Quantum Mechanical/Molecular Mechanical (QM/MM) studies of DNA Binding Drugs

SUMMARY SUBMITTED FOR THE AWARD OF THE DEGREE

of

Doctor of Philosophy

in

Applied Physics

Submitted by

Anamika Shukla

Enrollment No.: 673/15

Under the supervision of

Prof. Devesh Kumar

Department of Applied Physics
School for Physical Sciences
Babasaheb Bhimrao Ambedkar University
Vidya Vihar, Raebareli Road
Lucknow (U.P.), India – 226025

2022

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Chapter 1: Introduction

DNA is the base of genetic information in almost all organisms. It is responsible for genetic transfer among generations. Also, the functions of DNA help the body in proper growth and functioning. Any change in the functioning of DNA can have very challenging effects on whole body. In 1953, Watson and Crick gave the basic structure of DNA. According to them, DNA consists of two helical chains each coiled around each other along the same axis. The bases are on the inside of the helix and the phosphates on the outside. The planes of the bases are perpendicular to the axis of DNA. These bases are adenine, thymine, guanine and cytosine. Adenine pairs up with thymine while guanine pairs with cytosine. The bases are projected inwards and the spaces between the bases create the minor and the major groove of the DNA. As the names suggest, minor groove is small in size and major groove is larger.

In the field of drug designing and drug development, computational methods are playing a very important role. As the computer hardware, software and algorithms are developing rapidly, the process of drug screening and theoretical drug designing has reduced the time and cost dramatically. These computational methods are helping in effectively reducing animal models used in pharmacological research, supporting the designing of new, safe and potent drug candidates, and for displacing marketed drugs, assisting medicinal chemists and pharmacologists during the drug discovery course.

In this regard, drug-DNA interaction is also very important. The binding of small molecules to DNA is medically very important. Studies have shown that certain small molecules which interact and bind with DNA can be effective anticancer, antiviral and antibiotic therapeutic agents that affect the well-being of millions of people worldwide. Some DNA binder molecules are being used and new ones are designed and synthesised for the betterment of clinical efficiency. To target DNA

for the regulation of cell function via interfering with replication process sounds logical, intuitively appealing and conceptually uncomplicated.

There have been a lot of studies on drug-DNA interaction since about four decades but a clear and detailed mechanism of action of drug on DNA is still lacking. For the evolution of new drugs for the treatment of various diseases like cancer, tumour etc. it is mandatory to understand structural and mechanistic information in detail.

Based on the structure, shape and size, DNA is classified into different forms: B-type, A-type and Z-type. B form of DNA is most commonly found in nature. Transitions between these forms of DNA can be produced by salt concentration and solvent. The two main functions of DNA are transcription and replication.

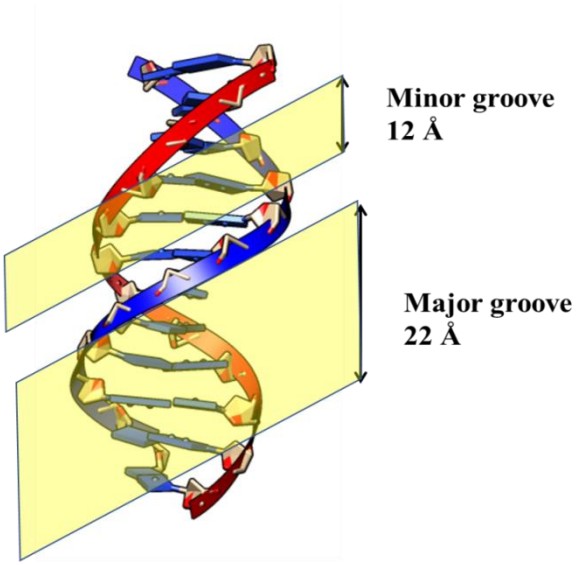

Figure 1. Double helical structure of DNA with major and minor grooves.

The process of transcription involves using the enzyme RNA polymerase to convert a DNA sequence into an RNA molecule. To create a complementary RNA strand, one of the DNA strands serves as a template. DNA replication is the process by which DNA makes a copy of itself during cell division. Transcription and replication are the vital processes essential for the survival of the living system.

Drugs attach to DNA in both covalent and non-covalent ways. In DNA, covalent binding is irreversible, but non-covalent binding is reversible. Further the binding

maybe groove binding or intercalation type. Groove binding is of two type's major groove binding or minor groove binding. Minor groove binders are compounds that bind to the minor groove of DNA in a non-covalent manner. It takes place by combination of directed hydrogen bonding to base pair edges. Major groove binders are molecules that bind to the major groove of DNA. In intercalation, intercalators which are planar heterocyclic groups stack between adjacent DNA base pairs.

The aim of proposed research is to understand mechanism of drug-DNA interactions. Computational chemical techniques such as Molecular Docking, Molecular Dynamics (MD) and QM/MM will be used for predicting new drug, binding of drug to DNA and stability of bonded drugs respectively. A thorough study of these interactions would provide theoretical techniques to complete experimental techniques and will also be helpful in the design of new drugs.

