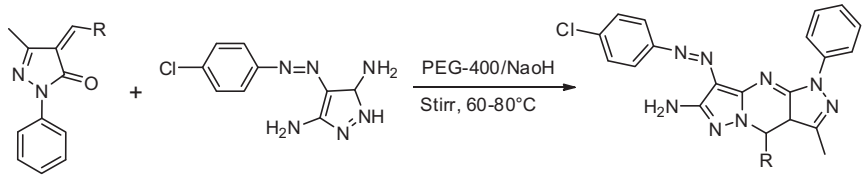


Figure 1. Structures of different substituted pyrrazole-5-one derivatives.



Scheme 3. Synthesis of pyrazolo [1,5-*a*]pyrimidines.

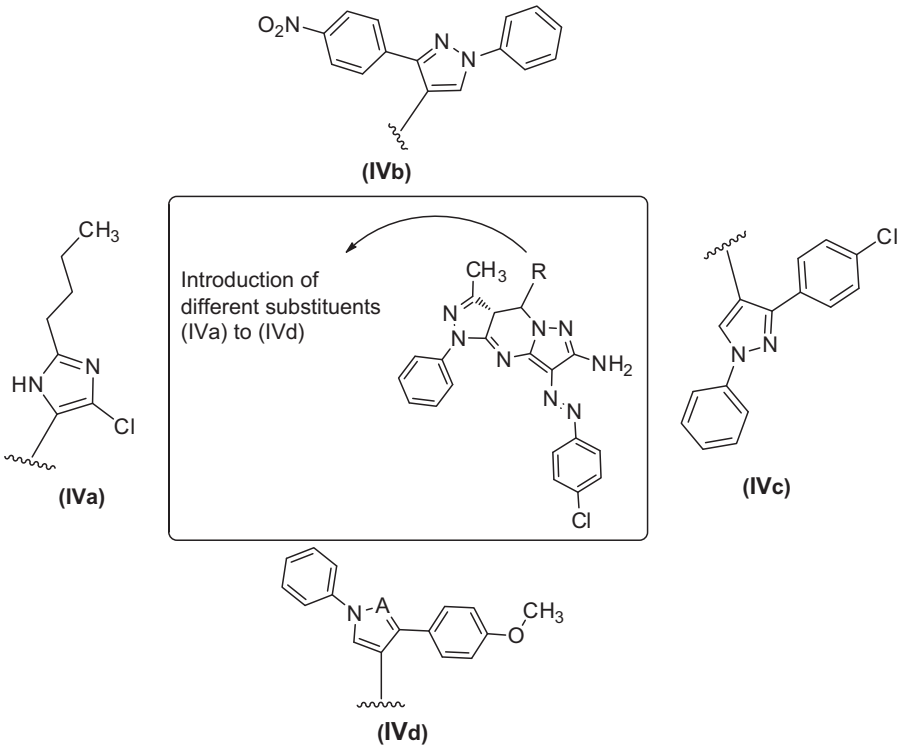


Figure 2. Structures of different substituted pyrazolo [1,5-*a*]pyrimidines.

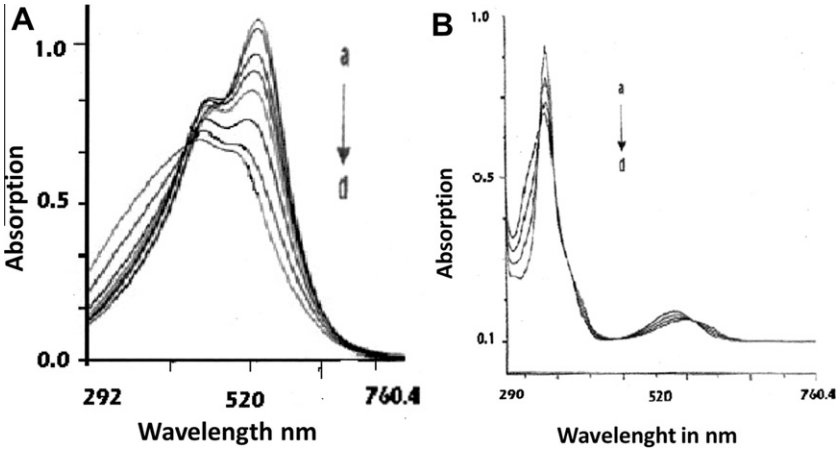


Figure 3. UV-absorption spectra in phosphate buffer upon addition of CT-DNA (**IIIa**) and (**IVa**).

