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

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Submitted by

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2022

THIS THESIS IS DEDICATED

TO

MY FAMILY

DECLARATION

I declare that the thesis entitled **"Quantum Mechanical/Molecular Mechanical (QM/MM) studies of DNA Binding Drugs"** has been prepared by me under the supervision of **Prof. Devesh Kumar**, Department of Applied Physics, School for Physical Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow. No part of this thesis has formed the basis for the award of any degree, diploma or fellowship previously. Further, I declare that the material embodied in the present work is based on original research work and the indebtedness to others has been duly acknowledged at relevant places. This is also declared that the thesis is essentially free from all kinds of plagiarism.

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CERTIFICATE

This is to certify that the thesis titled **"Quantum Mechanical/Molecular Mechanical (QM/MM) studies of DNA Binding Drugs"** submitted by **Ms. Anamika Shukla** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university or institutions. The thesis submitted to the Babasaheb Bhimrao Ambedkar University, Lucknow, satisfies all the requirements as stipulated in the ***Doctor of Philosophy (Ph.D.) regulations -1999 as amended in 2013*** and it is fit for submission and evaluation for the award of Doctor of Philosophy of the University.

Date: 15/07/2022

Supervisor

Head of the Department

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Anamika Shukla

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ABSTRACT

DNA is very important for heredity, protein coding and supplying guidelines for life and its activities in all living organisms. DNA controls a person's or an animal's growth, reproduction and final demise. The investigation of DNA interactions with various drugs is one of the most important areas of study in the domain of drug development and the pharmaceutical sector. The research towards using DNA as a target molecule for the study of anticancer, antimicrobial, antibacterial, and several other types of diseases is constantly expanding. These studies offer information that can be used to find more effective anticancer drugs. It is an even more popular target for researchers due to its binding specificity, thermodynamic stability, and limitless options for the selectivity of the DNA sequence towards a wide variety of drugs.

The basic structure of DNA is comprised of two polynucleotide chains coiled around each other in helical pattern. The backbone of DNA basically consists of sugar and phosphate residues which are arranged in alternating sequence. The bases are projected inwards and the spaces between the bases create the minor and the major groove of the DNA. DNA interacts with small molecules like drugs via two modes: covalent and non- covalent interactions. Non-covalent interactions have mainly three modes which are electrostatic interactions, groove binding and intercalative binding. Out of these modes of binding, groove binding can be classified as major and minor groove binding. In present study of drugs with DNA, intercalative and groove binding are of prime focus.

The research presented in this thesis is concerned with drug-DNA interactions, their many kinds, and the use of sophisticated computational approaches to examine these interactions and give underlying reasons for the binding of compounds of potential pharmacological relevance. In this research, the molecular interactions between various DNA binding compounds have been explained utilizing benchmarked computational approaches as molecular docking, molecular dynamics simulation, free energy calculations, and QM/MM calculations etc. Molecular docking is a probabilistic strategy to determining the potential drug binding location near a biological macromolecule. Molecular dynamics ensures the stability of drug-DNA complexes throughout time. Free energy calculations provide important details on the

molecular forces that govern and control the key factors responsible for stable complex formation. Quantum mechanical/molecular mechanical (QM/MM) approach is used for the modelling of reaction mechanism by computationally mimicking the biomolecular systems which are not well understood so far.

It is very important to acquire elaborated aspect of the drug-DNA complex system, to gain knowledge about the kinematics and mechanism of the binding between the two and to measure the energetics involved in these interactions. Even after so many years of investigation in this field, there are new and potent drugs still coming in light giving scope for study of novel interactions.

This research supports the idea that several non-covalent interactions are used by the minor groove binders to bind to DNA. Intercalators stack in between the bases of DNA mostly by hydrophobic bonding, whereas DNA minor groove binders establish hydrogen bonds between the drug and DNA bases. The recognition of DNA by drug molecules is based not only on base sequences, but also on sequence dependent conformation or DNA alterations and distortions. Through the use of sophisticated computational approaches, this work gives a thorough insight of drug-DNA interactions, sequence selectivity and sequence specificity.

PREFACE

The thesis entitled **"Quantum Mechanical/Molecular Mechanical (QM/MM) studies of DNA Binding Drugs"** encapsulate the results of theoretical investigations carried out in the Department of Applied Physics, Babasaheb Bhimrao Ambedkar University in between 2017-2022 under the supervision of Prof. (Dr.) Devesh Kumar, Applied Physics, Babasaheb Bhimrao Ambedkar University, Lucknow.

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.

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. Studies have proved 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 synthesized 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.

It is essential to investigate these biological areas of interest using benchmarked computational methods. Studies using computational modelling have a significant deal of value in this sector than use of experimental settings. This thesis will focus on the interaction of some common drugs like flavonoids, BPA derivatives etc. with DNA. Computational chemical techniques such as Molecular Docking, Molecular Dynamics (MD) and QM/MM will be used for predicting binding of these drugs to DNA and stability of bonded drugs respectively. A thorough study of these interactions would provide theoretical techniques to complement experimental techniques and will also be helpful in the design of new drugs.

The thesis is divided into seven chapters.

Chapter 1 describes the structure of DNA, various types of interactions between drugs and DNA, and the various methodologies used to analyze drug-DNA interactions. The previous research in this topic has been reviewed in this chapter as well.

Chapter 2 discusses the different molecular modelling techniques that are used in this thesis, giving a brief idea of the theoretical protocol being followed in their development and implementation.

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.

Chapter 4 discusses the interaction of natural antioxidant flavonols with DNA via molecular docking computational tool.

Chapter 5 gives the computational investigations on interactions between DNA and flavonols. Molecular docking, molecular dynamics and free energy calculations were performed to provide elaborative descriptions for the present interactions.

Chapter 7 uses elaborate and advanced computational technique like, QM/MM to study interactions between endocrine disruptive compound BPA and its analogs with DNA and thus explains the role of interactions with great sophistications.

The general conclusions of the thesis are drawn in **Chapter 7**.

Hopefully, a greater understanding of the interactions that occur between small molecules and DNA will allow for more efficient and successful computational drug design efforts in the future.

LIST OF ABBREVIATION

S.No.	Abbrv.	Full form
1.	A	Adenine
2.	AMBER	Assisted Model Building with Energy Refinement
3.	BPA	Bisphenol A
4.	BPAF	Bisphenol AF
5.	BPF	Bisphenol F
6.	BPS	Bisphenol S
7.	C	Cytosine
8.	CD	Circular Dichroism
9.	CHARMM	Chemistry at HARward Macromolecular Mechanics
10.	CHD	Cyclohexadiene
11.	DFT	Density Functional Theory
12.	DNA	De-oxy ribonucleic acid
13.	EDC	Endocrine Disrupting Compound
14.	G	Guanine
15.	GAFF	Generalized Amber Force Field
16.	GLIDE	Grid-based Ligand Docking with Energetics
17.	GOLD	Genetic Optimization for Ligand Docking
18.	GTOs	Gaussian Type Orbitals
19.	HF	Hartree-Fock
20.	IR	Infrared
21.	LGA	Lamarckian Genetic Algorithm
22.	MC	Monte Carlo
23.	MD	Molecular Dynamics
24.	MM	Molecular Mechanics
25.	MS	Mass Spectrometry
26.	MMGBSA	Molecular Mechanics Generalized-Born Surface Area
27.	MMPBSA	Molecular Mechanics Poisson-Boltzmann Surface Area
28.	NBD	Nucleic acid Data Bank

29.	NMR	Nuclear Magnetic Resonance
30.	PBC	Periodic Boundary Conditions
31.	PDB	Protein Data Bank
32.	PDBQT	Protein Data Bank, Partial Charge (Q), & Atom Type (T)
33.	PME	Fourier Transform-Ion Cyclotron Resonance
34.	QM	Quantum Mechanics
35.	QM/MM	Quantum Mechanics/Molecular Mechanics
36.	RMSD	Root Mean Square Deviation
37.	RMSF	Root Mean Square Fluctuations
38.	RNA	Ribonucleic Acid
39.	SCF	Self Consistent Field
40.	STOs	Slater Type Orbitals
41.	T	Thymine
42.	TBBPA	Tetrabromobisphenol A
43.	TCBPA	Tetrachlorobisphenol A
44.	U	Uracil
45.	VS	Virtual Screening

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CHAPTER-1

INTRODUCTION

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 use 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 [1-4].

The information of accurate biomolecular interactions is the prerequisite for structure based rational and effective therapeutic drug design. In particular, modelling studies on DNA, RNA, enzymes, proteins, lipids, etc. is certainly a huge interdisciplinary enterprise including biology, chemistry, physics, computer science and even mathematics [5-7].

Now both, academic research groups and pharmaceutical companies are using various virtual screening techniques for reducing the time and cost essential for the discovery of a novel and potent drug. Although there are various advances going rapidly in these tools, still continuous enhancements are crucial for upcoming drug discovery tools in future. Structure-based and ligand-based drug design raises the possibility that these approaches' complementary usage and combination with experimental techniques have a significant influence on rational drug design [8-11]. To upgrade and modify presently used in silico tools and techniques, continuous growth in the area of chemical and structural biology, bioinformatics, and computational technology is inevitable. This will alleviate various big and small limitations of virtual drug discovery techniques and the maximum utilization of computational drug design can be achieved [12].

In this regard, drug-DNA interaction is also very important. The binding of small molecules to DNA is medically very important [13, 14]. Studies have proved 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 [15, 16]. Some DNA binder molecules are being used and new ones are designed and synthesised for the betterment of clinical efficiency [17]. To target DNA for the regulation of cell function via interfering with replication process sounds logical, intuitively appealing and conceptually uncomplicated [18].

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 [19, 20].

In 1953, Watson and Crick gave the basic structure of DNA [21]. 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 fibre axis. These bases are adenine, thymine, guanine and cytosine. Adenine pairs up with thymine while guanine pairs with cytosine in the case of DNA [22].

Proteins also interact with DNA. Jonathan B. Chaires reported in 2006 that there are thermodynamics differences between the binding of protein with DNA and small molecules with DNA. Proteins that strongly distort DNA displayed net unfavourable enthalpy. Contrary to that, intercalators which distort DNA show net favourable enthalpy values [17].

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 [23-25]. 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.

1.1 Nucleic Acids

Nucleic acids are the major elements of all living creatures' cells. They are called nucleic acids because of their major occurrence and acidic character. A nucleic acid's unit cell is termed as nucleotide which means nucleic acids are made up of continuous polymeric chains of nucleotides [26]. DNA and RNA are two types of nucleic acids that store lengthy thread-like macromolecular structures and are involved in a variety of genetic processes. DNA makes up the majority of chromosomes and is found in minor amounts in chloroplast and mitochondria,

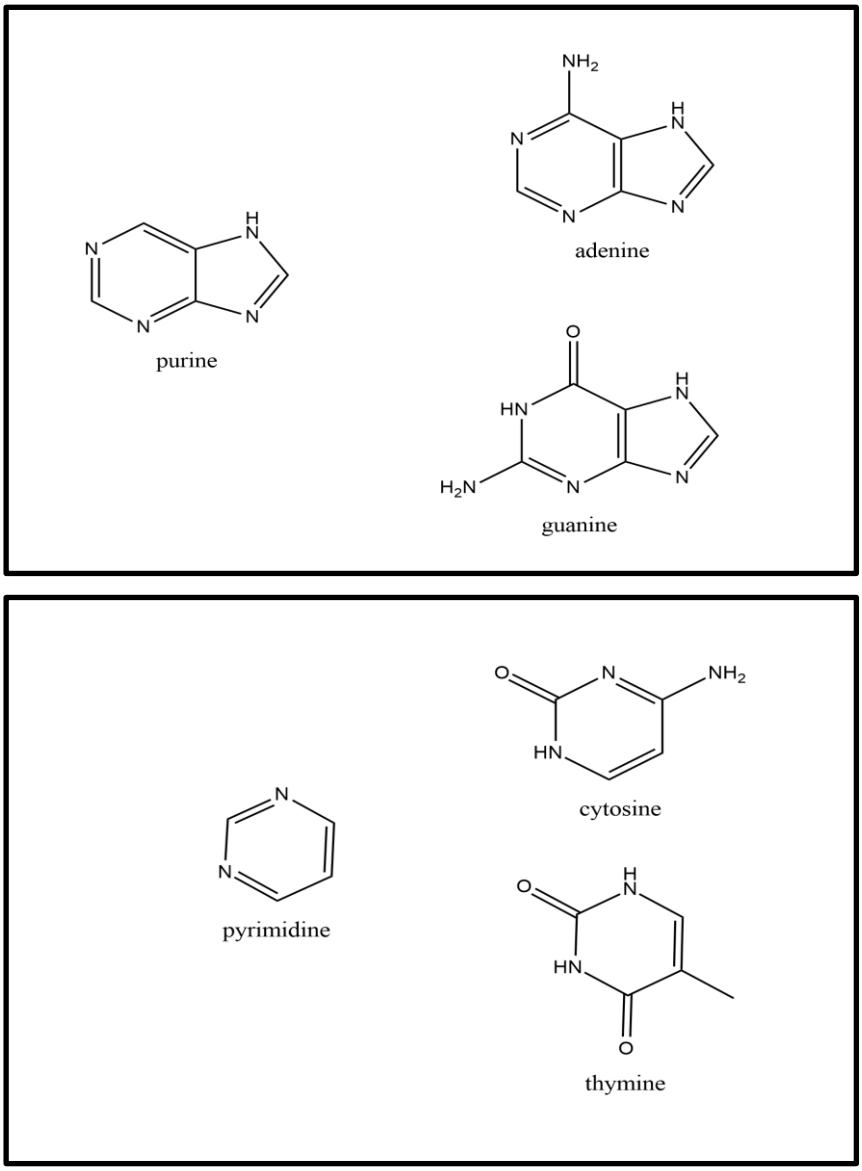

Figure 1.1. Purines and Pyrimidines.

whereas RNA is predominantly found in plant viruses and cytoplasm [27, 28].

A sugar-phosphate poly-deoxy/oxy ribonucleotide double helix entangled strand makes up each nucleic acid. Nucleic acids are primarily comprised of nitrogenous bases, phosphate groups, sugars and nucleotides which are described as follows:

1.1.1 Nitrogenous bases

The bases present in DNA are organic rings containing nitrogen, called nitrogenous bases. Purines and pyrimidines are the two types of bases found in DNA. Adenine and guanine are purines, while cytosine and thymine are pyrimidines. Purines are made up of double-ringed structures, as depicted in Figure 1.1. This basic structure is shared by adenine and guanine, although with different groups attached. Cytosine and thymine are also variations on the single-ringed structure depicted in Figure 1.1. In RNA, a different base called uracil (U) replaces thymine (T) [22].

1.1.2 Phosphates

D-electrons make up the phosphate group. A phosphorous atom is bonded to four oxygen atoms in a phosphate group by sp^3 hybridised tetrahedral connections. A phosphate group is depicted in Figure 1.2 below.

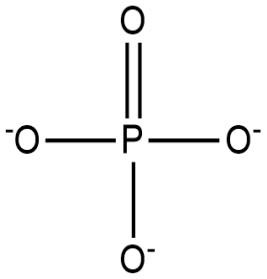

Figure 1.2. A sp3 hybridised phosphate group.

1.1.3 Sugar

Ribose and deoxyribose sugars are the sugar components of nucleic acids. These sugars are pentoaldoses which have the shape of furanose rings. Sugars are found in both DNA and RNA; however, they are made up of distinct sugar units.

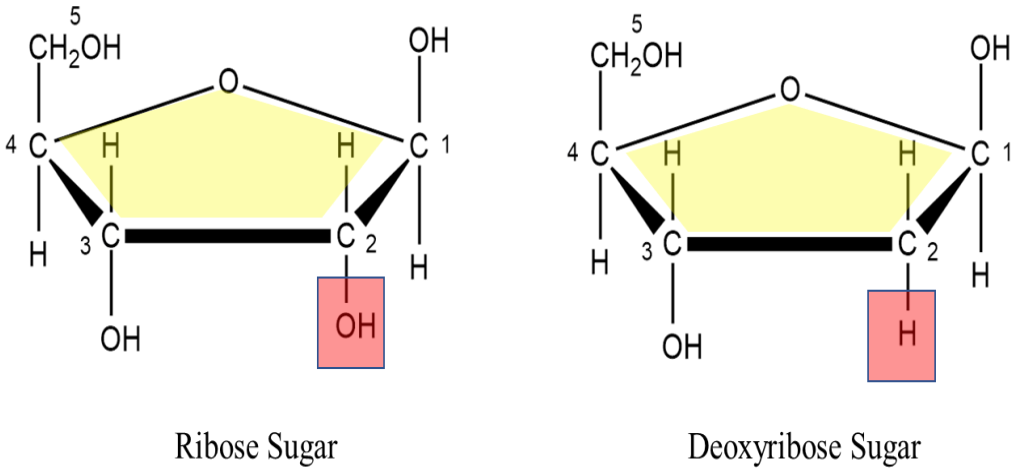

Figure 1.3. Ribose sugar and Deoxyribose sugar.

Deoxyribose sugar makes up DNA, whereas ribose sugar makes up RNA. The presence of a hydroxyl (–OH) group on the second carbon atom distinguishes these sugars. Because there is no hydroxyl at position 2, the sugar is called 2-deoxyribose (just two hydrogens). Both deoxyribose and ribose sugar are depicted in Figure 1.3.

1.1.4 Nucleoside and Nucleotide

A nucleoside is a deoxyribose or ribose sugar linked to a purine or pyrimidine.

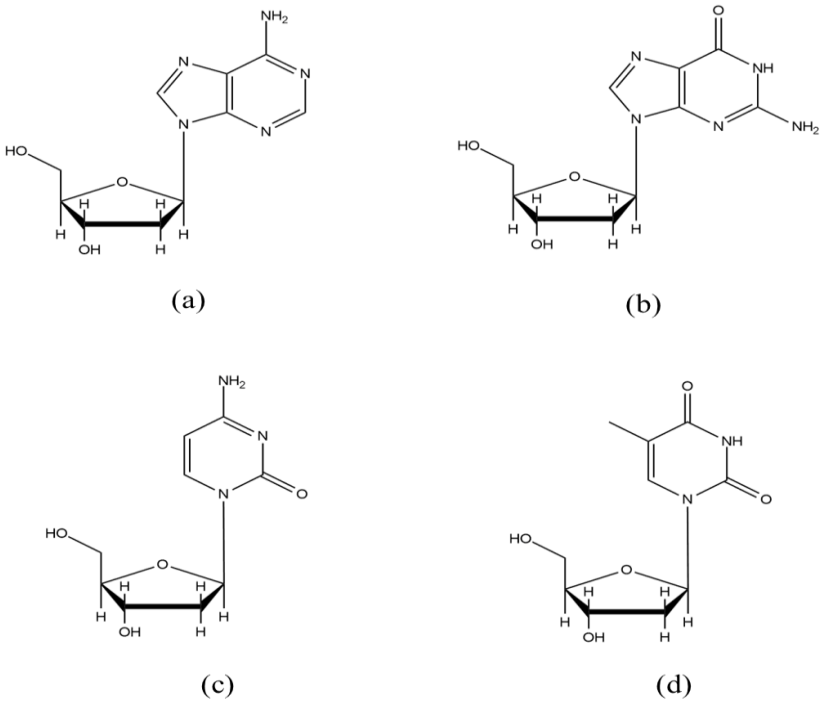

Figure 1.4. Nucleoside of deoxyribose with (a) Adenine, (b) Guanine, (c) Cytosine, (d) Thymine.

Adenosine is adenine nucleoside coupled to a ribose or deoxyribose, and guanosine is adenine nucleoside linked to a ribose or deoxyribose. Uridine, cytidine and thymidine are the three pyrimidine nucleosides. Similarly, oxyribose sugar forms nucleosides and is known as adenosine monophosphate (AMP), as well as GMP, TMP, CMP and UMP. Nucleotides are phosphate esters of nucleosides that are highly acidic. The main building block of DNA is nucleotide. The structure of nucleosides and nucleotides are shown in Figure 1.4 and Figure 1.5.

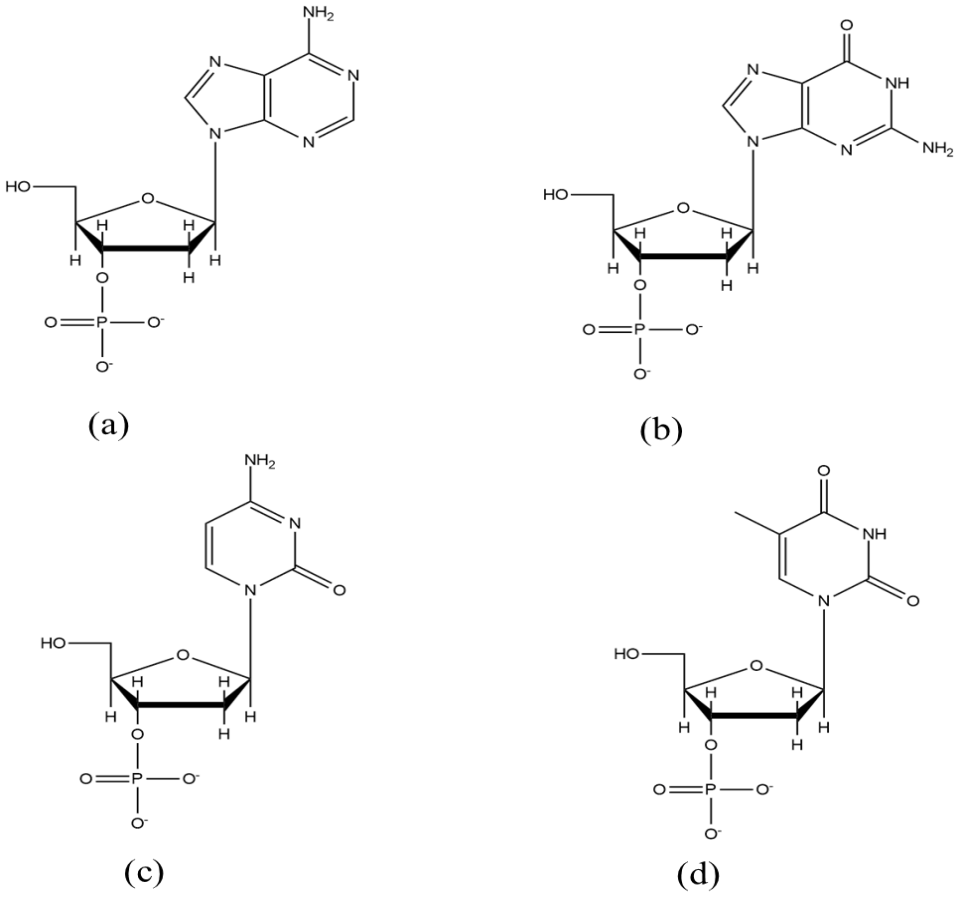

Figure 1.5. Nucleotides of deoxyribose with (a) Adenine, (b) Guanine, (c) Cytosine, (d) Thymine.

1.2 DNA

DNA is the basic building block of all the organisms including human beings. Providing instructions for life and its functions in all living species, DNA is essential for heredity, protein coding and ensuring genetic continuity. The development, reproduction, and death of an individual or an animal are all governed by DNA. It is

also target of many internal or external molecules. These molecules can be internal like RNA, proteins or external molecules like antibiotic, antioxidants drugs etc. Transcription and replication are the primarily important functions of DNA [29].

1.2.1 History of DNA

Although Friedrich Meischer made the initial discovery of DNA in 1868, its significance for heredity was not understood until 1944, when Avery and colleagues published their renowned finding that DNA, not proteins, was the primary genetic information carrier [30-31]. In 1950, Chargaff developed the complimentary base-pair rule [32]. The double helix structure of DNA, which Watson and Crick predicted in 1953 based on findings from X-ray fibre diffraction, was a significant development in the field [21]. The production of DNA complementary strands from parent strands made it instantly clear how information might be passed from one generation to the next. Together with Maurice Wilkins, they shared the 1962 Nobel Prize in Chemistry for their discovery.

However, it wasn't until the late 1970s, when synthetic DNA fragments were made commercially available, that DNA research really took off. In 1979, the left-handed d(CGCGCG) structure, commonly known as Z-DNA, was described as the first atomic resolution structure of a single crystal oligonucleotide. Dickerson and colleagues figured out the first B-form DNA structure a year later [22, 33].

1.2.2 DNA structure

Watson and crick were the first to give the structure of DNA. The basic structure of DNA is comprised of two polynucleotide chains coiled around each other in helical pattern. Since two polynucleotide chains are twisted about each other, this structure is called double helical structure. The bases of each strand of the helix are pushed inwards, while the backbone is made up of alternating sugar and phosphate residues. The two chains of DNA are complementary to each other. Figure 1.6 shows the double helical DNA structure. If a section of DNA is rotated by 180º, the resultant segment is identical to the later one. Although the two strands' helical shape is the identical, base pairing, which has the opposite polarity, keeps them together [21].

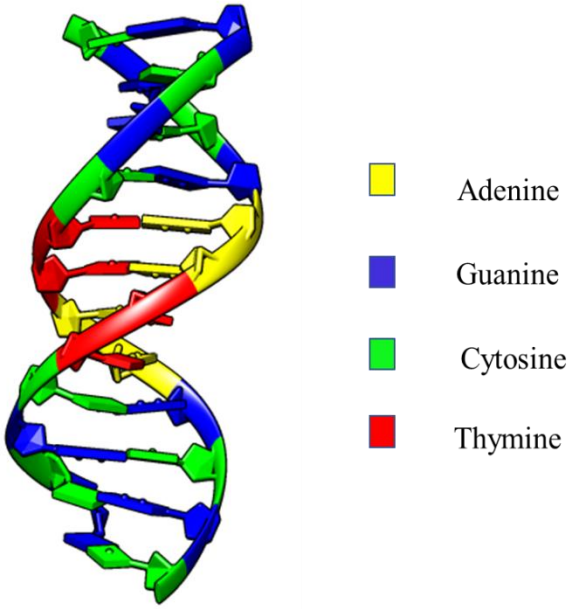

Figure 1.6. Double helical DNA structure.

The whole DNA segment is made up of two parts. One is the backbone of the DNA and the other part is the bases which are attached to the backbone of the DNA. The backbone of DNA basically consists of sugar and phosphate residues which are arranged in alternating sequence [22].

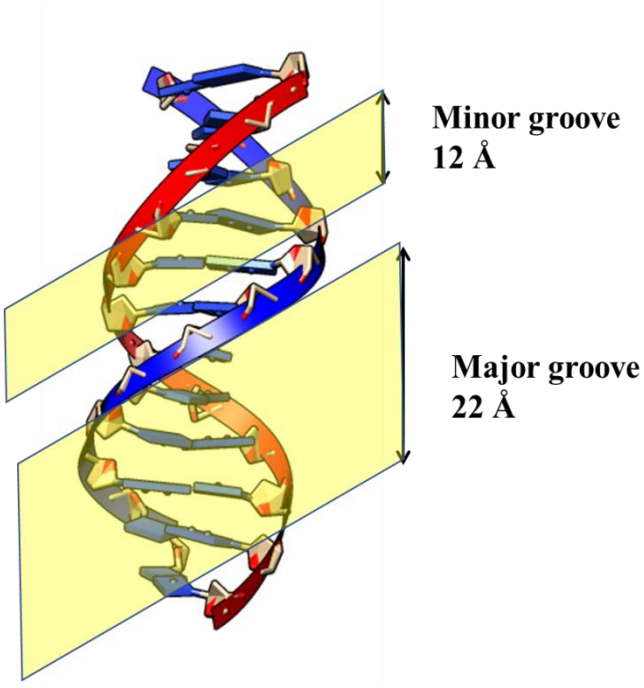

Figure 1.7. Minor groove and Major groove in DNA.

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. Figure 1.7 represents the minor groove and major groove in DNA. The minor and major grooves allow access to the bases.

Weak hydrogen bonds in between bases on the complementary backbone of the DNA hold the two helical strands together. The non- covalent bonds between the bases are accountable for the stable helical structure of the DNA. Out of the four bases Adenine, Guanine, Cytosine and Thymine, Adenine on one backbone always pairs up with Thymine attached to the other stand, whereas Guanine and Cytosine of the two chains form hydrogen bonds with each other. Adenine and thymine form 2 hydrogen bonds whereas Guanine and Thymine are attached by 3 hydrogen bonds as shown in Figure 1.8.

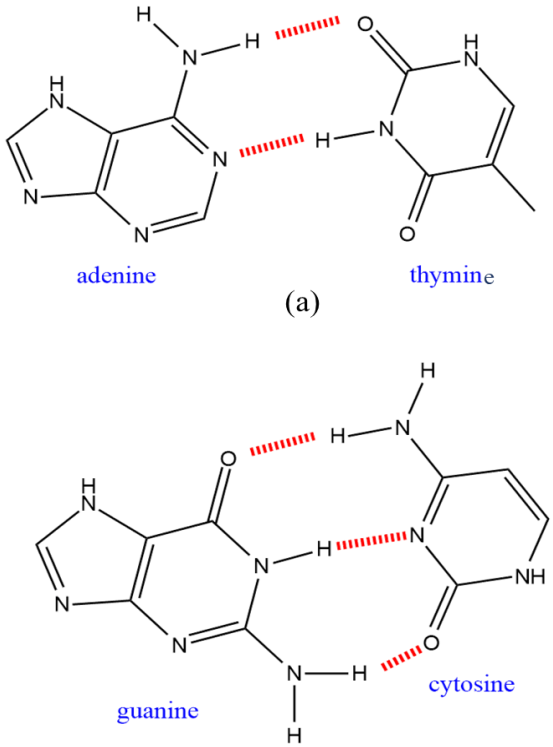

Figure 1.8. Hyrogen bonding between bases (a) Adenine and Thymine (b) Guanine and cytosine.

1.3 Common forms of DNA

Based on the structure, shape and size, DNA is classified into different forms which are B-type, A-type and Z-type. These are the forms of DNA which are most commonly found in nature. Transitions between these forms of DNA can be produced by salt concentration and solvent [33].

Figure 1.9 gives the diagram of A, B and Z form of DNA.

1.3.1 B-DNA

The most prevalent and predominant kind of structural conformation of DNA is B-DNA. It is formed under the natural physiological conditions in the cell. The rotation sense of B-DNA is right-handed. The helical diameter is 20Å. Each pair of base in B-DNA has the same width. It contains 10 pairs of bases per turn. It contains major grooves that are broad and deep, as well as minor grooves that are narrow and deep.

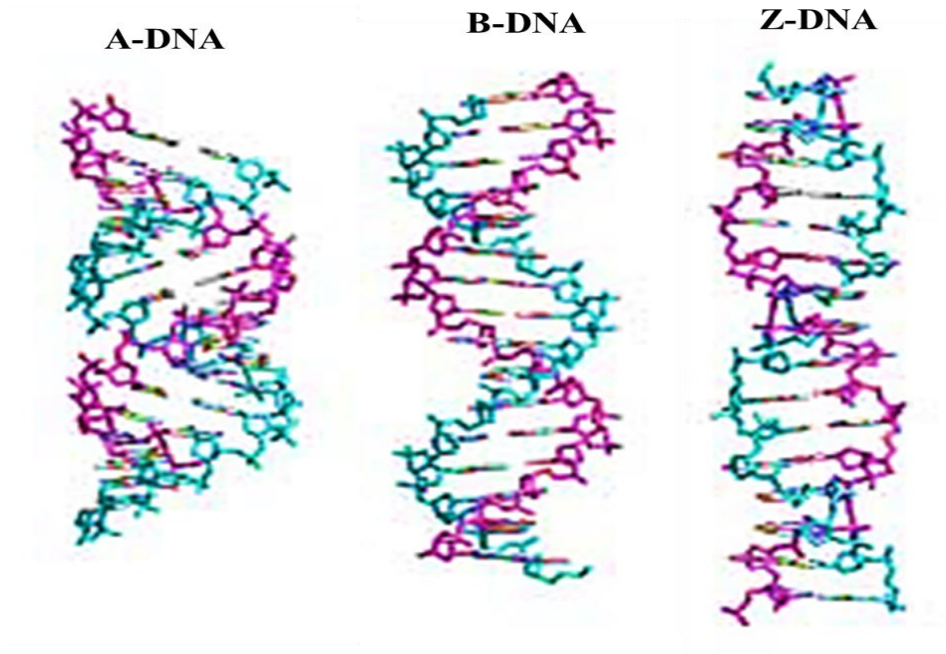

Figure 1.9. A, B and Z form of DNA.

Table 1.1. Properties of B-, A- and Z- forms of DNA.

Structural properties	Helix Type		
	B	**A**	**Z**
Overall portions	Longer and thinner	Short and broad	Elongated and slim
Rise per base pair	3.3 Å	2.3 Å	3.8 Å
Helix-packing diameter	23.7 Å	25.5 Å	18.4 Å
Helix rotation sense	Right-handed	Right-handed	Left-handed
Base pair per helix repeats	1	1	1
Base pair per turn of helix	10	11	12
Mean rotation per base pair	35.9º	33.6º	-30º
Pitch per turn of the helix axis	3.3 Å	3.3 Å	3.3 Å
Tilt of base normal to helix	-1.2º	+19º	-9º
Base pair mean propeller twist	+16º	+18º	0º
Helix axis location	Through base pair	Major Groove	Minor Groove

1.3.2 A-DNA

A-DNA: A-DNA is a double stranded helical structure almost similar to B-DNA but with a shorter and more compact structural organization. A-DNA is formed from B-DNA under dehydrating condition. It is a right handed helix. A-DNA is a rare type of structural conformation. Rosalind Franklin discovered A- DNA. A- DNA is formed from B- DNA in the state of dehydration. The rotation sense of A-DNA is right-handed. The helical diameter is 26 Å. It contains 11 base pairs per turn. It contains major grooves that are narrow and deep, as well as minor grooves that are wide and shallow.

1.3.3 Z-DNA

Z-DNA is a left-handed double helical conformation in which the double helix winds to the left in a zig-zag pattern. Wang and Rich have discovered Z-DNA. The helix

diameter is 18 Å. Each helix turn contains 12 nucleotides. It has somewhat flat major groove and a deep and narrow minor groove. It is among the biologically active DNA forms. It is frequently found upstream of a gene's start point. The rotation sense of Z-DNA is left-handed. The structural properties of B-, A- and Z- forms of DNA are compared in Table 1.1.

1.4 Functions of DNA

The two main functions of DNA are transcription and replication. 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 [34-37]

1.4.1 Transcription

In the process of transcription, ribonucleic acid, RNA retrieves information from DNA and utilizes it for the synthesis of proteins inside the body. These proteins participate in almost all body operations and play major role in the functioning of the body. For example, proteins act as hormones, carriers, enzymes, regulators, receptors etc. [38].

Only when DNA detects a signal, such as a regulatory protein attaching to a specific area of the DNA, it begin transcribing or replicating. As a result, if a tiny molecule can mimic the binding specificity and strength of this regulatory protein, DNA activity can be artificially regulated, blocked, or triggered by binding this molecule instead of the protein. As a result, when activation or inhibition of DNA activity is necessary to cure or control a disease, this synthetic/natural small molecule can operate as a drug [39].

1.4.2 Replication

To assure that each new cell obtains the appropriate amount of chromosomes, DNA, which is contained within the nucleus, must be reproduced. DNA replication is the process of duplicating DNA. Replication is carried out through a series of processes involving RNA and several proteins referred to as replication enzymes. For cell development, repair and reproduction in organisms, DNA replication is essential. A

single double-stranded DNA molecule may produce many identical DNA helices through the process of DNA replication. A strand from the original molecule plus a freshly generated strand make up each molecule as shown in Figure 1.10. The DNA uncoils and separates before replication. A replication template called a replication fork is created. DNA polymerases add new nucleotide sequences in the 5′ to 3′ orientation once DNA primers attach to the DNA [40, 41].

DNA starts the process of transcription or replication only after detecting a signal. This signal is in the form of a protein which binds to a particular portion of DNA. If it

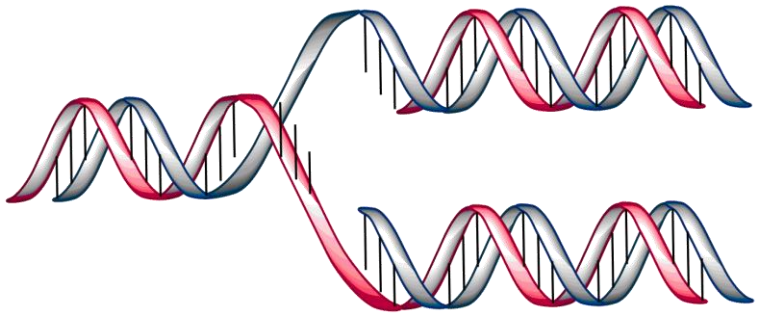

Figure 1.10. Figure representing DNA replication.

is possible to mimic the binding specificity of the protein by the help of a small molecule, then the functions of DNA can be artificially activated or modified because of the binding of small molecule to DNA. Thus, this small molecule can behave as a drug against a certain disease by altering, activating or inhibiting the function of DNA.

If the action of drug binding with DNA activates the DNA functions, it results in increment in the process of transcription or replication. Alternatively, if the binding inhibits the DNA to perform its functions, it will decrease the processes of transcription and replication, eventually leading to less cell growth or could induce cell death. Mostly the drugs are targeted to DNA for the inhibitory mode, to reduce cell growth and induce cell death for antitumor and antibiotic action [42, 43].

1.5 Drug-DNA interaction

1.5.1 Importance of drug-DNA interaction

The binding of small molecules to DNA is very important regarding the treatment of different types of cancer. The study of drug-DNA interaction has been crucial for

cancer chemotherapy for the last 30 years. The binding of drug to DNA can inhibit its fundamental functions which are replication, transcription and topoisomerase activity [44, 17]. This ability of DNA-binders make them special as they can prove to be potent inhibitors of disesases like cancer, tumor and they can be effective antiviral or antibiotic drugs. Because of these properties and functions, drug- DNA studies are most important for the well-being of millions of people. Researchers find it more efficient and effective to design drugs which have capability to bind at the DNA level rather than protein binding. Cis-platin is a platinum-based drug which has been used since years in the treatment of different types of cancer. It performs its action by binding with DNA causing the change in its length and shape thereby hindering replication and transcription [45, 46]. There is an interrelationship between the biological action of the drug and its binding properties with DNA [47, 48]. This relationship was established in mid-1950s by Peacocke AR et al [49].

It is very important to acquire elaborated aspect of the drug-DNA complex system, to gain knowledge about the kinematics and mechanism of the binding between the two and to measure the energetics involved in these interactions. Even after so many years of investigation in this field, there are new and potent drugs still coming in light giving novel interactions [50-52].

1.5.2 Types of bonding in drug-DNA interaction

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 [53, 54]. 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. Figure 1.11 gives the classification of various modes of binding of Drug-DNA.

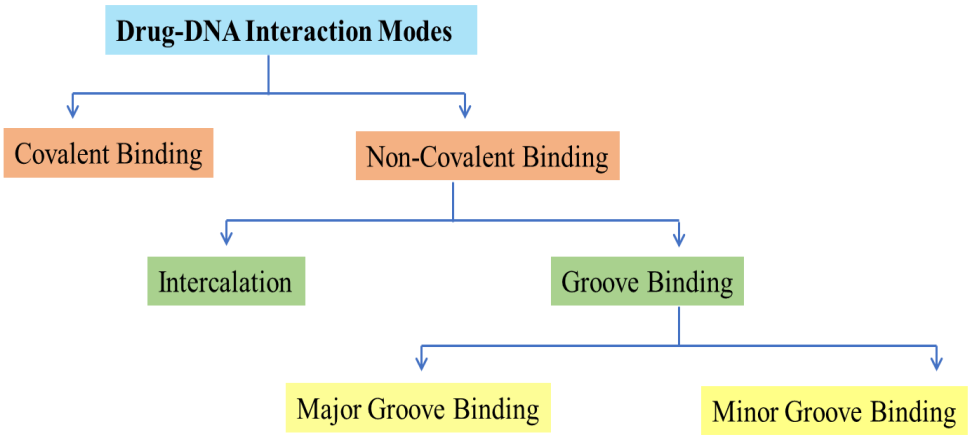

Figure 1.11. Different modes of Drug-DNA interaction.

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1.5.2.1 Covalent Binding

If the binding of any drug with DNA is covalent, it is totally irreversible. Covalent binding leads to total inhibition of DNA functions thus leading to complete cell death. Cisplatin is most widely used anticancer drug which binds covalently with DNA. Drugs that interfere with DNA function by chemically modifying specific nucleotides. There are two type of alkylating agents: mono functional (one reactive group) which cause single-strand breaks in DNA or damage bases and bi functional (two reactive group) which form cross-links. A great number of first generations of anti-cancer drugs were developed to combine a single alkylating function. Their general characteristic is that they form an initial physical complex with DNA before covalently bonding to it [55]. Many of them have also shown selective anti-tumor activity, this can be attributed to DNA binding specificity or to preferential metabolic activation by tumour cells. The mechanism of action of these anti-cancer agents is by alkyl group transfer and they cause alkylation of DNA at N7 or N2 position of guanine (other sites as well) and interaction main involve single strands or both strands. Anthramycin is an anti-tumor antibiotic that binds covalently to N2 of guanine located in the minor groove of DNA [56, 57].

1.5.2.2 Non-covalent Binding

Non-covalent binding drugs have the ability to alter DNA torsional tension, disrupt protein-DNA interactions, and cause DNA strand breakage. All of these factors can

have a significant impact on gene expression. The structure of biomolecules is determined by non-covalent interactions, particularly hydrogen bonding and stacking interactions (such as nucleic acids and proteins) [58, 59]. While hydrogen bonding is known to be vital for base pairing selectivity, stacking interactions between planar aromatic rings of nucleobases play an equally important role in the final stability of nucleic acid structures. Non-covalent binding is divided into two parts groove binding and intercalation [60].

Groove binders are a significant family of small compounds that attach to DNA and are essential in the development of drugs. Both the major groove and minor groove of DNA can bind the molecules [61]. Targeting the two grooves needs molecules with quite diverse shapes and sizes since the dimensions of the two grooves differ. The major groove, as its name suggests, is significantly broader than the minor groove; for averaged-sequence B-form ds-DNA, the groove width values are 11.6 and 6.0, respectively. Numerous DNA interacting proteins bind to the main grooves as a result of this dimensional disparity. There are few reports of non-protein compounds that bind to the DNA main groove.

1.5.2.2.1 Minor groove binding

In 1974, Wartel et.al, were the first to suggest the concept of minor groove binding. Regarding the study of AT-specific binder netropsin, the idea of minor groove binding was introduced [62]. Minor groove binders are generally crescent-shaped and they get placed in the minor groove of the DNA. In the case of minor groove binding, after binding of the ligand with DNA, there is just a minute change in the steric hindrance of the DNA structure. It does not perturb the helical structure of DNA to any significant range [63]. Nowadays very much attention is given to the study of low molecular weighted ligands which bind to the minor groove of DNA in the A-T rich region. As they recognize A-T rich part of DNA sequence, the interaction between the ligand and DNA is of considerable importance [64]. A number of reviews have been written on intercalators and groove binders [65-67].

It has been shown that neutral, single-charged, and multiple-charged ligands may bind to minor grooves. Electrostatic, van der Waals, hydrophobic, and hydrogen bonding forces are those that predominate in interactions between small molecules and minor

grooves. Key hydrogen-bonds between the base pairs and the small molecules are frequently cited as the cause of sequence specificity [68]. The small molecule's complementary crescent shape to the minor groove is another essential structural prerequisite (radius of curvature). Minor-groove binders frequently exhibit strong AT selectivity. This well-known tendency is influenced by a number of things. GC-filled grooves have a lower electrostatic potential than AT-rich ones. In most cases, this results in AT selectivity for the diionic compounds. In contrast, the minor groove dimensions at AT sites are smaller and deeper than at GC locations. The different ionic interactions in between two different types of base-pair tracts have been acknowledged as a significant factor in this dimensional variation. Small molecules can occupy spaces at AT sites more easily and make more van der Waals interactions.

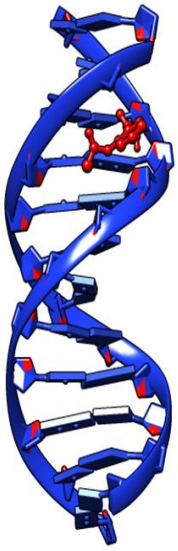


Figure 1.12. Minor groove binding of drug Aspirin with DNA.