Chapter 2: Methodology

Main methods used in the present research are discussed below:

Density functional theory

Density functional theory (DFT) is a computational quantum mechanical modelling method used to investigate the electronic structure (principally the ground state) of many-body systems, in particular atoms, molecules, and the condensed phases. Using this theory, the properties of a many-electron system can be determined by using functional, i.e. functions of another function, which in this case is the spatially dependent electron density. Hence the name density functional theory comes from the use of functional of the electron density. DFT is among the most popular and versatile methods available in condensed-matter physics, computational physics, and computational chemistry. The geometry optimization of the ligands was achieved using Gaussian 09 software package at B3LYP level and 6-31G basis set.

Molecular docking

Molecular docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Docking is a computational simulation of a ligand binding to a receptor.

It is a technique that determines which orientation a pair of molecules will choose to acquire when bonded together to form a stable combination. By applying scoring functions, for instance, knowledge of the preferred orientation may be utilised to forecast the strength of association or binding affinity between two molecules. Autodock 4.2 software was applied for molecular docking simulations. Autodock executes molecular dockings by computing energy grids around a target's region of interest. Energy assessments of the location of the ligand with regard to the target energy grids are carried out using a stochastic search method that makes use of the Lamarkian Genetic Algorithm (LGA) for exploring the grid space.

Molecular dynamics simulation

The special approaches like molecular dynamics simulation is required to understand the complex systems like nucleic acids. Molecular dynamics simulation is a computer simulation of physical movements of atoms and molecules. Biological activity of a molecule is the result of its time dependent interactions with other molecules or its environment and these interactions occur at the interfaces such as protein-protein, protein-ligand and DNA-ligand. These time dependent microscopic interactions can be calculated by molecular dynamics simulation. The atoms and molecules are allowed to interact for a period of time, giving a view of the motion of the atoms and the results are analyzed in the form of trajectories. In MD simulation, the complex is put into the water box and relaxed by a series of constrained energy minimization and MM runs at the MM level. MD simulations of the selected docked poses were performed using GROMACS or AMBER.

Free Energy Calculations

There are many computational methods available to determine binding free energy but only a few of them can be applied for bigger biomolecular systems. Molecular Mechanics/Poisson Boltzmann Surface Area (MM/PBSA) and Molecular Mechanics Generalized Born Surface Area (MM/GBSA) approaches are used extensively for the estimation of free energy. In the MM/PBSA method the Boltzmann equation of distribution of charges is combined with the Poisson's equation for electrostatic potential which results in the linearized Poisson-Boltzmann equation. The Generalized Born model is applied in MM/GBSA

method. After MD production runs, the binding energy between drug and DNA was calculated using MMPBSA/MMGBSA method.

Quantum mechanical/molecular mechanical

The QM/MM (quantum mechanics/molecular mechanics) approach is a molecular simulation method that combines the strengths of the QM (accuracy) and MM (speed) approaches, thus allowing for the study of chemical processes in solution and in proteins. An important advantage of QM/MM methods is their efficiency.

In QM/MM approach the active site is treated at quantum mechanical level (QM) and rest of the bimolecular system which includes DNA, solvent and the counter ions, is treated with molecular mechanics (MM).

Snapshots obtained from the MD simulation are taken as starting geometries for the QM/MM calculations. The QM/MM calculations were performed with two-level ONIOM method within the Gaussian 09 program suite. DFT method along with basis set 6-31+G is used for the high layer and AMBER force field for the low layer and all the water molecules are freezed. Geometry optimization of above system was performed and the energy of the high or QM region is calculated. Now, the co-ordinate of QM region from the above optimized system is extracted, which further subjected for geometry optimization and the single point energy calculations of QM region in gas phase using same method. The interaction energy between the DNA and drug molecule is calculated with the help of formula-

$\Delta E_{IE} = E_{QM\,(gp)} - E_{QM\,(pp)}$

Where,

ΔE_{IE} is the interaction energy between drug and the DNA,

$E_{QM\,(gp)}$ is the energy of QM region in nucleic acid phase,

$E_{QM\,(pp)}$ is the energy of QM region in the gas phase.

Chapter 3: Evaluation of binding properties of some common drugs with DNA as intercalator and groove binder

Chapter 3 is focused on the evaluation of binding properties of some common drugs with DNA as intercalator and groove binder. Molecular docking process discusses the underlying factors for their stability and interactions with DNA.

Present study is focused on the molecular docking of some common drugs with DNA. These drugs are Aspirin, Dephnetin, Eugenol, Ibuprofen and Idarubicin. They have been studied earlier and their interaction with DNA by experimental methods is also reported in many studies. This theoretical study will further enlighten the usefulness of these drugs.

The crystal structures of DNA sequences having PDB ID - 1BNA and 1N37 were sourced from RCSB 'Protein Data Bank' (PDB). The geometry optimization of the ligands was achieved using Gaussian 09 software package at B3LYP level and 6-31G basis set. 'Lamarckian Genetic Algorithm' (LGA) in Autodock 4.2 software was applied for molecular docking simulations. The DNA was prepared as the macromolecule and the flavonoids were modified as ligands. Files for both, receptor macromolecule and ligand were assembled for doing molecular docking by AutoDockTools (ADT). Next step was the formulation of grid maps. Grid boxes of varied dimensions were obtained to cover whole macromolecule for blind docking. For each drug-DNA docking, several poses of drug with DNA are docked. For each pose, binding energy is calculated and each pose is given an Autodock generated score function. The pose with lowest value of binding energy is considered as final binding mode.

