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Computational Investigations on Interactions Between DNA and Flavonols

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Abstract: Today, the main task of researchers is to study and develop drugs that are less toxic and have lesser side effects. The principal motive of this research is to study and analyze the interaction between naturally active compounds flavonoids with biomolecule DNA. Since the interaction between DNA and ligand is essential in drug designing, this study will provide a good base for further research and development of less toxic and more efficient drugs for various diseases. The selected compounds for this study are Kaempferide, Kaempferol, Morin, and Rutin. They all fall into the category 'flavonols' of flavonoids. Computational methods are implemented for theoretical drug designing. These are molecular optimization, molecular docking, and molecular dynamics. Computational results are compared with experimental data from previous studies. Molecular docking gives the most preferred orientation of ligands within DNA, and Molecular Dynamics provides the details about the DNA-ligand complex with respect to time. Free energy calculations were also performed by implementing MMPBSA and MMGBSA calculations.

Keywords: flavonoids; flavonols; DNA; molecular docking; molecular dynamics; MMPBSA/MMGBSA;

1. Introduction

The possible medication for cancer includes chemotherapy, radiation therapy, and surgery. But the main disadvantage of drugs used in treatment is that they are toxic [1-3]. Regular intake of these has various side effects [4]. It results in the starting of some other problem (disease). So, today the main task of researchers is to study and develop drugs that are less toxic and have lesser side effects. And here, the chosen compounds can be very beneficial. Flavonoids are natural compounds present in seeds, fruits, leaves of plants, and the bark of trees [5,6]. The human body takes them as daily nutrients. So, the main problem of toxicity is reduced a lot, using them as drugs for various diseases[7,8]. They will have minimum side effects. Flavonoids can prove a potent drug against cancer and other diseases [9-11].

Computational methods are beneficial and effective in predicting the nature and characteristics of various drugs as effective on diseases. Different research papers have predicted/concluded that computational methods are as predictive as experimental research [12]. We can rely on these systems for a good result. Theoretical research is essential because

it is an effective method to give results and complement experimental analysis. Theoretical, computational work is less costly, time-efficient, and more effective. Many studies are going on in various fields to understand the interaction of small drugs with DNA [13-15]. Therefore, the hour needs to investigate and study different compounds as potent anticancer or antitumor drugs.

Many studies have been done in the past on flavonoids[16-18]. These studies indicate that flavonoids have various pharmaceutical properties like anticancer, antitumor, antiallergic, anti-inflammatory, etc. [19-21]. They are better known for their antioxidant property. This property arises in the compounds due to their structure. Present result work focuses on investigating the interaction of flavonoids with DNA. DNA is the prime target of drugs like anticancer, antiviral drugs [22,23]. DNA is the primary element for almost every organism. The making of RNA is called transcription. In the process of replication, if the signal RNA is altered, the process of copying is disturbed. It results in the uncontrolled divisions of DNA, thereby uncontrolled cell division. This cell division is the ground level for the origin of diseases like cancer and tumor. So, the interaction of molecules with DNA is of significant importance.

Now, if the drug or ligand is targeted on DNA, it interacts with it and balances the replication process, thereby balancing the cell division. Thus our main problem is solved. The overproduction of cells and tissues stops, providing a good way to cure disease. It is most necessary to study and investigate the interaction of drug-DNA to predict the interaction mechanism. The mechanism will be clearer if the selected drug is potent for that particular disease. Inspired by this concept, the present study is the computational interaction of flavonoids [24] with DNA. Selected molecules are Kaempferide [25], Kaempferol [26], Morin [27], and Rutin [28].