Moreover, the amino group of G in GC positions protrudes into the groove, preventing van der Waals connections in comparison to those possible at AT sites. Since proteins and polymerases, which normally interact with the main groove, are less competitive at the minor groove, it is a particularly appealing target for small molecules. A small molecule can attach securely because the strands in the minor groove are closer than they are in the main groove [69–72]. Pentamidine, distamycin, netrospin, Hoechst 33258, DAPI and other antimalarial medications are examples of well-known and well-characterized minor groove binders [73].

1.5.2.2.2 Major groove binding

The major groove is broader than the minor groove; for the B-form of DNA, the groove width values are 11.6 and 6.0, respectively. The major groove is a destination for several DNA-interacting proteins because of this dimension discrepancy. The variety of hydrogen bond acceptor and donor given in the main groove interacts with

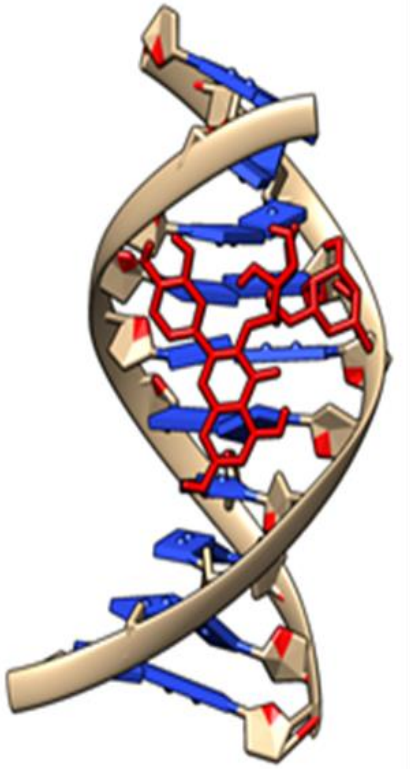


Figure 1.13. Major groove binding.

several biological macromolecules such as proteins [74-77]. It's critical for a significant groove binding molecule to be able to prevent proteins from recognising the same groove. Sequence affinity and sequence selectivity can help with this [78]. Figure 1.13 shows major groove binding of a drug with DNA. Oligomers can read DNA duplexes made up of polypurine–polypyrimidine sequences, which attach to the main groove and generate hydrogen bonds with nucleic bases. These oligonucleotides are known as triplex-forming oligonucleotides (TFOs). Various biomolecules, like proteins, tend to interact with the range of hydrogen-bond donor and acceptors provided in the major groove, implying that the major groove seems to be a more appealing therapeutic target. Some examples of DNA major groove binders are chloroquine, leinamycin, pluramycin etc. [79].

1.5.2.2.3 Intercalation

In 1961, Lerman was the first to introduce intercalation. His study on the aminoacridine frame-shift mutatgens was the milestone for proposing intercalation binding [65, 66]. In intercalation of drug to DNA, the planar, aromatic part of the drug find its position between the stacking of DNA base pairs. The intercalation binding results in the lengthening and unwinding of DNA helix, so that the molecule could fit

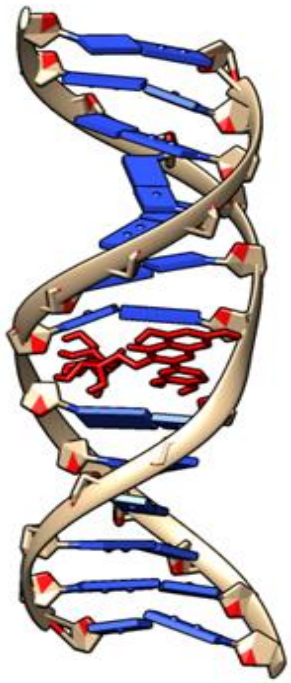


Figure 1.14. Intercalation binding between drug and DNA.

in the intercalation gap [79, 80]. This extension is different for each intercalator-DNA pair, but it is about same extent. All intercalators lengthen the helix in the same range, but they can unwind DNA varyingly. The change in the length of DNA after binding can be used to refer as the binding is groove binding or intercalation. Intercalators usually have a planar moiety comprised of several fused rings that interact with DNA by inserting the planar moiety between adjacent base pairs of the double helix. Ethidium bromide, quinacrine, dounomycin, doxorubicin, acridine orange etc. are some examples of intercalators [80-82].

Drug development has heavily relied on molecules that bind to double-stranded DNA in an intercalative manner. Planar aromatic rings that are inserted between DNA base pairs during the binding of these molecules to DNA are what give this binding its

identity. Van der Waals, hydrophobic and electrostatic forces control the stability of intercalation complexes. The sequencing of base pair has little effect on intercalation. Classical intercalation and threading intercalation are the two main type of intercalation-binding modes. There are several traditional intercalators that are utilised as anticancer drugs. G/C rich sequences are more likely to experience intercalation because they are more likely to get stacked. The native conformations of DNA are often more significantly altered by intercalators [83–85].

Typically, threading intercalators feature two-side chains on opposing sides of a planar aromatic ring structure. This explains why the creation of complexes with DNA is more difficult. In these circumstances, the complex must be formed by one of the side-chains passing through the intercalation cavity. The complicated stability of the threading intercalators is a result of the side-chains' favourable interactions with both the major and minor grooves [86-88].

1.6 Forces involved in drug-DNA interaction

The main type of forces which are involved in drug binding to DNA are electrostatic interaction, van der waals or packing interactions, hydrophobic interactions, solvation electrostatics, solvation van der Waals, ion effects and entropy terms. There is a major role of hydrogen bonding in the case of minor groove binding [89-93].

1.6.1 Hydrogen bonding

In drug-DNA complexes, the formation of hydrogen bonds is an important aspect that helps to determine the relevant interaction and anticipate the system's stability. It normally occurs between the drug molecule's functional groups and the bases of DNA. Because all hydrogen bonds are linear, their contribution to a beneficial energy change when a molecule interacts with DNA in solution is minimal. A penalty of 4kJ/mol is imposed when a non-linear or poorly aligned hydrogen bond is formed, as well as when no hydrogen bond is formed at all. As a result, the formation of hydrogen bonds indicates sequence-specific interactions between the drug and DNA, which contributes to the system's long-term stability [89].

1.6.2 Van der Waals interaction

A van der Waals interaction occurs when two atoms are sufficiently close for their outermost electron clouds to simply contact. This action causes charge oscillations, which provide an attraction that is non-directional and non-specific. These interactions are greatly influenced by distance and become less effective as the separation's sixth power increases. Only when several connections are linked does the energy of each interaction become significant, which is only around 4 kJ mol^{-1} (very low when compared to the typical kinetic energy of a molecule in solution, which is approximately 2.5 kJ mol^{-1}) (as in interactions of complementary surfaces). Under ideal circumstances, van der Waals interactions can reach bonding energies of up to 40 kJ mol^{-1}.

1.6.3 Hydrophobic interaction

This force is created by the presence of water molecules at the drug-DNA interaction surfaces. When a molecule is surrounded by water, it tends to form a sharp cusp-like curving surface of ordered molecules surrounding it. As water molecules accumulate at the contact, the system becomes disordered, increasing the entropy of the system. Water molecules left at the drug-DNA interaction site, on the other hand, tend to reduce the system's entropy [94]. As a result, it is critical that the non-aromatic drug chromophore's chemical structure be precisely complementary in such a way that no extra water molecules obstruct the moiety in which the drug-DNA interaction is to take place [95, 96].

1.7 Methods used to study interaction between drug and DNA

Serious attention is provided to the sequence recogniser compounds which interact and bind to specific region of the DNA, especially A-T rich minor grooves. A lot of techniques are used to study the interaction. In various studies both experimental and theoretical methods are useful for the successfully analysing and demonstrating intercalation, minor groove minding and major groove binding of the compounds with DNA sequences. These studies and investigations are utilized for the molecular modelling, designing and synthesis of different and efficient binders of DNA [97]. Figure 1.15 gives various experimental and theoretical methods used to study drug-DNA interactions.

1.7.1 Experimental methods

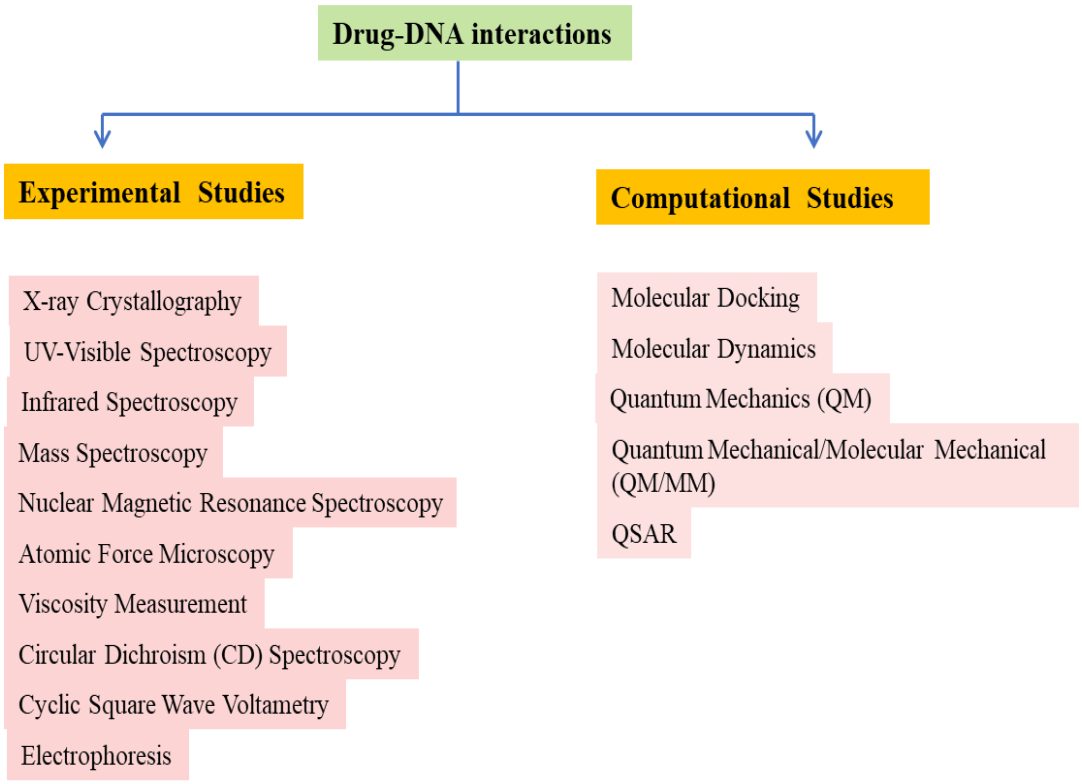


Figure 1.15. Experimental and theoretical methods used to study drug-DNA interactions.

There are several types of analytical techniques to study drug-DNA interactions. Some of these are Fourier transform, Infrared spectroscopy, UV-Visible spectroscopy, Nuclear Magnetic Resonance spectroscopy, Circular Dichroism (CD) spectroscopy, Mass spectroscopy, X-ray crystallography, Atomic Force Microscopy, Viscosity Measurement etc. These studies evaluate or examine the binding mode and binding constant between the drug and DNA [98-102].

Thermal stability: To investigate the function of a drug's stabilising or destabilising effects on the DNA transition, thermal stability is utilised. This may be computed by keeping track of how UV-absorption spectra change with temperature [103].

X-ray crystallography: For crystallographic investigation, X-ray crystallography requires a high-quality single crystal of the drug-DNA complex [104]. However, large molecular weight molecules like drug-DNA complexes are extremely challenging to crystallise in excellent quality. The main drawback of this method is that the

macromolecule is chosen in a certain conformational shape or structure under the effect of crystal packing pressures and underlying local ionic strengths.

Circular Dichroism (CD): In the investigation of drug-DNA interactions, circular dichroism (CD) spectroscopy is crucial. This method is used to determine if a drug is an intercalator or groove binder. Circular Dichroism may be used to determine the binding location as well as a number of other binding factors [105].

Electric Linear Dichroism: Regarding the preferred method of drug interaction with DNA and the base pair preferences of the nucleic acid during drug binding, Electric Linear Dichroism provides important information [106–109].

Nuclear Magnetic Resonance (NMR) is very useful for the characterization of drug-DNA interactions at molecular levels. The atomic nuclei which are available for the study of DNA are (^{1}H, ^{13}C, ^{15}N and ^{31}P); among them, ^{1}H is the very common for NMR studies, but NMR of ^{31}P is useful for studying the effect of binding of ligands to the phosphate groups of DNAs [104].

Surface Plasmon Resonance (SPR) is a technology using biosensor surfaces that has become very popular and largely-used mechanism for studying nucleic acid interactions. This technique is able to give kinetic and equilibrium characterization of the interactivity of biomolecules simultaneously [110].

1.7.2 Computational methods

Computational drug design methodologies have become of great importance and useful in the biomedical research and pharmaceutical field [111]. Figure 1.16 gives an overview of theoretical or computational drug designing method. Computational approach is the newest and most effective tool for drug-designing and analysis. There is not a single method which provides all the details required for the drug-designing. So a lot of techniques have been done and new tools are being developed to sincerely understand and examine drug-DNA interaction.

Some of the important theoretical techniques which have gained researcher's great attention in biophysics, biochemistry and biotechnological fields are Molecular Docking, Molecular Dynamics, Quantum Mechanics (QM) and Quantum Mechanical/Molecular Mechanical (QM/MM) tools, etc. [112-116]. These all

methods are utilized to study the interaction. These drug designing methods have become so important and valuable tools for the designing of new drugs prediction of their behaviour and binding modes. Various studies show good correlation between experimental and computational results [117].

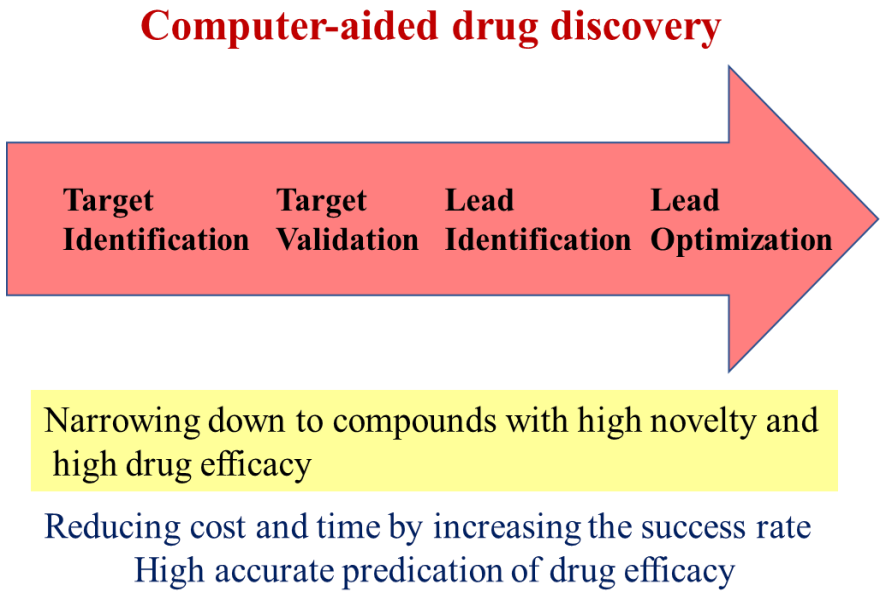


Figure 1.16. Computational drug-designing method pathway.

Chemical, structural and electronic properties drug-DNA complexes can be understood using Quantum Mechanical/Molecular Mechanical (QM/MM) tool. QM/MM technique is a combination of computational methods of varying efficiency and accuracy. In this method, chemically active part of the ligand-receptor system is treated quantum mechanically whereas remaining surrounding is treated with molecular mechanics [118, 119]. Elaborated discussion about each computational method is given in next chapter.

1.8 Thermodynamic Information for Drugs from Prior Research

Numerous research teams have been studying the interactions between drugs and DNA using experimental and computational methods and they have documented the thermodynamic data they have obtained in terms of numerous parameters including K, G, and T_m [120-154]. The table 1.2 below summarises the thermodynamic data as determined by review of the literature, along with their respective DNA binding:

Table 1.2. Experimental and theoretical data of DNA binding ligands collected from literature with their binding mode.

Ligand	**DNA Sequence**	**Exp. Data**	**Binding Mode**
Aspirin	CT-DNA	$\Delta T_m=6.4^0C$	I
Diflunisal	CT-DNA	$\Delta T_m=2.3^0C$	G
Ibuprofen	CT-DNA	ΔG= -6.96 kcal/mole	I
Eugenol	Salmon sperm DNA	$K= 7.28\times10^3\ M^{-1}$	I
Querceitn	Ds-DNA	K= 3.56×103M-1	I
Flavones	CT-DNA	ΔG= -29.9±1.2 kj/mol	I
3-Hydroxyflavone	CT-DNA	ΔG= -26.9±0.9 kj/mol	I
5-Hydroxyflavone	CT-DNA	ΔG= -27.5±1.0 kj/mol	I
6-Hydroxyflavone	CT-DNA	ΔG= -28.3±0.6 kj/mol	I
7-Hydroxyflavone	CT-DNA	ΔG= -28.0±0.1 kj/mol	I
Daphnetin	CT-DNA	ΔG= -25.19 kj/mol	I
6-Mercaptopurine	CT-DNA	K= 7.48×103M-1	G
Idarubicin	Ds-DNA	$K= 5.14\times10^5\ M^{-1}$	I
Elsculetin	CT-DNA	$K= 1.84\times10^4\ M^{-1}$	m
Methotrexate	Ds-DNA	$K= 1.0\times10^3\ M^{-1}$	G
Olanzapine	CT-DNA	$K= 2.0\times10^3\ M^{-1}$	m
Propidium	CT-DNA	ΔG= -7.5 kcal/mole	I
Daunorubicin	CT-DNA	ΔG= -7.9 kcal/mole	I
Ethidium	CT-DNA	ΔG= -6.7 kcal/mole	I
Chartreusim	CT-DNA	ΔG= -7.4 kcal/mole	I
Mesalamine	CT-DNA	ΔG= -4.2 kcal/mole	I
Doxorubicin	1R2L DNA	$K= 0.17\times10^5\ M^{-1}$	I
Epirubicin	1R2L DNA	$K= 0.14\times10^5\ M^{-1}$	G
Daunorubicin	1R2L DNA	$K= 1.80\times10^5\ M^{-1}$	I
Cisplatin	1R2L DNA	$K= 0.41\times10^5\ M^{-1}$	I
HT32258	A3T3 CT-DNA	ΔG= -7.7 kcal/mole ΔG= -7.9 kcal/mole	m

Netropsin	A3T3 CT-DNA	ΔG= -7.7 kcal/mole ΔG= -8.8 kcal/mole	m
Propamidine	A3T3 CT-DNA	ΔG= -7.0 kcal/mole ΔG= -8.2 kcal/mole	m
Berenil	A3T3 CT-DNA	ΔG= -8.0 kcal/mole ΔG= -8.6 kcal/mole	m
Distamycin	A3T3	ΔG= -10.5 kcal/mole	m
DB244	CT-DNA	ΔG= -9.9 kcal/mole	m
DB75	CT-DNA	ΔG= -9.0 kcal/mole	m
DB226	CT-DNA	ΔG= -8.5 kcal/mole	m
Benzidine	CT-DNA	$K= 6.2\pm0.4\times10^{3}\ M^{-1}$	I
Captopril	CT-DNA	$K= 6.2\pm0.4\times10^{3}\ M^{-1}$	m
Echinomycin	CT-DNA	ΔG= -7.5 kcal/mole	B
DAPI	AATT	$K= 1.2\times10^{5}\ M^{-1}$	I
Sperimine	CT-DNA	$K= 2.1\times10^{5}\ M^{-1}$	G
Proflavin	CT-DNA	$K= 2.5\times10^{6}\ M^{-1}$	I
HT32258	Poly{d(AT)} 2A3T3	ΔG= -11.8 kcal/mole ΔG= -11.7 kcal/mole	m
DB293	CT-DNA	ΔG= -9.6 kcal/mole	m
Doxorubicin	CT-DNA	ΔG= -8.9 kcal/mole	I
NB506	CT-DNA	ΔG= -5.9kcal/mole	I
WP631	CT-DNA	ΔG= -15.3 kcal/mole	B
WP762	CT-DNA	ΔG= -16.3 kcal/mole	B
Tau-Fluvalinate	CT-DNA	ΔG= -24.56 kJ/mole	m
Flumethrin	CT-DNA	ΔG= -24.80 kJ/mole	m
Flourouracil	1R2L DNA	$K= 8.7\times10^{5}\ M^{-1}$	G
Carboplatin	1R2L DNA	$K= 2.41\times10^{5}\ M^{-1}$	m
Etopside	1R2L DNA	$K= 0.14\times10^{3}\ M^{-1}$	G
Cyclophosphamide	1R2L DNA	$K= 4.21\times10^{5}\ M^{-1}$	m
Dactinomycin	1R2L DNA	$K= 3.61\times10^{5}M^{-1}$	I
Mitoxantrone	1R2L DNA	$K= 61.91\times10^{5}M^{-1}$	I

Intercalator (I) , Bisintercalator (B), Groove Binder (G), Major Groove Binder (M)

Minor Groove Binder (m)

1.9 Objective of present thesis

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 development of new drugs to treat various diseases like cancer, tumour etc it is mandatory to understand structural and mechanistic information in detail. An elaborated conception of the structural and electronic properties of DNA-drug complexes and their binding mechanism is crucial for the interpretation of main features of anticancer activity.

Here comes the role of theoretical research. Computational tools and technology play very important role in drug designing and pharmacology. There are various most widely used anticancer drugs but they have some limitations. They cause severe side effects which are hazardous over a long period of time. These limitations provoke researchers to develop more efficient and less toxic anticancer drugs. This study is a step towards the search for novel and potent drugs that bind with DNA and provokes anticancer or antitumour effect. The new drug should be less toxic, more efficient and cost effective. The objectives of the present study are:

- Structural analysis of available crystal structure of *drug*-DNA complexes.
- Docking studies of different *drugs* for design of some new and potent *drugs.*
- Molecular Dynamic studies to check stability of bounded *drug*, role of water molecules etc.
- QM/MM calculations for the studies of electronic structure, properties etc of drug-DNA complex.

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.

References

1 T. Yang, J.C. Wu, C. Yan, Y. Wang, R. Luo, M.B. Gonzales, K.N. Dalby, P. Ren, Virtual screening using molecular simulations. Proteins 79 (2011) 1940−1951.

2 G.D. Geromichalos, Importance of molecular computer modelling in anticancer drug development. J. B.U.ON. : Official journal of the Balkan Union of Oncology, 12 (2007) 101–118.

3 W. Jorgensen, The many roles of computation in drug discovery. Science 303 (2004) 1813–1818.

4 RM. Rydzewsky, Real world drug discovery — a chemist's guide to biotech and pharmaceutical research. Elsevier; 2008.

5 V.M. Robles, E. Ortega-Carrasco, E.G. Fuentes, A. Lledos, J.D. Marechal, What can molecular modelling bring to the design of artificial inorganic cofactors? Faraday Discuss. 148 (2011) 137−159.

6 M.D. Parenti, G. Rastelli, Advances and applications of binding affinity prediction methods in drug discovery. Biotechnol. Adv. 30 (2012) 244−250.

7 P. Leelananda, Steffen Lindert, J. Beilstein, Computational methods in drug discovery. Org. Chem. 12 (2016) 2694–2718.

8 I. Haq, J. Ladbury, Drug–DNA recognition: energetics and implications for design, J. Mol. Recognit. 13 (2000) 188–197.

9 H. Meirovitch, Recent developments in methodologies for calculating the entropy and free energy of biological systems by computer simulation. Curr. Opin. Struct. Biol. 17 (2007) 181−186.

10 C. Nantasenamat, C. Isarankura-Na-Ayudhya, V. Prachayasittikul, Advances in computational methods to predict the biological activity of compounds, Expert Opinion on Drug Discovery. 5 (7) (2010) 633-654.

11 T. Katsila, G.A. Spyroulias, G.P. Patrinos, M.T Matsoukas, Computational approaches in target identification and drug discovery, Comp. Struct. Biotech. J. 14 (2016) 177-184.

12 K.C. Nicolaou, Advancing the Drug Discovery and Development Process. Angew. Chem. 126 (2014) 9280 – 9292.

13 J.J. Bissler, Triplex DNA and human disease. Front Biosci., 12 (2007) 4536–46.

14 H. Han, L.H. Hurley, G-quadruplex DNA: a potential target for anticancer drug design. Trends Pharmacol. Sci. 21 (2000) 136–42.

15 S.M. Nelson, L.R. Ferguson, W.A. Denny, Non-covalent ligand/DNA interactions: minor groove binding agents. Mutat. Res. 623 (2007) 24–40.

16 L.H. Hurley, Secondary DNA structures as molecular targets for cancer therapeutics, Biochem. Soc. Trans. 29 (2001) 692–698.

17 J.B. Chaires, A thermodynamic signature for drug–DNA binding mode, Arch. Biochem. Biophys. 453 (2006) 26–31

18 L.H. Hurley, R.T. Wheelhouse, D. Sun, S.M. Kerwin, M. Salazar, O.Y. Fedoroff, F.X. Han, H. Han, E. Izbicka, D.D. Von Hoff, G-quadruplexes as targets for drug design, Pharmacol Ther. 85 (2000) 141–158.

19 L.H. Hurley, DNA and its associated processes as targets for cancer therapy. Nat. ReV. Cancer 2 (2002) 188–200.

20 W.D. Wilson, F.A. Tanious, A. Mathis, D. Tevis, J.E. Hall, D.W. Boykin, Antiparasitic compounds that target DNA. Biochimie. 90(7) (2008) 999-1014.

21 J.D. Watson, F.H. Crick, Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid, Nature, 171 (1953) 737-738.

22 J.D. Watson, F.H. Crick, Genetical implications of the structure of deoxyribo nucleic acid, Nature 171 (1953) 964-967.

23 E.C. Long, J.K. Barton, On demonstrating DNA intercalation, Acc. Chem. Res. 23 (1990) 271–273.

24 A. Pandey, R. Mishra, A. Shukla, A.K. Yadav, D. Kumar, Proceeding of international symposium on advances in functional and biological materials (ISAFBM-2019). ISAFBM Conference Proceedings (2019) 11-18.

25 U. Yadava, S.K. Yadav, R.K. Yadav. Electronic structure, vibrational assignments and simulation studies with A/T rich DNA duplex of an aromatic bis-amidine derivative. DNA repair. 60 (2017) 9-17.

26 A.N. Lane, T.C. Jenkins, Structures and Properties of Multi-stranded Nucleic Acids. Curr. Org. Chem. 5 (2001) 845–869.

27 H. Lodish, A. Berk, C.A. Kaiser, M. Krieger, M.P. Scott, A. Bretscher, H. Ploegh, P. Matsudaira, Molecular Cell Biology, W.H. Freeman and Company, 6th Ed. (2008).

28 R.R. Sinden, DNA Structure and Function, Academic Press, San Diego. (1994).

29 C.K. Peng, S.V. Buldyrev, A.L. Goldberger, S. Havlin, R.N. Mantegna, M. Simons, H.E. Stanley, Statistical properties of DNA sequences, Physica A: Statist. Mech. Appl., 221 (1995) 180-192.

30 Dahm, R. Discovering DNA: Friedrich Miescher and the early years of nucleic acid research. Hum. Genet. 122 (2008) 565–581.

31 R. Franklin, R.G. Gosling, Molecular Configuration in Sodium Thymonucleate Nature, 171 (1953) 740–741.

32 E. Chargaff, Some recent studies on the composition and structure of nucleic acids. J Cell Physiol. Suppl., 38 (1951) 41-59.

33 R.E. Dickerson, H.R. Drew, B.N. Conner, R.M. Wing, A.V. Fratini, M.L. Kopka, The anatomy of A-, B-, and Z-DNA. Science, 216 (1982) 475-85.

34 J.B. Chaires, Energetics of drug–DNA interactions. Biopoly., 44 (1997) 201-215.

35 J.B. Chaires, Drug--DNA interactions. Curr. Opin. Struc. Biol., 8 (1998) 314-320.

36 J.B. Chaires, Calorimetry and thermodynamics in drug design. Annu. Rev. Biophys., 37 (2008) 135-151.

37 J.C. Wang, A. Simon, Lynch, Transcription and DNA supercoiling, Current Opinion in Genetics & Development, 3 (5) (1993) 764-768.

38 L.H. Gregersen, J.Q. Svejstrup, The Cellular Response to Transcription-Blocking DNA Damage, Trends in Biochem. Scien. 43 (5) (2018) 327-341.

39 Y. Zou, Y. Liu, X. Wu, S.M. Shell, Functions of human replication protein A (RPA): From DNA replication to DNA damage and stress responses. J. Cell. Physiol., 208 (2006) 267-273.

40 M. Raschle, P. Knipscheer, M. Enoiu, T. Angelov, J. Sun, J.D. Griffith, T.E. Ellenberger, O.D. Scharer, J.C. Walter, Mechanism of Replication-Coupled DNA Interstrand Crosslink Repair, Cell. 134 (6) (2008) 969-980.

41 G.M. Li, Mechanisms and functions of DNA mismatch repair. Cell Res 18, (2008) 85–98.

42 J.L. Nitiss, Investigating the biological functions of DNA topoisomerases in eukaryotic cells, Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression,Volume 1400 (1998) Pages 63-81.

43 G.M. Spitzer, B. Wellenzohn, C. Laggner, T. Langer, K.R. Liedl, DNA minor groove pharmacophores describing sequence specific properties, J. Chem. Inf. Model. 47 (2007) 1580–1589.

44 C.N. N'soukpoé-Kossi, C. Descôteaux, E. Asselin, H.A. Tajmir-Riahi, G. Bérubé, DNA interaction with novel antitumor estradiol-platinum(II) hybrid molecule: a comparative study with cisplatin drug. DNA Cell Biol. 27(2) (2008)101-107.

45 N. Hadjiliadis, E. Sletten, Metal complex–DNA interactions, Blackwell Publishing Ltd., (2009) 138–139.

46 P.D. Grootenhuis, D.C. Roe, P.A. Kollman, I.D. Kuntz, Finding potential DNA-binding compounds by using molecular shape. J. Comput.-Aided Mol. Des., 8 (1994) 731–50.

47 P.D. Grootenhuis, P.A. Kollman,. G.L. Seibel, R.L. DesJarlais, I.D. Kuntz, Computerized selection of potential DNA binding compounds. Anti-Cancer Drug Des. 5 (1990) 237–42.

48 D. Gibson, Drug–DNA interactions and novel drug design The Pharmacogenomics Journal 2 (2002) 275–276.

49 M.R. Gill, J.A. Thomas, Ruthenium(II) complexes and DNA-from structural probes to cellular imaging and therapeutics, Chem. Soc. Rev. 41 (2012) 3179–3192.

50 K.X. Wan, T. Shibue, M.L. Gross, Non-covalent complexes between DNA binding drugs and double-stranded oligodeoxynucleotides: a study by ESI iontrap mass spectrometry, J. Am. Chem. Soc. 122 (2000) 300–307.

51 M. Waring, Complex formation between ethidium bromide and nucleic acids, J. Mol. Biol. 13 (1965) 269-282.

52 U. Pindur, M. Jansen, T. Lemster, Advances in DNA-ligands with groove binding, intercalating and/or alkylating activity: chemistry, DNA-binding and biology. Curr Med Chem.; 12(24) (2005) 2805-2847.

53 A. Pandey, A. Upadhyaya, S. Kumar, A.K. Yadav, Interaction, Dynamics and Stability Analysis of Some Minor Groove Binders with B-DNA Dodecamer 5’ (CGCAAATTTGCG)-3’ Drug Des, 10 (2020) 172.

54 J.B. Chaires, A thermodynamic signature for drug-DNA binding mode. Arch. Biochem. Biophys. 453 (2006) 26–31.

55 A. Paul, S. Bhattacharya, Chemistry and biology of DNA-binding small molecules S., Curr. Sci., 102 (2002) 212-231.

56 H.K. Liu, P.J. Sadler, Metal Complexes as DNA Intercalators, Acc. Chem. Res. 44 (2011) 349-359.

57 R.D. Snyder, L.B. Hendry, Toward a greater appreciation of noncovalent chemical/DNA interactions: application of biological and computational approaches, Environ. Mol. Mutagen. 45 (2005) 100–105.

58 Manalo, M.N., Pérez, L.M., LiWang, A., Hydrogen-bonding and π-π base-stacking interactions are coupled in DNA, as suggested by calculated and experimental trans H-bond deuterium isotope shifts, J. Am. Chem. Soc., 129, (2007) 11298–11299.

59 S. Rauf, J.J. Gooding, K. Akhtar, M.A. Ghauri, M. Rahman, M.A. Anwar, A.M. Khalid, Electrochemical approach of anticancer drugs–DNA interaction, J. Pharmaceut. Biomed. Anal. 37 (2005) 205–217.

60 J. Ren, J. B. Chaires, Sequence and structural selectivity of nucleic acid binding ligands. Biochemistry 38 (1999) 16067–16075.

61 R.M. Wartell, J.E. Larson, R.D. Wells, A specific probe for A-T regions of duplex deoxyribonucleic acid, J. Biol. Chem. 249 (1974) 6719–6731.

62 D. Suh, J.B. Chaires, Criteria for the mode of binding of DNA binding agents, Bioorg. Med. Chem. 3 (1995) 723–728.

63 N. Spink, D.G Brown, J.V Skelly, S. Neidle, Sequence-dependent effects in drug-DNA interaction: the crystal structure of Hoechst 33258 bound to the d(CGCAAATTTGCG)2 duplex. Nucleic Acids Res. 22(9) (1994) 1607-1612.

64 L.S. Lerman, Structural considerations in the interaction of DNA and acridines, J. Mol. Biol. 3 (1961) 18–30.

65 V. Luzzati, F. Masson, L.S. Lerman, Interaction of DNA and proflavine: A small-angle X-ray scattering study J. Mol. Biol. 3 (1961) 634–639.

66 M. Waring, Variation of the supercoils in closed circular DNA by binding of antibiotics and drugs: Evidence for molecular models involving intercalation, J. Mol. Biol. 54 (1970) 247–279.

67 U. Yadava, S.K. Yadav, R.K. Yadav, Investigations on bisamidine derivatives as novel minor groove binders with the dodecamer 5′(CGCGAATTCGCG) 3′. J Mol Liq. 280 (2019) 135-152.

68 M.J. Waring, C. Bailly, The purine 2-amino group as a critical recognition element for binding of small molecules to DNA, Gene 149 (1994) 69–79.

69 B.S. Reddy, S.M. Sondhi, J.W. Lown, Synthetic DNA minor groove-binding drugs. Pharmacol Ther. 84 (1999) 1–111.

70 D.E. Wemmer, Designed sequence-specific minor groove ligands. Annu. ReV. Biophys. Biomol. Struct. 29 (2000) 439–461.

71 P.G Baraldi, A. Bovero, F. Fruttarolo, D. Preti, M.A. Tabrizi, M.G. Pavani, R. Romagnoli, DNA minor groove binders as potential antitumor and antimicrobial agents. Med. Res. ReV. 24 (2004) 475–528.

72 M.L. Kopka, C. Yoon, D.S. Goodsell, P. Pjura, R.E. Dickerson, Binding of an antitumor drug to DNA: netropsin and C-G-C-G-A-A-T-T-BrC-G-C-G, J. Mol. Biol. 183 (1985) 553–563.

73 W.D. Sasikala, A. Mukherjee, Intercalation and de-intercalation pathway of proflavine through the minor and major grooves of DNA: roles of water and entropy, Phys. Chem. Chem. Phys. 15 (2013) 6446-6455.

74 X.J. Lu, W.K. Olson, 3DNA: a versatile, integrated software system for the analysis, rebuilding, and visualization of three-dimensional nucleic-acid structures Nat. Protoc., 3, (2008) 1213-1227.

75 D.A. Case, T.E. Cheatham III, T. Darden, H. Gohlke, R. Luo, J. Merz, A. Onufriev, C. Simmerling, B. Wang, R. Woods, The Amber biomolecular simulation programs, J. Computat. Chem., 26, (2005) 1668-1688.

76 J.W. Ponder, D.A. Case, Force fields for protein simulations, Adv. Prot. Chem., 66 (2003) 27-85.

77 S. Arnott, P.J. Campbell-Smith, R. Chandrasekaran, Handbook of Biochemistry and Molecular Biology,3rd ed. Nucleic Acids, Cleveland:CRC Press, 2 (1976).

78 H.K. Srivastava, M. Chourasia, D. Kumar, G.N. Sastry, Comparison of computational methods to model DNA minor groove binders. J Chem Inf Model. 51(3) (2011) 558-571.

79 J.B. Chaires, Drug-DNA interactions, Curr. Opin. Struct. Biol. 8 (1998) 314-320.

80 H. Ihmels, L. Thomas, Intercalation of organic ligands as a tool to modify the properties of DNA, in: J.-I. Jin, J. Grote (Eds.), Materials Science of DNA, CRC Press, Boca Raton, (2011) 49-76.

81 S.K. Pawar, S. Jaldappagari, Intercalation of a flavonoid, silibinin into DNA base pairs: Experimental and theoretical approach. J. Mol Recognit. (2020) 33:e2812

82 X. Shui, M.E. Peek, L.A. Lipscomb, Q. Gao, C. Ogata, B.P. Roques, C. Garbay-Jaureguiberry, A.P. Wilkinson, L.D. Williams, Effects of cationic charge on three-dimensional structures of intercalative complexes structure of a

bisintercalated DNA complex solved by MAD phasing, Curr. Med. Chem. 7 (2000) 59–71.

83 R. Martínez, L.C. García, The search of DNA-intercalators as antitumoral drugs:what it worked and what did not work, Curr. Med. Chem. 12 (2005) 127–151.

84 K. Nakamoto, M. Tsuboi, G.D. Strahan, Intercalating Drugs, in: Drug-DNA Interactions, John Wiley & Sons, Inc, (2008) 119-208.

85 H.K. Liu, P.J. Sadler, Metal complexes as DNA intercalators, Acc. Chem. Res. 44 (2011) 349–359.

86 N.J. Wheate, CR. Brodie, J.G. Collins, S. Kemp, J.R. Aldrich-Wright, DNA intercalators in cancer therapy: organic and inorganic drugs and their spectroscopic tools of analysis, Mini Rev. Med. Chem. 7 (2007) 627-648.

87 W.D. Sasikala, A. Mukherjee, Molecular mechanism of direct proflavine-DNA intercalation: evidence for drug-induced minimum base-stacking penalty pathway, J. Phys. Chem. B 116 (2012) 12208-12212.

88 S.A. Shaikh, S.R. Ahmed, B. Jayaram, DNA Drug Interaction, Arch. Biochem. Biophys., 81 (2004) 429.

89 G.L. Seibel, U.C. Singh, P.A. Kollman, Proc. Nat. Acad. Sci., U.S.A., 82, (1985) 755.

90 K.A. Sharp, R.A. Friedman, V. Misra, J. Hecht, B. Honig, Salt effects on polyelectrolyte–ligand binding: Comparison of Poisson–Boltzmann, and limiting law/counterion binding models Biopolymers, 36 (2) (1995) 245-262.

91 V.K. Misra, B. Honig, On the magnitude of the electrostatic contribution to ligand-DNA interactions, Proc. Nat. Acad. Sci., USA, 92 (1995) 4691-4695.

92 S.B. Singh, D.E. Wemmer, P.A. Kollman, Relative binding affinities of distamycin and its analog to d(CGCAAGTTGGC).d(GCCAACTTGCG): comparison of simulation results with experiment, Proc. Nat. Acad. Sci., USA, 91 (1994) 7673-7677.

93 J.L. Dashnau, K.A. Sharp, J.M. Vanderkooi, Carbohydrate Intramolecular Hydrogen Bonding Cooperativity and Its Effect on Water Structure J. Phys. Chem. B, 109 (2005) 24152−24159.

94 K.F. Sergey, K. Cestmir, K. Pavla, S. Larisa, S. Milena, P. Stepanek, Effect of Hydrophobic Interactions on Properties and Stability of DNA−Polyelectrolyte Complexes, Lanmuir, 26 (2010) 4999-5006.

95 G. Hummer, S. Garde, A.E. Garcia, L.R. Pratt, An information theory model of hydrophobic interactions Proc. Natl. Acad. Sci. USA. 93 (17) (1996) 8951-8955.

96 M. Hasanzadeh, N. Shadjou, Pharmacogenomic study using bio- and nanobioelectrochemistry: Drug-DNA interaction, Materials Science and Engineering: C, Volume 61 (2016) 1002-1017.

97 V.I. Ivanov, L.E. Michenkova, A.K. Schyolkina, A.I. Poletayev, Different conformations of double-stranded nucleic acid in solution as revealed by circular dichroism, Biopolymers. 12 (1973) 89–110.

98 F. Zsila, Z. Bikadi, M. Simonyi, Probing the binding of the flavonoid, quercetin to human serum albumin by circular dichroism, electronic absorption spectroscopy and molecular modeling methods, Biochem. Pharmacol. 65 (2003) 447 – 456.

99 J.M. Kelly, A.B. Tossi, D.J. McConnell, C.O. Uigin, A study of the interactions of some polypyridylruthenium(II) complexes with DNA using fluorescence spectroscopy, topoisomerisation and thermal denaturation, Nucl. Acids Res. 13 (1985) 6017–6034.

100 M. Sirajuddin, S. Ali, A. Badshah, Drug–DNA interactions and their study by UV–Visible, fluorescence spectroscopies and cyclic voltammetry J. Photochem. Photobiol. B: Biol. 124 (2013) 1–19.

101 V. Gonzalez-Ruiz, A.I. Olives, M.A. Martin, P. Ribelles, M.T. Ramos, J.C. Menendez, An overview of analytical techniques employed to evidence drug–DNA interactions. Applications to the design of genosensors, in: M.A. Komorowska, S. Olsztynska-Janus (Eds.), Biomedical Engineering, Trends, Research and Technologies. In Tech, (2011) 65–90.

102 B. Nordeni, F. Tjernald, High-sensitivity linear dichroism as a tool for equilibrium analysis in biochemistry- stability constant of DNA-ethidiumbromide complex Biophys. Chem. 4 (1976) 191-198.

103 V. Gonzalez-Ruiz, A.I. Olives, M.A. Martin, P. Ribelles, M.T. Ramos, J.C. Menendez, An Overview of Analytical Techniques Employed to Evidence Drug-DNA Interactions. Applications to the Design of Genosensors, Biomedical Engineering, Trends, Research and Technologies (2011).

104 S. Marchini, M. Broggini, C. Sessa, M. D'Incalci , Development of distamycin-related DNA binding anticancer drugs, Expert Opinion on Investigational Drugs. 10 (2001)1703-1714.

105 B. Norden, F. Tjerneld, High-sensitivity linear dichroism as a tool for equilibrium analysis in biochemistry- stability constant of DNA-ethidiumbromide complex Biophys. Chem., 4 (1976) 191-198.

106 C. Bailly, P. Colson, C. Houssier, F. Hamy, The Binding Mode of Drugs to the TAR RNA of HIV-1 Studied by Electric Linear Dichroism, Nucl. Acids Res., 24 (1996) 1460-1464.

107 C. Bailly, P. Colson, C. Houssier, Electric linear dichroism as a new tool to study sequence preference in drug binding to DNA, Biophys. Chem., 58 (1996) 125-140.

108 O. Kennard, W.N. Hunter, Single-Crystal X-Ray Diffraction Studies of Oligonucleotides and Oligonucleotide–Drug Complexes, Angew. Chem. Int. Ed. Engl., 30, (1991) 1254-1277.

109 K.R. Fox, Drug-DNA Interaction Protocols, Methods in Mol. Biol., 613, (2010).

110 I.M. Kapetanovic, Computer-aided drug discovery and development (CADDD): In silico-chemico-biological approach. Chem. Biol. Interact, 171 (2008) 165–76.

111 I. Halperin, B. Ma, H. Wolfson, R Nussinov, Principles of docking: An overview of search algorithms and a guide to scoring functions. Proteins 47 (2002) 409–443.

112 J. Field, P. A. Bash, and M. Karplus, "A combined quantum mechanical and molecular mechanical potential for molecular dynamics simulations," J. Comput. Chem., 11(6) (1990) 700–733.