Table 2
Physical and analytical data of synthesized compounds

Compound	Color	Yield (%)	Mp (°C)	Time (min)	Crystal solvent	Mol formula (mol/wt)	Glide docking score (kcal/mol)		
							226D (AT rich)	1QC1 (GC rich)	2K0V (Mixed)
IIIa	Yellowish	88	178–182	80	Ethanol	$C_{19}H_{20}ClN_3O$ (341)	*−7.644* (B)	*−7.00* (DG-6, DC-15)	*−5.830* (DC-18, DG-7, DG-7,B)
IIIb	Yellowish orange	82	167–169	72	Ethanol	$C_{28}H_{23}N_3O_2$ (433.50)	−3.663(B)	−5.271 (DC-5)	−3.860 (DC-19)
IIIc	Orange solid	84	160–162	70	Aq acetic acid	$C_{27}H_{20}ClN_3O$ (437.92)	−4.881 (0)	−5.054 (DC-5)	−3.842 (DC-19)
IIId	Brown	85	180–183	84	Aq acetic acid	$C_{27}H_{20}N_4O_3$ (448.47)	−7.013 (B)	−5.727 (DC-4, DG-16)	−3.785 (DA-15)
IVa	Yellowish powder	88	180–182	90	Ethanol	$C_{27}H_{26}Cl_2N_{10}$ (561.7)	*−6.441* (DA-18, B)	*−6.65* (DG-7, DG-7,B)	*−5.909* (B)
IVb	Orange	84	167–169	86	Ethanol	$C_{36}H_{29}ClN_{10}O$ (653.13)	−4.049 (B)	−4.577 (DC-5, DG-16,B)	−4.064 (DG-7)
IVc	Yellowish	86	156–159	78	Ethanol	$C_{36}H_{26}Cl_2N_{10}$ (657.55)	−3.734 (DT-6)	−4.042 (DC-5, DG-16)	−3.895 (DG-17)
IVd	Yellowish orange	85	161–163	89	Aq acetic acid	$C_{36}H_{26}ClN_{11}O_2$ (668.11)	−6.164 (DT-4)	−6.555 (DG-6, DC-15)	−5.152 (DG-7)

**Note: Grey background indicated pose in major groove of DNA.
Values in bracket indicates, (0) as no H-Bond, (B) as hydrogen bonding interactions with DNA backbone or name and residue number of nucleotide. The molecular docking scores of most potent compounds are shown in *Italics*.

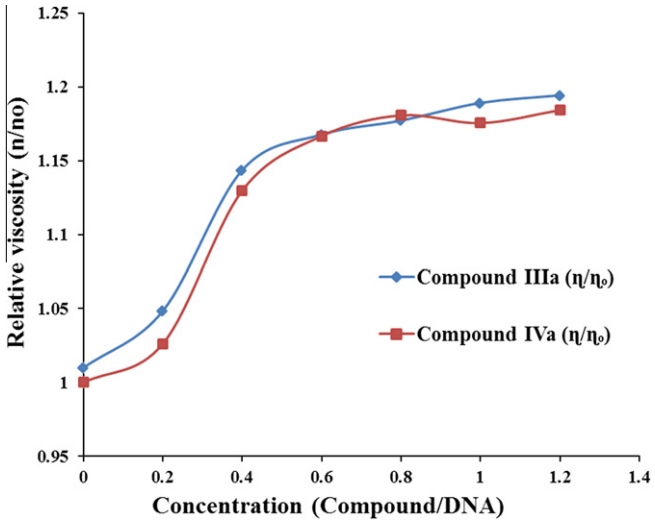


Figure 4. Effect of increasing amount of compound **IIIa** and **IVa** on the relative viscosity of CT-DNA.

ments. The presence of compound had an obvious effect on the relative viscosity of CT-DNA with an increase in concentration of the added compounds. This result further suggested an intercalative binding mode of the complex with DNA and also parallel to the above spectroscopic results, such as hypochromism and bathochromism of complexes in presence of DNA.