Flavonols are a class of flavonoids known for their antioxidant properties [28]. They are natural compounds found in plants. The present study is comprised of 4 parts. These are molecular optimization, molecular docking, molecular dynamics, and free energy calculation. Molecular optimization is used to get the minimized structure of selected drug molecules, molecular docking gives the most preferred orientation of ligands within DNA, and molecular dynamics provides the details about the DNA-ligand complex with respect to time. Free energy calculations were also performed by implementing MMPBSA and MMGBSA calculations. We are searching for better drugs. As a result of their structure and antioxidant properties, flavonols can prove to be a potent drug for cancer. The literature survey also indicates the same[3,26,29]. So here we are analyzing them with the help of computational techniques.

2. Computational Details

To study the interactions between DNA segment and selected flavanols, the following theoretical steps were taken.

2.1. Optimization of compounds.

Structures of compounds were obtained from a literature survey [26,28,30-31]. Figure 1 shows the chemical structure of all flavonols. All the compounds were designed using GaussView [32] and optimized by Gaussian 09. B3LYP level with basis set 6-31G was used for the optimization [33]. No imaginary frequencies were present after geometry optimization.

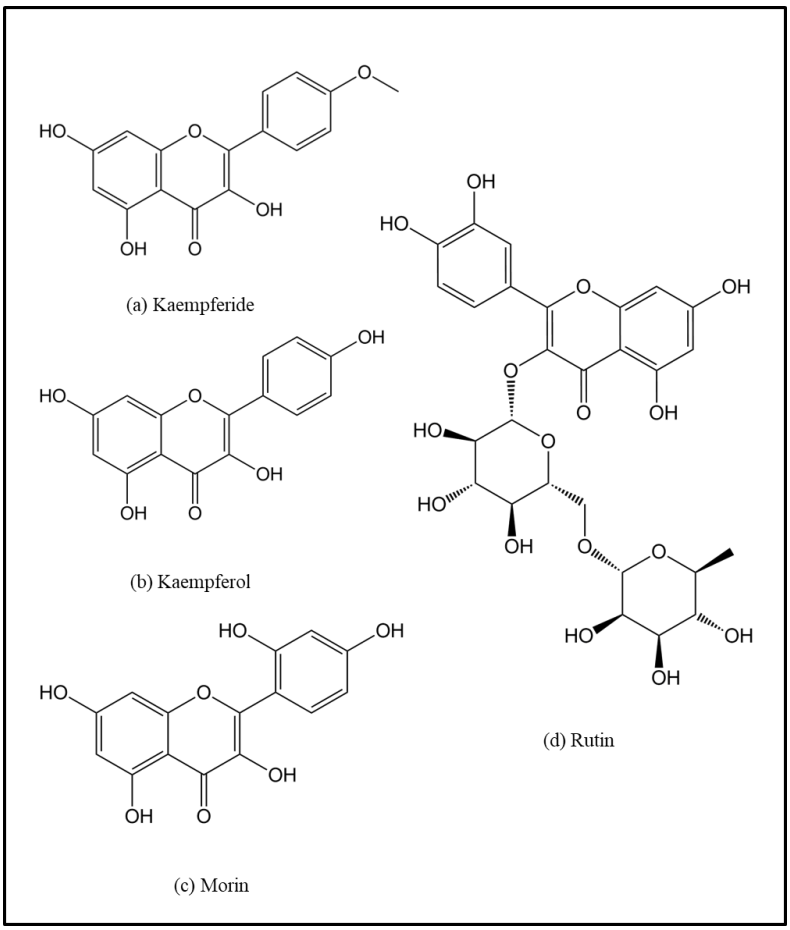

Figure 1. Chemical structure of selected ligands. (**a**) Kaempferide; (**b**) Kaempferol; (**c**) Morin; (**d**)Rutin.