113 M. Senn, W. Thiel, "QM/MM methods for biomolecular systems," Angew. Chem., Int. Ed., 48(7) (2009) 1198–1229.

114 A. Friesner, V. Guallar, "Ab initio quantum chemical and mixed quantum mechanics/molecular mechanics (QM/MM) methods for studying enzymatic catalysis," Annu. Rev. Phys. Chem., 56 (2005) 389–427.

115 M. Orozco, A. Pérez, A. Noy, F.J. Luque, Theoretical methods for the simulation of nucleic acids, Chem. Soc. Rev., 32 (2003) 350–364.

116 C. Li, J.X. Wang, Y. Le, J.F. Chen, Studies of Bicalutamide–Excipients Interaction by Combination of Molecular Docking and Molecular Dynamics Simulation, Mol. Pharm.10 (2013) 2362-2369.

117 Y. Liu, G. Lu, Z. Chen, N. Kioussis, “An improved QM/MM approach for metals,” Modell. Simul. Mater. Sci. Eng., 15(3) (2007) 275.

118 P. Sherwood, A.H. Vries, M. F. Guest, G. Schreckenbach, C. Richard, A Catlow, S.A. French, A.A. Sokol, S.T. Bromley, W. Thiel, A.J. Turner et al., “QUASI: A general purpose implementation of the QM/MM approach and its application to problems in catalysis,” J. Mol. Struct.: THEOCHEM 632(1) (2003) 1–28.

119 M.A. Husain, S.U. Rehman, H.M. Ishqi, S. Tarique, T. Mohammad, Spectroscopic and molecular docking evidence of aspirin and diflunisal binding to DNA: a comparative study, RSC Adv., 5 (2015) 64335-64345.

120 M.A. Husain, T. Sarwar, S.U. Rehman, H.M. Ishqi, M. Tabish, Ibuprofen causes photocleavage through ROS generation and intercalates with DNA: a combined biophysical and molecular docking approach, Phys. Chem. Chem. Phys. 2015(17) (21) (2015) 13837-13850.

121 S. Bi, L. Yan, Y. Wang, B. Pang, T. Wang, Spectroscopic study on the interaction of eugenol with salmon sperm DNA in vitro, J. Luminescence, 132 (2012) 2355-2360.

122 M. Mrksich, P.B. Dervan, Antiparallel side-by-side heterodimer for sequence-specific recognition in the minor groove of DNA by a distamycin/1-methylimidazole-2-carboxamide-netropsin pair, J. Am. Chem., Soc., 115 (1993) 2572-2576.

123 F.D. Mahvash, D. Gholamreza, M. Majid, A.H.F. Mohammad, DNA binding study of dihydropyrano [3, 4-C] chromene derivative by some spectroscopic techniques, Journal of Reports in Pharmaceutical Sciences, 5 (2016) 80-82.

124 J. Vitorino, M.J. Sottomayor, DNA interaction with flavone and hydroxyflavones, J. Mol. Struc., 975 (1-3) (2010) 292-297.

125 J. Barnali, S. Sudipta, G. Debanjana, B. Debosreeta, C. Nitin, Spectroscopic Exploration of Mode of Binding of ctDNA with 3-Hydroxyflavone: A Contrast to the Mode of Binding with Flavonoids Having Additional Hydroxyl Groups, J. Phys. Chem. B, 116 (2012) 639-645.

126 K.J. Naveed, S. Asima, Y. Azra, S. Sana, Q. Rumana, H.U. Sayed, Spectrophotometric analysis of flavonoid–DNA binding interactions at physiological conditions, Spectrochimica Acta Part A, 74 (5) (2009) 1135-1137.

127X. Zhou, G. Zhang, J. Pan, Groove binding interaction between daphnetin and calf thymus DNA, Inter. J. Bio. Macro. 74 (2015) 185-194,

128R. Bera, B.K. Sahoo, K.S. Ghosh, S. Dasgupta, Studies on the interaction of isoxazolcurcumin with calf thymus DNA, Inter. J. Bio. Macro. 42 (2008) 14-21.

129C. Ozluer, H.E.S. Kara, In vitro DNA binding studies of anticancer drug idarubicin using spectroscopic techniques, Journal of Photochemistry and Photobiology B: Biology, Volume138 (2014) 36-42.

130 T. Sarwar, M.A. Husain, S.U. Rehman, H.M. Ishqi, M. Tabish. Molecular BioSystems Multispectroscopic and molecular modelling thymus DNA. Mol Biosyst. Royal Society of Chemistry 11 (2015): 522-531.

131 R. Hajian, M. Tavakol, Interaction of anticancer drug methotrexate with ds-DNA analyzed by spectroscopic and electrochemical methods. E-Journal of Chemistry 9, no. 1 (2012) 471-480.

132 N. Shahabadi, S Bagheri, "Spectroscopic and molecular docking studies on the interaction of the drug olanzapine with calf thymus DNA." Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 136 (2015) 1454-1459.

133 J. Ren, T.C. Jenkins, J.B. Chaires. "Energetics of DNA intercalation reactions." Biochemistry 39, no. 29 (2000) 8439-8447.

134 B. Francisca, D. Capó, José Portugal. "Thermodynamic characterization of the multivalent binding of chartreusin to DNA." Nucleic acids research 30, no. 20 (2002) 4567-4573.

135 N. Shahabadi, S.M. Fili, Fahimeh Kheirdoosh. "Study on the interaction of the drug mesalamine with calf thymus DNA using molecular docking and spectroscopic techniques." Journal of Photochemistry and Photobiology B: Biology 128 (2013) 20-26.

136 P. Fouzia, R. Qureshi, F.L. Ansari, S. Kalsoom, S. Ahmed. "Investigations of drug–DNA interactions using molecular docking, cyclic voltammetry and UV–Vis spectroscopy." Journal of Molecular Structure 1004, no. 1-3 (2011) 67-73.

137 I. Haq, "Thermodynamics of drug–DNA interactions." Archives of biochemistry and biophysics 403, no. 1 (2002) 1-15.

138 S. Mazur, F.A. Tanious, D. Ding, A. Kumar, D.W. Boykin, I.J. Simpson, S. Neidle, W.D. Wilson, "A thermodynamic and structural analysis of DNA minor-groove complex formation." Journal of Molecular Biology 300, no. 2 (2000) 321-337.

139 L.A. Reis, E.B. Ramos, M.S. Rocha, "DNA interaction with diaminobenzidine studied with optical tweezers and dynamic light scattering." The Journal of Physical Chemistry B 117, no. 46 (2013) 14345-14350.

140 A. Mukherjee, B. Singh, "Binding interaction of pharmaceutical drug captopril with calf thymus DNA: a multispectroscopic and molecular docking study." Journal of Luminescence 190 (2017) 319-327.

141 X. Qu, J. Ren, P.V. Riccelli, A.S. Benight, J.B. Chaires, "Enthalpy/entropy compensation: influence of DNA flanking sequence on the binding of 7-amino actinomycin D to its primary binding site in short DNA duplexes." Biochemistry 42, no. 41 (2003) 11960-11967.

142 F. Leng, J.B. Chaires, M.J. Waring, "Energetics of echinomycin binding to DNA." Nucleic Acids Research 31, no. 21 (2003) 6191-6197.

143 W.D. Wilson, F.A. Tanious, H.J. Barton, R.L. Jones, L. Strekowski, D.W. Boykin, "Binding of 4', 6-diamidino-2-phenylindole (DAPI) to GC and mixed sequences in DNA: intercalation of a classical groove-binding molecule." Journal of the American Chemical Society 111, no. 13 (1989) 5008-5010.

144 L. Strekowski, D.B. Harden, R.L. Wydra, K.D. Stewart, W.D. Wilson, Molecular basis for potentiation of bleomycin-mediated degradation of DNA by polyamines. Experimental and molecular mechanical studies, J. Mol. Recog. 2 (1989) 158-166.

145 L.R. Ferguson, W.A. Denny, The genetic toxicology of acridines, Mutation Research/Reviews in Genetic Toxicology 258 (1991) 123-160.

146 I. Haq, J.E. Ladbury, B.Z. Chowdhry, T.C. Jenkins, J.B. Chaires, Specific binding of Hoechst 33258 to the d (CGCAAATTTGCG) 2 duplex: calorimetric and spectroscopic studies, J. Mol. Biol. 271 (1997) 244-257.

147 F.G. Loontiens, P. Regenfuss, A. Zechel, L. Dumortier, R.M. Clegg, Binding characteristics of Hoechst 33258 with calf thymus DNA, poly [d (AT)] and d (CCGGAATTCCGG): multiple stoichiometries and determination of tight binding with a wide spectrum of site affinities, Biochemistry 29 (1990) 9029-9039.

148 J. Ren, T.C. Jenkins, J.B. Chaires, Energetics of DNA intercalation reactions, Biochemistry. 39 (29) (2000) 8439-8447.

149 C. Carrasco, H. Vezin, W.D. Wilson, J. Ren, J.B. Chaires, DNA binding properties of the indolocarbazole antitumor drug NB-506, Anti-cancer drug design. 16 (2001) 99-107.

150 F. Leng, W. Priebe, J.B. Chaires, Ultratight DNA binding of a new bisintercalating anthracycline antibiotic, Biochemistry 37 (1998) 1743-1753.

151 J. Portugal, D.J. Cashman, J.O. Trent, N. Ferrer-Miralles, T. Przewloka, I. Fokt, W.Priebe, J.B. Chaires, A new bisintercalating anthracycline with picomolar DNA binding affinity, J. Med. Chem. 48 (26) (2005) 8209-8219.

152 M. Tao, G. Zhang, J. Pan, C. Xiong, Deciphering the groove binding modes of tau-fluvalinate and flumethrin with calf thymus DNA, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 155 (2016) 28-37.

153 F. Perveen, R. Qureshi, F.L. Ansari, S. Kalsoom, Safeer Ahmed, Investigations of drug–DNA interactions using molecular docking, cyclic voltammetry and UV–Vis spectroscopy. J. Mol. Struct. 1004 (2011) 67-73.

CHAPTER-2

METHODOLOGY

An overview of the various computational methods used to analyse and explain the structure, behaviour, and other physical and chemical characteristics of atomic and molecular systems is given in this chapter**.**

2.1 Quantum Mechanics

Atoms, which make up molecules, are made up of electrons, protons and neutrons. The nucleus is made up of protons and neutrons while electrons are found around the nucleus at different energy levels, referred as orbits or shells. This is the most fundamental concept of how subatomic particles are arranged inside an atom. Several distinct physical and chemical characteristics, such as stable geometry, charge distribution, dipole moment, vibrational frequency, etc., are brought about by the precise arrangement of the subatomic particles inside the atom. Quantum mechanical formulas can be used to exactly calculate these sets of distinct physical and chemical properties for a particular collection of atoms or molecules [1, 2].

One of the oldest mathematical expressions of theoretical chemistry is quantum mechanics. In terms of quantum mechanics (QM), the wave function Ψ, which is derived by solving the Schrödinger wave equation, is said to contain all the information that is feasible about a molecular system [3, 4]. The Schrödinger equation, which is the foundation of quantum mechanics and forms the basis for a comprehensive electronic description of the molecule, can be expressed as

$$H \Psi = E \Psi \tag{2.1}$$

Here, H is the system's entire Hamiltonian and consists of the components Kinetic Energy and Potential Energy of the system. E is the energy eigenvalue when H is operated on Ψ (wave function). Ψ is a mathematical function that behaves properly, and its inner product with its conjugate i.e., $\psi^*\psi$, indicates probability density. For hydrogen and atoms that are equivalent to hydrogen, this equation is easily solvable; unfortunately, for other atomic numbers, it cannot be solved. Although this equation

is straightforward or simple to express and is capable of accurately characterising any molecular system, its solution presents the main challenge. Despite being a very powerful and attractive equation that can accurately describe any system when solved, the fundamental issue is how to solve it.

The wave function of the molecule gives both its nuclear and electronic motions. Born Oppenheimer approximation is used to separate the electronic wave function from total wave function [5, 6].

When two or more atoms' nuclei are set at a specific distance, their electronic wave function is adequate to account for all of the molecule's physical and chemical properties. However, for a system with many electrons, the electronic component of the Schrödinger equation's Hamiltonian operator is described by

$$H_e = -\sum_p \frac{1}{2}\nabla_p{}^2 - \sum_A \sum_p \frac{Z_A}{r_{AP}} + \sum_{p<q} \frac{1}{r_{pq}} \tag{2.2}$$

In equation 2.2**,** the first term represents the kinetic energy; second term gives the potential energy whereas the third term gives the rise in potential energy due to inter-electron interactions. Thus, the modified Schrödinger wave equation for 'n' electron system [7] is given by:

$$H_e(1,2,...,n)\psi_e(1,2,...,n) = E_e\psi_e(1,2,...,n) \tag{2.3}$$

Here, Ψe denotes electronic part of the total wave function. Because a number of inter-electronic repulsion terms are present, the Schrödinger equation for a single atom is inseparable and so we attain the solution Ψ_0 by ignoring these inter-electronic repulsion terms. The wave function Ψ_0 for 'n' electrons is given by the product of 'n' single electronic wave functions. This product wave function is known as Hartree product of the wave function [8] and is given as:

$$\Psi_0 = \Psi_1(r_1,\theta_1,\varphi_1)\Psi_2(r_2,\theta_2,\varphi_2) .....\Psi_n(r_n,\theta_n,\varphi_n) \tag{2.4}$$

Since electrons are fermions, they follow half spin Fermi-Dirac statistics [9]. Single electron wave functions are the molecular orbitals which are the products of the spatial orbitals and the spin functions (α or β) [10]. In order to satisfy Pauli's exclusion principle, the wave function must be anti-symmetric in nature [11]. The anti-symmetric wave functions may be derived from Slater Determinants [12]. The spin orbital Slater determinant for 'n' electrons is given by:

$$\psi = \frac{1}{\sqrt{N!}} \begin{vmatrix} \phi_1(r_1)\alpha(s_1) & \phi_2(r_1)\beta(s_1) & \ldots & \ldots & \ldots & \phi_n(r_1)\beta(s_1) \\ \phi_1(r_2)\alpha(s_2) & \phi_2(r_2)\beta(s_2) & \ldots & \ldots & \ldots & \phi_n(r_2)\beta(s_2) \\ \ldots & \ldots & \ldots & & & \ldots \\ \ldots & \ldots & & \ldots & & \ldots \\ \ldots & \ldots & & & \ldots & \ldots \\ \phi_1(r_n)\alpha(s_n) & \phi_2(r_n)\beta(s_n) & & & & \phi_n(r_n)\beta(s_n) \end{vmatrix} \tag{2.5}$$

The solution to eq. 2.5 can be obtained by using perturbation over the solution Ψ_0 and a trial wave function is constructed with the help of Slater determinant by applying the Variational principle. The equation of energy of the system given below is written in form of Dirac notation given by:

$$E = \frac{\langle \psi \mid H \mid \psi \rangle}{\langle \psi \mid \psi \rangle} \tag{2.6}$$

2.1.1 Hartree Self -Consistent Field Method

The solution of the electronic Schrödinger wave equation of molecule, which is formed by writing the Slater determinant, consists of the nuclear–nuclear interaction energy, which has constant value for a given geometry, the nuclear-electron attraction, which is dependent on one electron coordinate and the electron–electron repulsion, which depends on two electron's coordinates [13].

The Hamiltonian is

$$H_e = \sum_{p}^{N} h + \sum_{i=1}^{N} \sum_{j>i}^{N} g_{ij} + V_{nn} \tag{2.7}$$

where,

$$h_p = -\frac{1}{2}\nabla^2 - \sum_a \frac{Z_a}{|R_a - r_p|} \tag{2.8}$$

and

$$g_{ij} = \frac{1}{|r_i - r_j|} \tag{2.9}$$

where one electron operator h_i describes the motion of ith electron in field of all nuclei, g_{ij} is two-electron operator giving the repulsion between two electrons while V_{nn} is the nuclear-nuclear interaction energy. The energy can be expressed as

$$E = \sum_{i}^{N} \langle \varphi_i \mid h_i \mid \varphi_i \rangle + \frac{1}{2} \sum_{ij}^{N} \left(\langle \varphi_j \mid J_i \mid \varphi_j \rangle - \langle \varphi_j \mid K_i \mid \varphi_j \rangle \right) + V_{nn} \tag{2.10}$$

$$J_{12} = \left\langle \varphi_1^{(1)} \varphi_2^{(2)} \mid g_{12} \mid \varphi_1^{(1)} \varphi_2^{(2)} \right\rangle \tag{2.11}$$

where, J operator represents the classical repulsion between the two charge distributions described by $\varphi_{12}(1)$ and $\varphi_{22}(2)$

$$K_{12} = \left\langle \varphi_1^{2} \varphi_2^{(2)} \mid g_{12} \mid \varphi_2^{(1)} \varphi_1^{(2)} \right\rangle \tag{2.12}$$

The K operator represents the exchange integral that has no classical analogue.[9] The Hartree Fock method is known as mean field approximation in which the average electron-electron repulsion is taken into account [14].

2.1.2 Density Functional Theory

In contradiction to wave function quantum mechanics, density functional theory (DFT) defines energy as a functional of electron density. The application of DFT theory in large and medium-sized molecular systems is well established. One can determine the electron density and, as a result, the system's total energy by using a set of one-electron equations that were developed by Kohn et al. His approach has lately been proved to be quite effective, with calculations utilising it being substantially less computationally expensive while still showing good correlation with experiment for very large chemical systems. [15, 16] In a DFT calculation, the total energy (E_{el}) is divided into four terms: kinetic energy, a term denoting electron-nucleus attractions, a term denoting electron Coulomb interaction, and a term denoting exchange-correlation (E_{xc}), as illustrated above, similarly to a Hartree-Fock calculation [17]. The first three terms, which are functions of the nuclear coordinates (R) and the electron coordinates, resemble the Hartree-Fock Hamiltonian presented in Eqs. 2.7, 2.8, and 2.9 above.

$$E_{el} = -\frac{1}{2} \sum_{i} \int \varphi_i(r_1) \nabla^2 \varphi_i(r_1) dr_1 + \sum_{A} \int \frac{Z_A}{|R_A - r_1|} \rho(r_1) dr_1 + \frac{1}{2} \frac{\rho(r_1)\rho(r_2)}{|r_1 - r_2|} dr_1 dr_2 + E_{XC} \tag{2.13}$$

Since the exchange-correlation functional is unknown, approximating equations have been developed to determine how much it contributes. Exc is often divided into a correlation functional (CF) and an exchange functional. While the correlation is the

pairing energy of electrons in the same orbital, the exchange functional mainly shows the interactions of two ferromagnetic spins in distinct orbitals [18].

$$E_X^{Slater} = -\frac{9}{4\alpha_{ex}}\left(\frac{3}{4\pi}\right)^{1/3}\sum_{\gamma}\int\left[\rho_1^{\gamma}(r_1)\right]^{4/3}dr_1 \tag{2.14}$$

In this equation α_{ex} is an exchange scale factor, which has the value ⅔ for an electron gas. Commonly used correlation energy functional E_C^{VWN} is due to Vosko, Wilk and Nusair [18] and represents the correlation energy per electron in a gas $\varepsilon_C\left[\rho_1^{\alpha}\rho_1^{\beta}\right]$ with spin densities ρ_1^{α} and ρ_1^{β}

$$E_C^{VWN} = \int \rho_1(r_1)\varepsilon_c\left[\rho_1^{\alpha}(r_1),\rho_1^{\beta}(r_1)\right]dr_1 \tag{2.15}$$

The Local Density Approximation is obtained by combining Slater exchange and Vosko-Wilk-Nusair correlation, both of which are directly derived from the homogeneous electron gas equations. Other (better) exchange and correlation functional have, however, been created to rectify the non-local terms. Perdew and Wang (PW91 correlation functional) [19] and Lee, Yang, and Parr (LYP correlation functional) [15] are two widely used methods, however there are many other functional methods available, each with unique benefits and/or disadvantages.

As a result, Becke performed highly accurate benchmarking of DFT techniques against a testing set of experimentally known ionisation energies, electron affinities, and proton affinities [20]. To estimate the contributions of the exchange and correlation functions, he developed a three parameter (hybrid) density functional technique. He then adjusted the values of these three fit parameters (A, B, and C) against the experimental data in the test set. There are other ways to combine different exchange and correlation functionals, but over time, the B3LYP technique has emerged as the most popular option, despite the fact that it is not always the most accurate [20]. Essentially the hybrid density functional method B3LYP has the following form:

$$E_{XC}^{B3LYP} = AE_X^{Slater} + (1-A)E_X^{HF} + B\Delta E_X^{Becke} + E_C^{VWN} + C\Delta E_C^{LYP} \tag{2.16}$$

Thus, it requires the Hartree-Fock exchange, a correction term for the exchange owing to Becke, the Lee-Yang-Parr correction for non-local correlation factors, the local density approximation functions of Slater and Vosko-Wilk-Nusair and the Hartree-Fock exchange. The coefficients A, B, and C are essentially fit-parameters found by

comparing the energies of B3LYP/6-31G* computations to ionisation energies and electron affinities discovered through experimentation. In essence, the B3LYP approach is not an ab initio method. Ab initio, when used strictly, refers to beginning an experiment from scratch without any prior experience. However, because of the accurate and affordable computational method that these fit-parameters have produced, B3LYP has grown to be one of the most widely utilised techniques in research during the past ten years. It has been demonstrated that the B3LYP approach, as well as other hybrid and non-hybrid DFT methods, are incredibly precise and flexible for computational chemists. DFT is a fairly well-known and effective methodology, despite the fact that its precision is generally inferior to that of high-level ab initio methods such as linked cluster methods. This is because of their speed and acceptable accuracy [21].

The B3LYP approach, which is frequently employed, fails to forecast dispersion energy. DFT is only applied sparingly and is employed in systems where the dispersion component dominates. In that instance, the values of the contact energy that are calculated are always too low. When Becke created the hybrid density functional methods, it marked a significant development in DFT and computational chemistry in general [20-21].

2.1.3 Basis Set

A basis set is a collection of mathematical formulas that describe the shapes of an atom's orbitals and are used to approximate theoretical calculations or modelling. The field of ab initio calculations is dominated by two different types of basis sets. Slater Type Orbitals (STO) and Gaussian Type Orbitals are those (GTO) [22]. Simple exponentials known as Slater type orbitals have the following functional form and precisely duplicate the eigen functions of the hydrogen atom:

$$S(r) = N_s e^{-\xi r^2} \tag{2.17}$$

where r is radial distance from the nucleus, N_s, is normalization constant, and ζ is a constant known as the orbital exponent, which governs with size of the orbital.

Slater functions are good approximations to atomic wave function. The introduction of GTOs to replace STOs in calculations made the evaluation of three and four centerintegrals more rapid. A Gaussian Type Orbital for an s-type atomic orbital with the same orbital exponent as STO has the form

$$g(r) = N_g e^{-\xi r^2} \tag{2.18}$$

where N_g is the normalization constant .

The STO basis set has an advantage that they have direct physical interpretation and thus are naturally good basis for molecular orbitals. The STOs have the shortcoming that most of the required integrals needed for the SCF procedure must be calculated numerically; hence it is computationally expensive whereas wave functions with GTO basis set are much easier to compute.

The minimal basis set is a smallest basis set which represent one basis function for each type of occupied orbital in the separated atoms.[10] At least three Gaussian functions are required to represent STO closely. STO-3G basis set is the most commonly used minimal basis set and the notation represents that basis set approximates shape of STO by single contraction three GTO orbitals. In general STO-nG basis set is minimal basis set in which n Gaussian functions are used to represent each orbital (in general n=2-6).

Another family of basis sets whose name is represented as 6-31G is the Pople basis set [23]. According to this, each core orbital is characterised by a single contraction of six GTO primitives, and each valence shell orbital is described by two further contractions, one with three primitives and the other with a single primitive. These basic sets are extremely common for organic compounds. It may occasionally be indicated as 6-31G* or 6-31G**. One asterisk (*) denotes the addition of a set of d primitives to atoms other than hydrogen, while two asterisks (**) denote the addition of a set of p primitives to hydrogen as well. Because they provide the wave function more freedom to change shape, these are known as polarisation functions. It is also possible to add one or more plus signs, for example, 6-31+G* or 6-31++G*. Atoms other than hydrogen have added diffuse functions when there is just one + sign. The second plus sign implies that all atoms are using diffuse functions.

2.1.4 Electron Correlation

Electrons avoid confronting themselves by repelling each other. Such effect of electron repulsion (force between electrons) is also called electron correlation. Thus the motion of each electron is correlated with the motion of the other electrons in the system. When the molecule forms there are also the possibility for the angular

correlation around the bond direction. The antisymmetric wavefunction is roughly represented by a single Slater determinant in the HF formalism, which excludes this Coulomb correlation. Therefore, the energy calculated with HF theory is different from the exact energy of the system. The difference between these two is called the correlation energy

$$E_{corr} = E_{exact} - E_{HF} \tag{2.19}$$

The development of methods to determine the correlation contributions accurately and efficiently leads to a broad categories of approaches called post HF methods.

Many post-HF calculations which include configuration interaction (CI), multi configurational self-consistent field (MCSCF), Moller-Plesset perturbation theory (MPn) and coupled cluster theory (CC) considers the electron correlation.

2.2 Molecular Mechanics

Even while semiempirical approaches are less useful for modelling the behaviour of big molecules or biomolecules, it is still feasible to completely sidestep quantum mechanics. The so-called molecular mechanics (MM) approaches do not need computing a wave function, total energy, or total electron density; instead, they establish a straightforward algebraic expression for the total energy of the compound. Molecular mechanics is based on the simple classical model where the atoms are treated as hard spheres and bonds as spring. In MM the energy of molecule is expressed as the energy due to the geometry of a molecule, from a few specific interactions within a molecule. These interactions include bonded terms such as bond stretching, bending and twisting and non-bonded terms such as van der Waals attractions or repulsions of atoms that come close together, and the electrostatic interactions between partial charges in a molecule due to polar bonds [24, 25]. The total potential energy can be written as the sum of energies of these two types of interactions:

$$E = E_{bonded} + E_{non-bonded} \tag{2.20}$$

$$E_{bonded} = E_{streching} + E_{bending} + E_{improper} + E_{dihedral} \tag{2.21}$$

$$E_{non-bonded} = E_{elec} + E_{vdw} \tag{2.22}$$

The approximations adopted in MM calculations make the computation less expensive. Due to the speed of the MM, the large biomolecular system such as protein and DNA are treated using this method. For the system where the database for parameterization is available, it is possible to predict the accurate geometries and corresponding energies in a short time. The shortcoming of this method is that it ignores electrons and there are many chemical properties which are not defined within this method such as electronic excited states, shape and energies of molecular orbitals. This method is for the limited class of molecules for which force field is parameterized.

2.2.1 Force Field

A force field is a mathematical function that represents the potential energy of a molecular system using a set of parameters (obtained both theoretically and experimentally from computationally complex quantum computations). For the purpose of calculating the potential energy of the system at a specific conformation, all the bonded and non-bonded variables in equation 2.16 are represented as a force field. The Simple MM energy expression will be

$$\boldsymbol{E_{MM}} = \sum_{\boldsymbol{bonds}} \boldsymbol{k_b}(\boldsymbol{d}-\boldsymbol{d_0})^{\boldsymbol{2}} + \sum_{\boldsymbol{angles}} \boldsymbol{k_\theta}(\boldsymbol{\theta}-\boldsymbol{\theta_0})^{\boldsymbol{2}} + \sum_{\boldsymbol{dihedrals}} \boldsymbol{k_\varphi}[\boldsymbol{1}+\boldsymbol{cos}(\boldsymbol{n\varphi}+\boldsymbol{\delta})] + \sum_{\substack{\boldsymbol{non-bonded}\\ \boldsymbol{pairsAB}}} \left\{\boldsymbol{\varepsilon_{AB}}\left[\left(\frac{\boldsymbol{\sigma_{AB}}}{\boldsymbol{r_{AB}}}\right)^{\boldsymbol{12}} - \left(\frac{\boldsymbol{\sigma_{AB}}}{\boldsymbol{r_{AB}}}\right)^{\boldsymbol{6}}\right] + \frac{\boldsymbol{1}}{\boldsymbol{4\Pi\varepsilon_0}}\frac{\boldsymbol{q_A q_B}}{\boldsymbol{r_{AB}}}\right\} \quad (2.23)$$

where d, θ, and ϕ are the bond distances, angles and torsions respectively. d_0 andθ_0 are equilibrium values; and η and δ are the torsion multiplicity and phase, respectively. K_b, K_θ and K_ϕ are bonded force constant. r_{AB} is the non-bonded distance, ε_{AB} and σ_{AB} are the van der Waals parameters between atoms A and B. q_A, q_B are atomic partial charges and ε_0 is the vacuum permittivity.

There are various types of force fields depending upon the level of accuracy. Prior to any molecular modelling calculation, it is important to first choose the required force field according to the calculation. The numerous force fields have been created for diverse purposes and for varied uses. AMBER [26], CHARMM [27], GROMOS [28] are the force fields which are used widely for the simulation of biological macromolecules (proteins and DNA). A thorough study of force fields revealed that

while some of them performed satisfactorily overall, all of them had issues with certain molecular systems. For this reason, it is crucial to thoroughly evaluate a force field before using it with molecules for which it was not originally designed.

2.2.2 Popular Force Fields

AMBER (Assisted Model Building with Energy Refinement) was first developed by Kollman et al. [http://ambermd.org/] has a historical significance on the parameter transferability and use of charges derived from electrostatic potential fitting [26]. The results obtained with this method can be very good for proteins and nucleic acids, but less so for other systems.

CHARMM (Chemistry at HARward Macromolecular Mechanics), created by Karplus et al. [http://www.charmm.org], was initially developed for proteins and nucleic acids [29] and is now used for a wide range of macromolecules for molecular dynamics, crystal packing, solvation, vibrational analysis, as well as for quantum mechanics/molecular mechanics (QM/MM) studies. Five valence terms are used in this force field, one of which is electrostatic and serves as the foundation for other force fields.

The Groningen Molecular Simulation, or GROMOS, was designed at the University of Groningen and the ETH (Eidgenössische Technische Hochschule) in Zurich and is relatively well known for its ability to forecast the dynamical motion of molecules and bulk liquids, as well as for modelling biomolecules. There are five valence terms used, including one that is electrostatic [30]. It is currently updating its parameters.

2.3 Molecular Docking

Molecular docking is one of the important techniques in molecular modelling. It is a very useful tool for designing and computational calculation for interaction of drug and DNA. A lot of docking studies have been done and many are still in progress. 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 [30-32].

Molecular docking is an efficient tool for understanding structural properties of DNA, mutation of genes, origin of some diseases and so on. Using the data calculated and docking score etc. we can draw conclusions as to which drug is preferable in which disease [33]. It helps us design new efficient DNA targeted drugs to deal with genetic diseases. The drugs interact with DNA in many ways, which include intercalation, non-covalent groove binding etc. The interaction results in the formation of a complex. Due to its formation, properties of DNA change. By studying these changes, we can advance towards more rational drug design. These studies are of immense interest in arena of medicinal or clinical chemistry. Many studies have been done till date and many are going on. Figure 2.1 illustrates the molecular docking of drug aspirin with DNA. These investigations aid in our comprehension of the ligand and target's binding characteristics, aiding our ability to forecast the three-dimensional structure of any complex. The score function is used to rank and classify the potential adduct structures. For the calculations, a variety of docking softwares including AUTODOCK, HEX, CHARM, etc. are used. The knowledge gained from the docking procedure is useful for determining complexes' binding energy, free energy, and stability [33, 34].

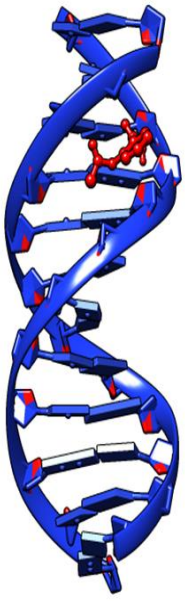


Figure 2.1. Molecular docking of drug aspirin with DNA.

The computational simulation of the molecular recognition process is the main goal of molecular docking research. In order to minimise the free energy of the entire system, it seeks to obtain an optimum conformation for the protein and ligand as well as their relative orientation. The identification and characterisation of different DNA docking types is incredibly exciting since it provides useful data for creating new, better agents for regulating gene expression [35, 36]. Spectroscopic methods that are selectively sensitive make it easier to ascertain how drugs interact with their intended DNA targets. Understanding the manner of interaction requires research on how drugs interact with DNA. It is also a huge assistance when developing drugs that target

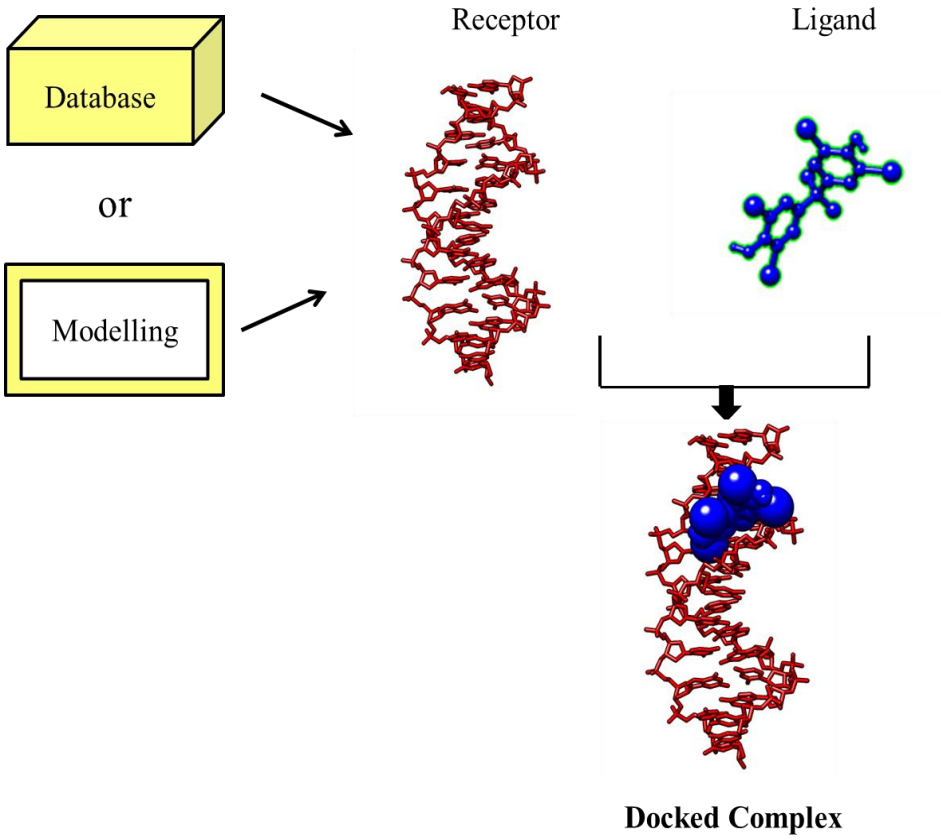


Figure 2.2. Flow chart of molecular docking process.

specific DNA. Targeting DNA and controlling transcription or replication to influence how cells operate seems appealing and conceptually candid.

Any biological reaction can be shown to be feasible via molecular docking. In particular, the activation or inhibition of an enzyme may be predicted by the interaction of small molecules with their protein targets. Figure 2.2 illustrates the process of molecular docking of a ligand with a macromolecule. Lead optimization,

hit detection, and drug-DNA interactions are some of the best applications of molecular docking [37, 38].

2.3.1 Approaches of Molecular Docking

For performing molecular docking, primarily two approaches are used.

2.2.1.1 Simulation approach

This method involves physically separating the ligand from the target before allowing the ligand to attach to the groove in the target's conformational space. Each time the ligand moves, total energy is released. This strategy is more suited to ligand flexibility acceptance. Accessing the molecular recognition between the ligand and the target is more realistic. Since a lot of energy is lost with each conformation, this method takes longer to estimate the ideal docked conformer. Fast optimization techniques and grid-based technologies have become more popular recently [39].

2.2.1.2 Shape complementarity approach

In this method, the surface structure characteristics of the ligand and target are used to give the molecular interaction between them. In this example, the molecular surface of the ligand is illustrated in terms of the surface area of the target that is accessible to solvents. Searching the corresponding groove for ligand on the target surface is made easier by the complementarity among two surfaces depending on shape matching illustration. For instance, the number of twists in the main-chain are used to determine the hydrophobicity of protein target molecules. This speedy method entails quickly scanning through thousands of ligands in a matter of seconds to determine whether any of them could have the ability to bind to the target molecular surface [40].

2.3.2 Types of docking

Basically, there are three types of docking.

Protein ligand docking: When a protein interacts with small molecules like ligands, its structure, location, and orientation are examined using a process called protein ligand docking. It may have a flexible ligand with a rigid receptor or a rigid receptor with a flexible ligand [41].

Protein protein docking: Protein-protein docking is the process of simulating the interaction between two protein molecules via computer software. In contrast to the ligand protein docking, the connections in this instance are essentially stiff [42].

DNA ligand docking: It is the binding of a ligand to the DNA. It is much similar to protein ligand docking.

2.3.3 Softwares for Molecular Docking

Docking algorithms are usually computationally demanding since they consist of generating and evaluating a large amount of different molecule conformations and placements. There are large number of docking programs and search algorithm that are available for carrying out docking calculations. Some popular docking softwares are given in table 2.1.

Table 2.1: Popular Docking Softwares.

S. No.	Software	Description	Ref.
1	AutoDock	Grid based scheme; quick computation of interaction energies of ligand-receptor complexes; incorporates evolutionary, genetic and Lamarckian Genetic algorithms; affinities predicted using autodock show great success	43
2	DOCK	ligands geometries and binding sites are described by sets of spheres; overlapping of spheres is done for binding; in recent versions, electrostatic & molecular-mechanics based interaction energies are incorporated	44
3	FlexX	Docked ligand conformers are classified using pose-clustering; it models the entire geometry of the protein-ligand complex and then estimates the binding affinity complex	45

4	GLIDE	Grid Based Ligand Docking with Energetics; uses a grid-based scheme is used for docking, representation the shape and properties of the receptor; whereas OPLS-AA force field is used to search for conformational, orientational and positional space of the docked ligand	46
5	GOLD	performs flexible docking; uses genetic algorithm for the conformational search; a powerful tool for screening of novel lead compounds; provides high accuracy and reliability	47
6	SURFLEX	performs fully automatic and flexible molecular docking; provides results evaluated for reliability and accuracy in comparison to the existing crystallographic experimental results	48

It is extremely important to visualize the results of the docking studies which we have performed. For the visualization of the results, we use software like Chimera, VMD (Visual Molecular Display), Discovery Studio etc [49, 50].

In order to find the "right" posture for the ligand, scoring functions are employed to evaluate and rank the final poses of the ligand after conformationally sampling the interactions between the ligand and target.

2.3.4 Docking score

The technique of rating a posture by measuring the amount of advantageous intermolecular interactions, such as hydrogen bonds and hydrophobic contacts, is known as scoring. The procedure of ranking follows that. It involves identifying the ligands that are most likely to bind favourably with a certain receptor. After two molecules have docked, scoring functions are mathematical operations that roughly estimate the binding affinity between them. In order to anticipate the intensity of the interactions between proteins or between proteins and DNA, scoring systems have also been devised [51].

2.3.5 Docking by Autodock

It has been demonstrated that Autodock can sometimes reproduce the crystallographic pose of ligand-protein complexes better than DOCK, FlexX, and GOLD, making it a viable choice for further investigation. 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. This method determines the lowest energy conformation in the target site after exploring the various orientations and conformations of the whole ligand in relation to the energy grids for the specified number of energy evaluations. Particularly useful for modelling systems with many of rotatable bonds and various conformations is the LGA [52-54].

2.4 Molecular Dynamics Simulations

To study the structure and dynamics of biomolecules, computer simulations are very significant tools. A molecular dynamics simulation of nucleic acids is now a field that has gained considerable growth. It utilizes classical approach using Newton’s law of motion and classical force fields. This relates the nuclear structure of the system with its energy.

The foundation of all molecular mechanics processes is Born-Oppenheimer approximation which allows the separation of nuclear and electronic motion. So the energy of a system can be expressed as a function of nuclear coordinates only. Molecular Dynamics (MD) is a classical tool based on already defined force fields [55].

For proteins to operate properly, their internal movements and the effects of their inherent dynamic nature are crucial. At various time scales, the dynamic properties, such as straightforward conformational changes, bending modes, ligand binding, protein folding, etc., vary. A molecule's time-dependent connections with other molecules or its surroundings lead to its biological activity. These time-dependent interactions were computed using molecular dynamics (MD) simulations, which are computer models of the physical movements of atoms and molecules across time. When compared to experimental methods, MD simulations offer an atomistic picture

of the dynamic characteristics of biomolecules at various time scales, which is sometimes impossible or difficult to compute experimentally [56, 57].

Molecular dynamics simulation is a significant computer method for describing physical motion of atoms and molecules, i.e. how the locations, velocities, and orientations of molecules change with respect to each other over time. MD is also known as a virtual experiment since it acts as a bridge between theory and laboratory experiment. When doing real-world trials would be too expensive, time-consuming, or impossible, MD is typically utilised.

Table 2.2: Different types of statistical ensembles used in MD simulation [58].

Ensemble	**Properties**	**Partition Function**
Microcanonical	Number of particles(N), volume (V) and energy (E) are constant	Ω(E) Where Ω(E) is the number of micro-states corresponding the system's energy E
Canonical	Number of particles(N), volume (V) and Temperature (T) are constant	$Z_{NVT} = \sum_i e^{-\beta E_i}$ Where $\beta = \frac{1}{KT}$ and E_i is the total energy of the system in the respective microstate
Grand canonical	Chemical potential (μ), volume (V) and Temperature (T) are constant	$Z_{\mu VT} = \sum_i e^{\frac{(N_i \mu - E_i)}{KT}}$ Where $\beta = \frac{1}{KT}$, N_i is total number of particles and E_i is the total energy of the system in the respective microstate

In a molecular dynamics simulation, atoms and molecules are prompted to interact for a specified amount of time. While the forces and potential energy are determined using force fields based on molecular mechanics, the trajectories of atoms and molecules are calculated by mathematically solving Newton's equations of motion [59]. Long-range MD simulations are theoretically challenging to simulate because they produce some mistakes in numerical integration. These errors can be reduced by applying certain procedures, but they cannot be completely eliminated. A crucial tool

for analysing structural and dynamical characteristics at various scales of time and space is molecular dynamics. The classical mechanics method and quantum molecular dynamics are the two primary families of molecular dynamics. The dynamics of the system are quantified using classical molecular dynamics, and the nature of chemical bonds is revealed by a quantum molecular dynamics technique. Numerous biological issues can benefit greatly from quantum molecular dynamics, but these calculations are expensive and demand greater processing power. In a simulation, the selection of the ensemble to be used is another concern.

Simulation can be done with a constant NPT and NVT ensemble. It appears that the NPT ensemble is a generally accepted method for modelling bio membranes and other structures. One may clearly grasp the various kinds of statistical ensembles utilised in MD simulation [60] from table 2.2.

2.4.1 Basic scheme of Molecular Dynamics

When the system under consideration contains large number of atoms then the quantum mechanical description is not sufficient. Hence classical mechanics describes a method which is used to approximate the motion executed by heavy atoms of molecule. The general scheme of MD simulation is obtained by numerically integrating Newton's equations of motions for any system of interest, given by:

$$\boldsymbol{F}_i = m_i.\boldsymbol{a} = m_i.\frac{d^2\mathbf{r_i}}{dt^2} = -\boldsymbol{\nabla_i}[U(r_1, r_2, r_3 \dots\dots\dots\dots r_n)] \quad \text{, i=1,N} \qquad (2.24)$$

By solving these equations of motion, the position and velocities as a function of time are obtained. Here m_i and r_i are the mass and position of particle i and U is the potential energy which depends on positions of N particles in the system. In the molecular system we can divide Newton's equation into two parts-

a) Evaluation of energies and underlying forces
b) Propagations of atomic positions and velocities

The force field equation contains several energy terms to represent each, chemical bonds, angles and improper torsions and also rotations about bonds and pair wise non-bonded interactions (Van der Waals and Columbic interactions). Sum of all pairs of effective potential [61], as shown in equation below:

$$U(r_1, r_2, r_3 \ldots r_n) = \sum \frac{1}{2} K_b (b - b_0)^2 + \sum \frac{1}{2} K_\theta (\theta - \theta_0)^2 + \sum \frac{1}{2} K_w (w - w_0)^2 +$$
$$\sum k_\phi [1 + \cos(nx - \tau)] + + \sum \{ 4_{ij} \left[\left(\frac{\sigma_{ij}}{r} \right)^{12} - \left(\frac{\sigma_{ij}}{r} \right)^{6} \right] + \frac{q_i q_j}{r} \} \qquad (2.25)$$

Here k_b, k_θ, k_w and k_φ are force constants.