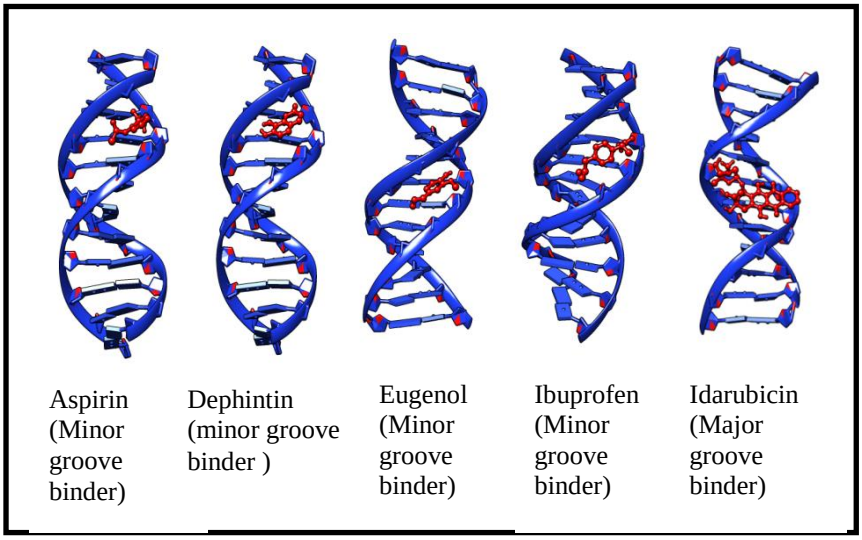

Figure 2. Molecular Docking between drugs and 1BNA.

Binding energies obtained from computational docking method for the DNA sequence with PDB ID 1BNA is tabulated in Table 1. From the resulting docking

complexes, as shown in Figure 2, it is clear that the mode of binding interaction of drugs with sequence 1BNA shows mainly minor groove binding.

Table 1: Binding energies and Binding constants of various drug-DNA complexes.

Ligand		**1BNA**	**1N37**		**Experimental Results**	
	Binding constant (K_B) (10^4 M^{-1})	Binding Energy (kcal/mol)	Binding constant (K_B) (10^4 M^{-1})	Binding Energy (kcal/mol)	Binding constant (K_B) (10^4 M^{-1})	Binding Energy (kcal/mol)
Aspirin	1.15	-5.54	0.14	-4.29	-	-4.71
Daphentin	3.11	-6.13	0.95	-5.43	-	-6.01
Eugenol	0.55	-5.11	0.18	-4.45	0.36	-
Ibuprofen	0.27	-4.70	2.04	-5.88	-	-6.96
Idarubicin	**30.96**	**-7.49**	**69.13**	**-9.33**	**51.40**	**-**

Figure 3 shows the interaction of drugs with 1N37. Small sized ligands find the place between the stacking of bases of 1N37 whereas in the case of Idarubicin which have larger size, some part of the ligand is also in the minor groove of DNA. The main reason for intercalative type of binding was mainly the structure of the DNA sequence. 1N37 DNA sequence has an intercalative gap between its base pairs. Therefore, all of the ligands bind with the intercalative site present in the DNA.

Idarubicin-DNA complex is most stable having strongest binding between them. On comparing the results and experimental data, it is clear that the complex having highest binding energy is same as reported in literature. It shows the capability of Autodock 4.2 software in the prediction of the right binding mode between any drug-DNA complexes.

. Hydrogen bonds were dominant in case of groove binding whereas intercalative type of binding mainly depends upon the hydrophobic bonds. Large number of hydrophobic bonds indicate high stability of drug-DNA complex in the intercalation gap.

Binding energies obtained from comparing the data, it is clear that the complex

having highest binding energy is same as reported in literature. It shows the capability of Autodock4.2 software in telling the right binding mode between any

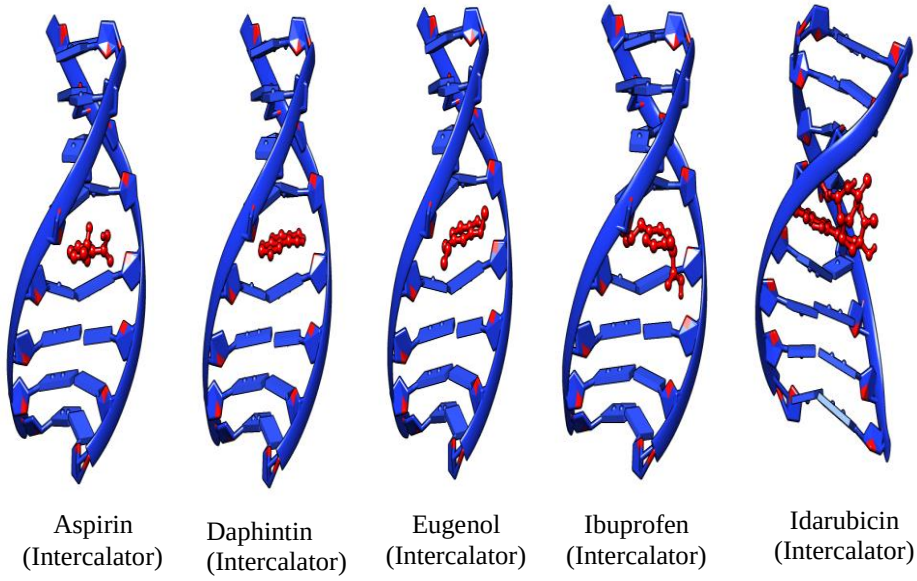

Figure 3. Molecular Docking between drugs and 1N37.

drug-recepto complexes. This will promote a theoretical procedure supporting experimental methods and database creation for drug-DNA complex structure-energy correlations.