2.2. Molecular docking studies with DNA.

For docking purposes, a segment of DNA was downloaded from the RCSB PDB website [34]. 2ROU is the PDB ID of the chosen DNA strand [35]. The sequence of this segment is 5'-ATCGCGCGGCATG-3'. 2ROU is selected as it has grooves as well as an intercalation gap. It will give a more clear view of the binding mode of the ligand with DNA. The attached ligand and water molecules were removed from the PDB ID 2ROU using the software Chimera [36]. The molecular docking process is done using the program set AUTODOCK4 [37]. The Genetic Algorithm was used to process the computational docking process [38]. Optimized compounds were made to dock with the 13mer DNA 2ROU giving the most preferred binding position of flavonoids within DNA. Both ligand and macromolecule were prepared in the form of PDBQT files for gird and dock calculations. Grid boxes with dimensions 60×84×126 were prepared for each pair of DNA and ligand to enclose the macromolecule. Docking calculations were done using 20 LGA runs and other default settings for docking run options like energy parameters, step size parameters, output format parameters, etc. The docked pose having minimum binding affinity was extracted and combined with the macromolecule for analysis and further study.

2.3. Molecular dynamics studies of the drug-DNA system.

Molecular dynamics simulations provide a great deal of information on nucleic acids and proteins' fluctuations, stability, and conformational changes. These methods are now routinely used to investigate the dynamics, structure, and thermodynamics of biomolecules and their complexes. In the present work, MD simulations were carried out using AMBER 15

software [39]. The best-docked poses of DNA-ligand complexes from the docking studies have been submitted to molecular dynamics simulations for the time-dependent study of the formation of the complexes and their stability.

For the preparation of ligands, 'leaprc.gaff' (generalized amber force field) was used, while the 'leaprc.ff03' was used to prepare DNA. Energy minimization of 500 steps was done to achieve the nearest stable low energy conformation. 50 ps of heating and 50 ps of density equilibrium was followed by 500 ps of constant pressure equilibrium at 300K was applied. Simulation of 5ns was done on these 4 complexes. RMSD plots were plotted to show the stability of the complex with time.

2.4. Free energy calculations.

Free energy calculations were performed by MMPBSA and MMGBSA. The MM-PBSA approach has come out to be a good and widely used scheme to calculate free energies and is frequently employed for the study of biomolecule complexes [40,41]. In MMPBSA, the interaction energies of MD simulations are combined with the solvation energy by Poisson-Boltzmann calculations and molecular surface area-based calculations of the non-polar part of the solvation free energy.

To determine the relative binding energy between DNA and ligand, mm_pbsa.pl script was used. MMPBSA/MMGBSA calculations were done using the script "extract_coords.mmpbsa". To calculate ΔG_{bind} , "binding_energy.mmpbsa" script was used. To solve the PB equation g_mmpbsa uses the APBS package whereas mm_pbsa.pl uses the PBSA program of the AMBER suite.

3. Results

3.1. Molecular docking.

Table 1 gives the binding energies and binding modes of used Flavonols with DNA sequence 2ROU. From the table, it is clear that Kaempferide, Kaempferol, and Morin bind in the minor groove of the DNA, i.e., they act as a minor groove binder. At the same time, rutin attaches itself between the base pairs of DNA and forms the intercalation binding. Obtained results are also compared with experimental data from previous studies. It was observed that the experimental and theoretical values of binding energy are in the same range, and they follow a similar trend. Figure 2 shows a similar trend in the variation of the binding energy for theoretical and experimental cases. Thus we can say that the molecular docking results agree with previous studies.

Table 1. Comparison of theoretical binding energies of used flavonols with DNA sequence 2ROU and experimental binding energy (from literature survey).

S.No.	Flavonoids	Binding mode	Binding energy (kcal/mol) molecular docking	Experimental Data		Ref.
				Binding constant (M^{-1})	Binding energy (kcal/mol)	
1.	Kaempferide	Minor Groove	-7.57	5.63×10^4	-6.40	[25]
2.	Kaempferol	Minor Groove	-6.98	3.60×10^4	-6.21	[26]
3.	Morin	Minor Groove	-7.22	7.04×10^4	-6.42	[27]
4.	Rutin	Intercalation	-6.43	2.10×10^4	-5.89	[27]