2.4.2 Steps in MD simulation

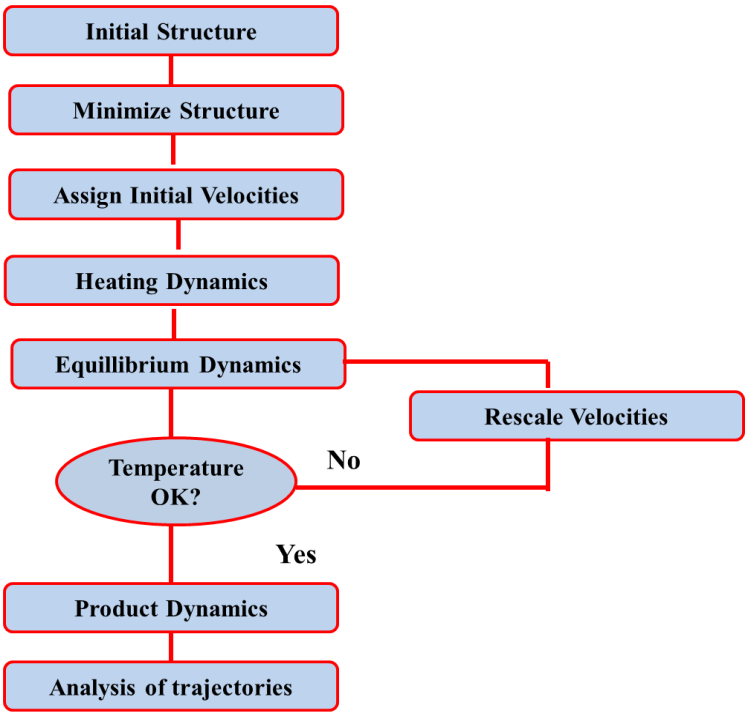


Figure 2.3. Steps in MD Simulation.

Before beginning the MD simulation, a starting set of coordinates or configuration for the simulated molecule is needed. In most cases, the structures retrieved from the protein data bank [62] come through X-ray scattering or NMR investigations. Pauli repulsions, a type of high energy interaction in these crystal formations, cause local distortion and an unstable simulation as a result. Therefore, these systems required energy minimization prior to the calculation, and the result of minimization is a structure with the lowest energy attainable. As a result, this condition corresponds to one where there is no motion and the temperature is close to 0 K [63]. The atoms of the minimised structure are not subjected to any motion or forces. The gradient of the potential energy vector equals zero at the energy minimum, indicating that there is no force acting on any atom in any direction and therefore no motion will be appearing inside the system.

To raise the temperature from 0K to the appropriate value and to supply energy to the system, initial velocities are assigned to the atoms matching to a Gaussian distribution. The system is then allowed to propagate through time by integrating Newton's equations of motion after that. The kinetic energy exhibits the exact same behaviour first, followed by the temperature. Since the total energy does not change during the equilibration process but the kinetic and potential energies behave differently, this indicates that the total energy is distributed amongst the system's components in a vibrating fashion.

After the system has been heated, the structure can be unstable, which might cause the temperature to decrease fast [61]. Therefore, the system must be adjusted before the production run. Equilibration refers to the process through which the system's kinetic and potential energies are distributed equally. The velocities are rescaled to desirable levels at the desired temperature for the constant amount of time. Repeating this approach until thermodynamic terms, such as temperature and energy, are held in a specific, narrow interval for a considerable amount of time causes the simulation to become stable with regard to time [64].

Table 2.3: Molecular Dynamics Softwares used in present research.

S. No.	Software	Description	Ref.
1	AMBER	provides a set of inbuilt modules to apply the AMBER forcefields for simulations of biomolecules viz., LEAP, antechamber, sander, pmemd, cpptraj, mmpbsa, etc.	65
3	GROMACS	excellent CUDA-based GPU acceleration; user-friendly, as topologies and parameter files are written as text format; all programs use a simple interface; keeps updating about the progress of the simulation; it’s a free software.	66

2.5 Free Energy Calculations

A system's free energy and the optimum amount of work it can perform under thermodynamic conditions are connected. A process's thermodynamic favorability or prohibition is shown by the sign of the change in free energy. There are two different forms of free energy; the first is the Helmholtz free energy 'A' for a system with a constant particle number, volume, and temperature. The second is the Gibbs free energy, which is appropriate for systems with constant pressure, temperature, and particle number. The majority of experiments are performed with constant NPT, with the Gibbs function serving as the suitable free energy quantity. Because liquids and macromolecules have lowest energy configurations divided by low energy barriers, it is particularly challenging to determine the free energy of these substances. For drug-DNA complexes, binding free energy is vital to find new drug candidates, which bind to the target DNA [67, 68].

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[69, 70] 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. Even FEP (Free Energy Perturbation) and TI (Thermodynamic Integration) are extensively laborious and need too much calculating power to be cast of on a large scale.

2.5.1 MMPBSA/MMGBSA method

MM/PBSA or MMGBSA methods depend on the calculations of average potential and solvation free energies which use MD simulations of free ligands, free receptor, and their complexes. In this approach, ΔG_{bind} is estimated using equation [71]

$$\Delta G_{bind} = G_{RL} - G_R - G_L \tag{2.26}$$

Where, G_{RL}, G_R and G_L are the free energies of receptor-ligand complex, ligand and receptor (DNA/Protein) respectively. The state free energy i.e, R, L or RL is estimated from the following expression.

$$G = G_{MM} + G_{pol} + G_{np} - TS \quad (2.27)$$

The first term in expression in expression 2.27 is the classical Molecular Mechanics energy which comprises of bonded (bond, angle and dihedral) terms and non-bonded (electrostatics and van der Waals) terms. Second and third terms represent the polar and nonpolar influences to the solvation free energies.

Polar energy term is assessed by deciphering Poisson-Boltzmann equation by generalized Born model whereas the nonpolar term is estimated by a linear equation to solvent-accessible surface area (SASA). The entropic contribution that depends on the degrees of freedom is included in the last term. Nonpolar solvation energy is responsible for the non-favorable cavity formation and promising van der waals interaction among solute atoms and solvent [72, 73].

$$E_{solv,np} = \gamma A + b \quad (2.28)$$

where A stands for SASA (solvent-accessible surface area), γ and b are empirical constants and they can have distinct values.

The MMPBSA method utilizes 200-500 frames of MD simulation in explicit water and then average is taken while in MMGBSA a single snapshot is used to calculate free energy. Implicit solvent studies have also been performed [74, 75]. The innovative submissions of the MMPBSA techniques can be found in recent reports[76]. Sometimes MM/QMSA method is used when MM/PBSA fails to calculate the binding free energy in which the whole receptor is described by QM [77, 78].

2.6 Hybrid QM/MM methods

In the field of molecular modelling and computational drug design, the electronic structure of macromolecular system is of utmost importance. Since macromolecular or biomolecules are large bodies having a very large no of electrons, the evaluation of electronic structure of such systems has extreme complexity. To treat the whole system with quantum mechanics require a large amount of cost and computational

time. To overcome this problem, Quantum Mechanical/ Molecular Mechanical approach is used extensively [79, 80].

The key idea behind this approach is the division of the system into two parts. One of the two parts is the small chemically active part and the other one is the larger chemically inactive part. The main chemically active part or the interacting part of the macromolecule is treated with Quantum Mechanics whereas the remaining part is treated with Molecular Mechanics. So the name is Quantum Mechanics/ Molecular Mechanics (QM/MM). If there are solvent molecules also present in the system then these molecules are treated molecular mechanically [81, 82].

QM method provides the accuracy whereas MM method gives the required efficiency and speed. The balance between accuracy and efficiency gives the method its

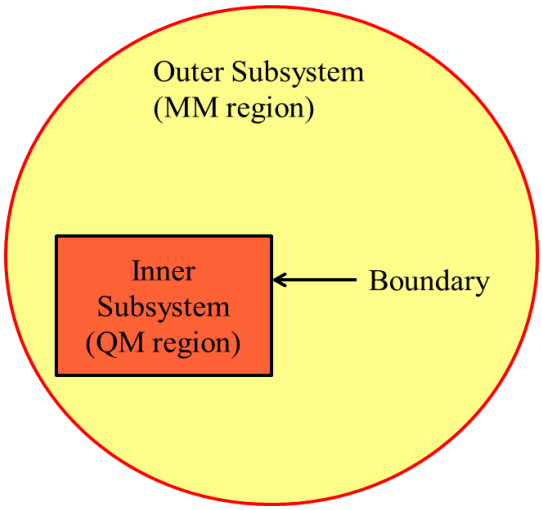


Figure 2.4. A typical QM/MM partitioning Scheme.

importance and need.

Warshel and Levitt introduced the QM/MM idea for the first time in 1976, using it to examine the chemical process in lysozyme [83]. An innovative method for simulating local electronic processes in massive biomolecular systems has emerged: combined QM/MM theory. The fundamental idea behind this approach is to accurately show the chemically active site using quantum mechanics, while molecular mechanics is used to characterise the impact of the bimolecular environment. Because of the combined QM/MM approaches' accuracy and speed, reactive bimolecular systems may be accurately modelled at a reasonable computing cost. The 2013 Nobel Prize in Chemistry was awarded to this discipline for its potential applications. The total energy of the system is calculated using the two available schemes, the additive and

the subtractive scheme [84, 85]. Regarding this boundary scheme, the labeling conventions are given in Figure 2.4.

2.6.1 Additive QM/MM Scheme

In this method, the total energy ($E_{QM\text{-}MM}$ (system)) only has three components: the E_{MM} (MM), the molecular mechanical energy of the MM area alone, the E_{QM} (QM), the quantum mechanical energy of the QM region, and the E_{QMMM} (QM-MM); this term is due to the inclusion of bound and non-bonded interaction between QM/MM regions. The non-bonded term explains the Van der Waals and electrostatic interactions, but the bonded term specifies and contains the bond stretching, bending, and twisting. Following equation 2.29 represents the additive scheme for obtaining QM/MM energy:

$$E_{QM\text{-}MM}\,(\text{system}) = E_{MM}\,(\text{system}) + E_{QM}\,(\text{QM}) - E_{QM\text{-}MM}\,(\text{QM-MM}) \qquad (2.29)$$

2.6.2 Subtractive QM/MM Scheme

It is given by following equation 2.30:

$$E_{QM\text{-}MM}\,(\text{system}) = E_{MM}\,(\text{system}) + E_{QM}\,(\text{QM}) - E_{MM}\,(\text{QM}) \qquad (2.30)$$

where $E_{QM\text{-}MM}$ (system), E_{MM} (system), E_{QM} (QM), E_{MM} (QM) are the total energy, the molecular mechanical energy of the entire system, the quantum mechanical energy of the QM region and the molecular mechanical energy of the QM region, respectively. This scheme's disadvantage is that it only takes into account interactions between the QM and MM regions at the MM level, which is unreliable. It also necessitates the use of MM parameters for the QM area.

The main objective of implying QM/MM methods is that they consider the pairing between the electric field from the surrounding and the Hamiltonian based on quantum mechanics from the region of active site and the boundary between QM and MM region is treated accurately [86, 87]. The most important part of QM/MM calculations is the partitioning of the systems. The basic considerations for QM/MM partitioning are:

a. The QM region should be chosen carefully as per the chemical problem; the QM region should be small which can be enlarged later to check the effect of the QM/MM results.

b. If a QM/MM partition is through such that the covalent bonds cannot be avoided, then only unconjugated single bonds are cut and avoided, preferably without electronically demanding substituents (e.g., cut unpolar C–C bonds).

A protein crystal structure from the protein data bank is frequently used as the starting point for QM/MM calculations. Next, missing hydrogens, then missing atoms, etc., are added. The system is then MM-equilibrated, followed by a run of molecular dynamics, and a few low energy snapshots are obtained and investigated. These final snapshots serve as the foundation for choosing the outside limits of QM/MM computations. The bimolecular system in these structures is solvated in water (20000–30000 atoms), and this setup necessitates a lot of preliminary effort to prevent mistakes and poor decisions during the actual QM/MM calculations.

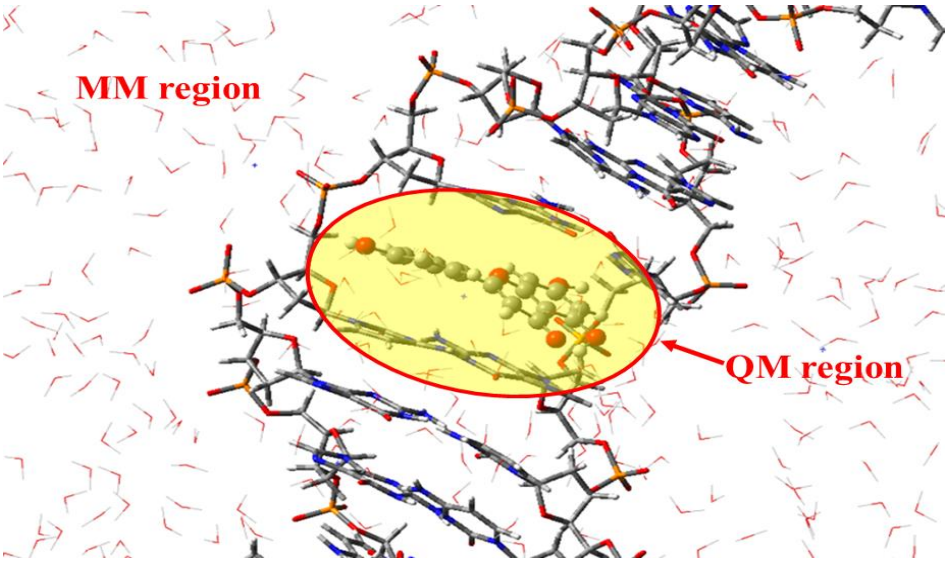


Figure 2.5: A two layered QM/MM Scheme.

ONIOM (*Our N-layered Integrated molecular Orbital + molecular Mechanics*) method is a hybrid computational method was developed by Morokuma and co-workers. This method was developed for lager macromolecular biomolecular systems. In the method, whole system split in layers depending upon chemically active areas [88]. The two layered ONIOM scheme is shown above in figure 2.5. In many studies, the ONIOM method is used for the study of DNA binding drugs [89, 90].

References

1. M. Born, J. R. Oppenheimer, Nuclear and eletronic motion, *Ann. Physik.*, 84 (1927) 457.
2. L.I. Shiff, Quantum Mechanics, McGraw- Hill, NewYork., 3rd edition (1968).
3. E. Schroedinger, Quantisierung als eigenwertproblem, Annalen der physik 385 (13) (1926) 437-490.
4. L. Pauling, E.B. Wilson, Introduction to Quantum Mechanics, McGraw-Hill, New York (1935).
5. B.T. Sutsliffe, The nuclear motion problem in molecular physics. Adv. Quantum. Chem., 28 (1997) 65-80.
6. W. Kolos, L. Wolniewicz, Accurate adiabatic treatment of the ground state of the hydrogen molecule. The Journal of Chemical Physics 41 (12) (1964) 3663-3673.
7. J.A. Pople, D.L. Beveridge, Approximate Molecular Orbital Theory, McGraw-Hill Book Co., New York (1970).
8. D.R. Hartree, The wave mechanics of an atom with a non-Coulomb central field. Part I. Theory and methods. Proc. Cambridge Phil. Soc., 24 (1928) 89-110.
9. J.E. Klepeis, Introduction to first-principles electronic structure methods: Application to actinide materials. Journal of materials research 21 (2006) 2979-2985.
10. J.A. Pople, D.L. Beveridge, P. A. Dobosh, Approximate self-consistent molecular-orbital theory. V. Intermediate neglect of differential overlap J. Chem. Phys., 47 (1967) 2026-2033.
11. W. Pauli, On the connexion between the completion of electron groups in an atom with the complex structure of spectra. Zeitschrift für Physik 31 (1925) 765.
12. J.C. Slater, Cohesion in monovalent metals. Physical Review 35(5) (1930) 509.
13. X. Assfeld, J.L. Rivail, Quantum chemical computations on parts of large molecules: the ab initio local self-consistent method, Chem. Phys. Lett. 263 (1996) 100–106.
14. I.N. Levine, Quantum Chemistry, Pearson, Fifth edition (2000).

15. C. Lee, W. Yang, R.G. Parr, Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density, Phys. Rev. B. 37 (1988) 785.
16. A.J. Cohen, P. Mori-S´anchez, W. Yang, Challenges for density functional theory, Chem. Rev. 112(1) (2011) 289–320.
17. F. Neese, A critical evaluation of DFT, including time-dependent DFT, applied to bioinorganic chemistry. JBIC, J. Biol. Inorg. Chem. 11 (2006) 702−711.
18. S.H. Vosko, L. Wilk, M. Nusair, Accurate spin-dependent electron liquid correlation energies for local spin density calculations: a critical analysis. Canadian Journal of physics, 58(8) (1980) 1200-1211.
19. J.P. Perdew, Y. Wang, Pair-distribution function and its coupling-constant average for the spin-polarized electron gas, Physical Review B 46 (20) (1992) 12947.
20. A.D. Becke, Perspective on ``Density functional thermochemistry. III. The role of exact exchange", J. Chem. Phys., 98 (1993) 5648.
21. R.G. Parr, W. Yang, Density Functional Theory, Oxford University Press (1989).
22. T. Helgaker, P.R. Taylor, Modern Electronic Structure Theory, Part II (D. Yarkony ed.), World Scientific, pp. 727 (1995).
23. M.J. Frisch, J.A. Pople, J.S. Binkley. Self-consistent molecular orbital methods 25. Supplementary functions for Gaussian basis sets, J. Chem. Phy. 80 (1984) 3265-3269.
24. M.J. Field, L.D. Moleculaire, C. Grenoble, Practical introduction to the simulation of molecular systems. Second edn. Cambridge University Press (2007).
25. J.W. Ponder, D.A. Case, Force fields for protein simulations, Adv. Prot. Chem. 66 (2003): 27-85.
26. W.D. Cornell, P. Cieplak, C.I. Bayly, I.R. Gould, K.M. Merz, D.M, Ferguson, D.C. Spellmeyer, T. Fox, J.W. Caldwell, P.A., Kollman, A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. Journal of the American Chemical Society, 117(19) (1995) 5179-5197.
27. S.J. Weiner, P.A. Kollman, D.A. Case, U.C. Singh, C. Ghio, G. Alagona, S. Profetaand, P. Weiner, A new force field for molecular mechanical simulation

of nucleic acids and proteins. Journal of the American Chemical Society, 106(3) (1984) 765-784.

28. X. Daura, A.E. Mark, W.F.V. Gunsteren, Parametrization of aliphatic CHn united atoms of GROMOS96 force field. J. Comp. Chem. 19(5) (1998) 535-547.
29. B.R. Brooks, R.E. Bruccoleri, B.D. Olafson, D.J. States, S. Swaminathan, M. Karplus, CHARMM:A program for macromolecular energy, minimization, and dynamics calculations, J. Comput. Chem. 4 (2) (1983) 187–217
30. Gunsteren van, W.F., Berendsen H.J.C., GROMOS 86: Groningen Molecular Simulation Program Package; University of Groningen: Groningen, The Netherlands (1986).
31. G.L.Warren, C.W. Andrews, A.-M. Capelli, B. Clarke, J. LaLonde, M.H. Lambert, M. Lindvall, N. Nevins, S.F. Semus, S. Senger, G. Tedesco, I.D. Wall, J.M. Woolven, C.E. Peishoff, M.S. Head, A Critical Assessment of Docking Programs and Scoring Functions. J. Med. Chem. 49 (2006) 5912−5931.
32. D.B. Kitchen, H. Decornez, J.R. Furr, J. Bajorath, Docking and scoring in virtual screening for drug discovery: methods and applications. Nature Reviews. Drug Discovery 3 (11) (2004) 935–949.
33. E. Yuriev, J. Holien, P.A. Ramsland, Improvements, trends, and new ideas in molecular docking: 2012−2013 in review. J. Mol. Recognit. 28 (2015) 581−604.
34. B.Q. Wei, L.H. Weaver, A.M. Ferrari, B.W. Matthews, B.K. Shoichet, Testing a flexible-receptor docking algorithm in a model binding site. Journal of Molecular Biology 337 (5) (2004) 1161–1182.
35. B.D. Bursulaya, M. Totrov, R. Abagyan, C.L. Brooks,Comparative study of several algorithms for flexible ligand docking. Journal of Computer-Aided Molecular Design 17 (11) (2003) 755–763.
36. X.Y. Meng, H.X. Zhang, M. Mezei, M. Cui, Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery. Curr. Comput. Aided Drug Des. 7 (2011) 146−157.
37. I.A. Guedes, C.S. de Magalhães, L.E. Dardenne, Receptor−ligand molecular docking. Biophys. Rev. 6 (2014) 75−87.

38. S. Agarwal, R. Mehrotra, An Overview of Molecular Docking. JSM Chem. 4 (2016) 1024.
39. F.N. Novikov, G.G. Chilov, Molecular docking: Theoretical background, practical applications and perspectives. Mendeleev Commun., 19 (5) (2009) 237−242.
40. N. Moitessier, P. Englebienne, D. Lee, J. Lawandi, C. Corbeil, Towards the development of universal, fast and highly accurate docking/scoring methods: a long way to go. Br. J. Pharmacol. 153 (2007) S7-S26.
41. S.F. Sousa, A.J.M. Ribeiro, J.T.S. Coimbra, R.P.P. Neves, S.A. Martins, N.S.H.N. Moorthy, P.A. Fernandes, M.J. Ramos, Protein-Ligand Docking in the New Millennium − A Retrospective of 10 Years in the Field. Curr. Med. Chem. 20 (2013) 2296−2314.
42. B.J. Ruppert, W. Welch, A.N. Jain, Automatic identification and representation of protein binding sites for molecular docking. Protein Sci. 6 (1997) 524–33.
43. G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A. J.Olson, AutoDock4 and AutoDockTools4 Automated Docking with Selective Receptor Flexibility. J. Comput. Chem. 30 (2009) 2785−2791.
44. K. Ronald, M.A. Irwin, D. Kuntz, C.M. Oshiro, Molecular docking to ensembles of protein structures. J. Mol. Biol. 266 (1997) 424-440.
45. R. Matthias, B. Kramer, T. Lengauer, G. Klebe, A fast flexible docking method using an incremental construction algorithm. J. Mol. Biol. 261 (1996) 470-489.
46. A. Friesner, J.L. Banks, R.B. Murphy, T.A. Halgren, J.J. Klicic, D.T. Mainz, M.P. Repasky, E.H. Knoll, M. Shelley, J.K. Perry, D.E. Shaw, P. Francis, P.S. Shenkin, Glide: a new approach for rapid, accurate docking and scoring. Method and assessment of docking accuracy. J. Med. Chem. 47 (2004) 1739–1749.
47. M.L. Verdonk, J.C. Cole, M.J. Hartshorn, C.W. Murray, R.D. Taylor, Improved protein−ligand docking using GOLD. Proteins: Struct., Funct., Genet. 52 (2003) 609−623.
48. A.N. Jain, Surflex: fully automatic flexible molecular docking using a molecular similarity-based search engine. J. Med. Chem. 46 (2003) 499–511.

49. E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, UCSF Chimera-A visualization system for exploratory research and analysis. J. Comput. Chem. 25 (2004) 1605−1612.
50. W. Humphrey, A. Dalke, K. Schulten, VMD: visual molecular dynamics. J Mol. Graph. 14(1) (1996) 33-38.
51. G.A. Balaji, V.N. Balaji, S.N. Rao, Utility of scoring function customization in docking-based virtual screening approaches. Curr. Sci. 104 (1) (2013) 86−97.
52. G. Jones, P. Willett, R.C. Glen, A.R. Leach, R. Taylor, Development and validation of a genetic algorithm for flexible docking1. J. Mol. Biol. 267 (1997) 727−748.
53. G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew, A.J. Olson, Automated Docking Using a Lamarkian Genetic Algorithm and an Empirical Binding Free Energy Function. J. Comput. Chem. 19 (1998) 1639–1662.
54. P.A. Holt, J.B. Chaires, J.O. Trent, Molecular Docking of Intercalators and Groove-Binders to Nucleic Acids Using Autodock and Surflex J. Chem. Inf. Model. 48 (2008) 1602–1615.
55. H. Wang, C. Laughton, Molecular modelling methods for prediction of sequence-selectivity in DNA recognition, Methods 42 (2007) 196-203.
56. D.L. Beveridge, T.E. Cheatham, M. Mezei, The ABCs of molecular dynamics simulations on B-DNA, J. Biosci. 37 (2012) 379-397.
57. P. Mark, L. Nilsson, Structure and dynamics of the TIP3P, SPC, and SPC/E water models at 298 K, J. Phys. Chem. A. 105 (2001) 9954-9960.
58. Frenkel D, Smit B.,Understanding molecular simulations: from algorithms to applications, 2nd edn. vol 1, Computational Science Series Academic Press, San Diego(2002).
59. G. Alagona, S. Profeta, P. Weiner, A new force field for molecular mechanical simulation of nucleic acids and proteins, J. Am. Chem. Soc. 106(3) (1984) 765–784.
60. M.P. Allen, D.J. Tildesley, Computer simulations of liquids. Clarendon Press, Oxford(1987).

61. B. Zeigler, H. Praehofer, T. Kim (eds) Theory of modeling and simulation: integrating discrete event and continuous complex dynamic systems, 2nd edn. Academic Press, NewYork (2000).
62. H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The Protein Data Bank. Nucleic Acids Research 28 (2000) 235-242.
63. V.E. Rudi, C.D. Hubbard, eds. Chemistry under extreme and non-classical conditions. John Wiley & Sons, (1996).
64. P. Várnai, K. Zakrzewska, DNA and its counterions: a molecular dynamics study, Nucl. Acids Res. 32 (2004) 4269-4280.
65. D.A. Case, T.A. Darden, T.E. Cheatham, C.L. Simmerling, J. Wang, R.E. Duke, R. Luo, K.M. Merk, D.A. Pearlman, M. Crowley, R.C. Walker, W. Zhang, B. Wang, S. Hayik, A. Roitberg, G. Seabra, K.F. Wong, F. Paesani, X. Wu, S. Brozell, V. Tsui, H. Gohlke, L. Yang, C. Tan, J. Mongan, V. Hornak, G. Cui, P. Beroza, D.H. Mathews, C. Schafmeister, W.S. Ross, P.A. Kollman, AMBER 9; AMBER: San Francisco, CA, 2008.
66. S. Pronk, S. Pall, R. Schulz, P. Larsson, P. Bjelkmar, R. Apostolov, M.R. Shirts, J.C. Smith, P.M. Kasson, D. van der Spoel, B. Hess, E. Lindahl, GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. Bioinformatics 29 (2013) 845−854.
67. P. Kollman, Free energy calculations: Applications to chemical and biochemical phenomena. Chem. Rev. 93 (1993) 2395−2417.
68. A. Wlodawer, Rational Approach to AIDS Drug Design Through Structural Biology, Annu. Rev. Med. 53 (2002) 595-614.
69. J.M. Wang, T.J. Hou, X.J. Xu, Recent advances in free energy calculations with a combination of molecular mechanics and continuum models. Curr. Comput.-Aided Drug Des. 2 (2006) 287-306.
70. W. Wang, O. Donini, C.M. Reyes, P.A. Kollman, Biomolecular simulations: recent developments in force fields, simulations of enzyme catalysis, protein-ligand, protein-protein, and protein-nucleic acid noncovalent interactions. Annu Rev Biophys Biomol Struct. 30 (2001) 211-43.
71. A. Perdih, U. Bren,T. Solmajer, Binding free energy calculations of N-sulphonyl-glutamic acid inhibitors of MurD ligase. Journal of molecular Modeling, 15(8) (2009) 983-996.

72. F. Fogolari, A. Brigo, H. Molinari, Protocol for MM/PBSA molecular dynamics simulations of proteins. Biophys. J. 85(1) (2003)159-166.
73. H. Gohlke, D.A. Case, Converging free energy estimates: MM-PB (GB) SA studies on the protein–protein complex Ras–Raf. J. Comp. Chem. 25(2) (2004) 238-250.
74. D.A. Pearlman, Evaluating the molecular mechanics Poisson− Boltzmann surface area free energy method using a congeneric series of ligands to p38 MAP kinase, J. med. chem. 48 (24) (2005) 7796-7807.
75. B. Tidor, M. Karplus, The contribution of vibrational entropy to molecular association: the dimerization of insulin, J. Mol. Biol. 238(3) (1994) 405-414.
76. U. Yadava, S.K. Yadav, R.K. Yadav, Electronic structure, vibrational assignments and simulation studies with A/T rich DNA duplex of an aromatic bis-amidine derivative. DNA repair 60 (2017) 9-17.
77. V.M. Anisimov, C.N. Cavasotto, Quantum mechanical binding free energy calculation for phosphopeptide inhibitors of the Lck SH2 domain, J. Comput. Chem. 32 (2011) 2254-2263.
78. F.M. Ytreberg, R.H. Swendsen, D.M. Zuckerman, Comparison of free energy methods for molecular systems. J. Chem. Phys. 125 (2006) 184114.
79. H.M. Senn, W. Theil, QM/MM methods for biological systems in Topics in Current Chemistry. M. Reiher (Ed.), Springer, Berlin, 268 (2007) 173.
80. A.E. Cho, D. Rinaldo, Extension of QM/MM docking and its applications to metalloproteins. J Comput Chem 30 (2009) 2609-2616.
81. P. Sherwood, H.V. Alex, F.G. Martyn, S. Georg, C. Richard, A. Catlow, S.A. French, A.A. Sokol, QUASI: A general purpose implementation of the QM/MM approach and its application to problems in catalysis." Journal of Molecular Structure: THEOCHEM 632 (2003) 1-28.
82. A. Ciancetta, S. Genheden, U. Ryde, A QM/MM study of the binding of RAPTA ligands to cathepsin B, J. Comput.-Aided Mol. Des. 25 (2011) 729−742.
83. A. Warshel, L. Michael, Theoretical studies of enzymic reactions: dielectric, electrostatic and steric stabilization of the carbonium ion in the reaction of lysozyme. J. Mol. Biol. 103 (1976) 227-249.

84. P. Sherwood, R.B. Bernard, S.P.S. Mark, Multiscale methods for macromolecular simulations. Current opinion in structural biology. 18 (2008) 630-640.
85. H.M. Senn, W. Thiel, QM/MM methods for biomolecular systems. Angewandte Chemie International Edition, 48(7) (2009) 1198-1229.
86. A. Monari, J.L. Rivail, X. Assfeld, Theoretical modeling of large molecular systems. Advances in the local self-consistent field method for mixed quantum mechanics/molecular mechanics, Acc. Chem. Res. 46 (2013) 596–603.
87. C. Curutchet, A. Munoz-Losa, S. Monti, J. Kongsted, G.D. Scholes, B. Mennucci, Electronic energy transfer in condensed phase studied by a polarizable QM/MM model, J. Chem. Theory Comp. 5 (2009) 1838–1848.
88. M.J. Frisch, Combining quantum mechanics methods with molecular mechanics methods in ONIOM, J. Chem. Theory. Comput. 2 (2006) 815-826.
89. H. M. Senn, W. Thiel, QM/MM Methods for Biomolecular Systems. Angew. Chem., Int. Ed. 48 (2009) 1198−1229.
90. A. Robertazzi, J.A. Platts, A QM/MM study of cisplatin–DNA oligonucleotides: from simple models to realistic systems. Chemistry–A European Journal, 12(22) (2006) 5747-5756.

CHAPTER-3

EVALUATION OF BINDING PROPERTIES OF SOME COMMON DRUGS WITH DNA AS INTERCALATOR AND GROOVE BINDER

3.1 Introduction

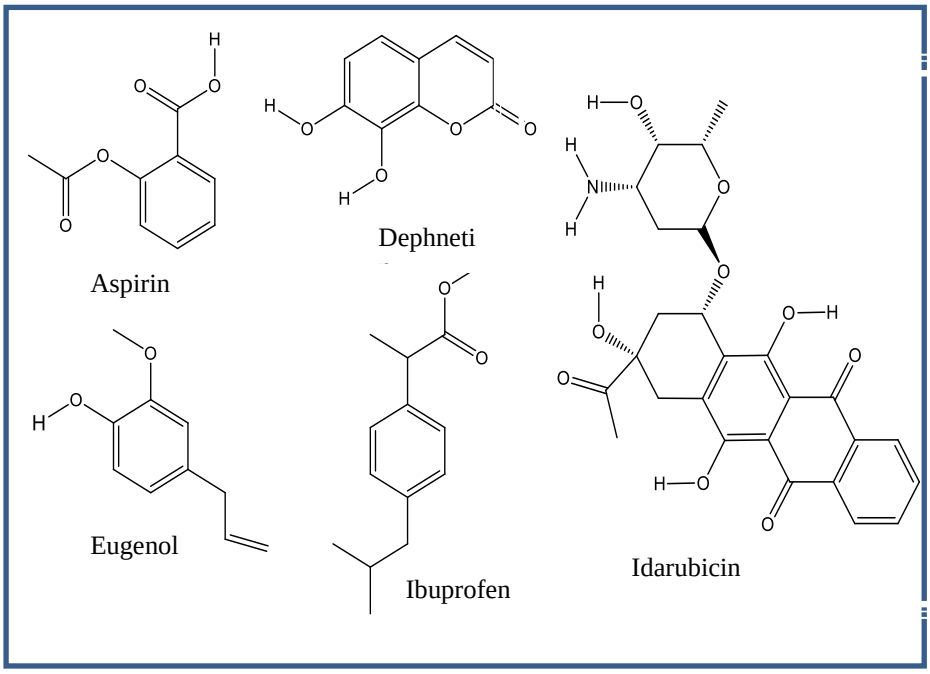
The importance of computational studies in present era is growing rapidly. Especially in medicine and pharmaceutical field. Since the advent of computational tools, enormous computational studies are going on to produce favourable results. Molecular modelling tools are easier, precise, cost and time efficient tools for research and studies. Molecular docking is one such platform which can predict the mode and position of binding of a targeted ligand into a macromolecule i.e, the preferential binding site of target for the ligand [1, 2]. Molecular docking is a technique of molecular modelling where the study, simulation and analysis of the interaction of two or more molecules is done. Binding energy, total energy, docking score etc. are calculated on the basis of docking simulations and then the docking with least energy is preferred [3, 4]. These computational studies are of enormous interest in the field of medicinal or clinical chemistry. Using the data calculated, like docking score etc. we can draw conclusions as to which drug is preferable in which disease. A large number of docking studies have been done to analyse and calculate the effect of different drugs on DNA [5-7].

Deoxyribonucleic acid (DNA) has a significant biological importance. DNA is composed of two polynucleotide chains which contribute in forming the double helical structure of DNA. The nature of the two strands is complementary. The bases are accessible through the major and minor groove [8]. DNA is also the central target of many anticancer and antibiotic drugs. Interaction between DNA and drug molecules is a very popular subject especially for the designing and modelling of novel DNA-aimed drugs and their in vitro screening. DNA interacts with small molecules like drugs via two modes, covalent and non- covalent interactions. Non-covalent interactions have mainly three modes which are electrostatic interactions,

groove binding and intercalative binding. Out of these modes of binding, groove binding can be classified as major and minor groove binding [9,10]. In present study of drugs with DNA intercalative and groove binding are of prime focus.

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 [11,12]. This theoretical study will further enlighten the usefulness of these drugs.

Non-steroidal anti-inflammatory medication aspirin has a variety of pharmacological and biological effects. Understanding how Aspirin interacts with DNA complexes can help in the development of new, more potent medications that can target DNA. Additionally, it expands the potential for efficient therapeutic agents for the regulation of gene expression. This analysis has been done by Zhu et.al. by experimental



Fi**gure 3.1**: Structure of used drugs.

techniques [13].

Zhou et.al. reported groove binding type interaction between the drug Daphnetin and calf thymus DNA [14]. In further studies, the detailed interaction study of Daphnetin with bovine serum albumin was done, and remarkable anti-cancer drug type behaviour of Daphnetin was revealed [15-17].

Eugenol, which is 4-allyl-2-methoxyphenol, a phytochemical, is a phenolic component of Syzigium aromaticum (cloves). It has been utilized as an analgesic, antiseptic and antibacterial durg. Eugenol is claimed to possess a number of biological charactertistics like antioxidant, antiviral, anti-inflammatory, etc. [18-20]. Shuyun Bi reported intercalative binding of Eugenol with DNA [21].

Ibuprofen is also an NSAID (nonsteroidal anti-inflammatory drug) having anticancer activities. Studies suggested intercalative mode of binding in Ibuprofen-DNA binding [22]. Measurement of size of binding site and viscosity confirmed the mixed binding mode of attachment as revealed in molecular docking investigation, i.e. intercalation as well as groove binding [23].

Can Ozluer et.al. studied the interaction of antitumor and anticancer drug, idarubicin with DNA by various spectroscopic methods and reported intercalative mode of binding [24, 25]. The DNA sequences used for molecular docking are having PDB ID 1BNA and 1N37. 1BNA is the PDB ID of crystal structure of a B-DNA dodecamer. 1N37 is a double stranded DNA molecule having intercalation gap between its base pairs. Binding energy for each pair of drug and DNA is determined. The docked results were compared with experimental data to test the accuracy of the system put under consideration. One more important approach of this study is to analyse and understand the dependency of interaction between a macromolecule and ligand on their respective structural shape and size. The main reason for taking two types of DNA is to check the differences in binding energies and binding sites of the drug-DNA system for two different types of DNA sequences.

3.2 Computational Details

3.2.1 Dataset: The crystal structures of DNA sequences having PDB ID - 1BNA and 1N37 were sourced from RCSB 'Protein Data Bank' (PDB) [26]. All crystallographic water and small molecules were extracted from the DNA sequences by the help of UCSF Chimera Software [27]. Hydrogen atoms were also added. Table 1 shows the PDB ID and sequence of the DNA used.

Drugs: The structures of Aspirin, Daphentin, Eugenol, Ibuprofen and Idarubicin were collected from literature [11-12, 28-29]. Figure 3.1 shows the chemical structures of these drugs.

3.2.2 Geometry Optimization: The geometry optimization of the ligands was

achieved using Gaussian 09 software package at B3LYP level and 6-31G basis set [30].

Table 3.1: PDB ID and sequence of the DNA used.

S.No.	PDB ID	DNA Sequence
1	1BNA	5'-CGCGAATTCGCG-3'
2	1N37	5'-AGACGTCT-3'

3.2.3. Molecular Docking

'Lamarckian Genetic Algorithm' (LGA) in Autodock 4.2 software was applied for molecular docking simulations [31]. 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) [32]. 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.

3.3 Result and Discussions

3.3.1 Optimized Geometries

It is very important to bring the system of consideration into lowest possible energy state before doing molecular docking process, so the step of geometry optimization is done. It gives the state of the system with minimum steric hindrances and charge repulsions. The system achieves maximum bond lengths, angles, and dihedral angles in this condition. Additionally, the electrostatic charges also have a tendency to be evenly dispersed across the entire system, which causes an electron transfer between a specific pair of atoms. As a result, no damage to the chemical structures is produced. Figure 3.2 represents the three-dimensional optimized geometries of all the drugs after optimization by Gaussian 09 software at B3LYP density functional with basis set 631-

G. These optimized ligands were used as initial inputs for the molecular docking

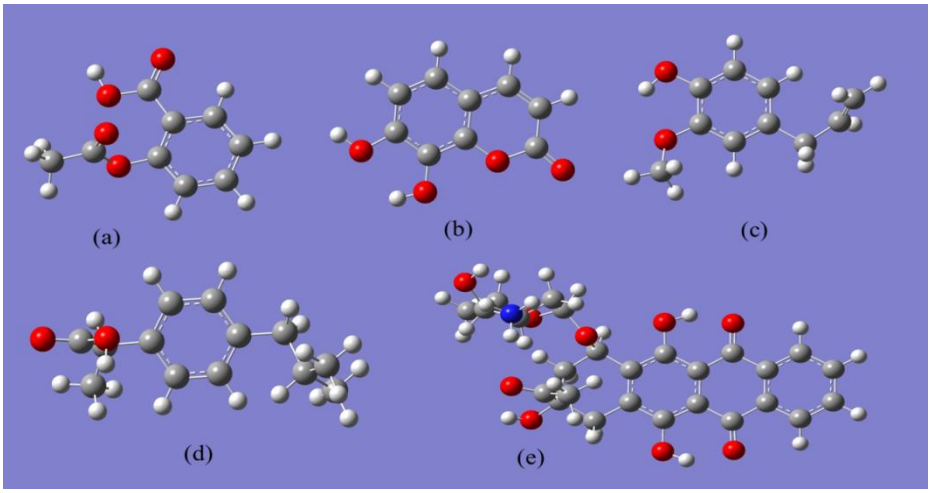


Figure 3.2. Optimized geometries of all the drugs. (a) Aspirin, (b) Daphentin, (c) Eugenol, (d) Ibuprofen, (d) Idarubicin

process.

3.3.2 Molecular Docking studies

The present work employed a molecular docking technique to determine how different drugs interact to DNA structures. It is an important and effective tool to gain information regarding the structural characteristics of ligand-macromolecule complexes and the efficiency of binding of a ligand with its receptor, which can support and verify the experimental results. The results obtained from molecular docking done by Autodock software for each DNA sequence are summarized in below sections.

Molecular docking studies were done for the two sets of DNA segments in search of their preferential binding modes, corresponding binding affinities and for gaining atomistic insights regarding the factors that contribute to the stable complex formation.

3.3.2.1 Drugs with 1BNA

Binding energies obtained from computational docking method for the DNA sequence with PDB ID 1BNA is tabulated in Table 3.2. From the resulting docking complexes, as given in Table 3.3 and shown in Figure 3.2, it is clear that the mode of binding

interaction of drugs with sequence 1BNA shows mainly minor groove binding whereas. The main reason for this type of difference in binding was mainly the structure of the DNA sequence. Idarubicin, being the largest compound binds to the major groove of 1BNA. All the results were compared with experimental data from previous studies [11, 14, 21-24]. For 1BNA, Idarubicin -DNA complex is most stable having strongest binding between them.

Clear cross-sectional view of the DNA-ligand complexes is visible in Figure 3.3. Small sized ligands find the place in the minor groove of 1BNA where Idarubicin which has larger size attaches itself to the major groove of DNA.

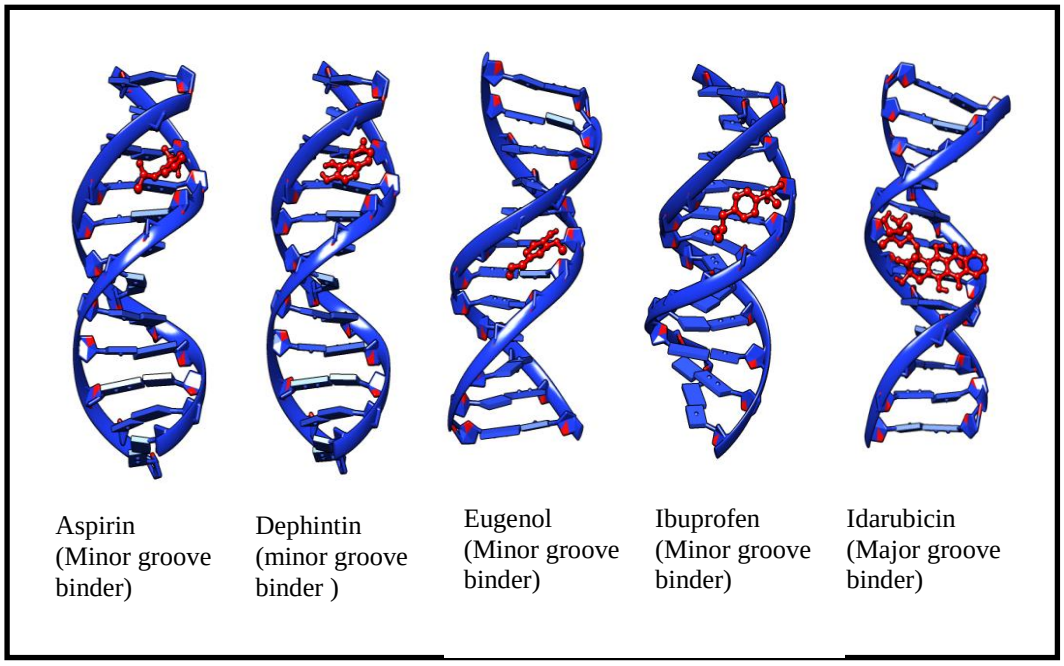


Figure 3.3. Molecular Docking between drugs and 1BNA.