Chapter 4: Interaction of flavonols with DNA: Molecular Docking studies

In this chapter, we are dealing with the prediction and investigation of binding properties of flavonols, a class of flavonoids, with DNA using molecular modelling. Molecular Docking is the technique which is used here. It gives details about the best pose of complexes formed between drug and DNA. It also tells the binding energy between drug and DNA. Flavonoids are dietary supplements well known for their antioxidative properties. They are found in various part of plant like fruits, seeds, bark etc. They are natural antioxidants. Various studies have been done on various types of flavonoids including flavonols because of their important use in medicine and pharmacology. Literature survey revealed that flavonoids have a wide range of biological activities like anticancer, antitumor,

antibiotic, antiviral, antiallergic, anti-inflammatory, etc. Flavonoids have potential to bind with DNA and show various pharmacological properties. Flavanols are also beneficial for human body and their interaction with DNA can provide various useful informational results. They can bind as groove binders as well as intercalate with DNA.

As they are natural products, flavonols can prove to be better anticancer drugs due to their low toxicity. The flavonols used for docking studies were Kaempferide, Karanjachromene, Quercetin and Rutin. These were docked with DNA sequences with PDB ID 1BNA, 195D, 1CP8, 1D66 and 1RMX. Docking studies provided details of the complexes formed between each of the drugs and DNA sequence. The pdb format file of DNA sequences with PDB ID 1BNA, 195D, 1CP8, 1D66 and 1RMX were extracted from RCSB Protein Data Bank. The drug molecules were taken to geometry optimization process using Gaussian 09 at B3LYP/6-31G level. AutoDock4.2 was utilized for the molecular docking process by using Lamarckian Genetic Algorithm (LGA). . For each of the docking cases, the docked conformation with the lowest energy, according to the Auto Dock scoring function, was selected as the binding mode.

Table 2: Binding energies (kcal/Mol) of flavonols with different DNA sequences.

Drug	1BNA	1CP8	1D66	1RMX	195D
Kaempferide	-8.75	**-7.52**	-8.90	**-7.16**	-8.77
Kranjachromene	**-8.98**	-6.78	**-8.96**	-6.70	**-9.23**
Quercetin	-8.13	-7.15	-8.65	-6.78	-8.23
Rutin	-5.10	-3.85	-6.08	-4.56	-5.22

Most of the flavonols bind in the minor groove of the DNA sequences. Three out of 20 complexes were found out to have major groove binding. These bindings are represented in Figure 4. As Rutin is biggest drug among all four, it binds in the major groove. This analysis concludes that the binding mode between drug and DNA depends on both, the structure and size of drug, as well as on the structure of DNA. The computationally calculated binding energies are listed in Table 2.

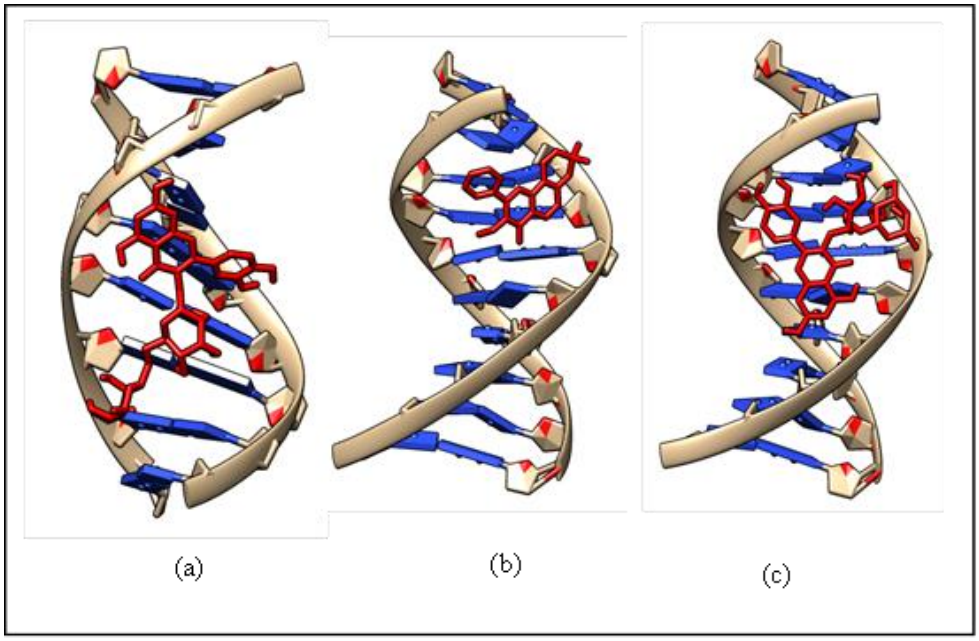

Figure 4. Major groove binding. (a) 1CP8 and rutin, (b) 1RMX and karanjachromene, (c) 1RMX and rutin

Most of the complexes showed minor groove binding with hydrogen bonding and pi-pi interactions as main interaction. Some complexes showed major groove binding. This suggests that the mode of binding between drug and DNA depends also on the structure of DNA sequence. Thus, our research aids in enhancing insight deeper about the mechanism of DNA binding and binding affinity of natural antioxidant flavonols with DNA.