Another strong evidence for intercalative binding of compound **IIIa** and **IVa** with double-helix DNA was obtained from DNA melting temperature (T_m), at which the DNA double-helix denatures into a single-stranded DNA, owing to increased stability of helix in presence of an intercalator ligand.[28] The molar extinction coefficient of DNA bases at 260 nm in double helical form is much less than the single-stranded form; hence, the melting of helix leads to an increase in absorbance at 260 nm. DNA melting studies were carried out with CT-DNA in presence as well as in absence of compounds **IIIa** and **IVa**. The results reveal that melting temperature of double helical CT-DNA was found to be 65 ± 2 °C but in presence of compound **IIIa** and **IVa** melting temperature increased by 10–15 °C (Fig. 5). This variation in DNA melting temperature strongly supports the intercalation of compounds into DNA double-helix.[36,37]

Energetically favorable DNA-ligand complexes for compounds **III**(**a**–**d**) and **IV**(**a**–**d**) were predicted by the systematic procedure as described in methods section, involving molecular docking

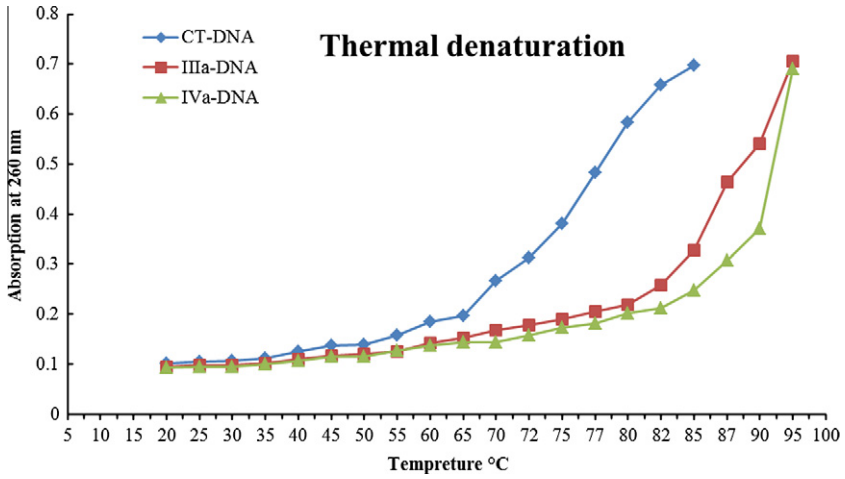


Figure 5. Melting temperature curve of CT-DNA in presence and absence of compound **IIIa** and **IVa**.