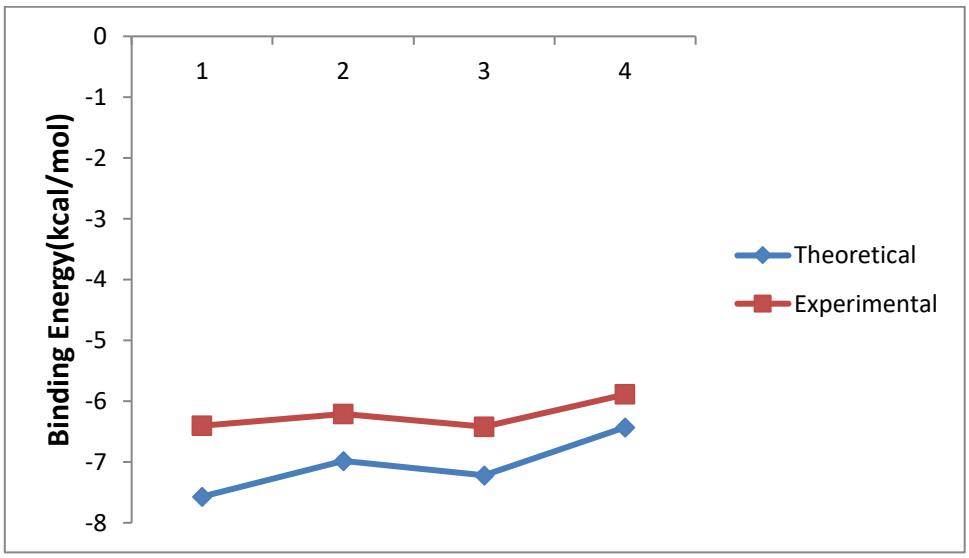

Figure 2. A similar trend of binding energy in theoretical and experimental cases.

All the details of the hydrogen bonds formed between the ligand and macromolecule are given in Table 2. We see that morin forms a maximum number of hydrogen bonds with the macromolecule. The non-planar optimized structure of morin makes it different from kaempferide and kaempferol, which is also the reason for its low binding energy than kaempferide despite having a maximum number of hydrogen bonding. The binding energy is maximum in the case of the Kaempferide-DNA complex, which is -7.57 kcal/mol. It suggests that Kaempferide binds with maximum strength with DNA. Figure 3 represents the binding position of ligands with DNA. These figures clearly show the binding mode of the complexes. Fig 4 represents the detailed interaction of ligands with DNA residues.

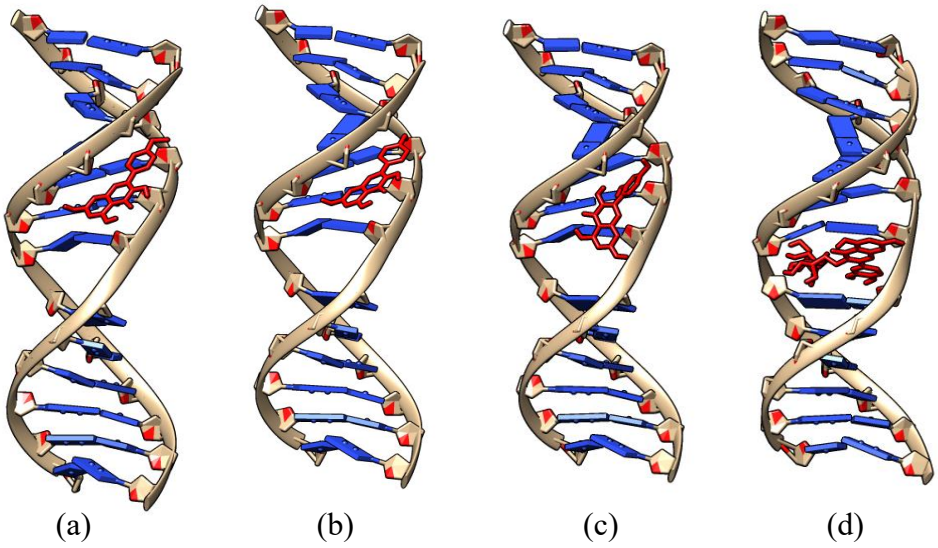

Figure 3. Binding sites of Flavonols with DNA. (**a**) kaempferide; (**b**) kaempferol; (**c**) morin; (**d**) rutin.