3.3.2.2 Drugs with 1N37

Binding energies obtained from computational docking method for the DNA sequences with PDB ID 1N37 is tabulated in Table 3.2. From the resulting docking complexes, as given in Table 3.3, shown in Figure 3.3 it is clear that the mode of binding interaction of drugs with sequence 1N37 is intercalation. The main reason for this 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, as shown in Figure 3.3. Idarubicin, the largest compound also binds in the intercalative gap of 1N37. All the results were compared with experimental data from previous studies [11, 14, 21-24]. 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.

Figure 3.4 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.

Table 3.2: 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**	**-**

3.3.3. Hydrogen binding analysis

For each best docked pose of the drug-DNA complex system, detailed hydrogen bond analysis was performed to get total information about the importance of hydrogen binding in the stability of the considered complex system. Both two dimensional and three-dimensional system configurations were obtained to identify the residues of the DNA and atoms of the ligands participating in the hydrogen bonding. This was done with the help of Discovery studio visualizer [33]. Figure 3.5 and figure 3.7 visualize the DNA residues and the atoms of the ligands involved in the hydrogen bonding for each complex in two dimensional figures. Figure 3.6 and figure 3.8 shows the donor and acceptor region between the ligand and DNA residues for the best docked pose of each DNA-ligand system.

Table 3.3. Mode of binding between drugs and DNA sequences.

S.no.	Drug	Binding mode with 1BNA	Binding mode with 1N37
1	Aspirin	Minor Groove binding	Intercalative binding
2	Daphentin	Minor Groove binding	Intercalative binding
3	Eugenol	Minor Groove binding	Intercalative binding
4	Ibuprofen	Minor Groove binding	Intercalative binding
5	Idarubicin	Major Groove binding	Intercalative binding

3.3.3.1. Drugs with 1BNA

The main type of binding in the case of drugs with 1BNA is groove binding and the main type of binding responsible for this behavior found out to be hydrogen bonding between the residues of DNA and atoms of the ligands. Figure. 3.5 represents the hydrogen bonding for each case of best docked pose of the complex in 2D. DNA residue name, atom name and bond length of the h-bond is also indicated. From

Figure. 3.5 we identify that the oxygen atoms present in each ligand are the main participants in the formation of hydrogen bonding.

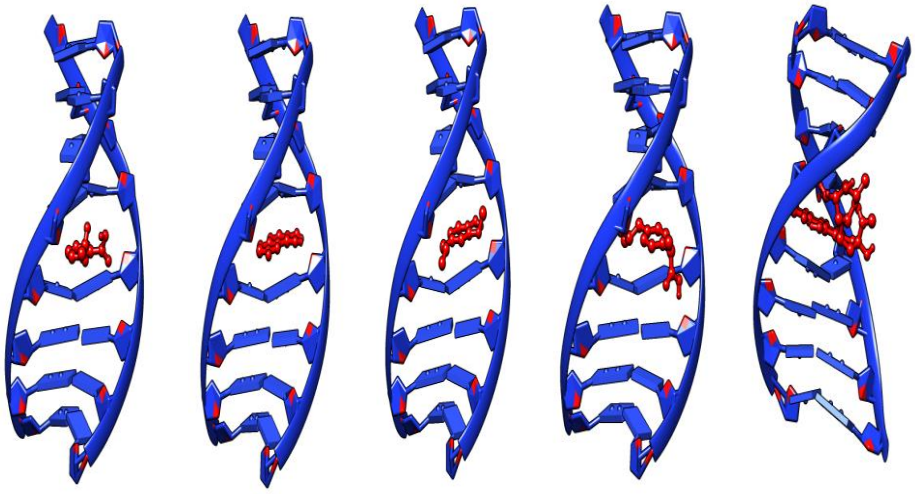


Figure 3.4. Molecular Docking between drugs and 1N37.

Table 3.4. The donor and the acceptor species and H-bond length formed between the ligands and 1BNA.

S.No.	Complex	No. of H-bonds	Interacting species	H-bond length(Å)
1.	1BNA + Aspirin	3	A:DG10:N2 - :UNK0:O B:DG16:N2 - :UNK0:O UNK0:H - B:DA17:O4'	2.671060 2.786127 2.077289
2.	1BNA + Daphentin	5	A:DG10:N2 - :UNK0:O A:DG10:N2-:UNK0:O B:DG16:N2-:UNK0:O UNK0:H - A:DC9:O2 UNK0:H - A:DG10:O4'	2.903734 2.950232 2.838476 2.956966 1.743174
3.	1BNA + Eugenol	2	UNK0:C-B:DT19:O3' A:DC9:C5'-UNK0:O	3.196279 3.224295
4.	1BNA + Ibuprofen	5	A:DG4:N2 - :UNK0:O UNK0:H - A:DA5:O4' A:DG4:N2 - :UNK0	2.773905 1.798620 3.520838
5.	1BNA + Idarubicin	6	UNK0:H- B:DA17:OP2 B:DA18:N6 - :UNK0 UNK0:H - B:DT19:O4 A:DA6:N6 - :UNK0:O UNK0:H - A:DA5:OP2 UNK0:H - A:DA5:OP2	2.391290 3.494988 2.024468 2.897006 1.852495 1.811564

Table 3.4 gives the donor and the acceptor species as well as the H-bond length formed between the ligands and 1BNA. The number of h-bonds formed for each complex is also indicated. Idarubicin forms 6 H-bonds with DNA residues which is maximum among all five of the complex systems. As it has the largest size and

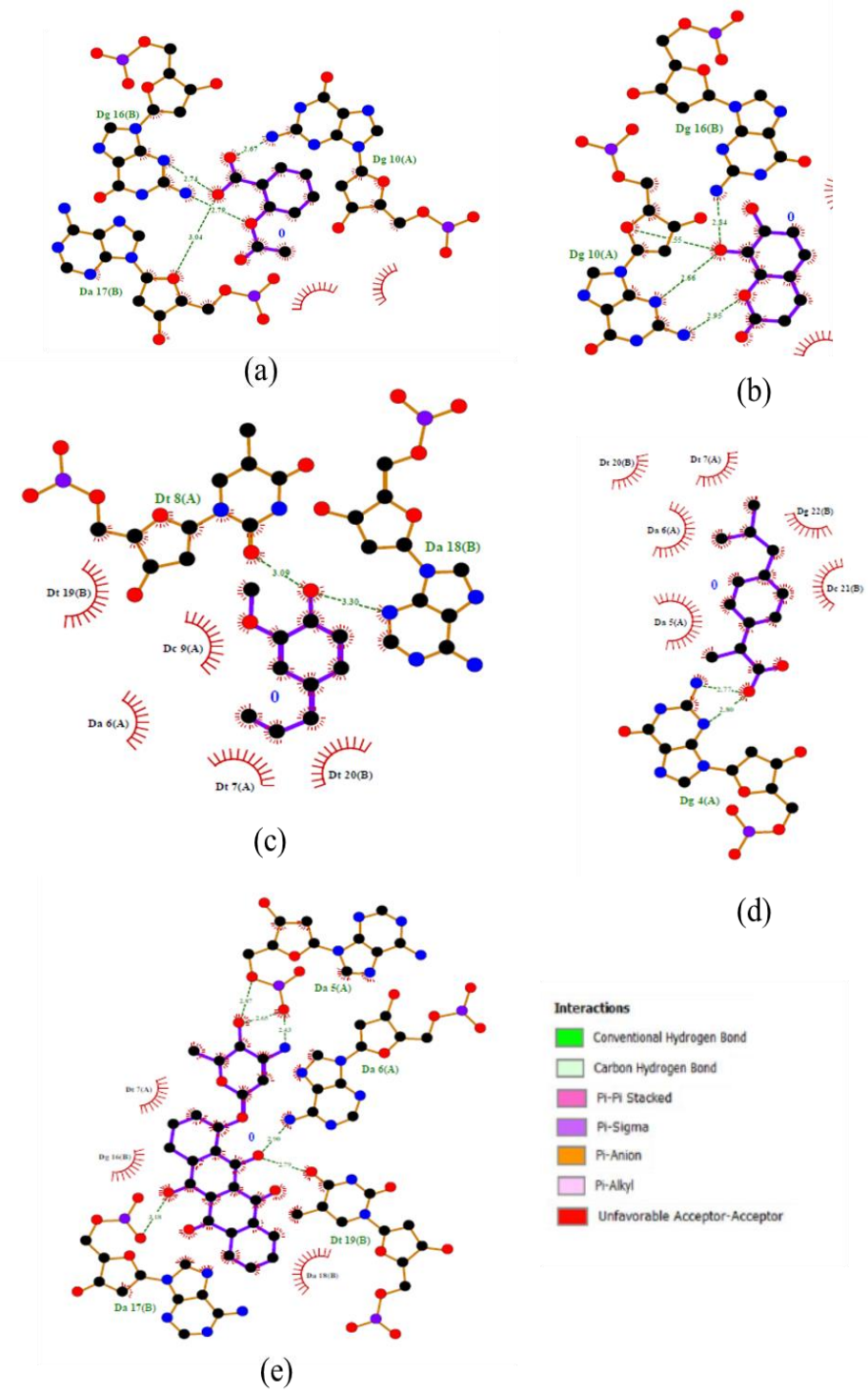


Figure 3.5. Interaction profile for ligands and 1BNA. (a) Aspirin+1BNA, (b) Daphentin +1BNA, (c) Eugenol +1BNA, (d) Ibuprofen +1BNA, (e) Idarubicin +1BNA

molecular mass, it forms maximum number of bonds thus giving the system maximum stability and maximum binding energy. Afterwards, Daphnetin has formed 5 H-bonds with the residues of DNA and these bonds give it the stability and binding

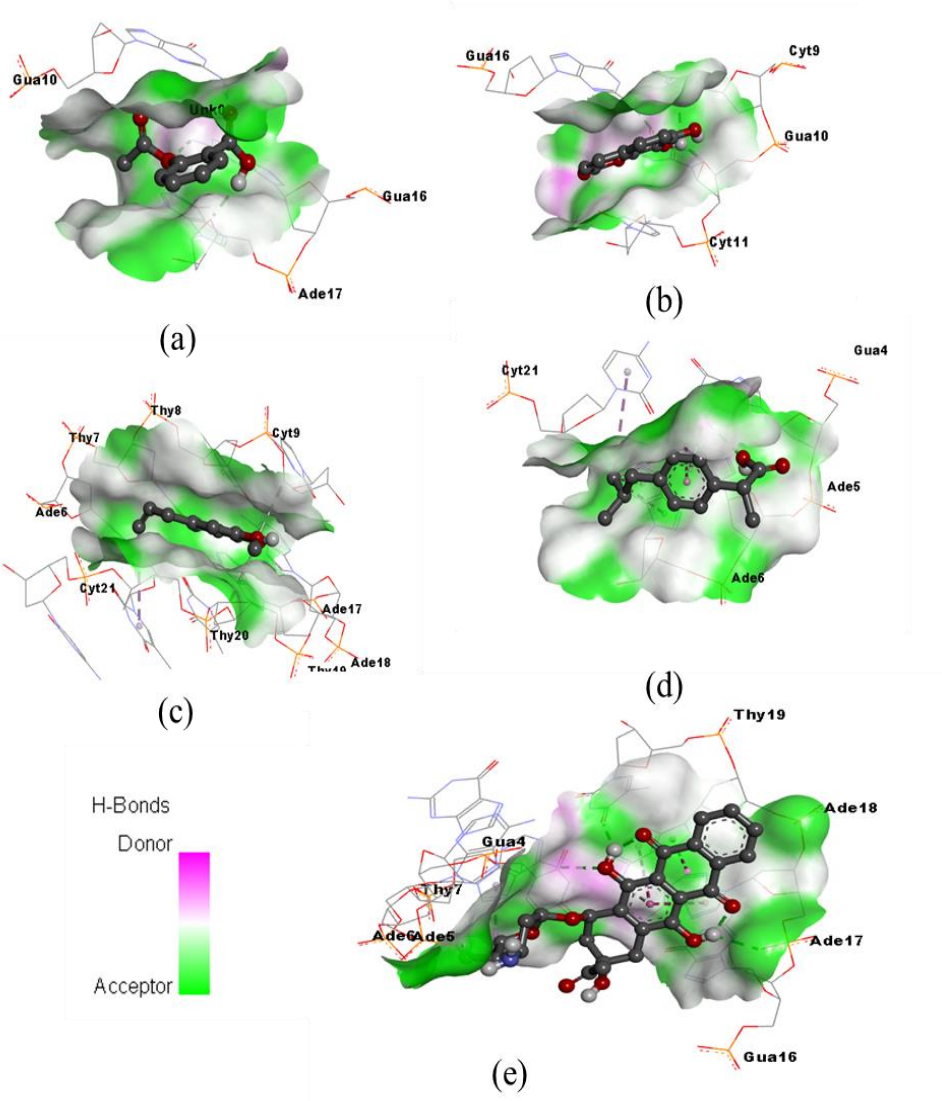


Figure 3.6. H-bonds acceptor and donor regions for drug-1BNA complex systems in three dimension. (a) Aspirin+1BNA, (b) Daphentin +1BNA, (c) Eugenol +1BNA, (d) Ibuprofen +1BNA, (e) Idarubicin +1BNA

energy on the second rank i.e, after Idarubicin.

To understand the hydrogen-bond acceptor and donor species more clearly, three-dimensional exploration was carried out using Discovery studio visualizer. Figure. 3.6. represents the H-bonds acceptor and donor regions for drug-1BNA complex systems in three dimensions. These regions help in determination of the electron deficient and electron rich locations in the drug-DNA complex system.

3.3.3.2. Drugs with 1N37

Two type of binding involved in the case of drugs with 1N37 is groove binding and hydrophobic bonding. Figure. 3.7 represents the hydrogen bonding for each case of best docked pose of the complex in two dimensions. DNA residue name, atom name and bond length of the h-bond is also indicated. From Figure. 3.7 we identify that the oxygen atoms present in each ligand are the main participants in the formation of hydrogen bonding.

Table 3.5. The donor and the acceptor species and H-bond length formed between the ligand and 1N37.

S.No.	Complex	No. of H-bonds	Interacting species	H-bond length(Å)
1.	1N37 + Aspirin	4	B:DG13:H1' - :UNK0:O UNK0:H - :UNK0:O A:DG5:H22 - :UNK0:O A:DT6:H5'1 - :UNK0:O	2.229808 2.116224 2.984705 2.438433
2.	1N37 + Daphentin	3	UNK0:H - B:DG13:O4' UNK0:H - B:DG13:O4' A:DG5:H22 - :UNK0:O	2.071562 2.077917 3.049086
3.	1N37 + Eugenol	3	B:DG13:H1' - :UNK0:O UNK0:H - B:DG13:O4' UNK0:C - B:DC12:O2	2.625393 1.994858 3.309219
4.	1N37 + Ibuprofen	3	B:DG13:H22 - :UNK0:O UNK0:H - B:DT14:O4' B:DG13:H1' - :UNK0:O	1.955134 1.988739 2.027019
5.	1N37 + Idarubicin	3	UNK0:H - B:DC12:OP2 UNK0:H - B:DA11:OP2 B:DC12:H42 - :UNK0:O	1.751039 1.772060 1.941024

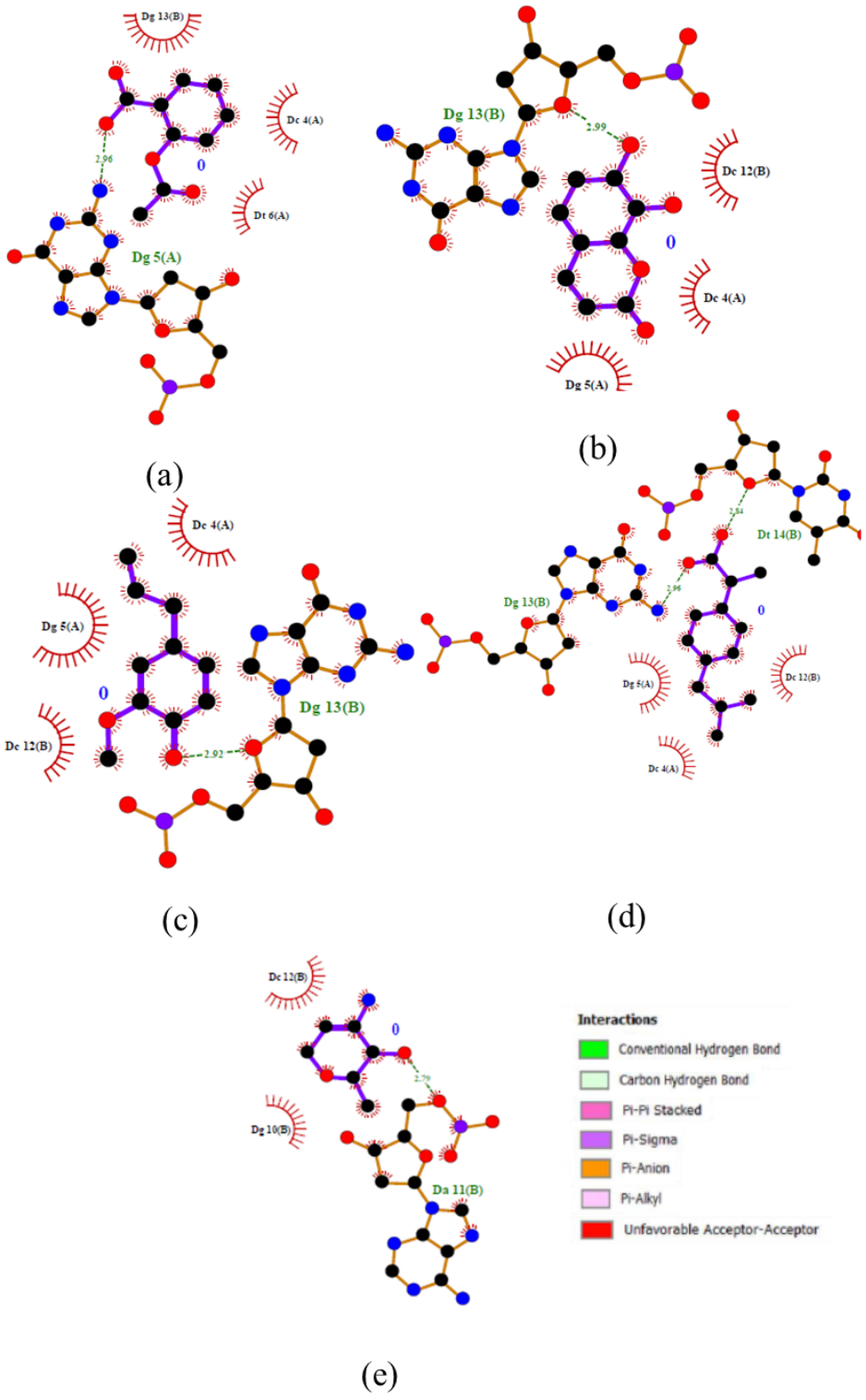


Figure 3.7. Interaction profile for ligands and 1N37.
(a) Aspirin+1N37, (b) Daphentin +1N37, (c) Eugenol +1N37, (d) Ibuprofen +1N37, (e) Idarubicin +1N37

Table 3.5 represents the donor and the acceptor species and H-bond length formed between the ligands and 1N37. The number of H-bonds formed for each complex is also indicated. Idarubicin forms 3 H-bonds with DNA residues. To understand the hydrogen-bond acceptor and donor species more clearly, three-dimensional exploration was carried out using Discovery studio visualizer. Figure. 3.6. represents the H-bonds acceptor and donor regions for drug-1BNA complex systems in three dimensions. These regions help in determination of the electron deficient and electron rich locations in the drug-DNA complex system.

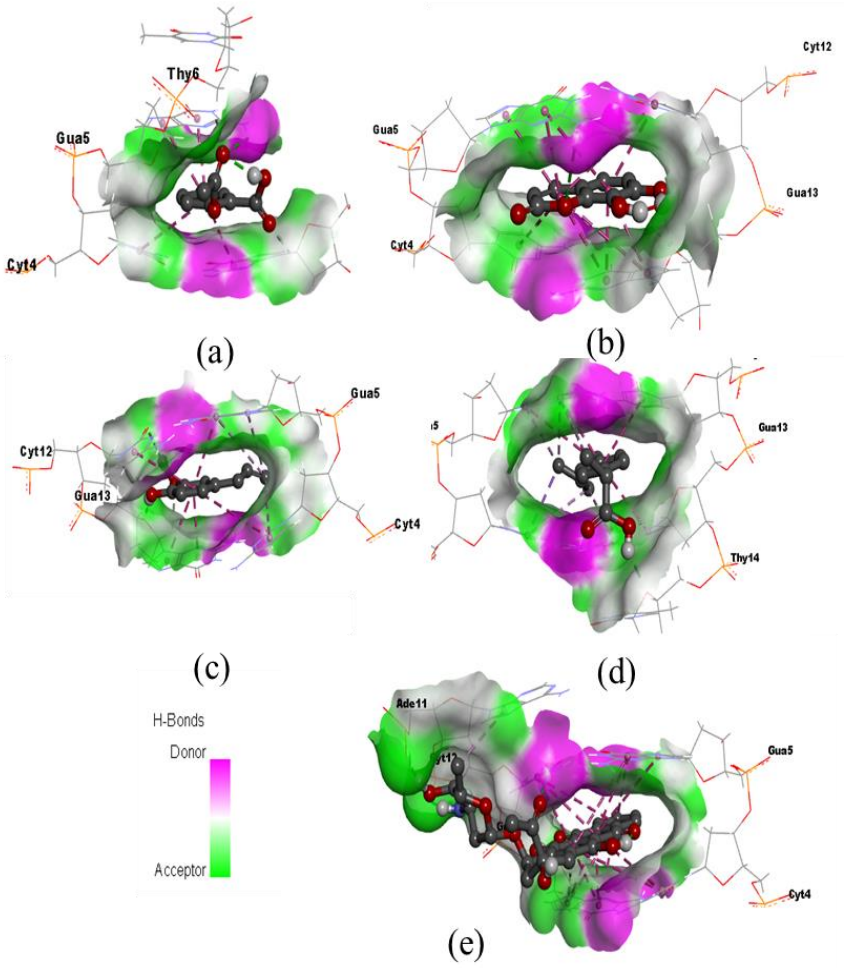


Figure 3.8. H-bonds acceptor and donor regions for drug-1N37 complex systems. (a) Aspirin+1N37, (b) Daphentin +1N37, (c) Eugenol +1N37, (d) Ibuprofen +1N37, (e)

Table 3.6. Hydrophobic bond details for 1N37-ligand complex systems.

S.No.	Complex	No. of Hydrophobic bonds	Interacting species	Bond length(Å)
1.	1N37 + Aspirin	4	B:DG13 - :UNK0 A:DC4 - :UNK0 A:DG5 - :UNK0 A:DG5 - :UNK0	4.211131 4.865555 4.042647 3.474699
2.	1N37 + Daphentin	9	A:DG5 - :UNK0 A:DG5 - :UNK0 A:DG5 - :UNK0 B:DC12 - :UNK0 B:DC12 - :UNK0 B:DG13 - :UNK0 B:DG13 - :UNK0 B:DG13 - :UNK0 A:DC4 - :UNK0	4.034621 3.314243 4.386686 5.839032 3.923474 3.673771 3.387277 3.900965 4.534557
3.	1N37 + Eugenol	8	B:DC12 - :UNK0 A:DG5 - :UNK0 A:DG5 - :UNK0:C A:DG5 - :UNK0:C A:DC4 - :UNK0:C A:DC4 - :UNK0 B:DG13 - :UNK0 B:DG13 - :UNK0	4.661951 3.920650 5.116148 4.611972 5.013350 5.973934 3.385699 3.992733
4.	1N37 + Ibuprofen	11	A:DG5 - :UNK0:C A:DG5 - :UNK0 A:DG5 - :UNK0 A:DG5 - :UNK0:C A:DG5 - :UNK0:C UNK0:C - A:DC4 B:DC12 - :UNK0 A:DC4 - :UNK0:C B:DG13 - :UNK0:C B:DG13 - :UNK0 B:DG13 - :UNK0	5.412972 5.298705 3.949191 4.219816 4.133090 3.798843 5.081958 4.837154 4.882732 4.177413 3.502345
5.	1N37 + Idarubicin	16	B:DA11 - :UNK0:C A:DC4 - :UNK0 A:DC4 - :UNK0 B:DG13 - :UNK0 B:DG13 - :UNK0 B:DG13 - :UNK0 B:DG13 - :UNK0 B:DG13 - :UNK0 B:DG13 - :UNK0 B:DC12 - :UNK0 B:DC12 - :UNK0	4.419006 5.780476 5.708797 4.208746 3.249211 3.961765 4.869578 3.815083 4.180830 4.127728 4.429075

			B:DC12 - :UNK0	5.865532
			A:DG5 - :UNK0	5.344392
			A:DG5 -UNK0	4.078766
			A:DG5 :UNK0	4.182771
			A:DG5 -UNK0	5.416361

3.3.4. Hydrophobic binding analysis

Hydrophobic binding is the main type of binding involved in the binding between 1N37 and ligands. As the binding mode in the case of 1N37 is intercalation with all of the drugs, hydrophobic interaction dominates in here. In the case of intercalative binding, small drugs get stacked in between the bases of DNA. In this stacking, hydrophobic bonds are responsible for the binding between the ligand and DNA. In Figure. 3.8. the hydrophobic bonds are shown.

Table 3.6. gives the hydrophobic bond details for 1N37-ligand complex systems. Number of hydrophobic bonds, involved species and bond length for each bond for all of the complexes is given. It is clear from the table that this binding is dominating over hydrogen binding in the complexes. Idarubicin, has 16 hydrophobic bonds with residues of 1N37. Being the largest compound, it has maximum number of bonds with 1N37 among all other ligand-DNA complexes. Other complexes also have a more hydrophobic bonds than hydrogen bonds.

3.4 Conclusions

Computational investigations were done to evaluate and understand the binding activity and energy of drugs with DNA. This study is focused to provide a wide outlook on the molecular level of drug-DNA interaction. This analysis can give an insight in designing inhibitors for the treatment of cancer and AIDS. 1BNA and 1N37 were the two type of DNA sequences used for the investigation. 1BNA is a normal B-DNA whereas 1N37 has intercalation gap between its base pairs. All the selected drugs behaved differently with these two types of DNA. The binding energy for each of the drug-DNA system was in same range but the mode of binding and binding pocket was dissimilar in each case. All of the complexes having large binding energy in negative value demonstrated a higher binding effinity of ligands with DNA.

For 1BNA, all the drugs except Idarubicin behaved as minor groove binders whereas Idarubicin acted like a major groove binder. This exceptional behavior of Idarubicin

can be understood from the fact that it has larger molecular weight, structural shape and size among all the drug molecules. Because of the larger size and unavailability of intercalation gap in 1BNA Idarubicin binded in the major groove of DNA. For 1N37, the binding mode comes out to be intercalation in each case of ligand-DNA complex. The planar structure of the ligands and presence of intercalation gap in between the pairs of bases is the prime reason for this type of binding. From the results, it is clear that the binding mode between a ligand and DNA does not only depend upon the size and structure of the ligand but also depends on the structure of the DNA sequence.

Detailed analysis of hydrogen bonding gives insight of the dependency of the stability of the complex system on hydrogen bonds. Large number of hydrogen bonds indicates large binding energy and more stability of the complex system. Idarubicin-1BNA complex has highest binding energy as well as largest number of hydrogen bonds in-between the complex system. 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 drug-receptor complexes. This will promote a theoretical procedure supporting experimental methods and database creation for drug-DNA complex structure-energy correlations.

References

1. A.M. Dar, S. Mir, Molecular Docking: Approaches, Types, Applications and Basic Challenges, J. Anal. Bioanal. Tech. 8:2 (2017) 1-7.
2. N.S. Pagadala1, K. Syed, J. Tuszynski, Software for molecular docking: a review, Biophys. Rev. 9 (2017) 91–102.
3. K.K. Chaudhary, N. Mishra, A Review on Molecular Docking: Novel Tool for Drug Discovery. JSM Chem 4(3) (2016) 1029
4. A. Pandey, R. Mishra, A. Shukla, A.K. Yadav, D. Kumar, In-silico docking studies of 2,5-bis(4-amidinophenyl) furan and its derivatives. Proceeding of ISAFBM-2019 Feb 28, Lucknow University (2019) 11-18.
5. A. Subastri, C.H. Ramamurthy, A. Suyavaran, R. Mareeswaran, P.L. Rao, M. Harikrishna, Spectroscopic and molecular docking studies on the interaction of troxerutin with DNA, International Journal of Biological Macromolecules 78 (2015) 122.
6. R. Mishra, A.S. Gaur, R. Chandra, D. Kumar, Molecular Docking and Molecular Dynamics Study of DNA Minor Groove Binders. International Journal of Pharmaceutical Chemistry and Analysis 2 (2015) 161.
7. A. Shukla, R. Mishra, A. Pandey, A.K. Dwivedi, D. Kumar, Interaction of Flavonols with DNA: Molecular Docking Studies. Proceeding of ISAFBM-2019 Feb 28, Lucknow University (2019) 4-10.
8. J.D. Watson, F.H.C. Crick, Molecular structure of nucleic acids. Nature, 171 (1953) 737-738.
9. S. Rauf, J.J. Gooding, K. Akhtar, M.A. Ghauri, M. Rahman & M.A. Anwar, Electrochemical approach of anticancer drugs – DNA interaction. Journal of Pharmaceutical and Biomedical Analysis, 37 (2005) 205-217.
10. A. Subastria, C.H. Ramamurthya, A. Suyavarana, R. Mareeswarana, P. Lokeswara, M. Harikrishna, M.S. Kumar, V. Sujathac, C. Thirunavukkarasua, Spectroscopic and molecular docking studies on the interaction of troxerutin with DNA, International Journal of Biological Macromolecules 78 (2015) 122-129.
11. M. Husain, S. Rehman, H. Ishqi, T. Sarwar, M. Tabish, Spectroscopic and molecular docking evidence of aspirin and diflunisal binding to DNA: A comparative study, RSC Advances, 5 (2015) 64335-64345.

12. S. Bi, L. Yan, Y. Wang, B. Pang, T. Wang, Spectroscopic study on the interaction of eugenol with salmon sperm DNA in vitro, Journal of Luminescence. 132 (2012) 2355–2360.
13. Y. Zhu, H. Zeng, J. Xie, L. Ba, X. Gao, Z. Lu, Atomic force microscopy studies on DNA structural changes induced by vincristine sulfate and aspirin, Microsc. Microanal. 10 (2004) 286–290.
14. X. Zhou, G. Zhang, J. Pan, Groove binding interaction between daphnetin and calf thymus DNA, International Journal of Biological Macromolecules. 74 (2015) 185-194.
15. X. Zhou, C. Zhang, G. Zhang, Y. Liao, Intercalation of the daphnetin–Cu (II) complex with calf thymus DNA. RSC Advances 6(7) (2016) 5408-5418.
16. J. Liu, J. Tian, W. He, J. Xie, Z. Hu, X. Chen, Spectrofluorimetric study of the binding of daphnetin to bovine serum albumin. J. Pharm. Biomed. Anal. 35 (2004) 671–677.
17. J. Chen, X. Liu, Y.P. Shi, Determination of daphnetin in Daphne tangutica and its medicinal preparation by liquid chromatography. Anal. Chim. Acta 523 (2004) 29–33.
18. M. Pisano, G. Pagnan, M. Loi, M.E. Mura, M.G. Tilocca, G. Palmieri, D. Fabbri, M.A. Dettori, G. Delogu, M. Ponzoni, Antiproliferative and pro-apoptotic activity of eugenol-related biphenyls on malignant melanoma cells. Mol. Cancer 6 (2007) 8–20.
19. M. Ogata, M. Hoshi, S. Urano, T. Endo, Antioxidant activity of eugenol and related monomeric and dimeric compounds. Chem. Pharm. Bull. 48 (2000) 1467–1469.
20. F. Benencia, M.C. Courreges, In vitro and in vivo activity of eugenol on human herpesvirus. Phytother. Res. 14 (2000) 495–500.
21. S. Bi, L. Yan, Y. Wang, B. Pang, T. Wang, Spectroscopic study on the interaction of eugenol with salmon sperm DNA in vitro. Journal of Luminescence 132 (2012) 2355–2360.
22. M.A. Husain, T. Sarwar, S.U. Rehman, H.M. Ishqi, M. Tabish, Ibuprofen causes photocleavage through ROS generation and intercalates with DNA: A combined biophysical and molecular docking approach. Physical Chemistry Chemical Physics: PCCP, 17(21) (2015) 13837–13850.

23. S.I. Farooqi, N. Arshad, P.A. Channar, F. Perveen, A. Saeed, F.A. Larik, A. Javed, M. Yamin: New aryl Schiff bases of thiadiazole derivative of ibuprofen as DNA binders and potential anticancer drug candidates, Journal of Biomolecular Structure and Dynamics 39:10 (2021) 3548-3564.
24. S. Charak, M. Ranjana, Structural investigation of idarubicin-DNA interaction: spectroscopic and molecular docking study, Int. J. Biol. Macromol. 60 (2013) 213–218.
25. C. Ozluer, H. Eda, S. Kara, In vitro DNA binding studies of anticancer drug idarubicin using spectroscopic techniques. Journal of Photochemistry and Photobiology B: Biology, 138 (2014) 36.
26. H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The Protein Data Bank, Nucleic Acids Research, 28 (2000) 235-242.
27. E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, UCSF Chimera--a visualization system for exploratory research and analysis, J. Comput. Chem. 25 (2004) 1605-1612.
28. S. Charak, R. Mehrotra, Structural investigation of idarubicin–DNA interaction: Spectroscopic and molecular docking study, International Journal of Biological Macromolecules, 60 (2013) 213-218.
29. M. Husain, T. Sarwar, S.U. Rehman, H.M. Ishqia, M. Tabish, Ibuprofen causes photocleavage through ROS generation and intercalates with DNA: a combined biophysical and molecular docking approach Phys. Chem. Chem. Phys., 17 (2015) 13837-13850.
30. Gaussian 09, Revision E.01, M.J .Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery, J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J.J. aramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J.

Dannenberg, S. Dapprich, A.D. Daniels, O. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski and D.J. Fox, Gaussian, Inc., Wallingford CT (2009).

31. G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility, J. Comput. Chem. 30 (2009) 2785-2791.
32. G.M. Morris, D. Goodsell, R. Halliday, R. Huey, W. Hart, R. Belew, A. Olson, Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function, J. Comput. Chem. 19 (1998) 1639-1662.
33. Dassault Systèmes BIOVIA, Discovery Studio Visualizer, 2020, San Diego, Dassault Systèmes, (2020).

CHAPTER-4

INTERACTION OF FLAVONOLS WITH DNA: MOLECULAR DOCKING STUDIES

4.1. Introduction

DNA is the most important genetic substance and basic building block of all organisms. It is responsible for all types of hereditary information. It is a nucleic acid which accommodates all the details necessary for describing the biological growth of all living bodies. The evaluation of interaction between small drugs and DNA is of prime importance for the designing of efficient and cost-effective chemotherapeutic drugs and potent anticancer medicines. In the field of drug design and drug discovery, interaction between DNA and drug molecules is a very popular and necessary subject [1]. The analysis of the detailed interactions between small molecules and DNA is an emerging important and interesting discipline. There are a lot of techniques available for the investigation of drug-DNA interactions and the efficiency of these tools and techniques is continuously expanding [2].

Watson and Crick were the first to give the basic and precise structure of DNA in 1953 [3]. According to them, DNA is comprised of two helical polynucleotide chains each coiled around the same axis. DNA naturally have conformation of a right-handed double helix. Each strand of the helix has a backbone and the backbone is made up of alternating sugar and phosphate residues that are joined with each other via phosphodiester linkages. The bases are on the inside of the helix and the phosphates on the outside. The planes of the bases are perpendicular to the fibre axis. These bases are: adenine and guanine, which are purines and thymine and cytosine, which are pyrimidines. Adenine pairs up with thymine and guanine pairs up with cytosine via hydrogen bonding. The two strands which comprise the double helical structure of DNA have complementary sequences. The complementary nature of the bases on the two helical DNA strands gives it its capacity for self-coding.

DNA is also the prime target of many anticancer and antibiotic drugs [4]. Studies have been done experimentally as well as theoretically to analyse and understand the interaction of various drugs with DNA. The investigation and its finding help the

researchers in the designing of efficient chemotherapeutic drugs and more potent anticancer medicines. As DNA is the main building block, a drug for any disease interacts with DNA. Drugs are mainly small molecules which can bind with DNA via two type of binding: groove binding and intercalation. On the basis of the mode of binding and binding energy between particular drug and DNA, their usefulness as a medicine for any specific disease can be predicted [5].

Table 4.1: PDB ID and sequence of the DNA used.

S.no.	PDB ID	DNA Sequence
1	1BNA	5'-CGCGAATTCGCG-3'
2	1CP8	5'-TTGGCCAA-3'
3	1D66	5'-CCGGAGGACAGTCCTCCGG-3'
4	1RMX	5'-CGACTAGTCG-3'
5	195D	5'-DCGCGTTAACGCG-3'

Here, we are dealing with the prediction and investigation of binding properties of flavonols, a class of flavonoid, 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 [6-8]. 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 [9, 10]. 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. To perform molecular docking AUTODOCK software was used. Best docked poses and binding energies of the drug-DNA complexes were calculated computationally. This study can be helpful in the designing of new and less toxic drugs and enhance their further applications in pharmacology.

4.2. Computational Details

4.2.1. Dataset

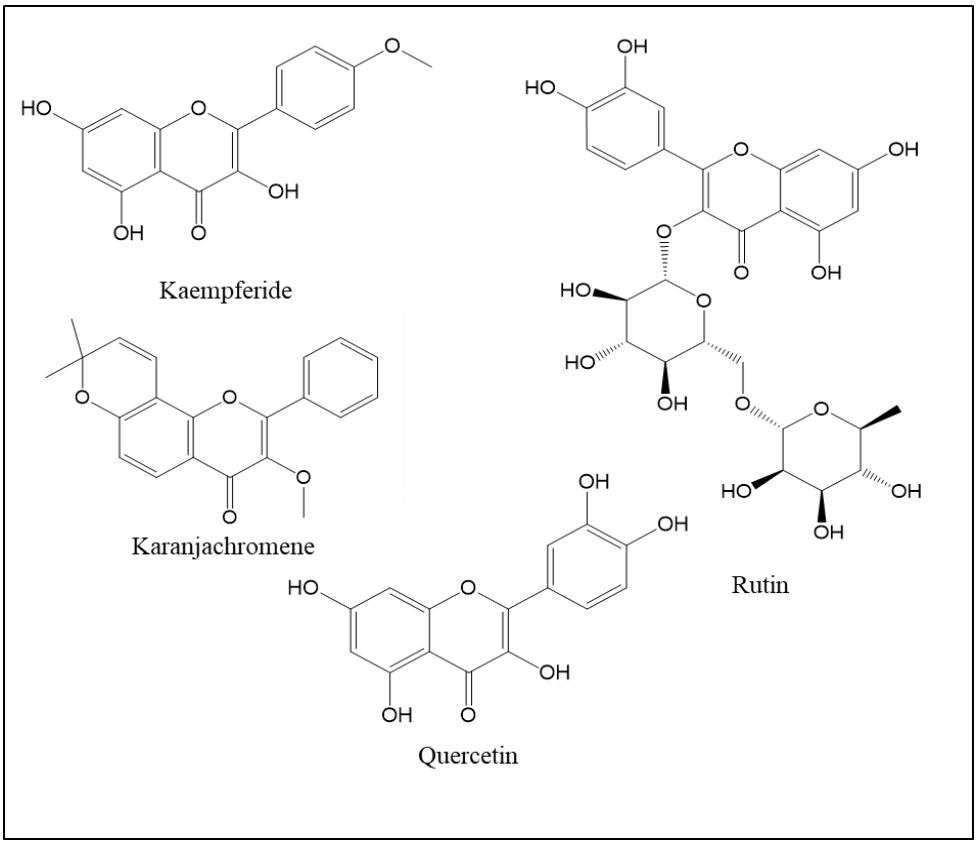


Figure 4.1. Structures of used flavonols.

DNA: The pdb format file of DNA sequences with PDB ID 1BNA, 195D, 1CP8, 1D66 and 1RMX were extracted from RCSB Protein Data Bank [11]. CHIMERA was used to remove ligands from the DNA sequences and delete all the water molecules from each of them [12]. PDB ID and sequence of the DNA used are shown in Table 4.1.

Drugs: The structures of Kaempferide, Karanjachromene, Quercetin and Rutinwere collected from literature [13-16]. Figure 4.1 represents the chemical structures of these flavonols. The drug molecules were taken to geometry optimization process using Gaussian 09 at B3LYP/6-31G level [17].

4.2.2. Molecular Docking

AutoDock4.2 was utilized for the molecular docking process by using Lamarckian Genetic Algorithm (LGA) [18]. The docking of the system was done taking DNA sequences as targeted receptor macromolecule, while flavonols were considered as flexible ligands. The receptor macromolecule and ligand files were assembled for the process of docking using Auto Dock Tools (ADT) [19]. Grid boxes of different dimensions for each DNA sequence were prepared by the help of Auto-Grid.

Gasteiger charges were also added to the macromolecule-ligand complex system by Auto Dock Tools (ADT) before starting docking calculations. Lamarckian genetic algorithms, as implemented in AutoDock, were utilized for performing blind docking calculations. 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.

4.3. Results and Discussions

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 4.2. 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 4.2.

The complex of Karanjachromene with 195D have lowest binding energy -9.23 kcal/Mol, this represents that the interaction between DNA sequence 5'-DCGCGTTAACGCG-3' and karajachromene is more stable when compared to other DNA-ligand complexes. So, it can prove to be an effective drug against cancer and other diseases. For each of the DNA sequences, non-covalent interaction for most stable complex is shown in Figure 4.3.