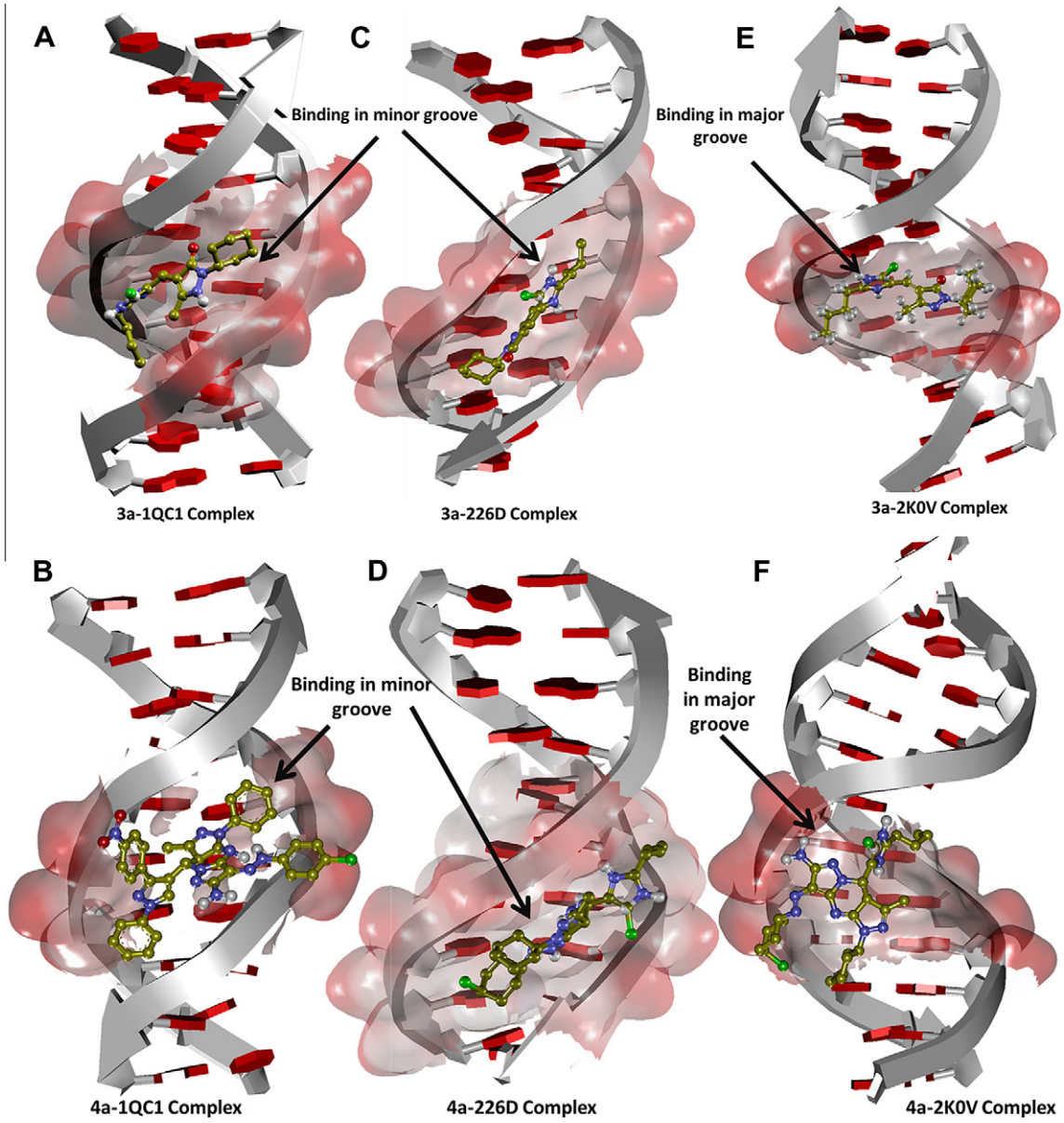


Fig. 6. DNA ligand binding pose of compound **IIIa** and compound **IVa** in minor groove of DNA crystal structures (A) and (B) GC rich (PDBID 1QC1); (C) and (D) AT rich (PDBID 226D); in major groove of (E) and (F) mixed sequence (PDBID 2K0V).

using Glide software. The results obtained from molecular docking of designed and synthesized analogues were summarized in Table 1. The molecular docking analysis showed that all ligands preferentially bind in minor groove of DNA in case of AT rich (PDB ID: 226D) and GC rich (PDB ID: 1QC1) nucleotide sequence, conversely major groove binding is preferred for mixed (PDB ID: 2K0V) nucleotide sequence. The highest docking scores were observed for compound **IIIa** (−7.644, −7.00 and −5.830 kcal/mol) and **IVa** (−6.441, −6.65 and −5.170 kcal/mol) in all three DNA structures (PDB ID: 226D, 1QC1 and 2K0 V) respectively. The strong hydrogen bonding interactions were observed with phosphate group of DNA backbone or adenine, guanine, thymine, cytosine nucleotides and ligands forming DNA intercalation as shown in Figure 6A–F. The molecular electrostatic surface analysis of DNA shows that, the ligand prefers minor groove for AT rich (226D) and GC rich (1QC1) nucleotide sequence. It is also clear that ligands bind preferentially in major groove for mixed (2K0V) nucleotide sequence. The above observation suggests that compound **IIIa** binds with DNA more strongly than **IVa** forming intercalation complex in support with previously described experimental results. Also the preference of major and minor groove of DNA for binding is based on its nucleotide sequences.

The detailed analysis of DNA intercalation by compound **IIIa** and **IVa** by UV–visible, viscosity measurements, thermal denaturation studies shows the perfect binding to DNA. The designed and synthesized compounds bind with DNA via interactions as well as intercalation with the base pairs of CT-DNA. It also suggests that the covalent binding of compound **IIIa** and **IVa** with DNA cause change in conformation of DNA as increase in viscosity as well as intensity of absorption band was generally observed with increase in intercalation. Moreover, the results described in this study showed that change in environment of molecule modulate the binding property of compound with DNA. The relative binding efficacy of the compound **IIIa** to DNA was much higher than that of compound **IVa** as predicted by molecular docking exercise. The preferential binding of compounds to minor groove of AT rich (PDB ID: 226D) and GC rich (PDB ID: 1QC1) DNA sequence as well as major groove of mixed (PDB ID: 2K0V) DNA sequence. The binding mode and molecular interactions of compounds with DNA will provide necessary information on mechanism of antitumor/antibiotic compounds with DNA and benefit the drug discovery process for designing of new molecules.