Dipole-dipole interactions, π-π stacking, and hydrogen bonds between the DNA base pairs and ligand were responsible for the stability of the docked poses. Being the biggest ligand, rutin forms hydrogen bonds as well as hydrophobic bonds with the DNA residues and intercalates with the DNA. Table 2 details the hydrogen bonds formed between the ligands and macromolecule.

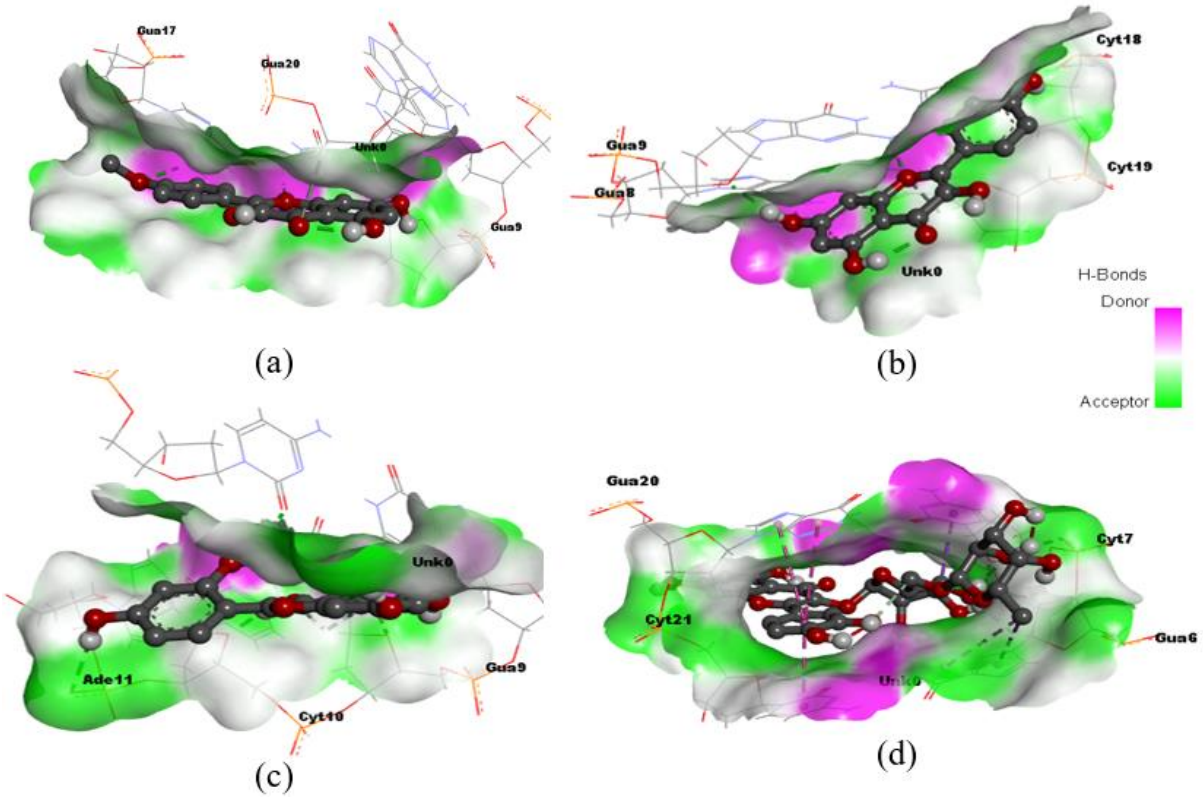

Figure 4. Figure representing the H-bond donor and acceptor regions in best docked posed complexes.