Chapter 5: Computational investigations on interactions between DNA and flavonols

Flavonols are a class of flavonoids known for their antioxidant properties. They are natural compounds found in plants. Present chapter is focused on the computational interaction of flavonoids with DNA. Selected molecules are Kaempferide, Kaempferol, Morin and Rutin .The 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. So here we are analysing them with the help of computational techniques.

All the compounds were designed using GaussView and optimized by Gaussian 09. B3LYP level with basis set 6-31G was used for the optimization. For docking purposes, a segment of DNA was downloaded from the RCSB PDB website. 2ROU is the PDB ID of the chosen DNA strand. 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 molecular docking process was done using the program set AUTODOCK 4.2. The Genetic Algorithm was used to process the computational docking process. Optimized compounds were made to dock with the DNA segment 2ROU giving the most preferred binding position of flavonoids within DNA.

Table 3. 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**	
				Binding constant (M^{-1})	**Binding energy (kcal/mol)**
1.	Kaempferide	Minor Groove	-7.57	5.63×10^4	-6.40
2.	Kaempferol	Minor Groove	-6.98	3.60×10^4	-6.21
3.	Morin	Minor Groove	-7.22	7.04×10^4	-6.42
4.	Rutin	Intercalation	-6.43	2.10×10^4	-5.89

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. 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.

Table 3 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 5).

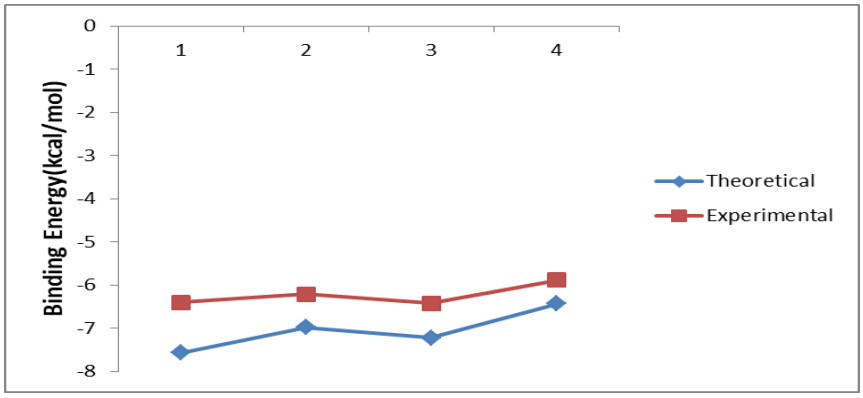

Figure 5. A similar trend of binding energy in theoretical and experimental cases.

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 6 represents the binding position of ligands with DNA. These figures clearly show the binding mode of the complexes. Figure 7 represents the detailed interaction of ligands with DNA residues.

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.

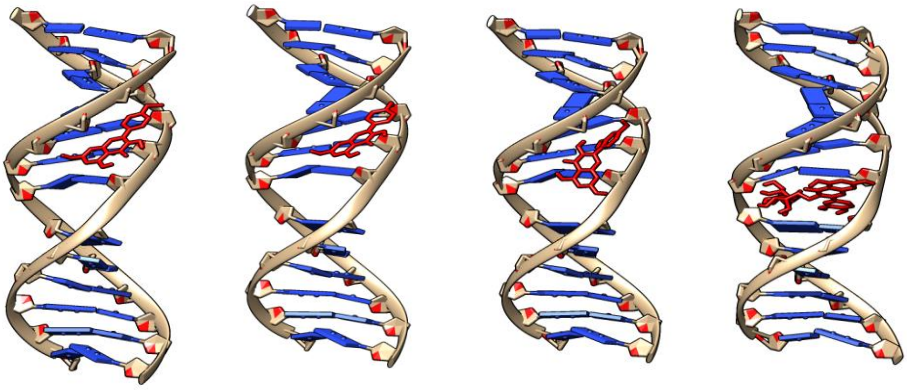

Figure 6. Binding sites of Flavonols with DNA (**a**) Kaempferide, (**b**) Kaempferol, (**c**) Morin, (**d**) Rutin.

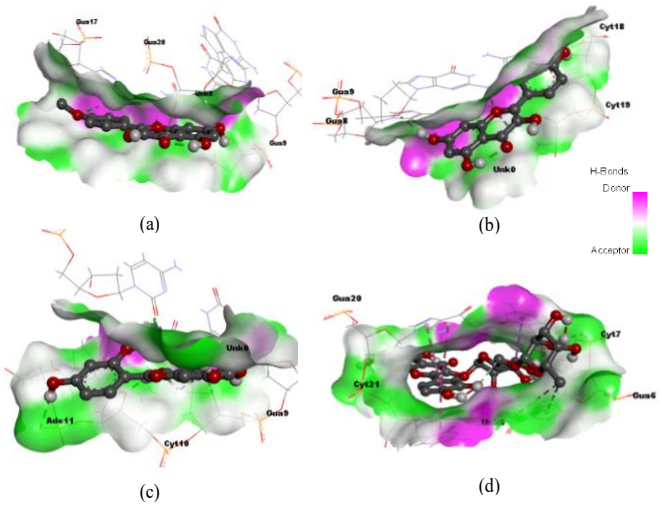

Figure 7. H-bond donor and acceptor regions in best docked posed complexes.