All melting points were uncorrected and determined in an open capillary tube. The chemicals and solvents used were of analytical grade. The completion of reaction was monitored by thin layer chromatography on precoated sheets of silica gel-G (Merck, Germany) using iodine vapor for detection. IR spectra were recorded in matrix of KBr on a Shimadzu spectrometer (Japan). ^{1}H NMR spectra was recorded in DMSO-d_6 on Advance spectrometer (300 MHz) using TMS as an internal standard. Mass spectra were recorded on an EI-Shimadzu QP 2010 PLUS GC–MS system (Shimadzu, Japan). Elemental analyses were performed on a Carlo Erba 106 Perkin-Elmer model 240 analyzer (Perkin-Elmer, USA).

General procedure for synthesis of 4-substituted benzylidene-3-methyl-1*H*-pyrazol-5(4*H*)-one **III**(**a**–**d**)[38] and of pyrazolo [1,5-*a*] pyrimidines **IV**(**a**–**d**)[39] used are mentioned in references. The spectral details of compounds synthesized viz **III**(**a**–**d**)and **IV**(**a**–**d**) are mentioned in reference footnotes.[40–47]

DNA-Binding assay: CT-DNA was purchase from Hi-Media, Mumbai, India. Pyrazolopyrimidine derivatives chemically were synthesized in our laboratory and anhydrous disodium hydrogen phosphate and monosodium hydrogen phosphate, used were of analytical grade for the preparation of buffer. The concentration of CT-DNA per nucleotide was measured using its known extinction coefficient at 260 nm for CT-DNA.[48] Absorbance at 260 nm for CT-DNA was measured in order to check the purity level. The concentration was found to be 496.2 μmol/L, indicating that CT-DNA was satisfactorily free from proteins. Buffer (0.2 M) phosphate, pH 7.2 was used for study. The absorbance measurement was calibrated by several researchers using ethidium bromide.[49] The previously calibrated results used in present study. Solution of Pyrazolo [1,5-*a*]pyrimidine derivatives were prepared using 50% concentration of each DMSO and phosphate buffer. The UV–visible absorption was measured in absences and in presences of various concentration of DNA, while maintaining the compound concentrations as constant.

The viscometer is cleaned thoroughly and DNA solution of 496.2 μmol/L is used to carry out viscometer titration, time of flow measurements for DNA solution was carried out at 25 °C using Oswald's viscometer. Typically, 10 ml DNA solution is transferred to measure the time required to flow. The increasing amount of ethidium bromide was used by Raja et al. in previous DNA biding study.[50] Solution was allowed to thermally equilibrate for 30 min and time of flow is again measured for each time. Each reading was taken three times and an average flow time was collected. Similar method was repeated for all compounds in present study.

DNA melting studies were carried out by monitoring the absorption of CT-DNA (50 μM) at 260 nm at various temperatures in the presence (5–10 μM) and in absence of compound **IIIa** and **IVa**. The melting temperature (T_m) at which 50% of double stranded DNA becomes single stranded and the curve width σT (temperature range where 10–90% of the absorption increase occurred) were calculated as previously described.[51–53]

The DNA-ligand binding affinity and DNA intercalating pose were predicted using molecular docking methodology in Glide software included in Schrodinger suite 9.0.211.[54,55] The molecular docking of designed and synthesized analogues was performed in three different DNA crystal structures having PDB ID: 226D, 1QC1 and 2K0V which were retrieved from protein data bank. (PDB).[56–58] The three crystal structures were selected on the basis of AT rich, GC rich and mixed nucleotide sequence, as it has significance in ligand binding.[59] DNA structures were prepared using preparation wizard for molecular docking as follows; hydrogen atoms were added, hydrogen bonding network was optimized, and protein was minimized to RMSD (Root Mean Square Deviation) 0.30 Å using OPLS (Optimized Potential for Liquid Simulations) 2005 force field. The designed ligands were prepared in LigPrep and minimized using OPLS 2005 force field using set of default parameters.[60] The whole DNA structure was defined as macromolecular grid for molecular docking. The ligand docking calculations were done in the standard precision mode of glide software. Ligands were passed through a scaling factor of 0.80 and partial charge cutoff of 0.15.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.10.027. These data include MOL files and InChiKeys of the most important compounds described in this article.