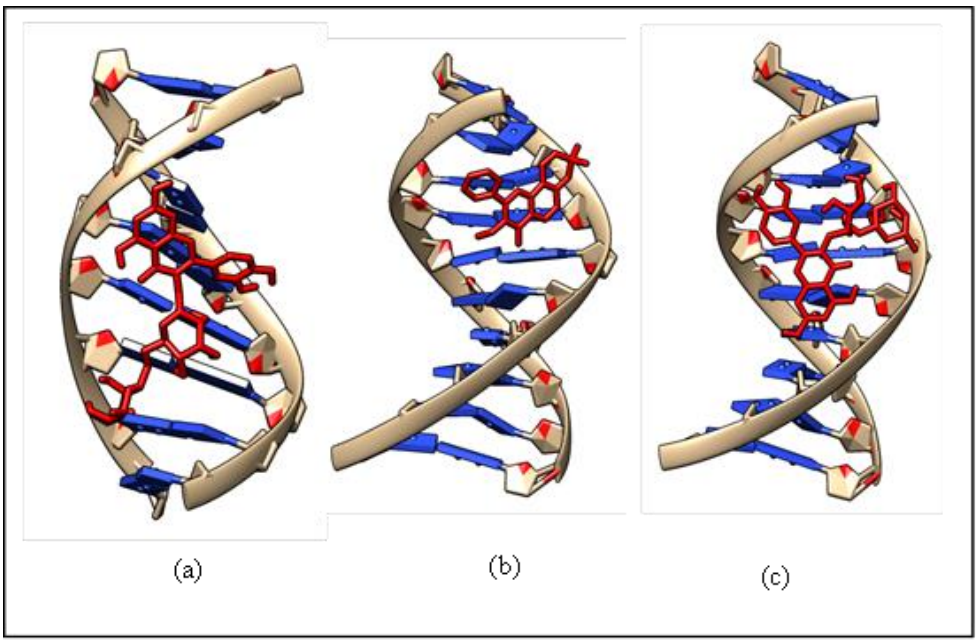


Figure 4.2. Major groove binding. (a) 1CP8 and rutin, (b) 1RMX and karanjachromene, (c) 1RMX and rutin

Table 4.2: 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

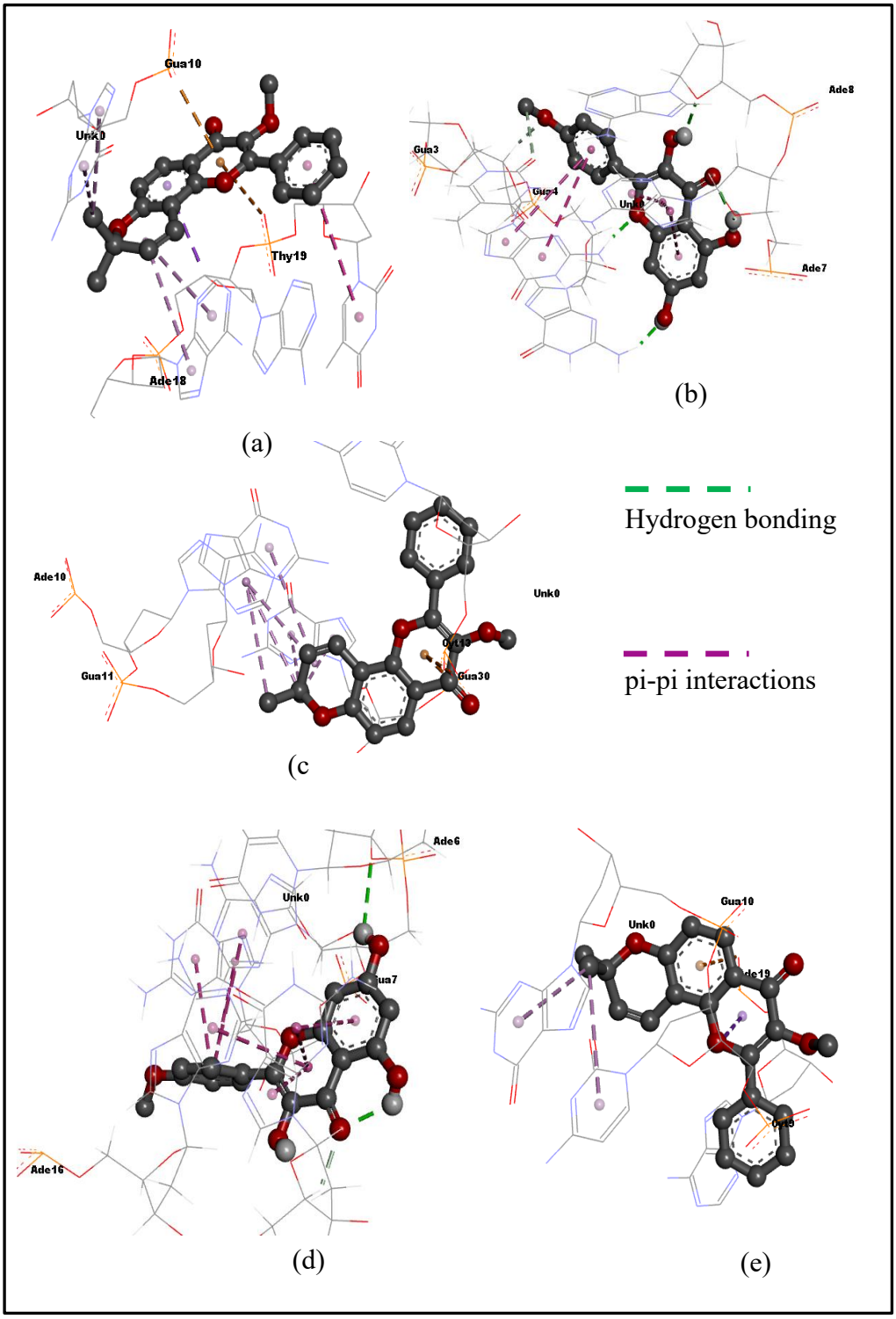


Figure 4.3. Non-covalent interaction between DNA and drug having lowest binding energy for each DNA sequence. (a) 1BNA and Karanjachromene, (b) 1CP8 and Kaempferide, (c) 1D66 and Karanjachromene, (d) 1RMX and Kaempferide, (e) 195D and Karanjachromene

4.4 Conclusion

Flavonols are a class of flavonoids having possible application in pharmacology. The computational study gives details about the interaction of flavonols Kaempferide, Karanjachromene, Quercetin and Rutin with DNA and binding energies of the complexes. 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. It can serve as an adequate database for structure-energy relationship of anticancer drug-DNA complexes.

References

1. A. Paul, S. Bhattacharya, Chemistry and biology of DNA-binding small molecules. Curr. Sci 02 (2012) 212–231.
2. J. Li, S. Shuang, C. Dong, Study on the phosphorescence characterizations of palmatine chloride on the solid substrate and its interaction with ctDNA. Talanta, 77 (2009) 1043–1049.
3. J.D. Watson, F.H.C. Crick, Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid. Nature 171 (1953) 737–738.
4. L.H. Hurley, DNA and its associated processes as targets for cancer therapy. Nat. Rev. Cancer 2 (2002) 188–200.
5. R. Mishra, A.S. Gaur, R. Chandra, D. Kumar, Molecular Docking and Molecular Dynamics Study of DNA Minor Groove Binders. Int. J. Phar. Chem. Analysis 2(4) (2015) 161-169.
6. P.A. Ragazzon, J. Iley, S. Missailidis, Structure-activity studies of the binding of the flavonoid scaffold to DNA. Anticancer Research 29 (2009) 2285-2294.
7. J.E.N. Dolatabadi, Molecular aspects on the interaction of quercetin and its metal complexes with DNA. Int. J. Biol. Macromol. 48 (2011) 227-233.
8. B. Tu, Z.-F. Chen, Z.-J. Liu, L.-Y. Cheng and Y.-J. Hu, Interaction of flavones with DNA in vitro: structure–activity relationships. RSC Adv.5 (2015) 33058-33066.
9. E. Middleton, C. Kandaswami T.C. Theoharides, The Effects of Plant Flavonoids on Mammalian Cells: Implications for Inflammation, Heart Disease, and Cancer Pharmacol. Rev. 52 (2000) 673–751.
10. M.J. Hannon, Supramolecular DNA recognition. Chem. Soc. Rev. 36 (2007) 280-295.
11. H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The Protein Data Bank. Nucleic Acids Research 28 (2000) 235-242.
12. E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng,T.E. Ferrin. UCSF Chimera—A visualization system for exploratory research and analysis. J. Comput. Chem. 25(13) (2004) 1605-1612.

13. S. Nafisi, M. Hashemi, M. Rajabi, H.A. Tajmir-Riahi, DNA Adducts with Antioxidant Flavonoids: Morin, Apigenin, and Naringin. DNA and cell biology 27(8) (2008) 433–442.
14. S. Balakrishnan, S. Jaldappagari, Binding of an anticancer *Rutaceae* plant flavonoid glycoside with calf thymus DNA: Biophysical and electrochemical studies. Journal of Luminescence 142 (2013) 17–22.
15. V. Mary, P. Haris, M.K. Varghese, P. Aparna, C. Sudarsanakumar, Experimental Probing and Molecular Dynamics Simulation of the Molecular Recognition of DNA Duplexes by the Flavonoid Luteolin. J. Chem. Inf. Model. 57 (2017) 2237−2249.
16. N. Arshad, N. Rashid, S. Absar, M.S.A. Abbasi, S. Saleem, B. Mirza, UV-absorption studies of interaction of karanjin and karanjachromene with ds. DNA: Evaluation of binding and antioxidant activity. Cent. Eur. J. Chem. 11(12) (2013) 2040-2047.
17. Gaussian 09, Revision E.01, M.J .Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery, J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J.J. aramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, O. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski and D.J. Fox, Gaussian, Inc., Wallingford CT (2009).
18. G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A. J. Olson, AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexiblity. J. Comput. Chem. 30 (2009) 2785–2791.
19. G.M. Morris, D. Goodsell, R. Halliday, R. Huey, W. Hart, R. Belew A. Olson, Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J. Comput. Chem. 19 (1998) 1639–1662

CHAPTER-5

COMPUTATIONAL INVESTIGATIONS ON INTERACTIONS BETWEEN DNA AND FLAVONOLS

5.1. 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 to be potent drugs 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 flavonols with DNA. Flavonols are a class of flavonoids known for their antioxidant properties. They are

natural compounds found in plants. 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

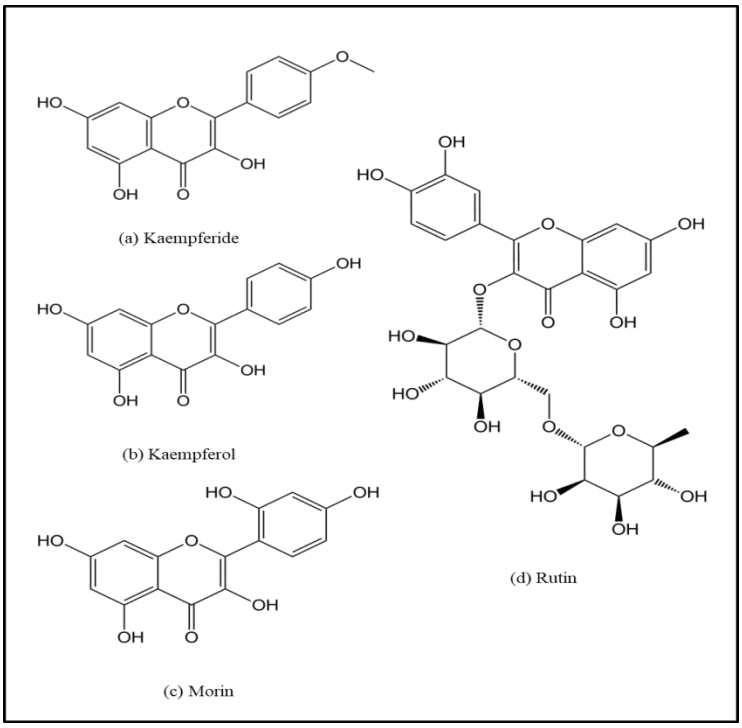


Figure 5.1. Chemical structure of selected ligands. (a) Kaempferide, (b) Kaempferol, (c) Morin, (d) Rutin.

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 flavonols [24] with DNA. Selected molecules are Kaempferide [25], Kaempferol [26], Morin [27] and Rutin [28].

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 analysing them with the help of computational techniques.

5.2. Computational Details

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

5.2.1. Optimization of compounds.

Structures of compounds were obtained from a literature survey [26, 28, 30-31]. Figure 5.1 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.

5.2.2. 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 AUTODOCK 4.2 [37]. The Genetic Algorithm was used to process the computational docking process [38]. Optimized compounds were made to dock with the DNA segment 2ROU giving the most preferred binding position of flavonoids within DNA. Both ligand and macromolecule were prepared in the form of

PDBQT files for grid 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.

5.2.3. 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.

5.2.4. 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.

5.3. Results and Discussions

5.3.1. Molecular docking

Table 5.1 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 5.2 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 5.1. 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]

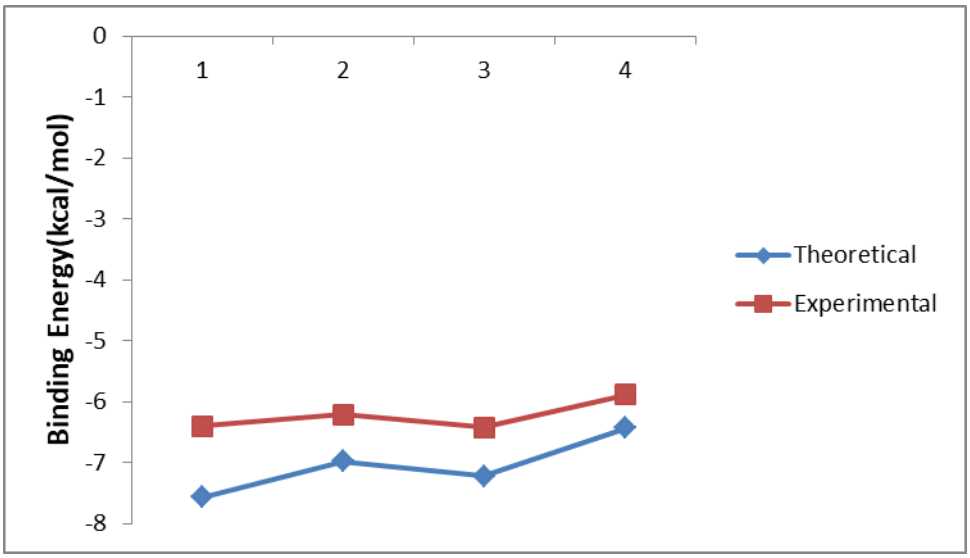


Figure 5.2. A similar trend of binding energy in theoretical and experimental cases.

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 5.3 represents the binding position of ligands with DNA. These figures clearly show the binding mode of the complexes. Figure 5.4 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. Table 5.2 details the hydrogen bonds formed between the ligands and macromolecule.

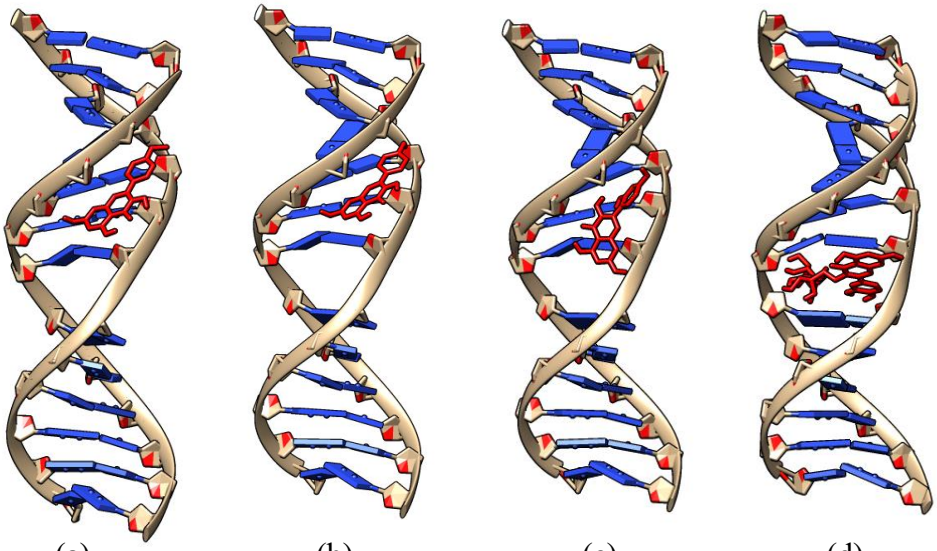


Figure 5.3. Binding sites of Flavonols with DNA (**a**) Kaempferide, (**b**) Kaempferol, (**c**) Morin, (**d**) Rutin.

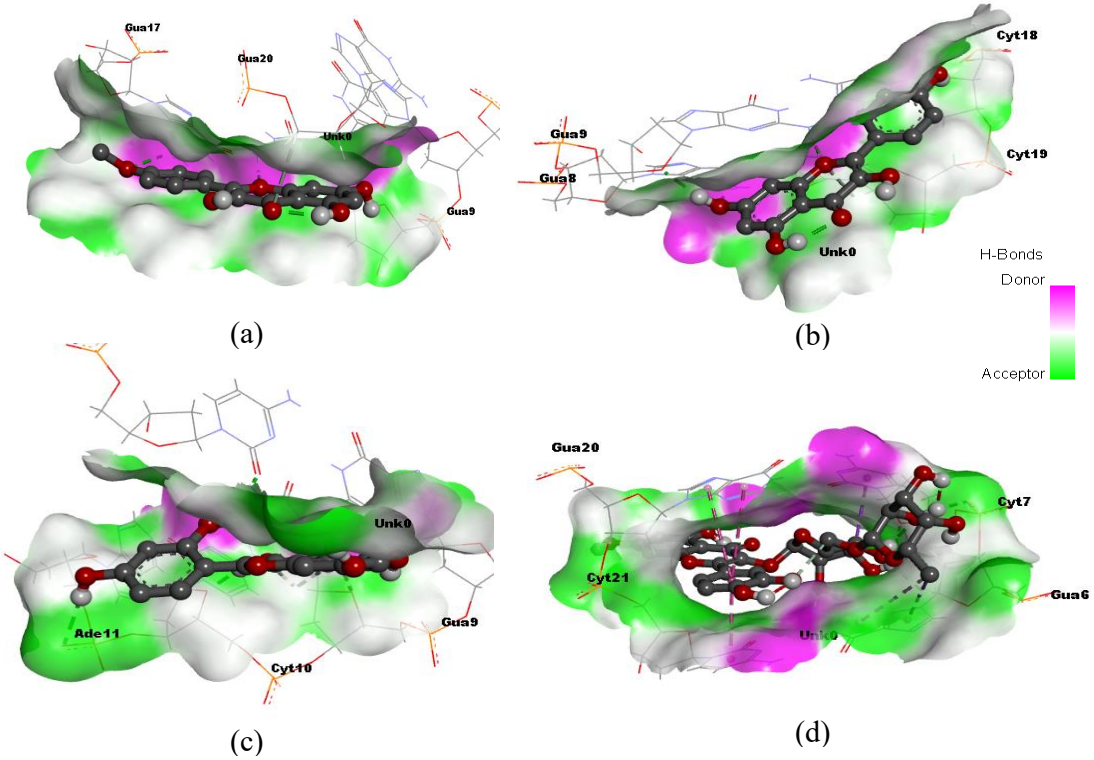


Figure 5.4. H-bond donor and acceptor regions in best docked posed complexes.

Table 5.2. 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

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.

5.3.2. Molecular dynamics

Table 5.3. 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

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 5.5. 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.

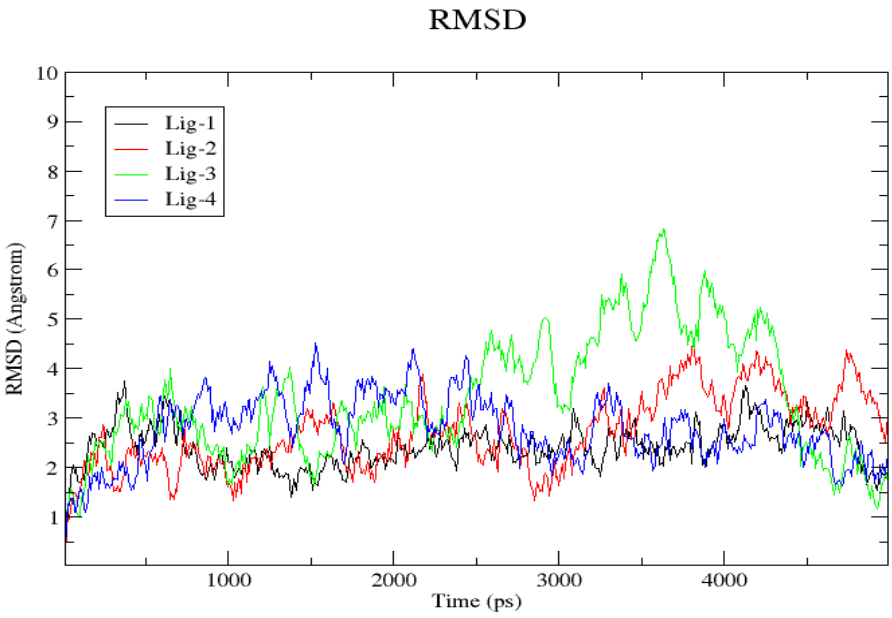


Figure 5.5. RMSD plot for drug-DNA

Figure 5.6 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.

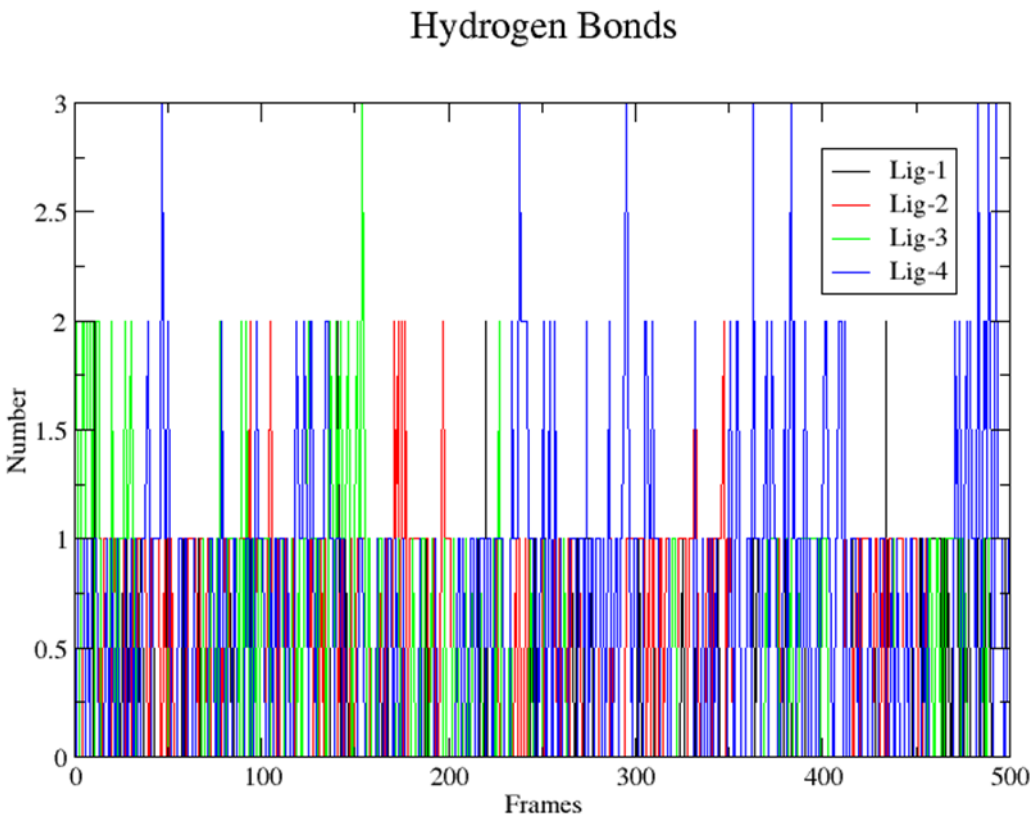


Figure 5.6. Hydrogen bonds for drug-DNA complexes.

As analyzed from molecular dynamics studies, Figure 5.5 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.

5.3.3. Free Energy Calculations

The MMPBSA and MMGBSA free energies for the DNA-ligand system are given in Table 5.4. 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 5.4 shows that morin has both minimum MMPBSA and MMGBSA energies.

Table 5.4. 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

5.4. 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.

References

1. M. Małecka, A. Skoczyńska, D.M. Goodman, C.G. Hartinger, E. Budzisz, Biological properties of ruthenium(II)/(III) complexes with flavonoids as ligands. Coord. Chem. Rev. 436 (2021) 213849.
2. K. Ahmad, M. Kalim, A. Khan, M H. Baig, M. Imran, G.K. Gupta, Role of Azoles in Cancer Prevention and Treatment: Present and Future Perspectives. Anti-Cancer Agents Med. Chem. 18 (2018) 46–56.
3. C. Busch, M. Burkard, C. Leischner, U.M. Lauer, J. Frank, S. Venturelli, Epigenetic activities of flavonoids in the prevention and treatment of cancer. Clin. Epigenetics 7 (2015) 1–18.
4. M. Mareel, A. Leroy, Clinical, cellular, and molecular aspects of cancer invasion. Physiol. Rev. 83 (2003) 337–376.
5. Z. Kejík, R. Kaplánek, M. Masařík, P. Babula, A. Matkowski, P. Filipenský, K. Veselá, J. Gburek, D. Sýkora, P. Martásek, Iron complexes of flavonoids-antioxidant capacity and beyond. Int. J. Mol. Sci., 22 (2021) 1–20.
6. J. Mierziak, K. Kostyn, A. Kulma, Flavonoids as Important Molecules of Plant Interactions with the Environment. Molecules, 19 (2014) 16240–16265.
7. A.R. Rubio, R. González, N. Busto, M. Vaquero, A.L. Iglesias, F.A. Jalón, G. Espino, A.M. Rodríguez, B. García, B.R. Manzano, Anticancer Activity of Half-Sandwich Ru, Rh and Ir Complexes with Chrysin Derived Ligands: Strong Effect of the Side Chain in the Ligand and Influence of the Metal. Pharmaceutics. 13 (2021) 1540.
8. F. Fusi, A. Trezza, M. Tramaglino, G. Sgaragli, S. Saponara, O. Spiga, The beneficial health effects of flavonoids on the cardiovascular system: Focus on K+ channels. Pharmacol. Res. 152 (2020) 104625.
9. P.M. Joyner, Protein adducts and protein oxidation as molecular mechanisms of flavonoid bioactivity. Molecules, 26 (2021) 5102.
10. M. Russo, S. Moccia, C. Spagnuolo, I. Tedesco, G.L. Russo, Roles of flavonoids against coronavirus infection. Chem. Biol. Interact. 328 (2020) 109211.
11. A. Tawani, A. Kumar, Structural Insight into the interaction of Flavonoids with Human Telomeric Sequence. Nat. Publ. Gr. 5 (2015) 17574.

12. H.K. Srivastava, M. Chourasia, D. Kumar, G.N. Sastry, Comparison of computational methods to model DNA minor groove binders. J. Chem. Inf. Model. 51 (2011) 558–571.
13. N. Sjakste, N. Djelić, M. Dzintare, L. Živković, DNA-BINDING and DNA-protecting activities of small natural organic molecules and food extracts. Chem. Biol. Interact. 323 (2020) 109030.
14. A. Pandey, A. Upadhyaya, S. Kumar, A.K. Yadav, Dynamics and Stability Analysis of Some Minor Groove Binders with B-DNA Dodecamer 5'(CGCAAATTTGCG)-3'. Drug Des. 10 (2020) 7–12.
15. A. Pandey, R. Mishra, A. Shukla, A.K. Yadav, D.Kumar, In-silico docking studies of 2 , 5-bis (4-amidinophenyl) furan and its derivatives. Proceeding of ISAFBM-2019 1 (2019) 11–18.
16. D. Atrahimovich, D. Avni, S. Khatib, Flavonoids-Macromolecules Interactions in Human Diseases with Focus on Alzheimer, Atherosclerosis and Cancer. Antioxidants 10 (2021) 423.
17. T. Hussain, B. Tan, G. Murtaza, G. Liu, N. Rahu, M. Saleem Kalhoro, D. Hussain Kalhoro, T.O. Adebowale, M. Usman Mazhar, Z Rehman, Flavonoids and type 2 diabetes: Evidence of efficacy in clinical and animal studies and delivery strategies to enhance their therapeutic efficacy. Pharmacol. Res. 152 (2020) 104629.
18. A. Shukla, R. Mishra, A. Pandey, A.K. Dwivedi, D. Kumar, Interaction of Flavonols with DNA : Molecular Docking Studies. Proceeding of ISAFBM-2019 (2019) 4–10.
19. A. Moulishankar, K. Lakshmanan, Data on molecular docking of naturally occurring flavonoids with biologically important targets. Data Br. 29 (2020) 105243.
20. R.K. Singh, A.K. Dwivedi, J.P. Pandey, R. Yadav, N. Awasthi, A. Shukla, A Review on the interaction of various types of Flavonoids with DNA. J. Sci. Comput. 9 (2020) 39–51.
21. C.D. Kanakis, P.A. Tarantilis, M.G. Polissiou, S. Diamantoglou, H.A. Tajmir-Riahi, An overview of DNA and RNA bindings to antioxidant flavonoids. Cell biochemistry and biophysics. 49(1) (2007) 29–36.
22. T. Zhang, T. Tian, R. Zhou, S. Li, W. Ma, Y. Zhang, N. Liu, S. Shi, Q. Li, X. Xie, Design, fabrication and applications of tetrahedral DNA nanostructure-based

multifunctional complexes in drug delivery and biomedical treatment. Nat. Protoc., 15 (2020) 2728–2757.

23. M. Sirajuddin, S. Ali, A. Badshah, DNA interactions and their study by UV – Visible, fluorescence spectroscopies and cyclic voltametry. J. Photochem. Photobiol. B Biol. 124 (2013) 1–19.
24. H.P.V. Rupasinghe, Special Issue "Flavonoids and Their Disease Prevention and Treatment Potential": Recent Advances and Future Perspectives. Molecules. 25 (2020) 1–7.
25. S. Bi, C. Qiao, D. Song, Y. Tian, D. Gao, Y. Sun, H. Zhang, Study of interactions of flavonoids with DNA using acridine orange as a fluorescence probe. Sensors Actuators B 119 (2006) 199–208.
26. C.D. Kanakis, P.A. Tarantilis, M.G. Polissiou, S. Diamantoglou, H.A. Tajmir-Riahi, DNA Interaction with Naturally Occurring Antioxidant Flavonoids Quercetin , Kaempferol , and Delphinidin DNA Interaction with Naturally. J. Biomol. Struct. Dyn. 22 (2005) 719–724.
27. N.K. Janjua, A. Siddiq, A. Yaqub, S. Sabahat, R. Qureshi, S. Ul Haque, Spectrophotometric analysis of flavonoid-DNA binding interactions at physiological conditions. Spectrochim. Acta - Part A Mol. Biomol. Spectrosc. 74 (2009) 1135–1137.
28. G.H. Kocharyan, S.H. Minasyan, L.A. Tavadyan, Interaction Of Flavonoids: Morin, Quercetin And Rutin, with DNA. Chem. Biol. 1 (2016) 49–54.
29. A.W. Septama, N. Simbak, E.P. Rahmi, Prospect of plant-based flavonoids to overcome antibacterial resistance: A mini-review. Walailak J. Sci. Technol. 17 (2020) 503–513.
30. S.A. Divakaran, P.S. Hema, M.S. Nair, C.K.K. Nair, Antioxidant capacity and radioprotective properties of the flavonoids galangin and kaempferide isolated from Alpinia galanga L (Zingiberaceae) against radiation induced cellular DNA damage. Int. J. Radiat. Res. 11 (2013) 81–89.
31. S.A. Payán-Gómez, N. Flores-Holguín, A. Pérez-Hernández, M. Piñón-Miramontes, D. Glossman-Mitnik, Computational molecular characterization of the flavonoid rutin. Chem. Cent. J. 4 (2010) 12.
32. R. Dennington, T.A. Keith, J.M. Millam, Semichem Inc., S.M. GaussView, Version 6.1. KS 2016, 2016.

33. Gaussian 09, Revision E.01, M.J .Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery, J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J.J. aramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, O. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski and D.J. Fox, Gaussian, Inc., Wallingford CT (2009).
34. H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The Protein Data Bank. Nucleic Acids Res. 28 (2000) 235–242.
35. Y. Wang, N.C. Schnetz-boutaud, H. Kroth, H. Yagi, M. Jane, S. Kumar, D.M. Jerina, Stone, M.P. 3′-Intercalation of a N2 -dG 1R-trans-anti-Benzo[c]phenanthrene DNA Adduct in an Iterated (CG)3 Repeat. Chem Res Toxicol. 21 (2009) 1348–1358.
36. E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, UCSF Chimera — A Visualization System for Exploratory Research and Analysis. J. Comput. Chem. 25 (2004) 1605–1612.
37. G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem. 30 (2009) 2785–2791.
38. G.M. Morris, D. Goodsell, R. Halliday, R. Huey, W. Hart, R. Belew A. Olson, J. Comput. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. Chem. 19 (1998) 1639–1662.
39. D.A. Case, T.E. Cheatham, T.O.M. Darden, H. Gohlke, R.A.Y. Luo, K.M. Merz, A. Onufriev, C. Simmerling, B. Wang, R.J. Woods, The Amber Biomolecular Simulation Programs. J. Comput. Chem. 26 (2005) 1668–1688.

40. H.K. Srivastava, G.N. Sastry, Efficient estimation of MMGBSA-based BEs for DNA and aromatic furan amidino derivatives. J. Biomol. Struct. Dyn. 31 (2013) 522–537.
41. R. Kumari, R. Kumar, A. Lynn, G-mmpbsa -A GROMACS tool for high-throughput MM-PBSA calculations. J. Chem. Inf. Model. 54 (2014) 1951–1962.

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

6.1 Introduction

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 [1]. It is an estrogenic EDC i.e., endocrine disrupting compound. BPA is called estrogenic EDC as it mimics estrogen [2]. Being a xenoestrogen, it disrupts the hormonal balance in the human body [3].

BPA and its derivatives have been used heavily in the manufacture of epoxy resins and polycarbonate plastics. They are mainly used in food packaging materials, dental sealants etc. Human beings are subjected to the exposure of BPA through their diet via consumption of packed food materials [4]. BPA which is used in food packaging materials can leak into the food or get mixed with it. Because of long preservation time period, storage condition like temperature, pressure or packing technique, the chemical constituents of packing material may get migrate into the food which may cause severe health issues [5].

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 [6-8].
A number of studies since a long time indicate the miserable effects due to BPA exposure in adults [9]. It may lead to various health associated problems like reduced

ovarian response and IVF success, decrease in fertilization success rate and embryo quality, miscarriage, abnormal and premature delivery, reduced male sexual function, reduction in sperm quality, PCOS, alteration in the concentration of thyroid hormone, type-2 diabetes, cardiovascular disease (i.e. heart disease, hypertension, and cholesterol levels), altered liver function, obesity, albuminuria, oxidative stress and many more. BPA has also been supposed to affect breast cancer and prostate cancer development [10-12].

The severe effect of BPA on the development of children and various health issues lead to the restriction of use of BPA in the manufacture of baby food containers and other food containers [13, 14]. After this several derivatives of BPA came in highlight which can act as an alternative of BPA. As a consequence, existence of bisphenol S (BPS), bisphenol F (BPF) and bisphenol AF (BPAF) in many daily use food and products including vegetables, meat, seafood and dairy products is very common now-a-days. But are these derivatives safe enough, is a question of concern.

If bromine or chlorine is substituted at the phenolic ring of BPA, we get halogenated derivatives of BPA. These are mainly used in the form of flame retardants (FRs) to decrease the flammability of polymers. The name of bromine substituted derivatives is TBBPA (Tetrabromobisphenol A) whereas chlorine substituted derivative is called TCBPA (Tetrachlorobisphenol A). TBBPA and TCBPA are generally used as FRs in the polymer industry. These derivatives are also dangerous for the environment as their continuous use enhances contamination in the environment [15].

Understanding and analysis of the mechanism by which BPA and its derivatives affects different functions of human body is a matter of great concern and it requires a lot of research work. A number of studies have been done on the effect of BPA on humans in the last few years but unfortunately the studies on its derivatives are not enough [16-18].

The alternatives of BPA which are currently used in the industries are just derivatives of BPA which can be as hazardous as BPA [19-22]. It is need of the hour to investigate the functionality, mechanism and effects of such compounds like BPS, BPAF, TBBPA, TCBPA etc. on human health.

The genotoxic and endocrine activities of BPF and its metabolites were examined by Cabaton et al. (2009) and it was found that the most toxic compound was BPF compared to its other metabolites identified in rat urine [23]. Another study tells that

alike BPA and BPS, other bisphenols derivatives BPF and BPAF have been found in the urine of humans [24].

BPS (bisphenol S) also acts as an EDC (endocrine disruptor compound) similar to BPA [25]. As BPS is more stable in response to heat and light effects than BPA, its degradation in sea water is very hard and remains a persistent toxic pollutant compared with BPA [26]. These properties of BPS indicate a high pollution potential for BPS as it is used increasingly [27].

However, no biotransformation studies have been performed for other BPA analogs such as bisphenol AF (BPAF), bisphenol Z (BPZ), TBBPA (Tetrabromobisphenol A), TCBPA (Tetrachlorobisphenol A) etc.

Some of the previous studies indicate the interaction of BPA and its analogs with DNA [28]. An oxidation product of 3-hydroxy-BPA is ortho-quinone BPA, which has been reported to form adducts with DNA in vitro and in vivo [29]. The reaction of bisphenol A 3,4-quinone with DNA is studied by Edmonds et.al. [30]. Wang et.al., in 2014 studied the interaction of BPS with DNA and concluded the minor groove binder nature of BPS [31].

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 [32–34]. 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 [35].

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 [36, 37]. There are only a few works which are dedicated to the interaction of BPA and BPA derivatives with DNA [38, 39]. 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. Molecular Docking is a tool to investigate the interactivity between a macromolecule and a ligand [40]. This will talk about the possible interaction of the selected 5 BPA compounds with DNA. The result from docking process provides the input for molecular dynamics simulation which investigates the time dependent characteristics of the system. Finally, Quantum Mechanical/Molecular Mechanical (QM/MM) was done to take the investigation at deeper level. Such investigations would aid in better understanding of the toxic mechanism of BPA and bring about new scientific insights about other Bisphenol A analogues.

6.2 Computational Details

6.2.1 Dataset: The pdb format file of DNA sequences with PDB ID - 1BNA, 1DSC, 1RMX, 2ROU and 195D were sourced from RCSB 'Protein Data Bank' (PDB) [41]. All crystallographic water and small molecules were removed from the DNA sequences by the help of UCSF Chimera Software [42]. Hydrogen atoms were

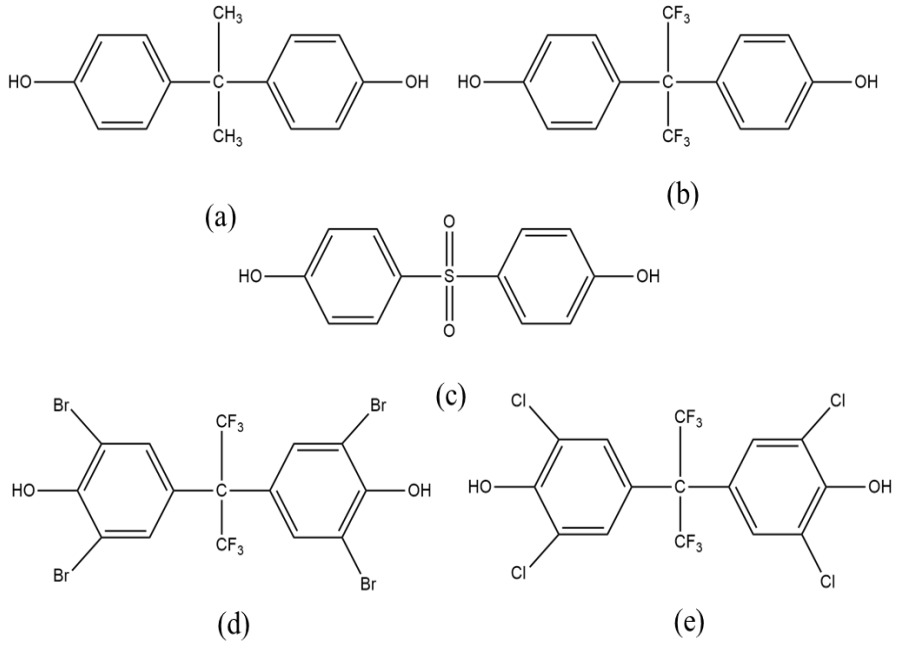


Figure 6.1. Structure of BPA and BPA derivatives. (a) BPA, (b) BPAF, (c) BPS, (d) TBBPA, (e) TCBPA

also added. Table 1 shows the PDB ID and sequence of the DNA segment used.

Drugs: The structures of BPA, BPAF, BPS, TBBPA and TCBPA were collected from literature [43, 44]. Figure 6.1 shows the chemical structures of these drugs. These structures were designed using Gaussview5.0 software [45].

6.2.2 Geometry Optimization: The Gaussian 09 software tool was used to optimize the geometry of the ligands at the B3LYP/6-31G level [46].

Table 6.1. PDB ID and sequence of the DNA used.

S.no	PDB ID	DNA Sequence
1	1BNA	5'-CGCGAATTCGCG-3'
2	1DSC	5'-GAAGCTTC-3'
3	1RMX	5'-CGACTAGTCG-3'
4	2ROU	5'-ATCGCGCGGCATG-3'
5	195D	5'-CGCGTTAACGCG-3'

6.2.3. Molecular Docking

Molecuar Docking process between the ligands and DNA segments was performed using AUTODOCK4.2 software [47]. Autodock tools with Lamarckian Genetic Algorithm (LGA) was implemented. Firstly, macromolecule was prepared in the form of PDBQT file [48]. Then, ligand was also prepared and saved as a PDBQT file. After preparation of macromolecule and ligand, grid boxes of various dimensions were prepared for each complex system and grid calculations were performed. Second step is the docking calculations. Docking was performed and dlg type file is the output file which gives the details of the docking process.

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.

6.2.4. Molecular Dynamics

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 [49]. In the present work, MD simulations were carried out using GROMACS 5.0.4 software [50]. 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.

After the analysis of molecular docking process, two complex systems namely BPS and TBBPA with DNA segment 1BNA were subjected to molecular dynamics simulations of 100ns each. The AMBER force fields [51] appear to be good for nucleic acid simulation due to the presence of unusual topologies for the terminal nucleotides, despite the fact that there are numerous studies on force fields accessible for molecular dynamics simulations of nucleic acids. For the purpose of creating a topology file for the chosen DNA sequence, the GROMACS software suite's Amber03 force field was used. To generate an octahedral box for the ligand-DNA solvation at 298K, the software's TIP3P water model was used [52]. The system was then neutralised by adding sodium ions to the solvated box holding the DNA-ligand complex by randomly replacing the water molecules. Long-range electrostatic interactions were handled using Particle Mesh Ewald (PME) [53]. Using the Steepest Descent leap-Frog Integration Method, the entire system's energy minimization was done in 25000 steps. This was followed by NVT ensemble equilibration at a constant temperature of 300K for 50ns using a Berendsen thermostat [54]. The system was then equilibrated using a steepest descent leap-frog integrator with an NPT ensemble at a constant pressure of 1 atm over a period of 25000 steps. [55]. XMgrace software was used to plot the graphs [56].

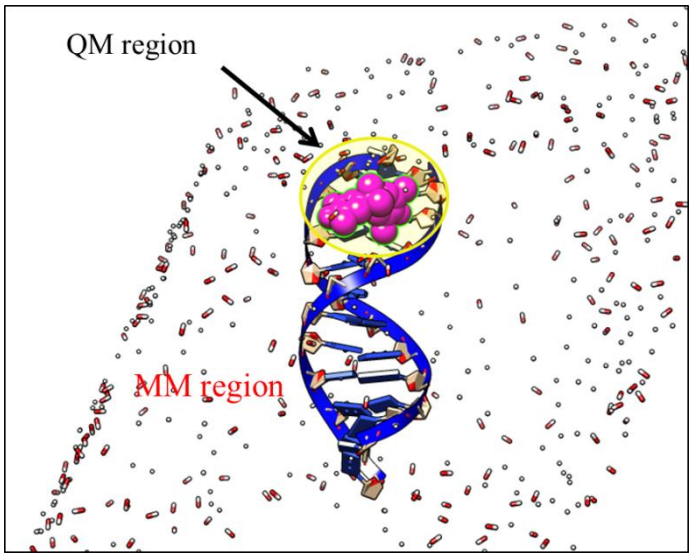


Figure 6.2. QM and MM regions for QM/MM

6.2.5 QM/MM calculations

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.

A total of 3 snapshots at 2ns difference from the molecular dynamics results as obtained from GROMACS, for the TBBPA-1BNA complex, were obtained using VMD and that constructed the base of the starting point of the QM/MM calculations. These structures contained the bimolecular system surrounded by water droplets of around 20000-30000 atoms in number and this setup requires a lot of prior work to avoid errors and wrong choices regarding the actual QM/MM calculations.

Figure 6.2, depicts the two layered ONIOM scheme of Gaussian09 used in the current study. The mechanical-embedding version of ONIOM was used for geometry optimization and the energy of the high or QM region is calculated. Now, the co-ordinate of high layer i.e., QM region from the optimized system is extracted. This high layer extracted system is now put through geometry optimization and the energy of QM region in the gas phase is calculated. The interaction energy between the DNA and drug molecule is calculated with the help of equation:

$$\Delta E_{IE} = E_{QM\,(gp)} - E_{QM\,(pp)} \qquad (6.1)$$

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.