The most preferred binding mode obtained from molecular docking calculations was taken as initial structure 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 Figure 8. 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.

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

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

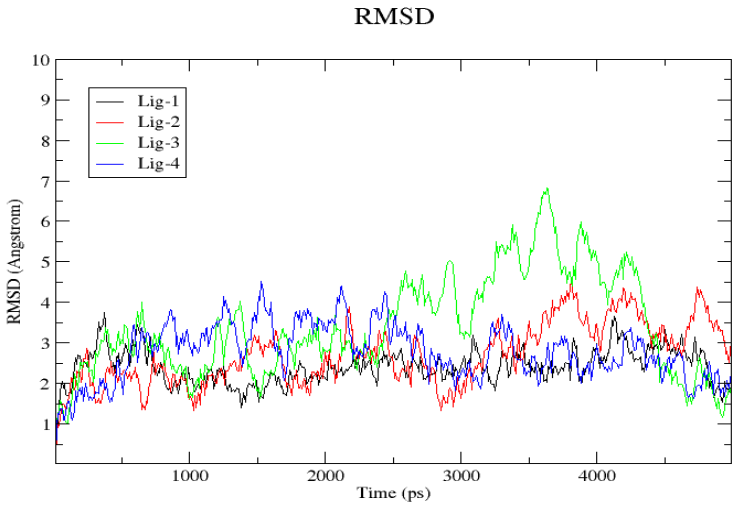

Figure 8. RMSD plot for drug-DNA complexes.

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.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.

Chapter 6: Investigation of the interaction of endocrine disruptive compound BPA and its analogs with DNA via Molecular Docking, Molecular Dynamics and Quantum Mechanical/Molecular Mechanical (QM/MM) calculations.

Bisphenol A is a compound which falls in the class of chemicals generally called as diphenylmethanes. Compounds of this class contain two benzene rings separated by one central carbon atom, usually with a para-hydroxy group on both benzene rings. It is an estrogenic EDC i.e., endocrine disrupting compound. BPA is called estrogenic EDC as it mimics estrogen. Being a xenoestrogen, it disrupts the hormonal balance in the human body. Previous studies have revealed diverse disadvantages of BPA and its halogen derivatives on plants growth and also on human body. Due to their tendency to affect photosynthesis, they are a risk for the development and growth of plants and because of endocrine disrupting activities they are a threat to human body also.

In order to comprehend the toxic effects or chemotherapeutic effects of small compounds, much attention has recently been paid to their binding interactions with DNA. Bisphenol A and its analogues' molecular interactions with serum albumin have been researched up to this point. As an illustration, Xie et al investigation of the interaction between BPA and human serum albumin revealed the presence of hydrophobic forces in the BPA–HSA interaction. DNA is the macromolecular target of many drugs. Interaction with DNA is a significant part of the study of a compound for being a useful drug against certain disease.

Present work is dedicated to the detailed study of interaction between DNA and BPA derivatives through computational tools. Five different BPA derivatives namely BPA, BPAF, BPS, TBBPA and TCBPA were selected as ligands for the study whereas 5 DNA sequences with PDB ID 1BNA, 1DSC, 1RMX, 2ROU and 195D were taken as macromolecular targets. Firstly, geometry optimization of the ligands was performed and then they were subjected to three computational

analysis methods, Molecular Docking, Molecular Dynamics and Quantum Mechanics/Molecular Mechanics (QM/MM) respectively.

The pdb format file of DNA sequences with PDB ID - 1BNA, 1DSC, 1RMX, 2ROU and 195D were sourced from RCSB 'Protein Data Bank' (PDB). The Gaussian 09 software tool was used to optimize the geometry of the ligands at the B3LYP/6-31G level. Molecuar Docking process between the ligands and DNA segments was performed using AUTODOCK4.2 software. Autodock tools with Lamarckian Genetic Algorithm (LGA) was implemented. In the present work, MD simulations were carried out using GROMACS 5.0.4 software. 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.

To further elaborate the study to a better extent, Quantum Mechanical/Molecular Mechanical (QM/MM) technique was adopted. It gives more clear and elaborated understanding of the system at molecular level. In this method, the main interacting portion of the structure is treated quantum mechanically whereas remaining whole macromolecular environment is treated molecular mechanically. QM/MM was performed on TBBPA-DNA complex system. After the process of molecular dynamics, interaction energies were calculated using two levels ONIOM scheme by employing B3LYP method. The geometry optimization was performed using the basis set 6-31G for the QM region and Amber force field for MM region.

Table 5: Binding energies (kcal/Mol) of ligands with different DNA sequences.