Table 2. Details of the hydrogen bonds formed between the ligands and macromolecule.

S.No.	Complex	No. of H-bonds	Interacting species	H-bond length(Å)
1.	2ROU+Kaempferide	4	B:DG17:H21 - UNK0:O A:DG9:H22 - UNK0:O A:DG8:H22 - UNK0:O B:DG20:H5'2 - UNK0:O	2.664911 2.090667 1.734066 3.017154
2.	2ROU+Kaempferol	5	UNK0:H - B:DC18:O4' B:DC19:H1' - UNK0:O A:DG9:H22 - UNK0:O A:DG8:H22 - UNK0:O UNK0:H - A:DG9:O4'	2.200747 2.994567 2.147357 1.673198 2.118409
3.	2ROU+Morin	8	UNK0:H - A:DA11:OP1 UNK0:H - A:DC10:O4' A:DG9:H22 - UNK0:O UNK0:H - B:DC19:O2 A:DG9:H1' - UNK0:O A:DG8:H22 - UNK0:O A:DG9:H1' - UNK0:O A:DG8:H22 - UNK0:O	2.403048 1.919445 1.908579 2.303229 2.172761 2.568482 2.972384 1.647879
4.	2ROU+Rutin	5	UNK0:H - A:DC7:OP2 A:DG6:H1' - UNK0:O UNK0:H - A:DC7:O4' UNK0:C - UNK0:O A:DG6:H21 - UNK0:O	2.174341 2.612094 2.381427 2.811063 1.962604

Table 3. Hydrophobic bonds between Rutin and DNA.

Hydrobhobic bonds of rutin	S.no.	Interacting species	Type of bond	Bond length(Å)
	1.	UNK0:C - A:DG6	Pi-Sigma	3.625665
	2.	A:DG6 - UNK0:C	Pi-Alkyl	4.940303
	3.	UNK0:C - A:DC7	Pi-Sigma	3.334250
	4.	B:DC21 - UNK0	Pi-Pi Stacked	3.864755
	5.	B:DG20 - UNK0	Pi-Pi Stacked	4.952077
	6.	B:DG20 - UNK0	Pi-Pi Stacked	4.632748

Kaempferide has a minimum number of hydrogen bonds, i.e., 4 hydrogen bonds. At the same time, the morin-DNA complex has a maximum number of hydrogen bonds. Table 3 gives the information about all the hydrophobic bonds between Rutin and DNA. As the other three ligands bind as groove binders, hydrogen bonding is the main bonding between ligand and DNA. In the case of groove binding, hydrogen bonds are responsible for the attachment between ligand and DNA. No hydrophobic bonds were seen in Morin, Kaempferide and Kaempferol complexes with DNA. Molecular Docking studies have also revealed that these compounds bind to DNA base pairs by hydrogen-bonding and the planar structure of the ligands is crucial for DNA intercalation.

3.2. Molecular dynamics.

The most preferred binding mode obtained from molecular docking calculations was taken as initial structures for MD simulations for the stability study of DNA-ligand complexes. All simulations were carried out using AMBER, as discussed in the method section. RMSD curves for all 4 systems are shown in Figure5. The RMSD value for kaempferide, kaempferol, and rutin complexes lies in the range 1Å - 4Å, whereas, for morin complex, RMSD reaches well above 7Å, showing low stability.

Figure 6 represents the average number of hydrogen bonds formed for each system during the simulation process. As clear from the figure, kaempferide and kaempferol form an average of 2 hydrogen bonds with DNA, whereas morin and rutin have a maximum of 3 hydrogen bonds with DNA during the simulation process.

