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Molecular Dynamics Simulation Study of Binding Affinity of Thieno[2,3-*b*]benzo[1,8]naphthyridine Derivatives to DNA[1]

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Abstract—DNA binding position and binding affinity of drugs are important information that helps medicinal chemists in synthesis of new drugs. We used molecular docking and molecular dynamics simulation to reveal binding strength of thieno[2,3-*b*]benzo[1,8]naphthyridine derivatives to DNA. Molecular docking showed that molecules with more steric hindrance select groove position in DNA structure. Other molecules are intercalated between base pairs of GC and AT. Restrained electrostatic potential (RESP) charges, root mean square deviation (RMSD), and total potential analyses were performed. RMSD and total potential analyses showed that all simulations have stability for MMGBSA analysis. Binding affinity of all drugs was derived via MMGBSA analysis. Thermodynamics analysis showed that binding affinity of groove binding drugs is less than that of intercalating ones. Also, it was found that a linear relationship exists between RESP charges and ΔG_{pred}. Additionally, our results demonstrated the highest affinity for molecules carrying substituent groups of $-OCH_3$ and $-CH_3$.

Keywords: molecular dynamics, docking, calfthymus DNA, Gibbs free energy of binding

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INTRODUCTION

Naphthyridine derivatives have been widely studied for their biological activities. Among these compounds, 1,8-naphthyridine derivatives have attracted a great deal of interest from researchers, since these compounds have been shown good bioactivities such as anti-cancer [1], anti-inflammatory [2], anti-protozoals [3], and anti-mycobacterial activity [4].

It has been shown that anti-cancer activity of these compounds is due to their intercalation into DNA [5]. Binding of these compounds to DNA motivated researchers for syntheses of new drug analogues [6–8]. Benzo[1,8]naphthyridine derivatives are examples of new synthetic 1,8-naphthyridine drugs that have been synthesized by Naik and co-workers [9]. Naik and co-workers showed that compound (**I**) (see Fig. 1) binds to DNA via intercalation mode but binding of other molecules in Fig. 1 has not been studied.

In this study, we used molecular docking and molecular dynamics simulation to obtain binding position and binding affinity of compounds (**I**) through (**VII**) in Fig. 1. Also, thermodynamics analysis was used to calculate ΔG_{pred}.

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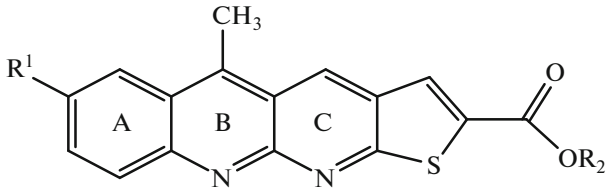
EXPERIMENTAL

The initial structures of all drugs were optimized with B3LYP [10] level and 6-31G* basis set using Gaussian 03 package [11]. The optimized structures were used for calculation of electrostatic potential using Hartree–Fock level and 6-31G* basis set. Restrained electrostatic potential (RESP) fit method [12] was applied to calculate the atomic charges. Initial structures of the seven drugs and DNA structure were constructed with docking procedure in the Arguslab 4.0.1 software [13]. A DNA sequence of $d(CACCCAAAACC)_2$,which is similar to a section of real calf thymus DNA [14],was constructed for molecular dynamics simulations. MD simulations were carried out using AMBER 9 [22] software and with ff99bsc0 [15, 16] and GAFF [17] force fields. A periodic cubic box with solute wall at adistance of 6 Å was used for all simulations. All simulation boxes were filled with 3000 SPC water molecules [18]. Also, temperatures of all systems were gradually increased from 0 to 300 K for 100 ps. Then, temperatures were kept at 300 K using Langevin algorithm [19]. Simulations were carried on for 15000 ps.