Drug	1BNA	1DSC	1RMX	2ROU	195D
BPA	-5.34	-5.10	-6.02	-5.33	-5.45
BPAF	-5.48	-4.52	-5.49	-5.35	-4.95
BPS	-5.25	-4.68	-5.87	-5.91	-5.68
TBBPA	**-7.53**	**-6.91**	**-6.16**	**-7.16**	**-8.12**
TCBPA	-7.06	-6.42	-5.72	-6.24	-7.18

Binding energies obtained from computational docking method for the DNA sequences is tabulated in Table 5.TBBPA-DNA complex has largest binding energy for each set of DNA sequence which means this complex is most tightly bonded and it is most stable. Table 6 gives the binding mode of ligand-DNA complex systems.

Table 6. Binding mode of ligand-DNA complex system. (MI-minor groove binding, MA-major groove binding, IN-intercalation).

Ligand	1BNA	1DSC	1RMX	2ROU	195D
BPA	MI	**MI**	MI	**MI**	MI
BPAF	**MI**	MI	**MI**	MI	**MI**
BPS	MI	MI	MI	MI	MI
TBBPA	MI	IN & MI	MA	IN	MI
TCBPA	MI	MI	MI	IN	MI

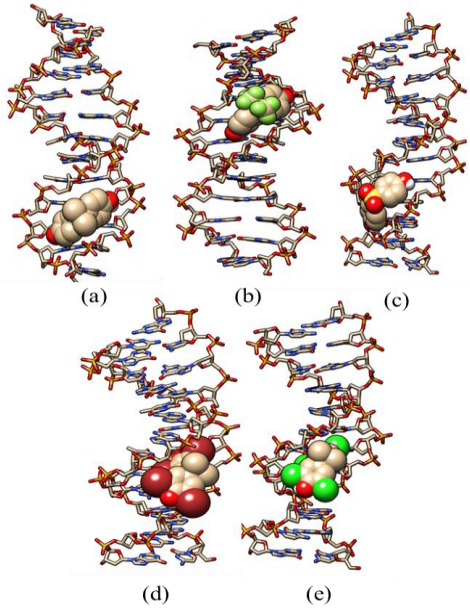

Figure 9. Best docked posed complexes for 195D (a) BPA, (b) BPAF, (c) BPS, (d) TBBPA, (e) TCBPA.

Figure 9 represents the docked poses for all the complex systems with 195D. They mainly show minor groove binding.

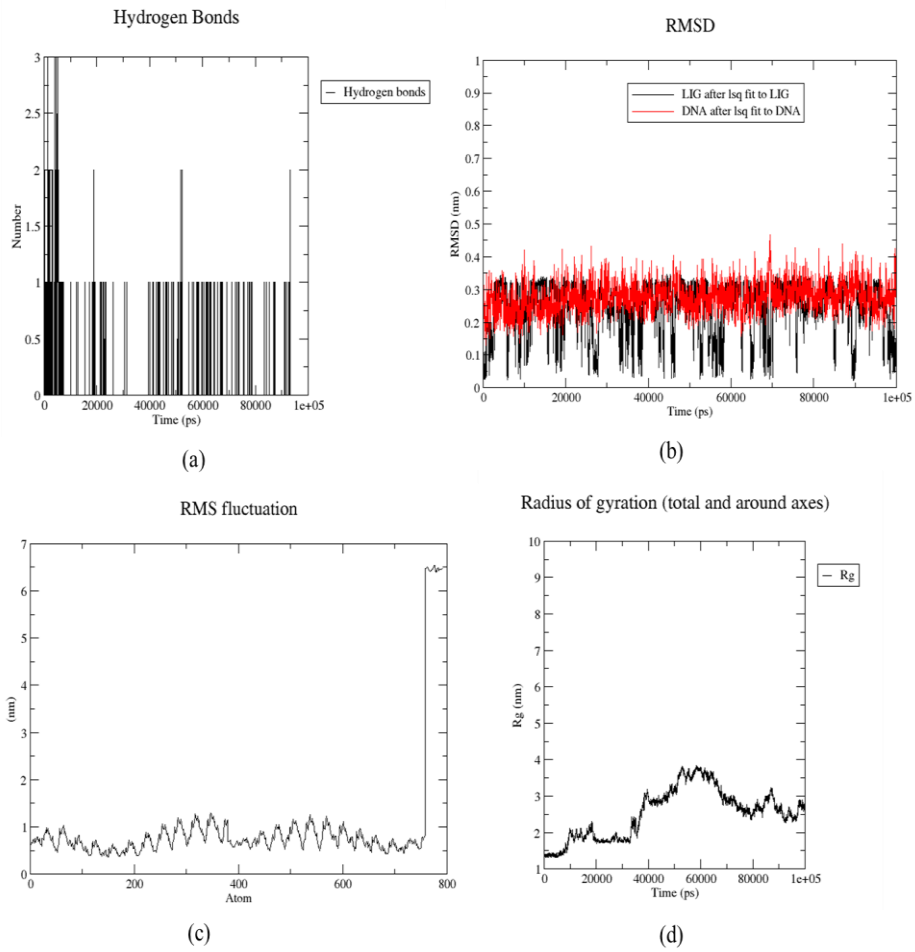

Figure 10. Trajectories for 1BNA-TBBPA complex (a) Variation in H-bond, (b) RMSD curve, (c) RMSF curve, (d) Variation in radius of gyration.