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38. General procedure for synthesis of 4-substituted benzylidene-3-methyl-1*H*-pyrazol-5(4*H*)-one **III**(**a**–**d**): A mixture of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (1 mmol), hetero carbaldehyde (1 mmol) and sodium acetate (4 mmol) was stirred in PEG-400 (10 ml) at 60–80 °C for 1–2 h. The completion of reaction was checked by TLC and the reaction mixture was extracted with diethyl ether (2 × 20 ml). The combined organic layers were dried over anhydrous Na_2SO_4, and solvent was evaporated under reduced pressure. The crude product was recrystallized from proper solvent to furnish the desired product.
39. Synthesis of pyrazolo [1,5-*a*]pyrimidines **IV**(**a**–**d**): A mixture of **IIIa** (0.340 g, 1 mmol), 5-amino pyrazole (0.236 g, 1 mmol) and 1–2 pallets of NaOH were dissolved in Polyethylene glycol (PEG-400 (15 mL). The reaction mixture was stirred for the period as shown in Table 1. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was extracted with diethyl ether (2 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was recrystallized from proper solvent to give the corresponding product **IVa**. Similarly, **IVb**, **IVc**, **IVd** were synthesized following the same procedure.
40. 3-(2-Butyl-4-chloro-1*H*-imidazol-5-yl-methylene)-4-methyl-1-phenyl-1*H*-pyrrol-2(3*H*)one (**IIIa**): Colour: Yellowish, mp (°C): 178–182, yield-88, IR (KBr, cm^{-1}): 3404, 3300, 3188, 2926, 1616,1564, 690 cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.93 (t, 3H, $-CH_3$), δ 1.31 (m, 2H, $-CH_2-$), δ 1.65 (m, 2H, $-CH_2-$), δ 2.76 (t, 2H, $-CH_2$), δ 1.93 (t, 3H, $-CH_3$), δ 7.24–7.86 (m, 5H, Ar-H + s,1H,CH), δ 8.19 (s, 1H, –NH, D_2O exchangeable), ppm;. EIMS (*m*/*z*) MS (*m*/*z*): 341 (M+), 343 (M+2), Anal. Calcd for $C_{19}H_{20}N_3ClO$: C, 66.76; H, 5.90; N, 12.29%. Found: C, 66.82; H, 5.78; N, 12.16%.
41. 3-[(4-Methoxyphenyl)-1-phenyl-1*H*-pyrrol-4-yl]methylene-4-methyl-1-phenyl-1*H*-pyrrol-2(3*H*)one (**IIIb**): Color: Yellowish orange, mp(°C): 167–169, yield-82, IR (KBr, cm^{-1}): 3325, 3158, 1620, 1574, 1435 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.94 (t, 3H, CH_3), δ 3.76 (t, 3H, OCH_3), δ 7.1–7.98 (m, 15H, ArH + s,1H,CH), ppm; EIMS (*m*/*z*) MS (*m*/*z*): 433 (M+), Anal. Calcd for $C_{28}H_{23}N_3O_2$: C, 77.58; H, 5.35; N, 9.69%. Found: C, 77.64; H, 5.28; N, 9.62%.
42. 3-[(4-Chlorophenyl)-1-phenyl-1*H*-pyrrol-4-yl]methylene-4-methyl-1-phenyl-1*H*-pyrrol-2(3*H*)one (**IIIc**): Color: Orange solid, mp (°C): 167–169, yield-84, IR (KBr, cm^{-1}): 3108, 3072, 2902, 1624, 1573,743 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.89 (t, 3H, CH_3), δ 7.2–7.98 (m, 15H, ArH + s,1H,CH), ppm; EIMS (*m*/*z*) MS (*m*/*z*): 438(M+), 440(M+2), Anal. Calcd for $C_{28}H_{23}ClN_3O$: C, 74.05; H, 4.60; N, 9.60%. Found: C, 73.92; H, 4.46; N, 9.65%.