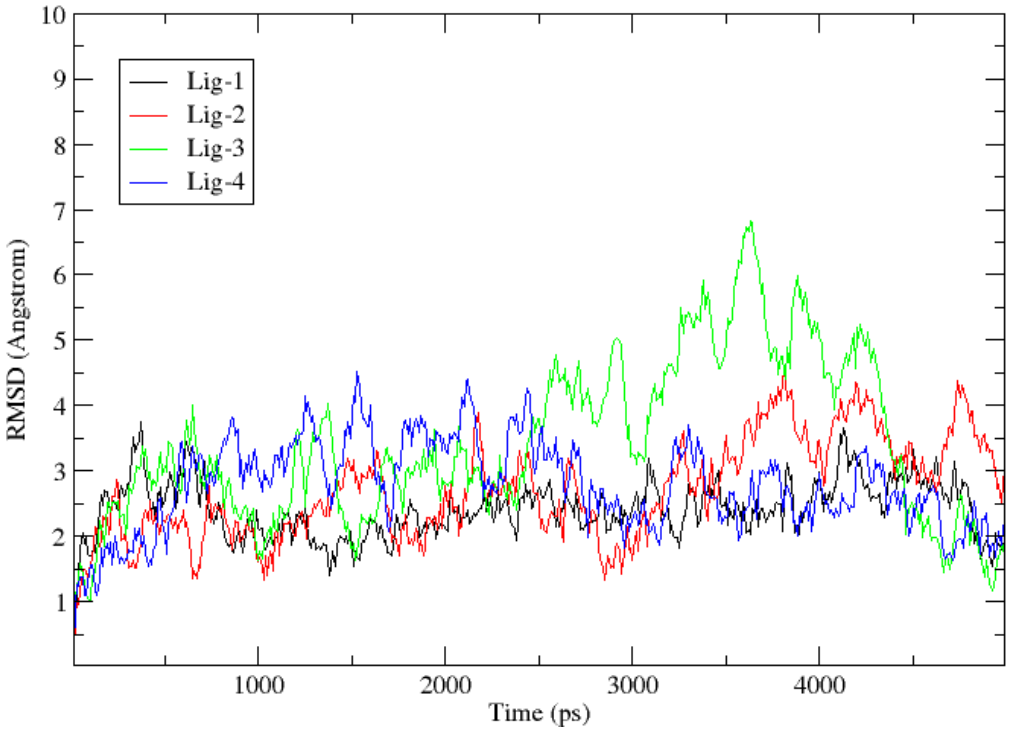

Figure 5. Figure representing RMSD plot for drug-DNA complexes.

As analyzed from molecular dynamics studies, figure 5 represents the RMSD curves for all drug-DNA complexes. RMSD curves show the stability of the drug DNA complex with respect to time. The deviations in the case of morin show minimum stability of the morin-DNA complex. RMSD plots show maximum fluctuations in the case of the DNA-morin complex. The non-planar optimized structure of morin, which makes it different from kaempferide and

kaempferol, could be a reason for the morin-DNA complex's low binding and low stability. Other complexes have convergence which shows the stability of the complex.