6.3 Results and Discussion

6.3.1 Geometry Optimization

It is very important to bring the system of consideration into lowest possible energy state before doing molecular docking process, so the step of geometry optimization has been done. It gives the state of the system with minimum steric hindrances and

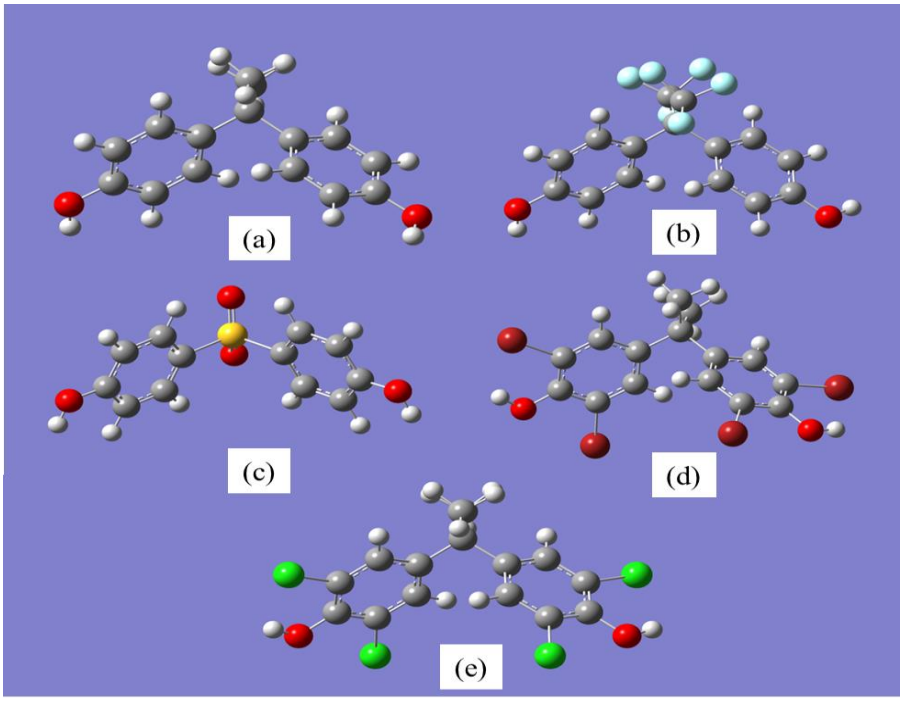


Figure 6.3. Optimized structures of the selected ligands. (a) BPA, (b) BPAF, (c) BPS, (d) TBBPA, (e) TCBPA

charge repulsions. In this state the system attains maximized bond lengths, angles and dihedrals angles. Additionally, the electrostatic charges also have a tendency to be evenly dispersed across the entire system, which causes an electron transfer between a specific pair of atoms. As a result, no damage to the chemical structures is produced. [57].

Figure 6.3 represents the three-dimensional optimized geometries of all the ligands after optimization by Gaussian 09 software at B3LYP density functional with basis set 631-G. These optimized ligands were used as initial inputs for the molecular docking process.

6.3.2. Molecular Docking

In an attempt to find the mode of binding of various drugs with DNA structures, molecular docking tool was used in the present study. It is an important and effective tool to gain information regarding the structural characteristics of ligand-

macromolecule complexes and the binding efficiency of a ligand with its receptor, which can support and verify the experimental results. The results obtained from molecular docking done by Autodock software for each DNA sequence are summarized in below sections.

Molecular docking studies were done for the five sets of DNA segments in search of their preferential binding modes, corresponding binding affinities and for gaining atomistic insights regarding the factors that contribute to the stable complex formation.

Table 6.2: 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

6.3.2.1 Drugs with 1BNA

Binding energies obtained from computational docking method for the DNA sequence with PDB ID 1BNA is tabulated in Table 6.2. From the resulting docking complexes, as given in Table 3.3 and shown in figure 6.4, it is quite clear that the manner of binding interaction of BPA derivatives with sequence 1BNA shows mainly minor groove binding. The main reason for this type of binding was mainly the structure of the DNA sequence. TBBPA-DNA complex has largest binding energy which means this complex is most

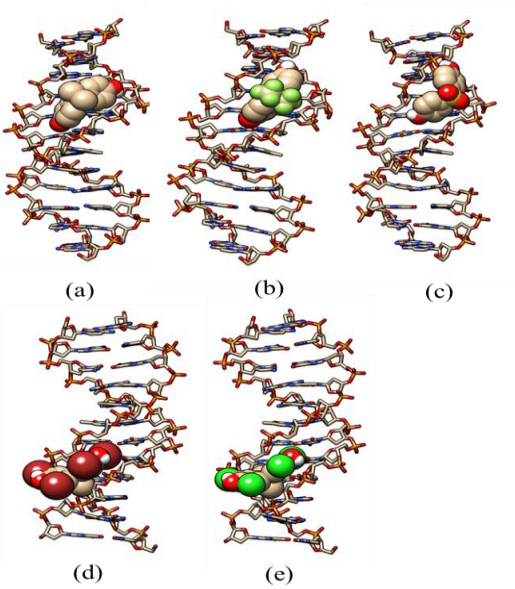


Figure 6.4. Best docked posed complexes for 1BNA (a) BPA, (b) BPAF, (c) BPS, (d) TBBPA, (e) TCBPA.

tightly bonded and it is most stable.

6.3.2.2 Drugs with 1DSC

Binding energies obtained from computational docking method for the DNA sequence with PDB ID 1DSC is tabulated in Table 6.2. From the resulting docking complexes, as given in Table 3.3 and shown in Figure 6.5, it is quite clear that the mode of binding interaction of BPA derivatives with sequence 1DSC shows mainly minor groove binding. TBBPA-DNA complex has largest binding energy which means this complex is most tightly bonded and it is most stable. The mode of binding of TBBPA with DNA is different from others. It is bound in the minor groove as well as in the intercalation gap of the DNA sequence.

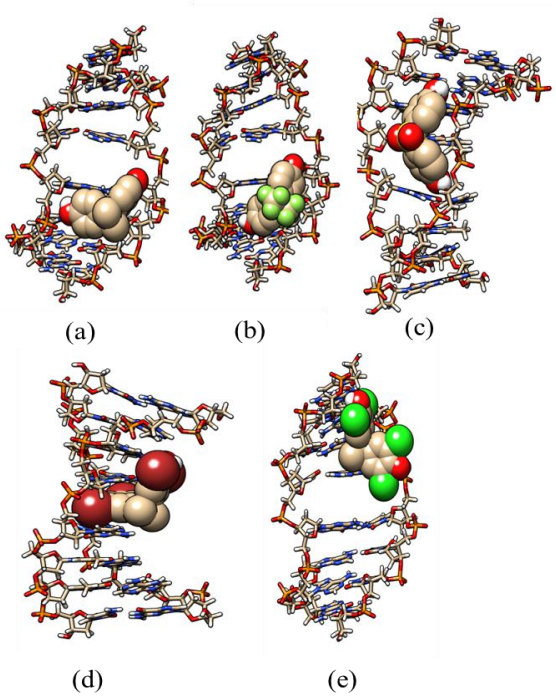


Figure 6.5. Best docked posed complexes for 1DSC (a) BPA, (b) BPAF, (c) BPS, (d) TBBPA, (e) TCBPA

6.3.2.3 Drugs with 1RMX

Binding energies obtained from computational docking method for the DNA sequence with PDB ID 1RMX is tabulated in Table 6.2. From the resulting docking complexes, as given in Table 3.3 and shown in Figure 6.6., it is quite clear that the manner of binding interaction of BPA derivatives with sequence 1RMX shows mainly minor groove binding. TBBPA-DNA complex has largest binding energy which means this complex is most tightly bonded and

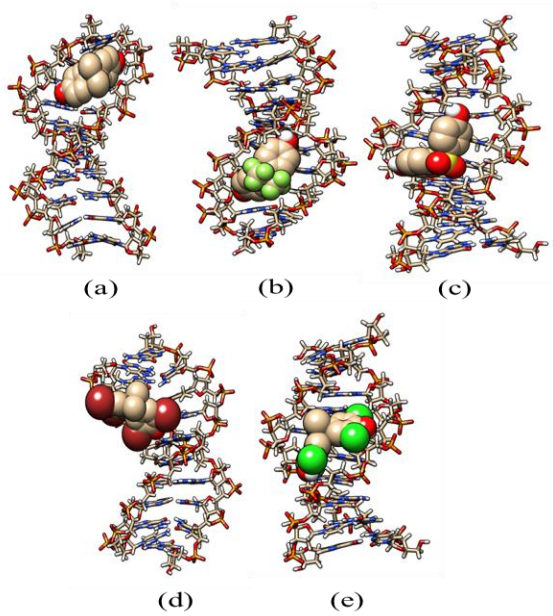


Figure 6.6. Best docked posed complexes for 1RMX (a) BPA, (b) BPAF, (c) BPS, (d) TBBPA, (e) TCBPA.

it is most stable. The mode of binding of TBBPA with 1RMX is major groove binding which is different from others. It is bound in the major groove of the DNA segment.

6.3.2.4 Drugs with 2ROU

Binding energies obtained from computational docking method for the DNA sequence with PDB ID 2ROU is tabulated in Table 6.2. From the resulting docking complexes, as given in Table 3.3 and shown in Figure 6.7, it is simply clear that the mode of binding interaction of BPA derivatives with sequence 2ROU shows both minor groove binding and intercalation. TBBPA and TCBPA forms intercalative complex with 2ROU. The availability of intercalation gap makes the larger size compounds to attach there. Here also TBBPA has largest binding energy which means this complex is most tightly bonded and it is most stable.

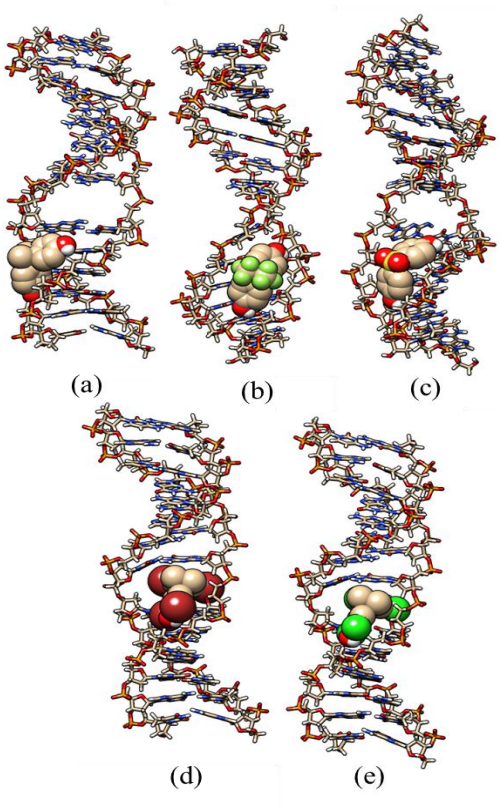


Figure 6.7. Best docked posed complexes for 2ROU (a) BPA, (b) BPAF, (c) BPS, (d) TBBPA, (e) TCBPA.

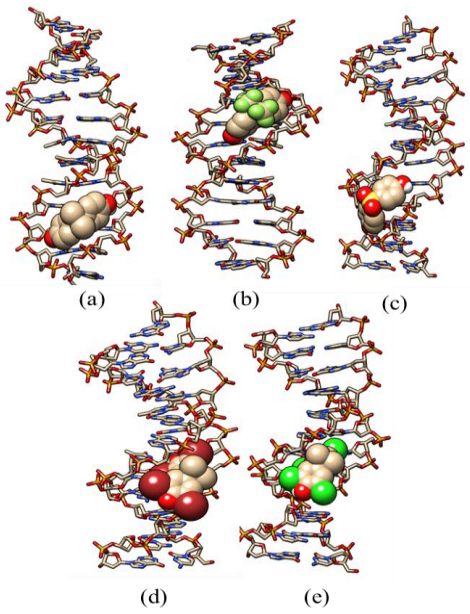


Figure 6.8. Best docked posed complexes for 195D (a) BPA, (b) BPAF, (c) BPS, (d) TBBPA, (e) TCBPA.

6.3.2.5 Drugs with 195D

Binding energies obtained from computational docking method for the DNA sequence with PDB ID 195D is tabulated in Table 6.2. From the resulting docking complexes, as given in Table 3.3 and shown in Figure 6.8., it is simply clear that the mode of binding

interaction of BPA derivatives with sequence 195D shows minor groove binding. Here also TBBPA has largest binding energy which means this complex is most tightly bonded and it is most stable.

Table 6.3. 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

6.3.3 Hydrogen binding analysis

For each drug-DNA complex system's optimal docked pose, detailed hydrogen bond analysis was performed to get total information about the importance of hydrogen binding in the stability of the considered complex system. Three-dimensional system configuration was obtained to identify the residues of the DNA and atoms of the ligands connected in the hydrogen bonding. This was done with the help of Discovery Studio visualizer [58]. Figure 6.9-6.13 shows the donor and acceptor region between the ligand and DNA residues for the best docked pose of each DNA-ligand system. In order to establish stability during the docking computations, the clouded patches in the figure depict the atoms or groups of atoms that have a capacity to give or take electrons for hydrogen bonding.

Table 6.4. The donor species, acceptor species and H-bond length formed between the ligands and 1BNA.

S.No.	DNA segment	Ligand	No. of H-bonds	Interacting species	H-bond length(Å)
1.	1BNA	BPA	2	UNK0:H - B:DT20:O2 A:DG4:N2 - :UNK0	2.146658 3.425313
		BPAF	5	A:DA6:C5' - :UNK0:F UNK0:H - B:DC23:O4' B:DG22:C5' - :UNK0:F B:DG22:C5' - :UNK0:F UNK0:H - B:DT20:O2	2.953399 1.974467 3.322622 2.920749 2.107035
		BPS	2	A:DA6:C5' - :UNK0:O B:DG22:N2 - :UNK0:O	3.107792 3.027773
		TBBPA	4	UNK0:H - A:DC9:O3' B:DT19:C5' - :UNK0:O B:DA17:C2 - :UNK0:Br B:DA17:C2 - :UNK0:Br	2.653264 3.460350 3.688278 3.688278
		TCBPA	4	UNK0:H - A:DC9:O3' B:DT19:C5' - :UNK0:O B:DA17:C2 - :UNK0:Cl B:DA17:C2 - :UNK0:Cl	2.496548 3.467059 3.422616 3.422616
2.	1DSC	BPA	3	B:DA11:H1' - :UNK0:O UNK0:H - B:DG12:O4' UNK0:H - A:DC5:O4'	2.592223 1.948967 1.985355
		BPAF	6	UNK0:H - A:DC5:O4' A:DT6:H4' - :UNK0:F A:DT6:H4' - :UNK0:F A:DT6:H1' - :UNK0:F A:DT7:H5'1 - :UNK0:F UNK0:H - B:DG12:OP	1.846534 2.478208 2.400654 2.124977 2.018987 1.616678
		BPS	4	B:DG12:H21 - UNK0:O UNK0:H - A:DC5:O4' UNK0:H - A:DG4:OP1 B:DT15:H1' - :UNK0:O	3.082238 1.795986 2.093244 2.868413
		TBBPA	1	A:DG4:H1' - :UNK0:Br	2.858970
		TCBPA	3	UNK0:H - A:DC5:OP1 UNK0:H - B:DT15:O3' B:DC16:H5'1 -UNK0:Cl	1.920369 1.848536 2.829143
3.	1RMX	BPA	4	B:DG17:H22 - UNK0:O UNK0:H - B:DT18:O2 UNK0:H - A:DG2:N3 UNK0:H - A:DA3:O4'	2.205445 1.954476 2.844300 2.000574
		BPAF	8	UNK0:H - B:DC14:O4' UNK0:H - B:DA16:O4' A:DT8:H1' - :UNK0:F A:DT8:H4' - :UNK0:F A:DG7:H22 - :UNK0 B:DT15:H5'1 - UNK0:F B:DT15:H4' - :UNK0:F A:DG7:H22 - :UNK0	1.849773 1.942170 2.607677 2.646219 2.326485 2.638398 2.456756 2.326485
		BPS	3	A:DG7:H4' - :UNK0:O A:DA6:H1' - :UNK0:O	2.739161 2.368616

				B:DG17:H22 - :UNK0:O	2.511983
		TBBPA	4	UNK0:H - A:DG2:OP2 UNK0:H - B:DG17:O6 A:DC4:H42 - :UNK0:O A:DA3:H8 - :UNK0:Br	1.649261 1.933400 2.766324 2.457550
		TCBPA	2	UNK0:H - A:DG7:O3' A:DT5:H4' - :UNK0:Cl	1.920755 2.829540
4.	2ROU	BPA	4	UNK0:H - B:DC23:O4' UNK0:H - B:DG24:N3 UNK0:H - B:DA25:O4' B:DA25:H5'1 - UNK0:O	2.112552 2.593854 2.288214 2.083864
		BPAF	7	UNK0:H - B:DC23:O4' A:DC5:H1' - :UNK0:F A:DC5:H4' - :UNK0:F A:DG4:H21 - :UNK0 B:DG24:H4' - :UNK0:F B:DG24:H22 - UNK0:O UNK0:H - B:DA25:O4'	1.971740 2.301018 2.471182 2.795444 2.566168 1.897726 2.059780
		BPS	4	B:DG22:H22 - UNK0:O UNK0:H - B:DC23:O4' B:DG24:H22 - UNK0:O UNK0:H - B:DA25:O4'	1.811454 2.086173 1.759489 1.931819
		TBBPA	2	UNK0:H - B:DG22:O3' B:DG22:N3 - :UNK0:Br	1.907082 3.364273
		TCBPA	1	UNK0:H - B:DG22:O3'	1.956736
5.	195D	BPA	6	UNK0:H - A:DG10:O4' UNK0:H - A:DC9:O2 B:DG16:N2 - :UNK0 A:DG10:N2 - :UNK0 B:DG14:N2 - :UNK0:O UNK0:H - B:DC15:O2	2.024700 3.010265 3.366872 3.732183 2.963752 2.255529
		BPAF	6	UNK0:H - A:DA7:O4' UNK0:H - A:DT6:O2 A:DT6:C4' - :UNK0:F B:DC21:C4' - :UNK0:F B:DG22:C5' - :UNK0:F B:DG22:C5' - :UNK0:F	2.914469 2.242876 3.275043 3.445188 3.440485 3.112585
		BPS	6	B:DT18:C5' - :UNK0:O UNK0:H - A:DC11:O2 A:DG10:N2 - :UNK0 B:DG16:N2 - :UNK0 B:DG16:N2 - :UNK0 UNK0:H - A:DC9:O4'	3.131961 1.868998 3.904925 3.360563 4.150626 2.103495
		TBBPA	3	UNK0:H - A:DG10:OP1 B:DA19:C5' - :UNK0:Br UNK0:H - B:DT18:O2	1.671796 3.493976 2.026440
		TCBPA	3	UNK0:H - A:DG10:OP1 UNK0:H - B:DT18:O2 A:DA7:C2 - :UNK0:O	1.779204 1.929722 2.838304

6.3.3.1. Drugs with 1BNA

The main type of binding in the case of drugs with 1BNA is groove binding and the main type of binding responsible for this behavior found out to be hydrogen bonding linking the residues of DNA and atoms of the ligands.

Table 6.4 gives the donor and the acceptor species as well as the H-bond length formed between the ligands and the DNA sequences. The number of H-bonds formed for each complex is also indicated. The number of hydrogen bonds for BPA, BPAF, BPS, TBBPA and TCBPA are 2,5,2,4 and 4 respectively.

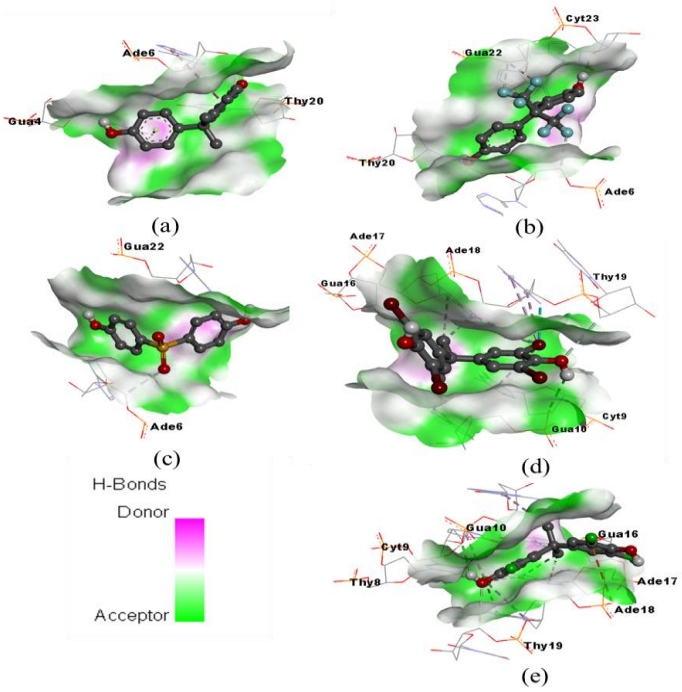


Figure 6.9. H-bonds acceptor and donor regions for 1BNA-ligand complex systems in three dimensions.

To understand the hydrogen-bond acceptor and donor species more clearly, three-dimensional exploration was carried out using Discovery studio visualizer. Figure 6.9 represents the H-bonds acceptor and donor regions for drug-1BNA complex systems in three dimensional view. These regions help in determination of the electron deficient and electron rich locations in the drug-DNA complex system.

6.3.3.2. Drugs with 1DSC

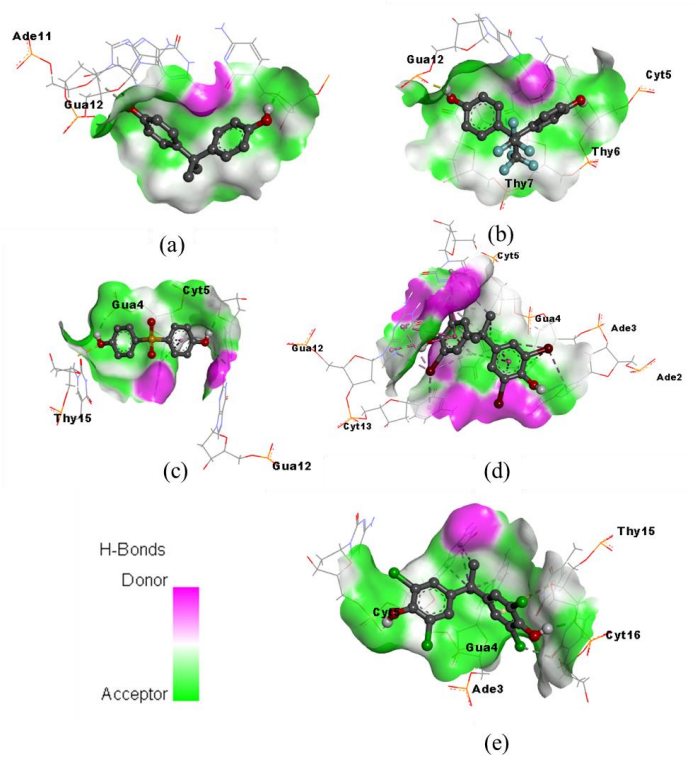


Figure 6.10. H-bonds acceptor and donor regions for 1DSC-ligand complex systems in three dimensions.

The main type of binding in the case of drugs with 1DSC is groove binding and the main type of bonding responsible for this behavior found out to be hydrogen bonding between the residues of DNA and atoms of the ligands. The number of hydrogen bonds for BPA, BPAF, BPS, TBBPA and TCBPA are 3, 6, 4, 1 and 3 respectively. As TBBPA intercalates in the DNA segment so hydrophobic bonding dominates and the number of H-bonds is minimum.

To understand the hydrogen-bond acceptor and donor species more clearly, three-dimensional exploration was carried out using Discovery studio visualizer. Figure 6.10 represents the H-bonds acceptor and donor regions for drug-1DSC complex systems in three-dimensional view.

6.3.3.3. Drugs with 1RMX

The main type of binding in the case of drugs with 1RMX is groove binding and the main type of bonding responsible for this behavior was found out to be hydrogen bonding between the residues of DNA and atoms of the ligands. The number of hydrogen bonds for BPA, BPAF, BPS, TBBPA and TCBPA are 4,8,3,4 and 2 respectively.

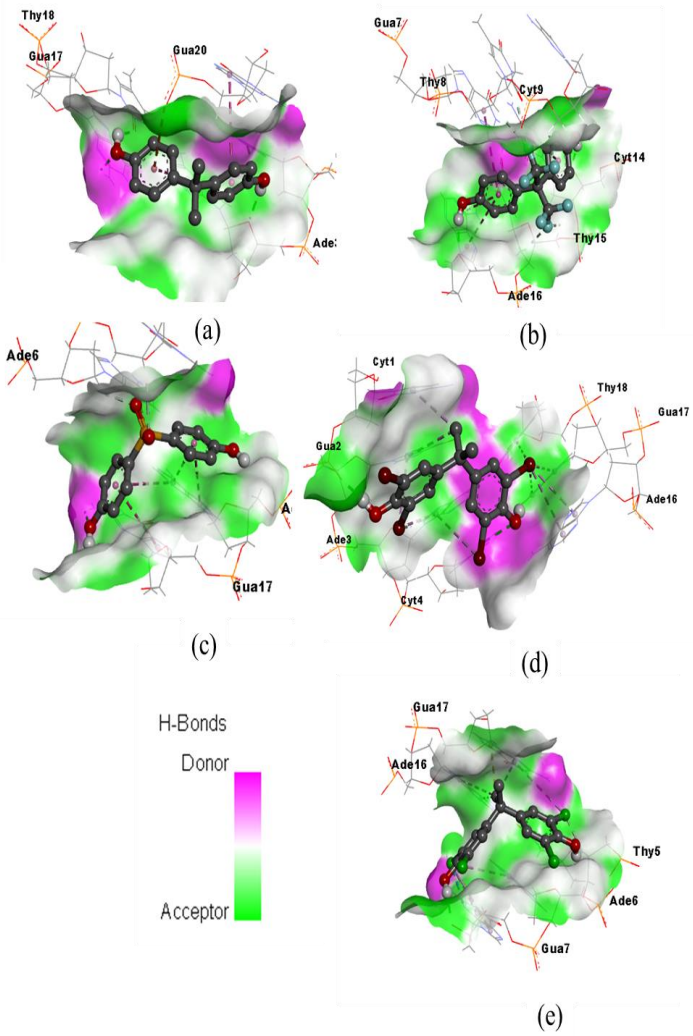


Figure 6.11. H-bonds acceptor and donor regions for 1RMX-ligand complex systems in three dimensions.

To understand the hydrogen-bond acceptor and donor species more clearly, three-dimensional exploration was carried out using Discovery studio visualizer. Figure 6.11 represents the H-bonds acceptor and donor regions for drug-1RMX complex systems in three-dimensional view.

6.3.3.4. Drugs with 2ROU

Both type of binding, groove binding and intercalation were present in the case of

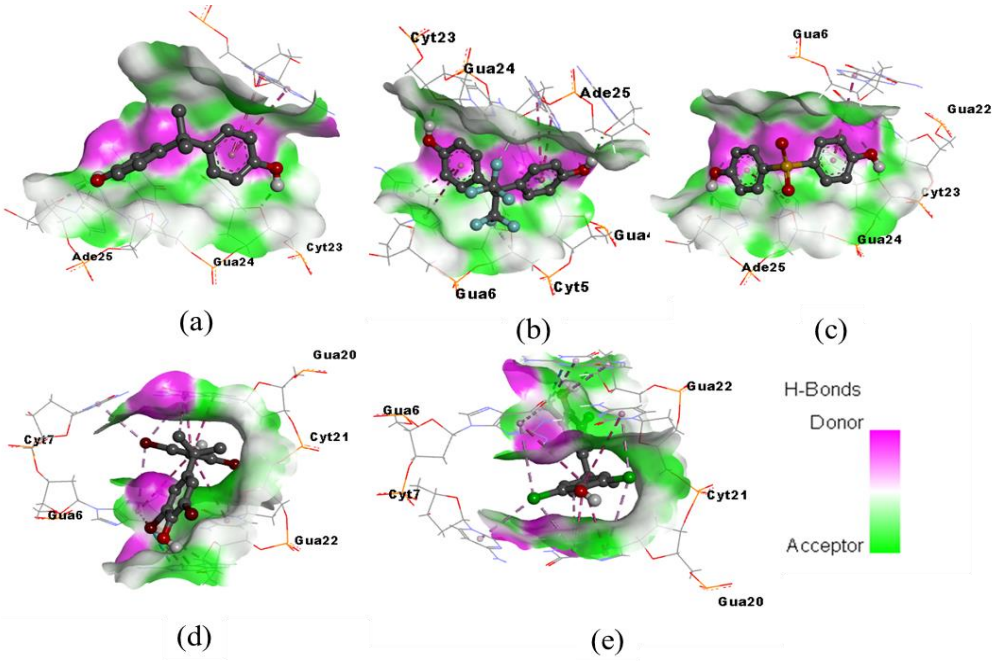


Figure 6.12. H-bonds acceptor and donor regions for 2ROU-ligand complex systems in three dimensions.

drugs with 2ROU. Hydrogen bonding and hydrophobic bonding both are present between the residues of DNA and atoms of the ligands. The number of hydrogen bonds for BPA, BPAF, BPS, TBBPA and TCBPA are 4,7,4,2 and 1 respectively. Since TBBPA and TCBPA are intercalated so H-bonds for them are minimum.

To understand the hydrogen-bond acceptor and donor species more clearly, three-dimensional exploration was carried out using Discovery studio visualizer. Figure 6.12 represents the H-bonds acceptor and donor regions for drug-2ROU complex systems in three-dimensional view. More H-bond donor region can be seen from the figure.

6.3.3.5. Drugs with 195D

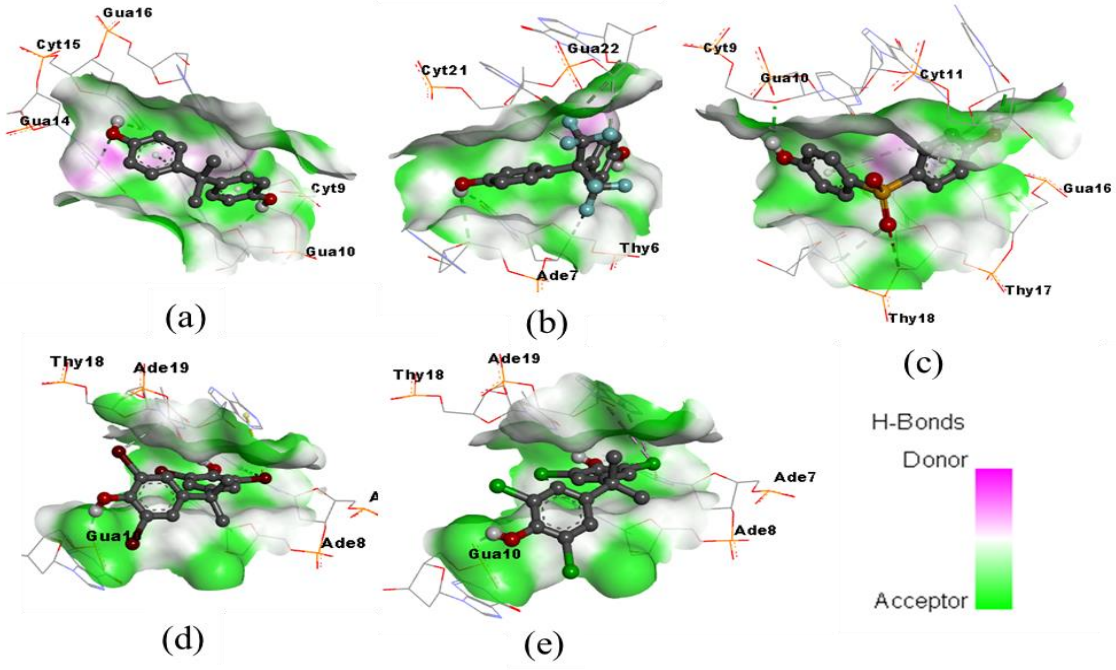


Figure 6.13. H-bonds acceptor and donor regions for 195D-ligand complex systems in three dimensions.

The main type of binding in the case of drugs with 195D is groove binding and the main type of binding responsible for this behavior is found out to be hydrogen bonding between the residues of DNA and atoms of the ligands. The number of hydrogen bonds for BPA, BPAF, BPS, TBBPA and TCBPA are 6, 6, 6, 3 and 3 respectively.

To understand the hydrogen-bond acceptor and donor species more clearly, three-dimensional exploration was carried out using Discovery studio visualizer. Figure 6.13 represents the H-bonds acceptor and donor regions for drug-195D complex systems in three-dimensional view.

6.3.4. Hydrophobic binding analysis

Hydrophobic binding is the main type of binding involved in the case of intercalation between ligand and DNA. As TBBPA interacts with 1DSC and 2ROU as an intercalator, hydrophobic bonding dominates there. In the case of intercalative binding, small drugs get stacked in between the bases of DNA. In this stacking, hydrophobic bonds are responsible for the binding between the ligand and DNA.

Table 6.5 gives the hydrophobic bond details for DNA-ligand complex systems. Number of hydrophobic bonds, involved species and bond length for each bond for all of the complexes is given. It is clear from the table that this binding is dominating over hydrogen binding in the case of intercalation. TBBPA-1DSC has maximum number of hydrophobic bonds (15) between them.

Table 6.5. Hydrophobic bond details for DNA-ligand complex systems.

S.No.	DNA segment	Ligand	No. of H-bonds	Interacting species	H-bond length(Å)
1.	1BNA	BPA	1	A:DA6 - :UNK0	5.640001
		BPAF	-	-	-
		BPS	-	-	-
		TBBPA	8	A:DG10 - :UNK0:C B:DA17 - :UNK0:C B:DA17 - :UNK0:C B:DG16 - :UNK0:C A:DC9 - :UNK0:Br B:DA17 - :UNK0:Br B:DA18 - :UNK0:Br B:DA18 - :UNK0:Br	5.107532 4.789819 4.629728 5.231530 5.100588 4.855225 4.953848 4.583980
		TCBPA	5	A:DG10 - :UNK0:C B:DG16 - :UNK0:C B:DA18 - :UNK0:Cl A:DC9 - :UNK0:Cl B:DA18 - :UNK0:Cl	5.057249 5.290743 4.919593 4.826548 4.515906
2.	1DSC	BPA	-	-	-
		BPAF	-	-	-
		BPS	2	A:DG4:H1' - :UNK0 A:DG4 - :UNK0	2.688382 5.428517
		TBBPA	15	A:DG4 - :UNK0 A:DG4 - :UNK0 B:DG12 - :UNK0 B:DG12 - :UNK0 A:DC5 - :UNK0 B:DG12 - :UNK0:Br B:DG12 - :UNK0:Br A:DG4 - :UNK0:Br A:DG4 - :UNK0:Br A:DG4 - :UNK0 A:DG4 - :UNK0 A:DA3 - :UNK0 A:DA3 - :UNK0:Br A:DA2 - :UNK0:Br A:DG4 - :UNK0:C	3.660076 3.472163 5.044223 4.137928 5.078582 4.615677 4.092550 4.413255 5.348134 4.214586 5.243034 5.851540 4.566702 4.848568 4.685772
		TCBPA	6	A:DG4 - :UNK0:C UNK0:C - A:DG4 A:DG4 - :UNK0:C A:DA3 - :UNK0:C	4.577710 3.956262 4.170467 4.723995

				A:DA3 - :UNK0:C B:DT15 - :UNK0	3.924687 5.804858
3.	1RMX	BPA	2	B:DG20 - :UNK0 A:DA3 - :UNK0	5.322433 5.746136
		BPAF	4	B:DA16 - :UNK0 B:DA16 - :UNK0 B:DC14 - :UNK0 A:DG7 - :UNK0	5.847956 5.701454 5.233760 5.442357
		BPS	4	B:DG17 - :UNK0 B:DA16 - :UNK0 B:DA16 - :UNK0 B:DA16 - :UNK0	5.245217 5.593453 4.614065 5.226543
		TBBPA	10	A:DC1 - :UNK0:C A:DG2 - :UNK0:C A:DG2 - :UNK0:Br A:DA3 - :UNK0:Br A:DA3 - :UNK0:Br B:DT18 - :UNK0:Br B:DG17 - :UNK0:Br B:DG17 - :UNK0:Br B:DA16 - :UNK0:Br B:DA16 - :UNK0:Br	4.059302 4.762154 4.163649 4.444690 5.062381 5.369911 5.394669 4.700506 5.406580 4.253863
		TCBPA	1	A:DG7 - :UNK0:Cl	4.936818
4.	2ROU	BPA	4	UNK0:H- :DC23:O4' UNK0:H- B:DG24:N3 UNK0:H- :DA25:O4' B:DA25:H5'1- NK0:O	2.112552 2.593854 2.288214 2.083864
		BPAF	7	UNK0:H- B:DC23:O4' A:DC5:H1' - :UNK0:F A:DC5:H4' - :UNK0:F A:DG4:H21 - :UNK0 B:DG24:H4'- UNK0:F B:DG24:H22-NK0:O UNK0:H- :DA25:O4'	1.971740 2.301018 2.471182 2.795444 2.566168 1.897726 2.059780
		BPS	4	B:DG22:H2-UNK0:O UNK0:H- :DC23:O4' B:DG24:H2-UNK0:O UNK0:H- :DA25:O4'	1.811454 2.086173 1.759489 1.931819
		TBBPA	2	UNK0:H- :DG22:O3' B:DG22:N3-UNK0:Br	1.907082 3.364273
		TCBPA	1	UNK0:H- :DG22:O3'	1.956736
5.	195D	BPA	6	UNK0:H- :DG10:O4' UNK0:H - A:DC9:O2 B:DG16:N2 - :UNK0 A:DG10:N2 - :UNK0 B:DG14:N2- UNK0:O UNK0:H- B:DC15:O2	2.024700 3.010265 3.366872 3.732183 2.963752 2.255529
		BPAF	6	UNK0:H - A:DA7:O4' UNK0:H - A:DT6:O2 A:DT6:C4' - :UNK0:F B:DC21:C4'- UNK0:F B:DG22:C5'- UNK0:F B:DG22:C5'- UNK0:F	2.914469 2.242876 3.275043 3.445188 3.440485 3.112585
		BPS	6	B:DT18:C5'- UNK0:O UNK0:H- A:DC11:O2 A:DG10:N2 - :UNK0	3.131961 1.868998 3.904925

				B:DG16:N2 - :UNK0 B:DG16:N2 - :UNK0 UNK0:H - A:DC9:O4'	3.360563 4.150626 2.103495
		TBBPA	3	UNK0:H - A:DG10:OP1 B:DA19:C5' - :UNK0:Br UNK0:H - B:DT18:O2	1.671796 3.493976 2.026440
		TCBPA	3	UNK0:H - A:DG10:OP1 UNK0:H - B:DT18:O2 A:DA7:C2 - :UNK0:O	1.779204 1.929722 2.838304

6.3.5. Molecular Dynamics

TBBPA has maximum binding energy amongst all of the compounds whereas BPS has lowest for 1BNA complexes. So, molecular dynamics simulations were performed for both TBBPA-1BNA complex and BPS-1BNA complex. The time for each simulation was taken to be 100 ns. As 1BNA is a B-type DNA which is prominent in human body so the simulations are carried out using complexes of 1BNA and ligands. 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 Gromacs, as discussed in the method section. For both systems, variation of hydrogen bonds, RMSD curves, RMSF curves and radius of gyration variation curves were plotted. The details are discussed below.

6.3.5.1 MD results for TBBPA-1BNA complex

The results from MD simulations were analysed in the form of trajectories. Figure 6.14 gives the trajectories for 1BNA-TBBPA complex. Figure 6.14(a) represents the average number of hydrogen bonds formed for the system during the simulation process. As clear from the figure, TBBPA forms an average of 2 hydrogen bonds with DNA during the simulation process.

Figure 6.14(b) represents the RMSD (root mean square deviation) curve for both DNA and the ligand. The RMSD serves as a measure for biological macromolecules' structural stability. It is clear from the figure that the deviation for both DNA and ligand lie well in the range 1Å - 4Å, showing stability of the complex for the 100 ns of simulation.

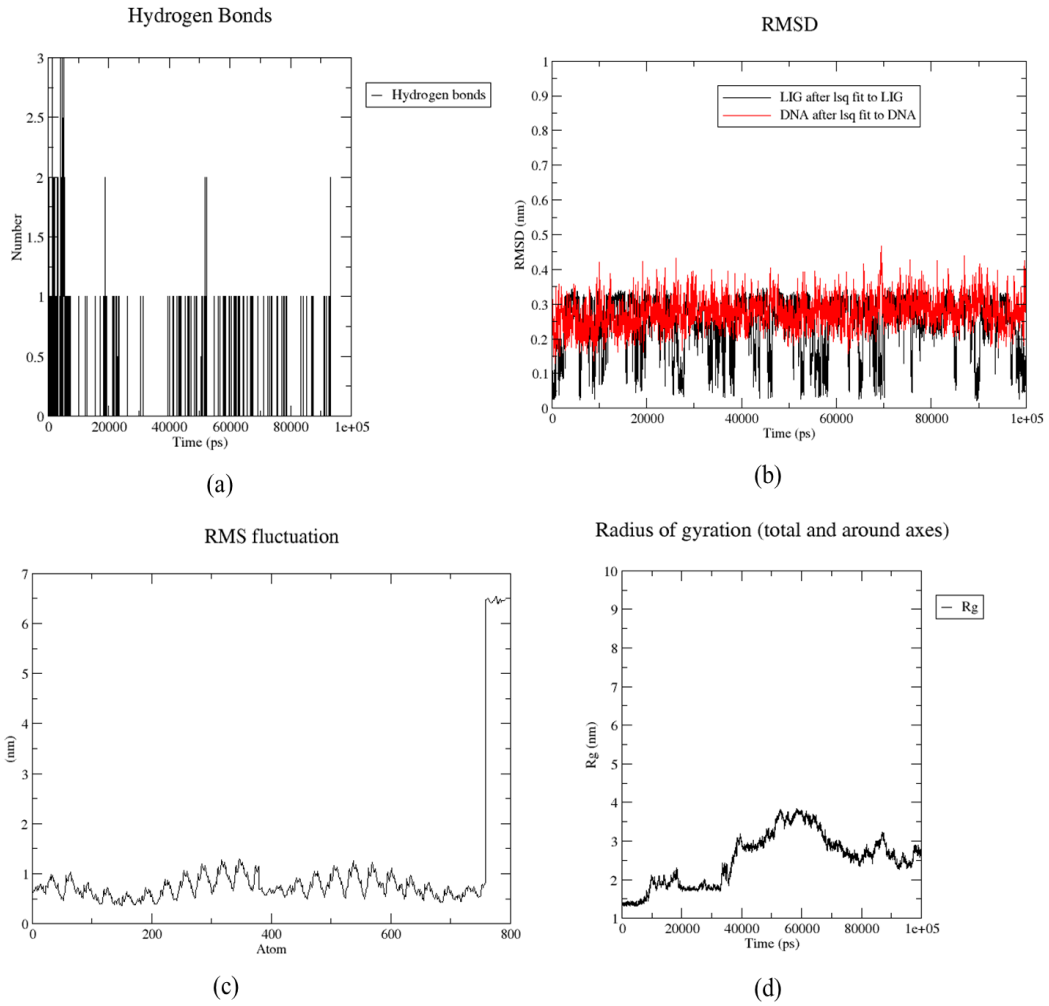


Figure 6.14. Trajectories for 1BNA-TBBPA complex (a) Variation in H-bond, (b) RMSD curve, (c) RMSF curve, (d) Variation in radius of gyration.

Figure 6.14(c) represents the RMSF (root mean square fluctuation) curve for the system. . RMSF keeps track of changes in each amino acid base pair over the course of the MD simulation, as well as changes in the nucleic acid's flexible areas. High- and low root mean square fluctuations levels, respectively, distinguish loosely bound and tightly bound sections in the DNA strands. The figure gives the RMSF curve which represent insignificant fluctuations.

Figure 6.14(d) represents the variations in the radius of gyration for the complex system. To understand the compactness of DNA and the dynamical stability of DNA-ligand complex systems, values of radii of gyration were obtained. These variations in energies were obtained through gmx_gyrate programme inbuilt in GROMACS software suite.