As BPS and TBBPA are mostly used as an alternative to BPA, further study was focused on these two compounds. Molecular Dynamics simulations of 100ns was performed for two systems, BPS+1BNA and TBBPA+1BNA for the time dependent study of stability of the system. Best poses of the system obtained from molecular docking having least energy were taken as the input for molecular dynamics simulations. After analysing trajectories obtained from MD simulations, it was confirmed that the BPS+1BNA complex loses its stability after a few nanoseconds whereas TBBPA+1BNA sustain stability during the whole time of 100ns. The results from MD simulations were analysed in the form of trajectories. Figure 10 gives the trajectories for 1BNA-TBBPA complex.

For the complex i.e., 1BNA+TBBPA, the interaction energy between drug & DNA was obtained by the help of previously discussed ONIOM method. B3LYP hybrid functional was utilized for the calculations. For the quantum mechanical (QM) region (high layer) the geometry optimization was performed using the basis set 6-31G basis set whereas amber force field was applied for molecular mechanical (MM) region (low layer). Different snapshots were taken at the difference of 2ns by the help of VMD software and were taken as the initial structure for ONIOM calculations.

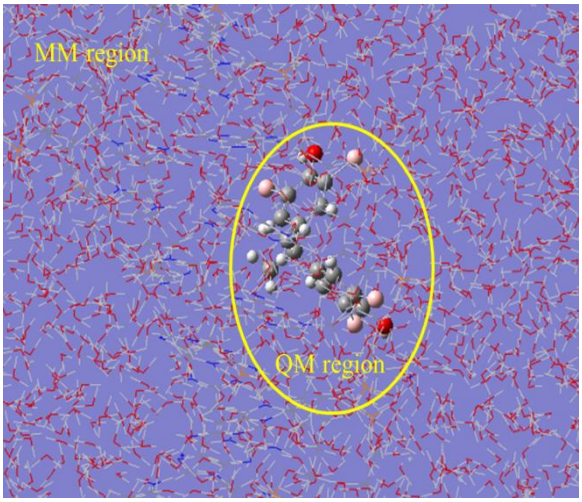

Figure 11. Snapshot of drug-DNA complex for QM/MM calculation.

The optimized geometry of complex having the least interaction energy is shown in Figure 11. The calculated interaction energies from QM/MM calculation using ONIOM scheme are mentioned below in Table 7**.**

Table 7. Obtained energies through QM/MM calculations.

S.No.	Time Scale	$E_{QM\ (gp)}$ (AU)	$E_{QM\ (pp)}$ (AU)	ΔE_{IE} (AU)	ΔE_{IE} (kcal/mol)
1.	0ns	-827.957989031	-827.957388180	-0.000600851	-0.37704011
2.	2ns	-827.957988999	-827.957666976	-0.000322023	-0.20207265
3.	4ns	-827.957816791	-827.957792811	-0.000023980	-0.01503905

It was observed that the interaction energy of DNA-Ligand complexes does not only depend on the chemical structure of ligand but also depends on the DNA sequence and its specificity.

Results confirmed that TBBPA binds with DNA with maximum binding energy; it means that the TBBPA-DNA complex formed is most stable amongst all the complexes of BPA derivatives with different types of DNA. The larger size of TBBPA may be the reason for the high binding affinity between the ligand and DNA. TCBPA-DNA complex system has binding energy next to TBBPA-DNA system. Hydrogen bond analysis and hydrophobic bond analysis of each system provides more details about the bonding in each complex. This study concluded that as TBBPA interacts with DNA strongly as compare to BPA and other derivatives, this compound may be as hazardous as BPA for the human body. As there are very few studies regarding this subject, current chapter provides a base for more explicit and elaborated investigation of this compound theoretically and experimentally.

Chapter 7: Conclusion and future scope

The present thesis highlights following points as general conclusions:

- The docking outcomes were satisfactorily explained by a number of analyses, and they were confirmed to have a good correlation with the findings reported in the literature.
- A portion of research in the thesis was dedicated to the interaction between DNA and natural antioxidants flavonoids. It can be concluded that various type of flavonoids interact with DNA as minor groove binders and intercalators. The mode of binding depends upon the shape and size of DNA as well as small molecules i.e., flavonoids. Further research can give more details about the anticancer properties of flavonoids.
- By assessing various parameters, including variations in energy, variations in radii of gyrations, variations in the number of hydrogen bonds, RMSD and RMSF, it was possible to critically analyse the system's dynamical behaviour

and gain detailed information about the stability of the drug-DNA complex with time.

- By including electrostatic effects, the free energy calculations increased the computational rigour of the calculations and their efficiency led to more detailed results, including energy contributions for each components (van der Waals energy, electrostatic energy, polar solvation energy, binding energy, and SASA energy), as well as per-residue contributions, which allowed for a critical analysis of the results.
- Due to the inclusion of DFT, QM/MM calculations yield significantly superior results than those of other molecular modelling techniques like molecular docking and molecular dynamics.
- In the future, further investigations on the systems analysed in this thesis may be done using computational and experimental tools. The information of accurate biomolecular interactions is the prerequisite for structure based rational and effective therapeutic drug design. So it will provide more elaborated details for the drug designing of new and potent drugs against fatal diseases.