Free energy of binding was computed using MMGBSA method [20, 21] as follows:



(**I**): $R^1 = H$ $R^2 = CH_3$
(**II**): $R^1 = H$ $R^2 = C_2H_5$
(**III**): $R^1 = Br$ $R^2 = CH_3$
(**IV**): $R^1 = Br$ $R^2 = C_2H_5$
(**V**): $R^1 = CH_3$ $R^2 = CH_3$
(**VI**): $R^1 = CH_3$ $R^2 = C_2H_5$
(**VII**): $R^1 = OCH_3$ $R^2 = C_2H_5$

Fig. 1. The structures of thieno[2,3-*b*]benzo[1,8]naphthayridine derivatives.

$$\Delta G_{binding} = \Delta G_{complex} - \Delta G_{DNA} - \Delta G_{drug}, \quad (1)$$

where ΔG is defined as follows:

$$\Delta G = \Delta E_{MM} - T\Delta S_{MM} + \Delta G_{solvent}, \quad (2)$$

$$E_{MM} = E_{bond} + E_{angle} + E_{torsion} + E_{vdW} + E_{ELE}, \quad (3)$$

$$S_{MM} = S_{rotation} + S_{translation} + S_{vibration}, \quad (4)$$

$$G_{solvent} = G_{GB} + G_{GBSUR}, \quad (5)$$

where G_{GB} and G_{GBSUR} are polar and non-polar contributions to solvation free energy. Entropies were derived with normal mode calculations by using the N mode module, which computes rotational, translational, and vibrational entropies [22–26]. Normal mode calculations were performed in equilibrium region of simulation. The entropy term S_{MM} is decomposed into rotational, $S_{rotation}$, translational, $S_{translation}$, and vibrational, $S_{Vibrational}$, contributions.

RESULTS AND DISCUSSION

Our molecular docking results show that molecules (**I**), (**II**), (**V**), (**VI**), and (**VII**) bind to DNA structure via intercalation. All of the above-mentioned molecules were located between base pairs of GC and AT (see Fig. 2). Experimental data of viscosity, thermal denaturation, and absorption spectra, which are available only for molecule (**I**), show that molecule (**I**) binds to DNA via intercalation. Viscosity data show that molecule (**I**) increases the viscosity of sonicated rod-like DNA fragments. Thermal denaturation is another strong evidence for the binding of compound (**I**) with the double-stranded DNA. The intercalation of compound (**I**) into the double helix increased the DNA melting temperature (T_m), at which the double helix denatures into single stranded DNA, by about 4°C by increasing the stability of the helix in the presence of an intercalator. Electronic absorption spectroscopy is one of the most useful techniques to study the DNA–drug binding. Hypochromicand bathochromic shifts in the absorption spectrum, which are the result of intercalation between drug and DNA, were observed for compound (**I**) [9, 27–29]. On the other hand, molecules of (**III**) and (**IV**) bound minor groove of DNA in the AT region. This minor groove binding mode has been reported for similar compounds of benzo[*b*][1,8]naphthyridin-5(5aH)-one, 5-chlorobenzo[*b*][1,8] naphthyridine, and benzo[*b*][1,8]naphthyridine-5-thiol [30]. We believe that the choice of the binding mode (intercalation or groove) depends on the volume of considered molecules. Among the molecules under study, molecules (**III**) and (**IV**) are bigger than other molecules due to bromide substitute.

Key tools for determination of stability in MD simulation are root mean square deviation (RMSD) and total potential energy plots. Stability is also important because MMGBSA analysis is done on stability part of simulation. Figure 3 demonstrates RMSD plots of all considered molecules. As seen, all simulations reach the equilibrium region after approximately 500 ps. Therefore, we can use this region for thermodynamics analysis. Another piece of information derived from RMSD plots is the effect of substrate on stability of the complexes. Comparison of average RMSD value for complex of compound (**I**) (3.594) and that of compound (**II**) (4.008) shows that molecule (**II**) decreases the stability of DNA structure more than molecule (**I**) does. Also, average RMSD values for complexes of compounds (**V**) and (**VI**) (3.892 and 4.053, respectively) demonstrate that DNA stability decreases in complex with compound (**VI**) compared to that with compound (**V**). Moreover, RMSD average for molecule (**VII**) is less than for other intercalator molecules.

Figure 4 shows total potential energy versus simulation time. Stability of all simulations also agrees with these plots.