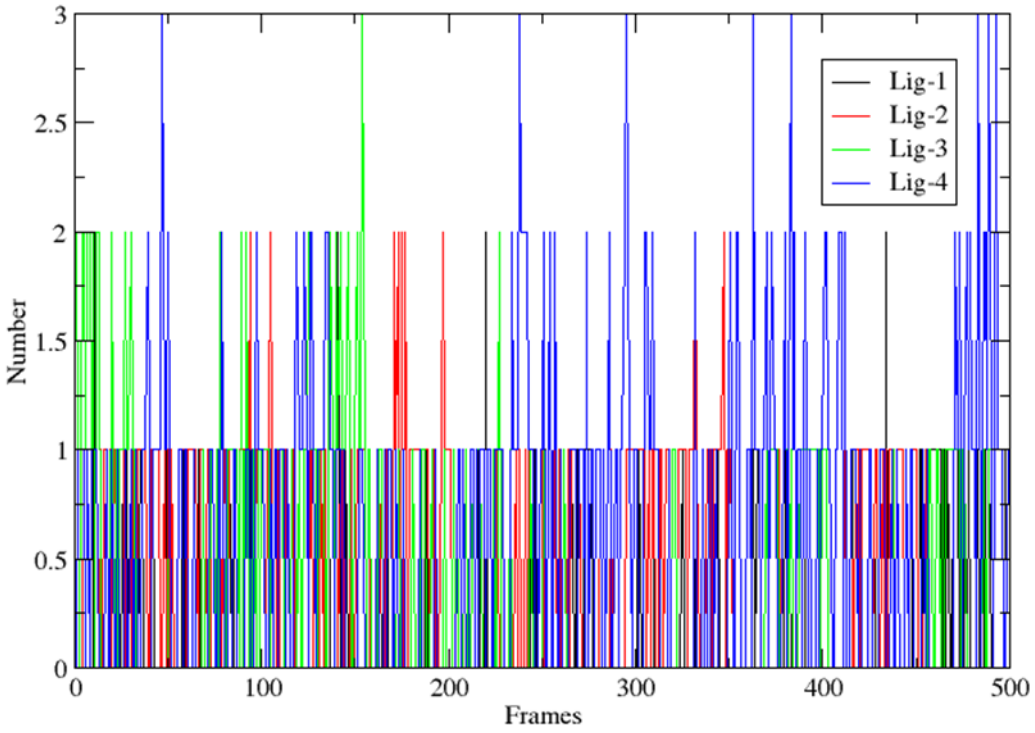

Figure 6. Figure representing hydrogen bonds for drug-DNA complexes.

3.3. Free Energy Calculations.

The MMPBSA and MMGBSA free energies for the DNA-ligand system are given in Table 4.

Morin has minimum MMPBSA and MMGBSA energies, and the plot also suggests minimum stability with time. Morin has the least interaction with DNA with respect to time. The results of molecular Dynamics were verified by the energy calculations. Table 4 shows that morin has both minimum MMPBSA and MMGBSA energies.

Table 4. MMPBSA and MMGBSA free energies ΔG_{bind} (kJ/mol) of DNA-ligand system.

S.No.	Flavonoids	No. of heavy atoms in ligand	MMPBSA energy	MMGBSA energy
1.	Kaempferide	22	-20.25	-17.99
2.	Kaempferol	21	-12.54	-10.89
3.	Morin	22	-4.16	-1.65
4.	Rutin	43	-29.39	-29.45

4. Conclusions

This paper studied the interaction of flavonols with DNA via computational techniques, namely molecular docking, molecular dynamics, and free energy calculations. The main objective was to investigate the stability of the ligand-macromolecule complex. Molecular Docking study carried out in the current work was done to achieve the DNA binding affinities of the flavonols. Kaempferide, kaempferol, and morin bind in the minor groove of the DNA, i.e., they act as a minor groove binder. At the same time, rutin attaches itself between the base pairs of DNA and forms the intercalation binding. The experimental (obtained from literature) and theoretical binding energy values are in the same range and follow a similar trend.

Molecular docking results are in agreement with previous studies. RMSD plots showed convergence which states that the complex formed by drug and DNA is stable. Morin-DNA complex has minimum stability, which is also verified by free energy calculations. Morin-DNA complex has maximum deviations in the RMSD plot. The energy obtained from MMPBSA and MMGBSA calculations also gave minimum energy for the Morin-DNA complex system. The non-planar optimized structure of morin, which makes it different from kaempferide and kaempferol, could be a reason for the morin-DNA complex's low binding and low stability. Whereas maximum free energy is in the case of the Rutin-DNA complex, the high molecular mass of rutin could be a reason for this high energy. The present study gives detailed insight on the interaction of flavonols with DNA and will be helpful in further study of flavonols as effective anticancer agents.

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Conflict of Interest

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version. This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue. The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

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