43. 3-[(4-Nitrophenyl)-1-phenyl-1*H*-pyrrol-4-yl]methylene-4-methyl-1-phenyl-1*H*-pyrrol-2(3*H*)one (**IIId**): Colour: brown, mp (°C): 178–182, yield-85, IR (KBr, cm^{-1}): 3539, 312, 3152, 1625, 1590, 778 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.90 (t, 3H, $-CH_3$), δ 7.18–7.96 (m, 15H, Ar-H + s,1H,CH), ppm;. EIMS (*m*/*z*) MS (*m*/*z*): 448(M+), Anal. Calcd for $C_{19}H_{20}N_3ClO$: C, 72.31; H, 4.49; N, 12.49%. Found: C, 72.18; H, 4.42; N, 12.38%.
44. 4-(2-Butyl-5-chloro-4,5-dihydro-1*H*-imidazol-4-yl)-8(4-chlorophenyl)-diazenyl)-3-methyl-1-phenyl-1*H*-dipyrazolo(1,5-*a*:3′,4′-*d*)pyrimidin-7-amine (**IVa**): Colour: Yellowish powder, mp (°C): 180–182, yield-88, IR (KBr, cm^{-1}): 3541, 3355, 3162, 2315 1640, 1556, 758 cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.90(t, 3H, $-CH_3$), δ 1.34 (m, 2H, $-CH_2-$), δ 1.62 (m, 2H, CH_2), δ 2.78 (t, 2H, $-CH_2$), δ 1.96 (t, 3H, CH_3), δ 3.43 (dd, 1H, HA), δ 4.04 (dd, 1H, HB) δ 4.92 (br s, 2H, NH_2) δ 7.14–7.92 (m, 9H, Ar-H), δ 8.17 (s, 1H, –NH, D_2O exchangeable), ppm; EIMS (*m*/*z*) MS (*m*/*z*): 560(M+), 562(M+2), 564(M+4) Anal. Calcd for $C_{27}H_{26}C_{l2}N_{10}$: C, 57.95; H, 4.29; N, 25.03%. Found: C, 57.86; H, 4.24; N, 25.12%.
45. 8(4-Chlorophenyl)-diazenyl)-4,5-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-dipyrazolo(1,5-*a*:3′,4′-*d*)pyrimidin-7-amine (**IVb**):Colour: Orange, mp (°C): 167–169, yield-84, IR (KBr, cm^{-1}): 3567, 3390, 3180, 2338, 1610, 1545 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.90 (t, 3H, CH3), δ 3.39 (dd, 1H, HA), δ 3.89 (t, 3H, $-OCH_3$), δ 4.10 (dd, 1H, HB) δ 4.89 (br s, 2H, NH_2) δ 7.02–8.21 (m, 18H, ArH), ppm; EIMS (*m*/*z*) MS (*m*/*z*): 653(M+), 655(M+2) Anal. Calcd for $C_{36}H_{29}C_lN_{10}O$: C, 66.38; H, 4.16; N, 21.50%. Found: C, 66.32; H, 4.10; N, 21.36%.
46. 8(4-Chlorophenyl)-diazenyl)-4,5-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-dipyrazolo(1,5-*a*:3′,4′-*d*)pyrimidin-7-amine (**IVc**): Colour: Yellowish, mp (°C): 156–159, yield-86, IR (KBr, cm^{-1}): 3447, 3344, 3153, 2934, 1648, 854,709 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.14 (t, 3H, CH_3),δ 3.22(dd, 1H, HA), δ 4.70(dd, 1H, HB), δ 3.86 (br s, 2H, $-NH_2$) δ 7.2–8.10 (m, 18H, ArH), ppm; EIMS (*m*/*z*) MS (*m*/*z*): 656(M+), 658(M+2), 660(M+4) Anal. Calcd for $C_{35}H_{26}C_{l2}N_{10}$: C, 64.12; H, 3.68; N, 21.36%. Found: C, 64.06; H, 3.57; N, 21.28%.
47. 8(4-Chlorophenyl)-diazenyl)-4,5-(4-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-dipyrazolo(1,5-*a*:3′,4′-*d*)pyrimidin-7-amine (**IVd**): Colour: Yellowish orange, mp (°C): 161–163, yield-85, IR (KBr, cm^{-1}): 3478, 3150, 2929, 1649, 834 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.89 (t, 3H, CH_3), δ 3.47(dd, 1H, HA), δ 4.15 (dd, 1H, HB), 4.86 (br s, 2H, $-NH_2$) δ 7.10–8.24 (m, 18H, ArH), ppm; EIMS (*m*/*z*) MS (*m*/*z*): 667(M+), Anal. Calcd for $C_{35}H_{26}C_lN_{11}O_2$: C, 63.09; H, 3.62; N, 23.11%. Found: C, 62.95; H, 3.54; N, 22.98%.
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