Table 1 presents results of MMGBSA energy analysis for all complexes. To represent a consensus view of all energies, we plotted change of different energies for each molecule in Fig. 5. As seen, the major contribution in predicted binding free energy belongs to van der Waals energy term. This finding indicates that size and shape of molecule is an important factor in binding process. The van der Waals contacts intensify with increasing atom size in molecules. Among considered molecules, the strongest van der Waals contact belongs to molecule (**VII**). This finding is related to the presence of an oxygen atom in $-OCH_3$ group. Thermodynamics data show that $-C_2H_5$ group in

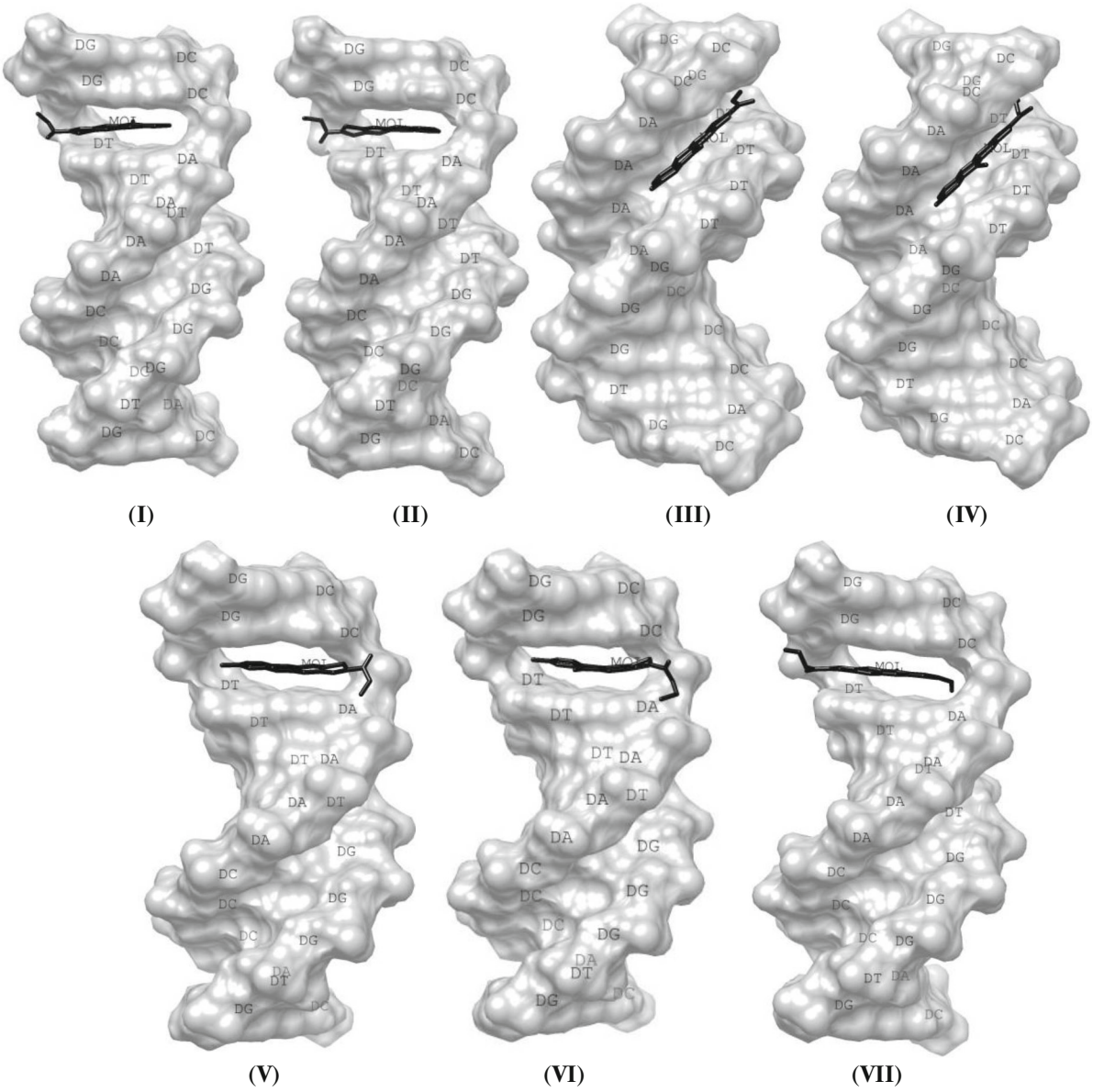


Fig. 2. The structures of docked complexes used for molecular dynamics simulation.

molecules **(II)** and **(VI)** decreases van der Waals contacts compared to molecules **(I)** and **(V)**, respectively. This finding demonstrates the role of shape complementarity in drug binding to DNA. Packing is another factor which increases contacts. Because of packing factor, van der Waals contacts increase for intercalator drugs with respect to groove binding ones. Therefore, as we expected, contribution of van der Waals energy for groove binding molecules is less than for the intercalator ones. Also, thermodynamics data show that polar contribution to solvation free energy or ΔG_{GB} is unfavorable to complexion. Moreover, this unfavorable energy term increases for intercalator drugs with respect to the groove-binding ones. This finding shows that polar interactions with solvent molecules for intercalator drugs are stronger than for groove binding drugs. On the other hand, non-polar energy term or ΔG_{GBSUR} is favorable to binding. This energy term is proportional to solvent accessible surface area (SAS). The negative value of ΔG_{GBSUR} shows that SAS for the formed DNA–drug complexes is less than for DNA and drug separately. Also, it can be seen that ΔG_{GBSUR} for groove-binding drugs is less than for the intercalator ones. This finding shows that SAS for groove-binding complexes decreases with respect to the other ones.

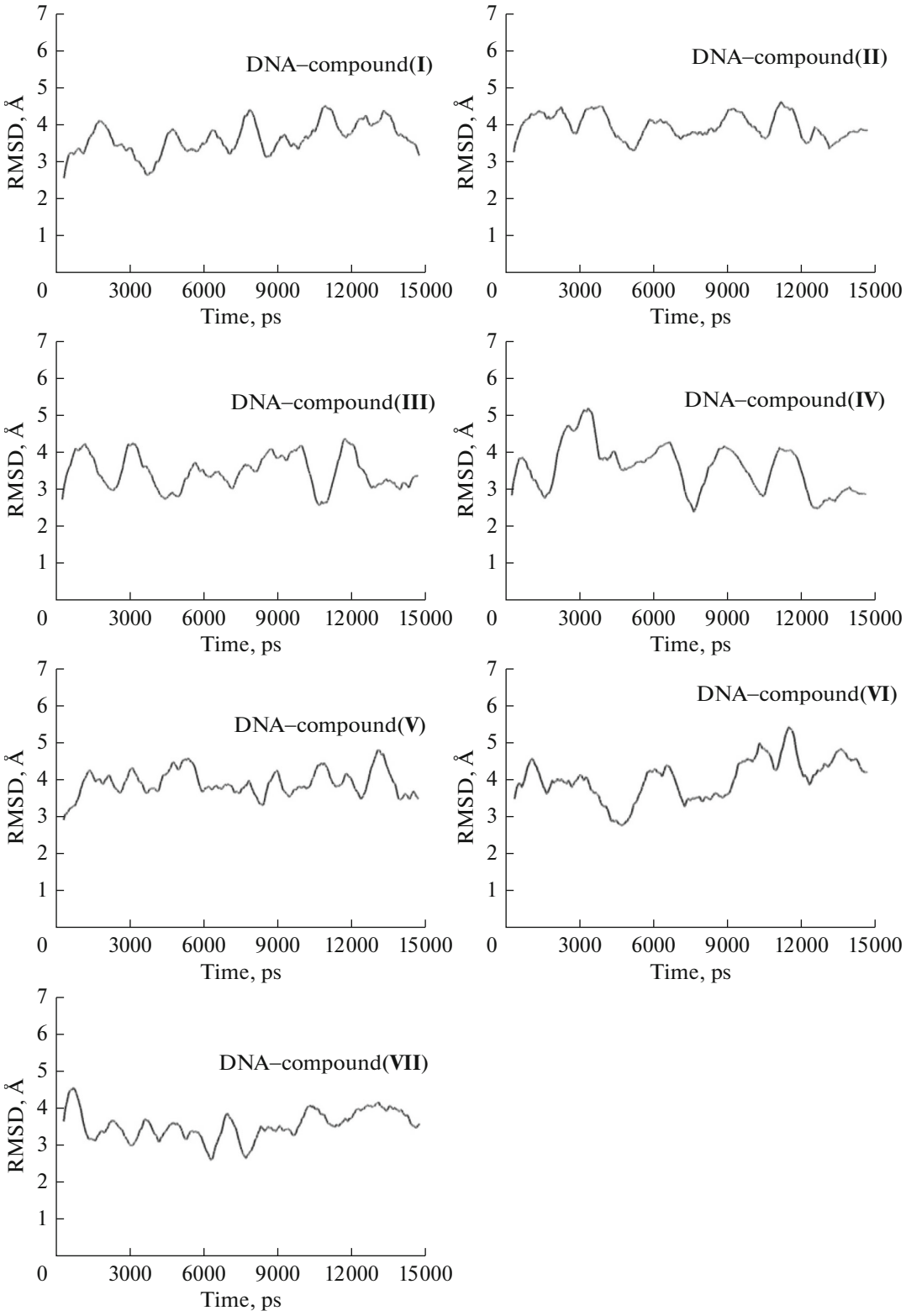


Fig. 3. RMSD plots versus simulation time for all studied complexes.

Entropy term is essential for calculation of binding affinity. So, we represented the values of entropy for all complexes in Table 2 and Fig. 6. Rotational and translational entropy components, which are calculated using equations for rigid body translation and rotation, are unfavorable to complexion. On the other hand,

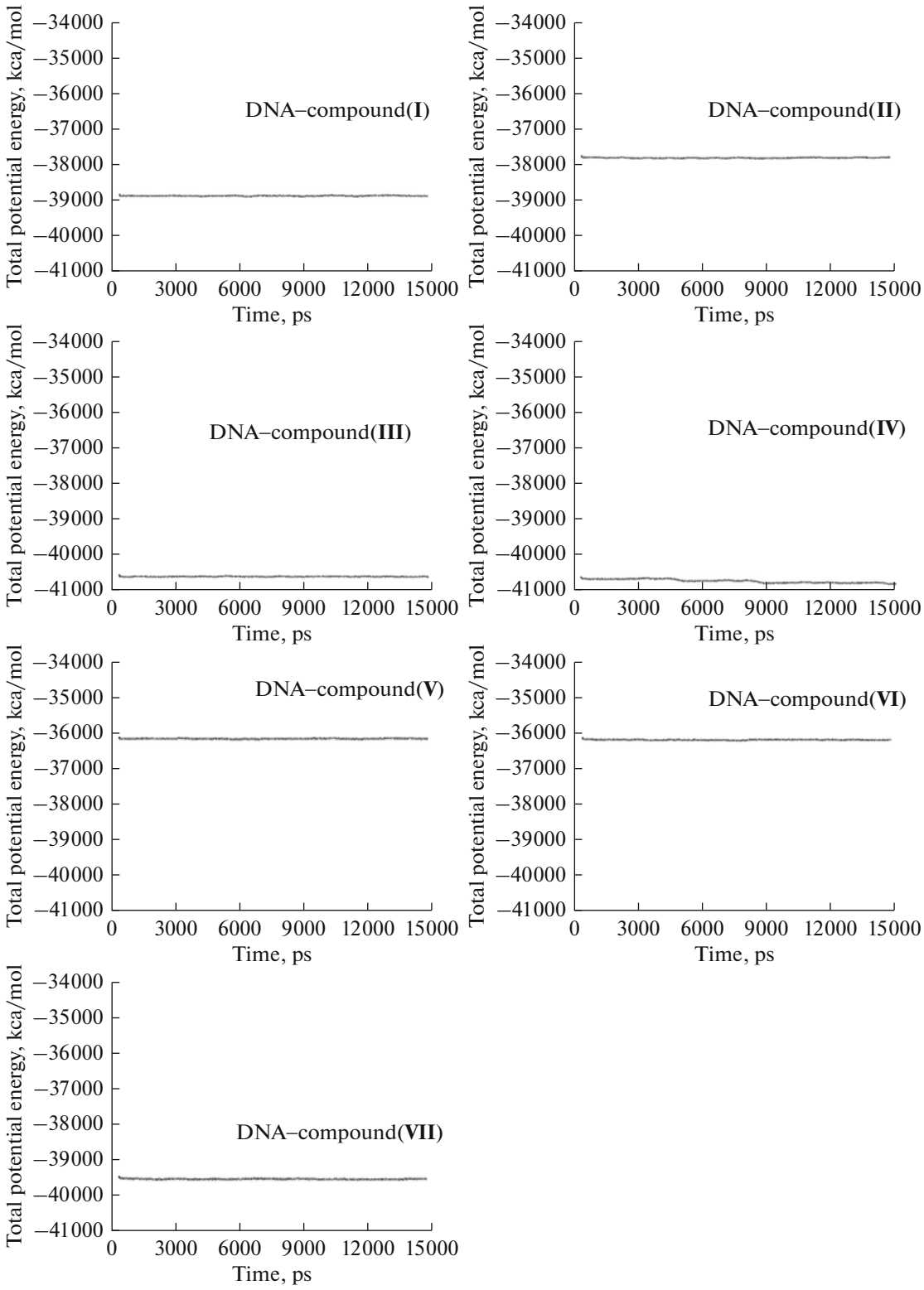


Fig. 4. Total potential energy plots with respect to simulation time.

Table 1. The results of MMGBSA energy analysis for drug complexes with DNA

Drug in complex with DNA	ΔE_{ele}	ΔE_{vdW}	ΔE_{INT}	ΔG_{GBSUR}	ΔG_{GB}	ΔG_{pred}
(I)	−16.91	−45.06	−0.32	−4.63	29.46	−19.75
(II)	−14.07	−42.04	−0.37	−4.35	26.03	−15.34
(III)	9.88	−31.14	−0.55	−3.63	0.61	−11.97
(IV)	8.45	−29.25	−0.41	−3.43	0.54	−10.25
(V)	11.76	−45.83	−0.31	−4.54	1.35	−27.11
(VI)	5.04	−40.82	−0.36	−4.06	9.05	−12.13
(VII)	4.67	−49.24	−0.53	−4.95	12.06	−31.28

Table 2. Calculated entropy contribution used for deriving ΔG_{pred}

Drug in complex with DNA	$T\Delta S_{TRA}$	$T\Delta S_{ROT}$	$T\Delta S_{VIB}$	$T\Delta S_{TOT}$
(I)	−12.88	−10.39	5.56	−17.71
(II)	−12.91	−10.49	3.94	−19.47
(III)	−13.07	−10.75	10.96	−12.86
(IV)	−13.10	−10.71	9.96	−13.85
(V)	−12.91	−10.49	12.93	−10.47
(VI)	−12.95	−10.57	4.51	−19.02
(VII)	−12.99	−10.67	16.95	−6.71

vibrational part of entropy, which is derived using normal mode analysis, is a favorable entropy term.

The value of ΔG_{pred} was calculated using equations (1) through (5) mentioned in computational details section. Comparison of ΔG_{pred} demonstrates that binding affinity of molecule **(I)** is higher than that of molecule **(II)**. Also, it was found that molecule **(V)** binds to DNA structure stronger than molecule **(VI)**. Molecule **(VII)** had the highestaffinity for DNA among studied molecules. As we expected, the values of ΔG_{pred} for the

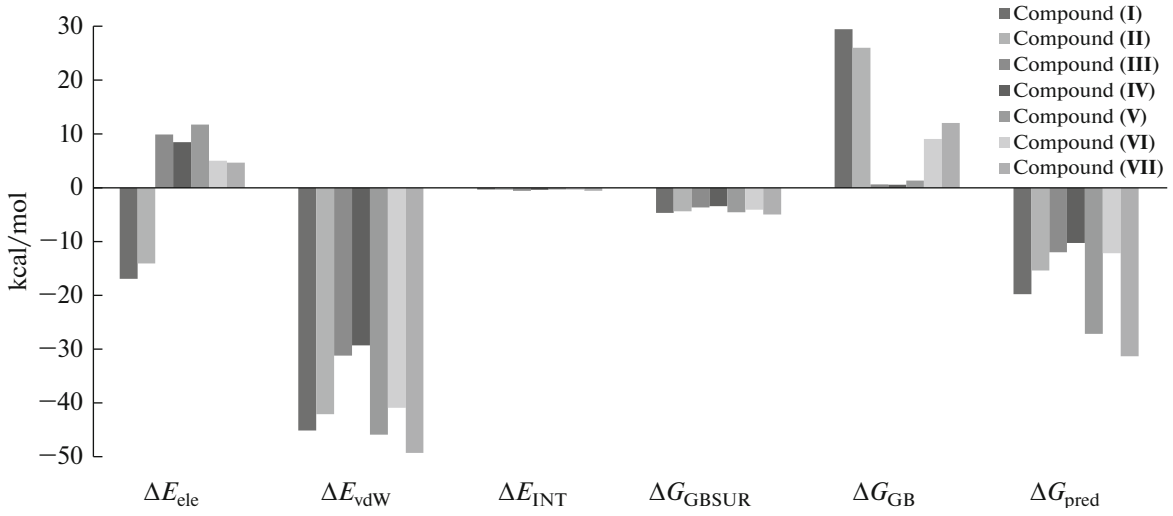


Fig. 5. Histograms of the various energy components contributing to free energy of binding. Histograms for molecules **(I)** through **(VII)** are shown from left to right for each energy component, respectively.

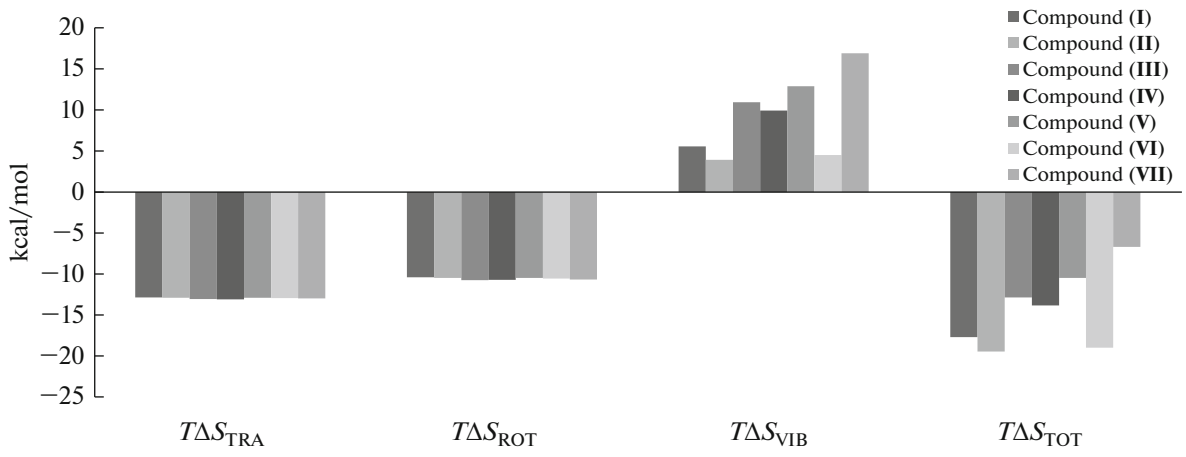


Fig. 6. Histograms of the various entropy components contributing to the total entropy term. Histograms for molecules (**I**) through (**VII**) are shown from left to right for each entropy component, respectively.

groove-binding molecules (**III**) and (**IV**) are lower than for the other ones.

CONCLUSIONS

In this work, we used molecular docking to determine binding position of thieno[2,3-*b*]benzo[1, 8]naphthyridine derivatives in complexes with DNA. Docking showed that molecules (**III**)and (**IV**) bound to minor groove, while other molecules selected intercalation position between base pairs of GC and AT. We found that steric hindrance of molecules (**III**) and (**IV**) was an important factor that did not allow for intercalation mode. Also, plots of RMSD and total potential energy versus time showed that our simulations were stable enough for MMGBSA analysis. Thermodynamics analyses were performed on all simulations in equilibrium region. Binding affinity (ΔG_{pred}) was calculated for all complexes. ΔG_{pred} showed that $-OCH_3$ and $-CH_3$ substituents increase binding affinity due to decreases of atomic charges on π-stacking atoms and increasing van der Waals interactions between the drug molecule and DNA.

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