6.3.5.2 MD results for 1BNA-BPS complex

The results from MD simulations were analysed in the form of trajectories. Figure 6.15 gives the trajectories for 1BNA-BPS complex. Figure 6.15(a) represents the average number of hydrogen bonds formed for the system during the simulation process. As clear from the figure, BPS form maximum number of 3 hydrogen bonds with DNA during the simulation process.

Figure 6.15(b) represents the RMSD (root mean square deviation) curve for both DNA and the ligand. The RMSD serves as a measure for biological macromolecules' structural stability. It is clear from the figure that the deviation for ligand lie well in the range 1Å - 4Å, but the case for DNA is not similar. After 40ns, the curve for DNA shows deviations inconsistent with previous data. For 40ns-100ns, deviations are above 4Å showing instability of the complex for the 100 ns of simulation. RMSD plot suggest that the complex is stable up to 40 ns but it loses its stability onwards.

Figure 6.15(c) represents the RMSF (root mean square fluctuation) curve for the system. RMSF keeps track of changes in each amino acid base pair over the course of the MD simulation, as well as changes in the nucleic acid's flexible areas. High- and low root mean square fluctuations levels, respectively, distinguish loosely bound and tightly bound sections in the DNA strands. The figure gives RMSF curve which represent insignificant fluctuations.

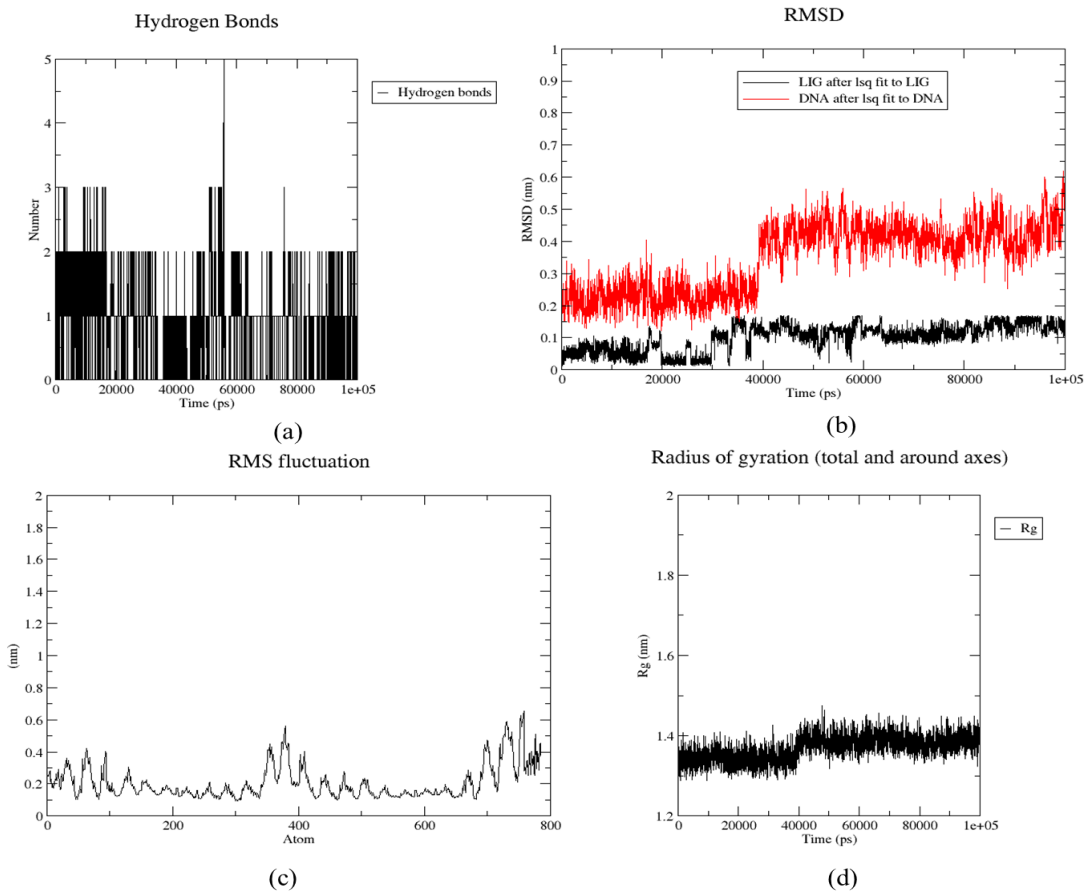


Figure 6.15. Trajectories for 1BNA-BPS complex (a) Variation of H-bond (b) RMSD curve (c) RMSF curve (d) Variation in radius of gyration.

Figure 6.15(d) represents the variations in the radius of gyration for the complex system. To understand the compactness of DNA and the dynamical stability of DNA-ligand complex systems, values of radii of gyration were obtained. These variations in energies were obtained through gmx_gyrate programme inbuilt in GROMACS software suite.

6.3.6. QM/MM

From Molecular dynamics simulation studies, it was evident that TBBPA-DNA complex shows maximum stability. It means TBBPA can bind and interact with DNA present in the human body.

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.

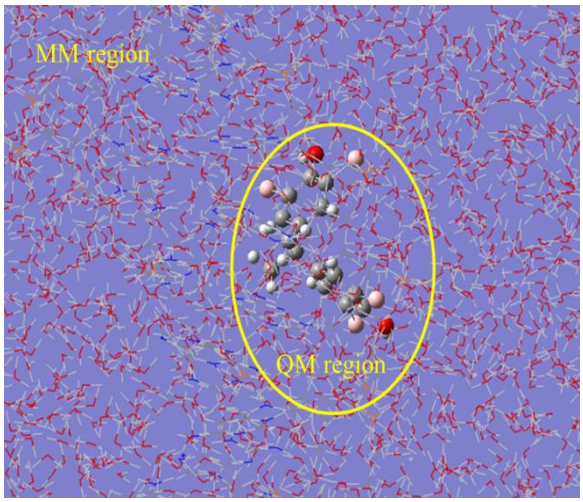


Figure 6.16. Snapshot of drug-DNA complex for QM/MM calculation.

The optimized geometry of complex having the least interaction energy is shown in Figure 6.16. The calculated interaction energies from QM/MM calculation using ONIOM scheme are mentioned below in Table 6.6**.** 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. QM/MM calculations also predict the similar binding mode predicted from Docking and Molecular dynamics.

Table 6.6. 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

6.4 Conclusion

Computational tools and techniques play an important and key role in drug designing and Pharmacy Corporation. New and potent drugs are designed and existing drugs are studied for their relative action tendencies using various computational methodologies. In this chapter, interaction, stability and dynamics of BPA and its derivatives compounds in the vicinity of DNA was studied. Detailed investigation of the interaction of BPA derivatives with DNA was performed by the help of computational tools. Molecular Optimization, Molecular Docking, Molecular Dynamics and Quantum Mechanical/Molecular Mechanical (QM/MM) methods were adopted to analyse the whole system.

Firstly, all the compounds were optimized for to start the process. Molecular Docking was performed on 25 systems of DNA-ligand. It was observed that most of the compounds bind with DNA as minor groove binders and binds to AT-rich region, except TBBPA. TBBPA intercalates with 1DSC and 2ROU as well as binds in the major groove of 1RMX. 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. In the case of groove binding, H-bonds were main bonds between the target and the ligand whereas for intercalation, hydrophobic bonding dominates. TBBPA forms maximum hydrophobic bonds with different segments of DNA.

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. Different

graphs showing variation of H-bonds, RMSD curve, RMSF curve and variation in radius of gyration confirmed the stability of the complex with time. For TBBPA-1BNA complex system, all the curves showed insignificant deviations which confirmed the stability of the complex system with time.

To further investigate the interaction at more detailed level, Quantum Mechanical/Molecular Mechanical (QM/MM) computational calculations were executed. As TBBPA-DNA system comes out to be the most stable system from previous calculations, QM/MM analysis was conducted on this system. The macromolecular-ligand complex from MD simulations was chosen as the initial structural system. QM/MM was performed on three systems taken at a difference of 2ns. The main interacting part was taken at the high level whereas remaining surrounding was given the low layer. Obtained energies again showed the stability of the complex.

All the computational tools and techniques combined together predicted the stability of compound TBBPA with DNA. Present study tells that TBBPA interacts with DNA with maximum affinity among all BPA derivatives and this interaction is stable with time, we can conclude that TBBPA compounds which come in the human body may interact with DNA. 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.

References

1. H. Fang, W. Tong, L.M. Shi, R. Blair, R. Perkins, W. Branham, B.S. Hass, Q. Xie, S.L. Dial, C.L. Moland, D.M. Sheehan, Structure-activity relationships for a large diverse set of natural, synthetic, and environmental estrogens. Chem. Res. Toxicol. 3 (2001) 280-294.
2. L.N. Vandenberg, M.V. Maffini, C. Sonnenschein, B.S. Rubin, A.M. Soto, Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. Endocr. Rev. 1 (2009) 75-95.
3. P. Kovacic, How safe is bisphenol A? Fundamentals of toxicity: metabolism, electron transfer and oxidative stress. Med. Hypotheses. 75 (2010) 1–4.
4. P.D. Bailin, M. Byrne, S. Lewis, R Liroff, Public awareness drives market for safer alternatives: bisphenol A market analysis report. IEHN (2008) 1-37.
5. J.R. Rochester, Bisphenol A and human Health: A review of the literature., Reproductive Toxicology 42 (2013) 132-55.
6. S. Yoshihara, T. Mizutare, M. Makishima, N. Suzuki, N. Fujimoto, K. Igarashi, S. Ohta, Potent estrogenic metabolites of bisphenolA and bisphenol B formed by rat liver S9 fraction: their structures and estrogenic potency. Toxicol. Sci. 78 (2004) 50-59.
7. S.V. Fernandez, Y. Huang, K.E. Snider, Y. Zhou, T.J. Pogash, J. Russo, Expression and DNA methylation changes in human breast epithelial cells after bisphenol A exposure. Int. J. Oncol. 41 (2012) 369-77.
8. G. Ginsberg, D.C. Rice, Does rapid metabolism ensure negligible risk from bisphenol A? Environ. Health Perspect. 117 (2009) 1639-1643.
9. S. Yoshihara, M. Makishima, N. Suzuki, S. Ohta, Metabolic activation of bisphenol A by rat liver S9 fraction. Toxicol. Sci., 62 (2001) 221-227.
10. S. Derouiche, M. Warnier, P. Mariot, P. Gosset, B. Mauroy, J.L. Bonnal, C. Slomianny, P. Delcourt, N. Prevarskaya, M. Roudbaraki, Bisphenol A stimulates human prostate cancer cell migration remodeling of calcium signaling. Springerplus, 2 (2013) 54-61.
11. J. Wang, S. Jenkins, C.A. Lamartiniere, Cell proliferation and apoptosis in rat mammary glands following combinational exposure to bisphenol A and genistein. BMC Cancer, 14 (2014) 365-379.

12. K. Moriyama, T. Tagami, T. Akamizu, T. Usui, M. Saijo, N. Kanamoto, et al. Thyroid hormone action is disrupted by bisphenol A as an antagonist. J Clin Endocrinol Metabol. 87 (2002) 5185-90.
13. Commission Directive 2011/8/EU of 28 January 2011 amending Directive 2002/72/EC as regards the restriction of use of Bisphenol A in plastic infant feeding bottles Text with EEA relevance.
14. J.M. Braun, A.E. Kalkbrenner, A.M. Calafat, K. Yolton, X. Ye, K.N. Dietrich, et al. Impact of early-life bisphenol A exposure on behavior and executive function in children. Pediatrics. 128 (2011) 873-82.
15. E. Danzl, K. Sei, S. Soda, M. Ike, M. Fujita, Biodegradation of bisphenol A, bisphenol F and bisphenol S in seawater. Int. J. Environ. Res. Public Health. 4 (2009) 1472–1484.
16. R. Yadav, N. Awasthi, D. Kumar, Biotransformation of BPA via epoxidation catalyzed by Cytochrome P450, Inorg. Chem. Comm. 139 (2022) 109321.
17. S. Lee, Y.K. Kim, T.Y. Shin, S.H. Kim, Neurotoxic effects of bisphenol AF on calcium-induced ROS and MAPKs. Neur. Res., 23 (2013) 249-259.
18. Y.B. Wetherill, B.T. Akingbemi, J. Kanno, J.A. McLachlan, A. Nadal, C. Sonnenschein et al. In vitro molecular mechanisms of bisphenol A action. Reprod Toxicol. 24 (2007) 178-98.
19. A. Miodovnik, S.M. Engel, C. Zhu, X. Ye, L.V. Soorya, M.J. Silva, et al. Endocrine disruptors and childhood social impairment. Neurotoxicology, 32 (2011) 261-7.
20. L.Y. Gao, X.M. Ren, Ch-H., Li, J. Zhang, W.P. Qin, Y. Yang, B. Wan, L.H. Guo, Bisphenol AF and bisphenol B exert higher estrogenic effects than bisphenol A via G protein-coupled estrogen receptor pathway. Environ. Sci. Technol., 51 (2017) 11423-11430.
21. D. Chen, K. Kannan, H. Tan, Z. Zheng, Y.L. Feng, Y. Wu, Bisphenol A analogues other than BPA: Environmental occurrence, human exposure, and toxicity—a review. Environ. Sci. Technol., 50 (2016) 5438-5453.
22. J. Schmidt, P. Kotnik, J. Trontelj, Ž. Knez, L. P. Mašič, Bioactivation of bisphenol A and its analogs (BPF, BPAF, BPZ and DMBPA) in human liver microsomes Toxicology in Vitro, 27 (2013) 1267-1276.

23. N. Cabaton, D. Zalko, E. Rathahao, C. Canlet, G. Delous, M.C. Chagnon, J.P. Cravedi, E. Perdu, Biotransformation of bisphenol F by human and rat liver subcellular fractions. Toxicol. In Vitro., 22 (2008) 1697-1704.
24. Y. Yang, J. Guan, J. Yin, B. Shao, H. Li. Urinary levels of bisphenol analogues in residents living near a manufacturing plant in south China. Chemosphere. 112 (2014) 481-486.
25. K. Mokra, K. Woźniak, B. Bukowska, P. Sicińska, J. Michałowicz, Low-concentration exposure to BPA, BPF and BPAF induces oxidative DNA bases lesions in human peripheral blood mononuclear cells, Chemosphere. (2018).
26. K. Ji, S. Hong, Y. Kho, K. Choi, Effects of bisphenol S exposure on endocrine functions and reproduction of zebrafish, Environ. Sci. Technol. 47 (2013) 8793-8800.
27. R. Viñas, C.S. Watson, Bisphenol S disrupts estradiol-induced nongenomic signalling in a rat pituitary cell line: effects on cell functions, Environ. Health Perspect. 121 (2013) 352-358.
28. A. Atkinson, D. Roy, In vitro conversion of environmental estrogenic chemical bisphenol A to DNA binding metabolite(s). Biochem. Biophys. Res. Commun. 210 (2005) 424-433.
29. D. Zalko, A.M. Soto, L. Dolo, C. Dorio, E. Rathahao, L. Debrauwer, R. Faure, J.P. Cravedi, Biotransformations of bisphenol A in a mammalian model: answers and new questions raised by low-dose metabolic fate studies in pregnant CD1 mice. Environ. Health Perspect. 111 (2003) 309-319.
30. J.S. Edmonds, M. Nomachi, M. Terasaki, M. Morita, B.W. Skelton, A.H. White, The reaction of bisphenol A 3,4-quinone with DNA. Biochem. Biophys. Res. Commun. 319 (2004) 556-561.
31. Y.Q. Wang, H.M. Zhang, J. Cao, B. P. Tang, Binding of a new bisphenol analogue, bisphenol S to bovine serum albumin and calf thymus DNA Journal of Photochemistry and Photobiology B: Biology. 138 (2014) 182-190.
32. A. Pandey, A. Upadhyaya, S. Kumar, A.K. Yadav Interaction, Dynamics and Stability Analysis of Some Minor Groove Binders With B-DNA Dodecamer 5'-(CGCAAATTTGCG)-3'. Drug Des. 10 (2020) 172.
33. A. Shukla, R. Mishra, R.Yadav, N. Awasthi, D. Kumar. Computational Investigations on Interactions Between DNA and Flavonols. 12 (2022) 8117-8127.

34. A. Rescifina, C. Zagni, M. G. Varrica, V. Pistarà, A. Corsaro, Recent advances in small organic molecules as DNA intercalating agents: Synthesis, activity, and modelling European Journal of Medicinal Chemistry. 74 (2014) 95-115.
35. X. Xie, X. Wang, X. Xu, H. Sun, X. Chen, Investigation of the interaction between endocrine disruptor bisphenol A and human serum albumin, Chemosphere. 80 (2010) 1075-1080.
36. T. Zhang, T. Tian, R. Zhou, S. Li, W. Ma, Y. Zhang, N. Liu, S. Shi, Q. Li, X. Xie, et al., Design, fabrication and applications of tetrahedral DNA nanostructure-based multifunctional complexes in drug delivery and biomedical treatment. Nat. Protoc. 15 (2020) 2728-2757.
37. M. Sirajuddin, S. Ali, A. Badshah, DNA interactions and their study by UV – Visible, fluorescence spectroscopies and cyclic voltametry. J. Photochem. Photobiol. B Biol., 124 (2013) 1-19.
38. Y.L. Zhang, X. Zhang, X.C. Fei, S.L. Wang, H.W. Gao, Binding of bisphenol A and acrylamide to BSA and DNA: insights into the comparative interactions of harmful chemicals with functional biomacromolecules, J. Hazard. Mater. 182 (2010) 877-885.
39. M. Mathew, S. Sreedhanya, P. Manoj, C.T. Aravindakumar, U.K. Aravind, Exploring the interaction of bisphenol-S with serum albumins: a better or worse alternative for bisphenol A? J Phys. Chem. B. 118 (2014) 3832-3848.
40. A.M. Dar, S. Mir Molecular Docking: Approaches, Types, Applications and Basic Challenges. J Anal Bioanal Tech. 8 (2017) 356.
41. H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The Protein Data Bank, Nucleic Acids Research. 28 (2000) 235-242.
42. E F. Pettersen, T.D. Goddard, C.C. Huang, G. S. Couch, D.M. Greenblatt, E.C. Meng and T.E. Ferrin. J. Comput. Chem. 25 (2004) 1605-12.
43. H. Ezoji, M. Rahimnejad, Electrochemical behavior of the endocrine disruptor bisphenol A and in situ investigation of its interaction with DNA, Sensors and amp; Actuators: B. Chemical. 274 (2018) 370-380.
44. H. Luo, C. Li, H. Fang, X. Wang, Comparative study of the interactions between bisphenol analogues and serum albumins by electrospray mass spectrometry and fluorescence spectroscopy Rapid Commun. Mass Spectrom. 30 (2016) 162–167.

45. R. Dennington, T. A. Keith, J. M. Millam, Semichem Inc., S.M. GaussView, Version 6.1. KS 2016.
46. Gaussian 09, Revision E.01, M.J .Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery, J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J.J. aramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, O. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski and D.J. Fox, Gaussian, Inc., Wallingford CT (2009).
47. G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility, J. Comput. Chem. 30 (2009) 2785-2791.
48. G.M. Morris, D. Goodsell, R. Halliday, R. Huey, W. Hart, R. Belew, A. Olson, Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function, J. Comput. Chem. 19 (1998) 1639-1662.
49. H. Zhao, A. Caflisch, Molecular dynamics in drug design. Eur J Med Chem., 91 (2015) 4-14.
50. M.J. Abraham, T. Murtola, R. Schulz, S. Pall, J.C. Smith, B. Hess, E. Lindahl, GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX, 1 (2015) 19–25.
51. J.W. Ponder, D.A. Case, Force Fields for Protein Simulations. Adv. Prot. Chem., 66 (2003) 27-85.
52. P. Mark, L. Nilsson, Structure and Dynamics of the TIP3P, SPC, and SPC/E Water Models at 298 K. Journal Physical Chemistry-A, 105 (2001) 9954-9960.
53. T. Darden, D. York, L. Pedersen, Particle mesh Ewald: An Nlog(N) method for Ewald sums in large systems. J Chem Phys., 98 (1993) 10089-10092.

54. J.P. Ryckaert, G. Ciccotti, H.J. Berendsen, Numerical integration of the cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes J Comput Phys., 23 (1977) 327-341.
55. B. Hess, H. Bekker, H.J.C. Berendsen, J.G.E.M Fraaije, LINCS: A Linear Constraint Solver for Molecular Simulations. Journal of Computational Chemistry, 18 (1997) 1463-1472.
56. P.J. Turner, XMGRACE, Version 5.1. 19. Center for Coastal and Land-Margin Research, Oregon Graduate Institute of Science and Technology, Beaverton, OR; (2005).
57. R. Shastri, N. Awasthi, D. Kumar, A.K. Yadav, D. Roy, S.P. Goutam, A. Pandey, A Density Functional Theory Study on Structural Stability and Electronic Properties of CoxOy (x + y = 4 - 12) Nanoclusters. Adv. Sci. Eng. 10 (2018) 1-5.
58. BIOVIA, Dassault Systèmes, BIOVIA Workbook, Release 2020; BIOVIA Pipeline Pilot, Release 2020, San Diego: Dassault Systèmes.

CHAPTER-7

CONCLUSION AND FUTURE SCOPE

This chapter gives the basic conclusions drawn from the analyses conducted in the chapters of present thesis.

- The goal of present research is to validate the relevance of DNA sequence and specificity in the formation of complex at molecular level. Our research aims to provide information on the complexities of small molecule binding mechanisms to DNA.
- 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.
- It is anticipated that the research presented in this thesis work will help to bridge the gap between computational and experimental research on drug-DNA interaction.
- 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.

List of Publications

1. **Anamika Shukla**, Ruchi Mishra, Rolly Yadav, Nidhi Awasthi, Devesh Kumar, Computational investigations on interactions between DNA and Flavonols, Biointerface Research in Applied Chemistry. 12(6) (2022) 8117 – 8127.

2. **Anamika Shukla**, Ruchi Mishra, Anwesh Pandey, A. K. Dwivedi, Devesh Kumar, Interaction of Flavonols with DNA: Molecular Docking Studies., Proceeding of ISAFBM-2019, ISBN: 978-93-5351-824-0 (2019) 4-10.

3. Gulam Abbas, Kijay Bahadur, Narinder Kumar, **Anamika Shukla**, Devesh Kumar, Gajanan Pandey, Efficient Anticarcinogenic Activity of α-Fe2O3 Nanoparticles: In-vitro and Computational Study on Human Renal Carcinoma Cells HEK-293. Materials Science & Engineering C. 26 (2021) 102175.

4. Rolly Yadav, **Anamika Shukla**, Devesh Kumar. (2022). Interplay Between Theory and Experiment: A Future Approach for Biomedical Research. In: Sobti, R., Sobti, A. (eds) Biomedical Translational Research. Springer, Singapore.

5. Anwesh Pandey, Ruchi Mishra, **Anamika Shukla**, Anil Kumar Yadav, Devesh Kumar, In-silico docking studies of 2,5-bis(4-amidinophenyl) furan and its derivatives. Proceeding of ISAFBM-2019, ISBN: 978-93-5351-824-0 (2019) 11-18.

6. Anwesh Pandey, Rolly Yadav, **Anamika Shukla** and Anil Kumar Yadav, Unveiling the Antimicrobial Activities of Dicationic Carbazoles and Related Analogs through Computational Docking. Advanced Science, Engineering and Medicin*e* Vol. 11 (2019) 1–5.

7. Nidhi Awasthi, Rolly Yadav, **Anamika Shukla**, Devesh Kumar, Interplay between two degenerate spin state determines the hydroxylation of 4-nitrophenol catalyzed via Cytochrome P450. Inorganic Chemistry Communications. 132 (2021) 108857.

8. Rolly Yadav, Anwesh Pandey, Nidhi Awasthi, **Anamika Shukla**, Molecular Docking Studies of Enzyme Binding Drugs on Family of Cytochrome P450 Enzymes. Advanced Science, Engineering and Medicine. 11 (2019) 11-18.

9. Rolly Yadav, Nidhi Awasthi, **Anamika Shukla**, Devesh Kumar, Modeling the hydroxylation of estragole via human liver cytochrome P450, Journal of Molecular Modeling. 27 (2021) 199.

List of Communicated Manuscripts

1. **Anamika Shukla**, Ruchi Mishra, Anwesh Pandey, Asheesh Kumar, Devesh Kumar, Evaluation of binding properties of some common drugs with DNA as intercalator and groove binder, *Indian Journal of Biochemistry and Biophysics (IJBB)*
2. **Anamika Shukla**, Anwesh Pandey, Devesh Kumar, 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, *Journal of the Indian Chemical Society.*
3. Ruchi Mishra, **Anamika Shukla**, The role of Quantum Mechanics/Molecular Mechanics calculations in understanding the binding of DNA minor groove binders, *Journal of Molecular Modeling.*

List of Conferences/Seminars/Workshops

1. North Indian Science Congress (NISC-2018) & International Conference On Science and Technology for Sustainable Future on 10th & 11th at Babasaheb Bhimrao Ambedkar University, Lucknow.
2. National Symposium on Advanced Materials Science, Department of Physics Deen Dyal Upadhyay Gorakhpur University, Gorakhpur, 7th -8th December, 2018.
3. International Confrence on Chemical Sciences : National and Global Perspective, Department of Chemistry, Christian Degree Collage, Uttar Pradesh, Lucknow, 29th-31st,October, 2018.
4. International Conference on Advanced Chemical and Structural Biology (ICACSB-2019) during February 19th-21th, 2019 at Thanjavur, Chennai.
5. International Symposium on Advances in Functional and Biological Materials (ISAFBM-2019) Feb. 28th, 2019 in Lucknow University, UP
6. National Conference on Smart Materials, Devices and Sustainable Technologies (SMDST-2019) during March 15th-16th, 2019 in MMM University of Technology, Gorakhpur (UP).
7. National Conference on Recent Advances in Chemical Sciences (NCRACS-2019) during March 29-30, 2019 in MMM University of Technology, Gorakhpur (UP).
8. Workshop on Applications of Gaussian and GaussView Software during July 18th-19th, 2019 in Lucknow University, UP.
9. International conference on Ultrasonics and Materials science for advanced technology (ICUMSAT-2019), November 16th-18th, Department of Physics, Veer Bahadur Singh Purvanchal University, Jaunpur.
10. Workshop on Tools and Techniquies to perfrom molecular modeling and computeraided drug design (MMTT-2021) January 11th-17th, 2021 organized by NIPER GUWAHATI.
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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.

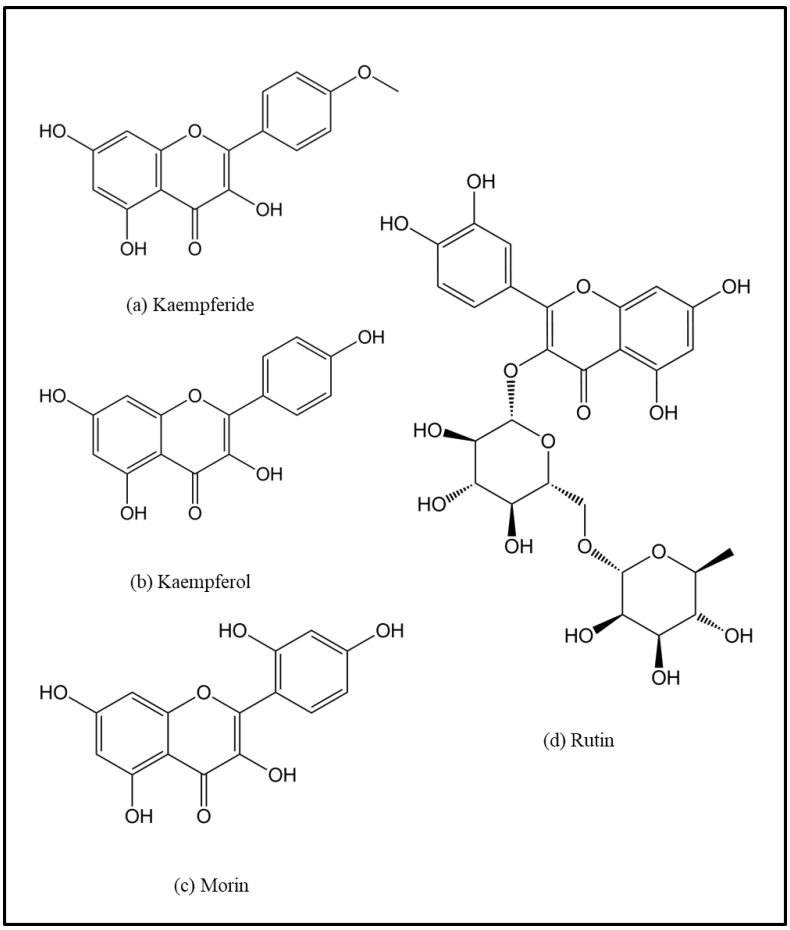

Figure 1. Chemical structure of selected ligands. (**a**) Kaempferide; (**b**) Kaempferol; (**c**) Morin; (**d**)Rutin.

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]

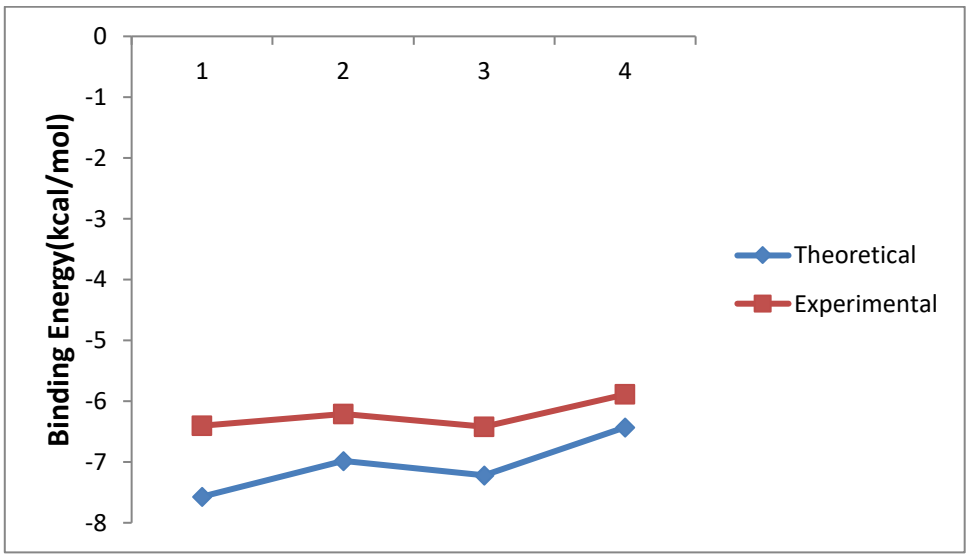

Figure 2. A similar trend of binding energy in theoretical and experimental cases.

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.

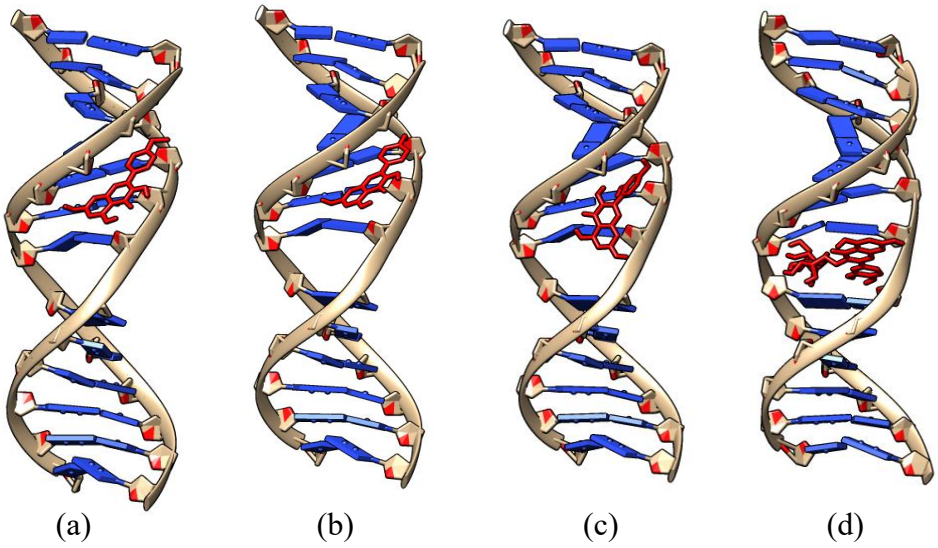

Figure 3. Binding sites of Flavonols with DNA. (**a**) kaempferide; (**b**) kaempferol; (**c**) morin; (**d**) rutin.

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.

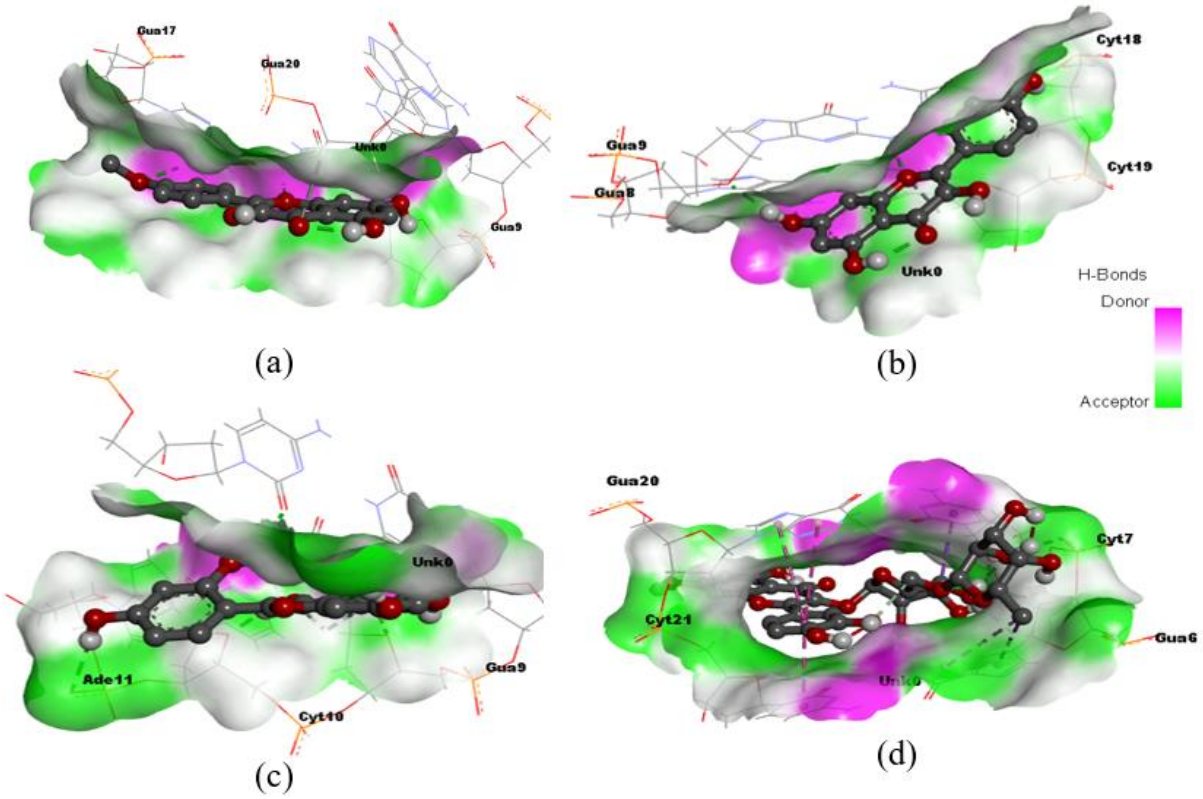

Figure 4. Figure representing the H-bond donor and acceptor regions in best docked posed complexes.

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.

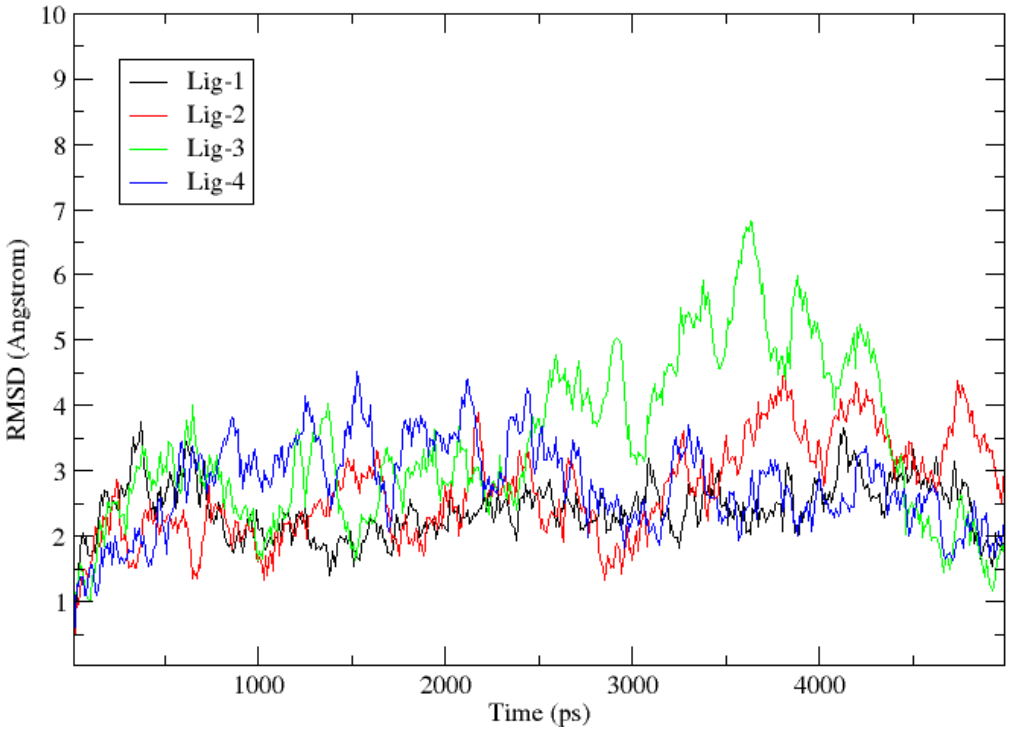

Figure 5. Figure representing RMSD plot for drug-DNA complexes.

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.

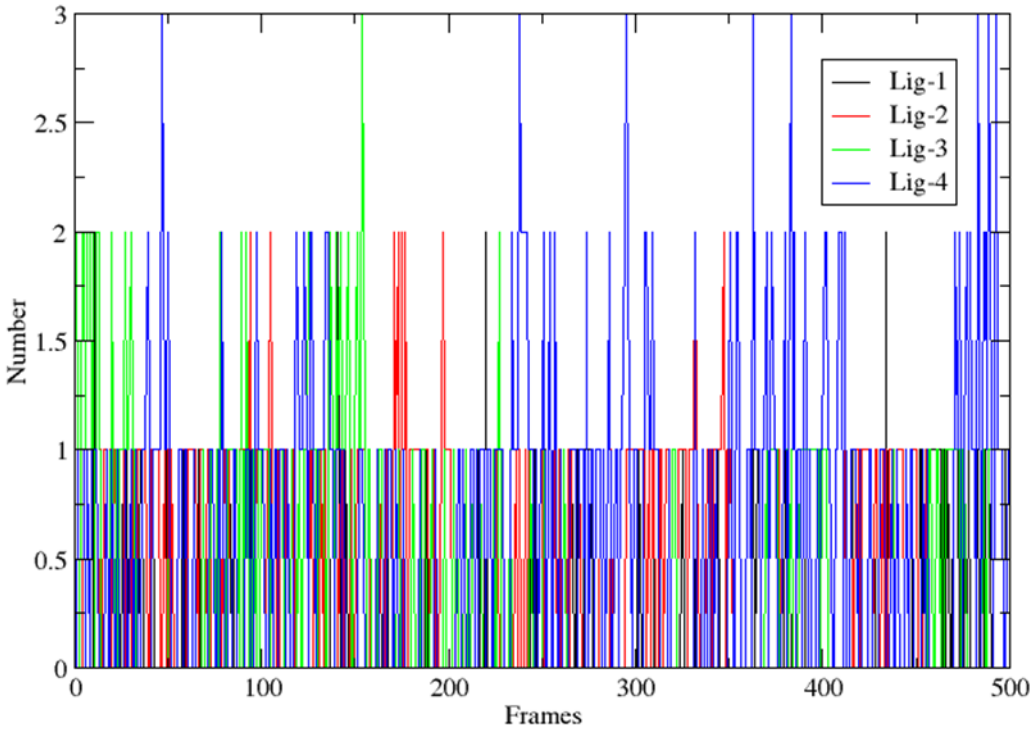

Figure 6. Figure representing hydrogen bonds for drug-DNA complexes.

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.

References

1. Małecka, M.; Skoczyńska, A.; Goodman, D.M.; Hartinger, C.G.; Budzisz, E. Biological properties of ruthenium(II)/(III) complexes with flavonoids as ligands. *Coord. Chem. Rev.* **2021**, *436*, https://doi.org/10.1016/j.ccr.2021.213849.
2. Ahmad, K.; Kalim, M.; Khan, A.; Baig, M.H.; Imran, M.; Gupta, G.K. Role of Azoles in Cancer Prevention and Treatment: Present and Future Perspectives. *Anti-Cancer Agents Med. Chem.* **2018**, *18*, 46–56, https://doi.org/10.2174/1871520616666161221112042.
3. Busch, C.; Burkard, M.; Leischner, C.; Lauer, U.M.; Frank, J.; Venturelli, S. Epigenetic activities of flavonoids in the prevention and treatment of cancer. *Clin. Epigenetics* **2015**, *7*, 1–18, https://doi.org/10.1186/s13148-015-0095-z.
4. Mareel, M.; Leroy, A. Clinical, cellular, and molecular aspects of cancer invasion. *Physiol. Rev.* **2003**, *83*, 337–376, https://doi.org/10.1152/physrev.00024.2002.
5. Kejík, Z.; Kaplánek, R.; Masařík, M.; Babula, P.; Matkowski, A.; Filipenský, P.; Veselá, K.; Gburek, J.; Sýkora, D.; Martásek, P.; et al. Iron complexes of flavonoids-antioxidant capacity and beyond. *Int. J. Mol. Sci.* **2021**, *22*, 1–20, https://doi.org/10.3390/ijms22020646.
6. Mierziak, J.; Kostyn, K.; Kulma, A. Flavonoids as Important Molecules of Plant Interactions with the Environment. *Molecules* **2014**, *19*, 16240–16265, https://doi.org/10.3390/molecules191016240.
7. Rubio, A.R.; González, R.; Busto, N.; Vaquero, M.; Iglesias, A.L.; Jalón, F.A.; Espino, G.; Rodríguez, A.M.; García, B.; Manzano, B.R. Anticancer Activity of Half-Sandwich Ru, Rh and Ir Complexes with Chrysin Derived Ligands: Strong Effect of the Side Chain in the Ligand and Influence of the Metal. *Pharmaceutics* **2021**, *13*, 1540, https://doi.org/10.3390/pharmaceutics13101540.
8. Fusi, F.; Trezza, A.; Tramaglino, M.; Sgaragli, G.; Saponara, S.; Spiga, O. The beneficial health effects of

flavonoids on the cardiovascular system: Focus on K+ channels. *Pharmacol. Res.* 2020, *152*.
9. Joyner, P.M. Protein adducts and protein oxidation as molecular mechanisms of flavonoid bioactivity. *Molecules* **2021**, *26*, https://doi.org/10.3390/molecules26165102.
10. Russo, M.; Moccia, S.; Spagnuolo, C.; Tedesco, I.; Russo, G.L. Roles of flavonoids against coronavirus infection. *Chem. Biol. Interact.* **2020**, *328*, 109211, https://doi.org/10.1016/j.cbi.2020.109211.
11. Tawani, A.; Kumar, A. Structural Insight into the interaction of Flavonoids with Human Telomeric Sequence. *Nat. Publ. Gr.* **2015**, 1–13, https://doi.org/10.1038/srep17574.
12. Srivastava, H.K.; Chourasia, M.; Kumar, D.; Sastry, G.N. Comparison of computational methods to model DNA minor groove binders. *J. Chem. Inf. Model.* **2011**, *51*, 558–571, https://doi.org/10.1021/ci100474n.
13. Sjakste, N.; Djelić, N.; Dzintare, M.; Živković, L. DNA-BINDING and DNA-protecting activities of small natural organic molecules and food extracts. *Chem. Biol. Interact.* **2020**, *323*, 109030, https://doi.org/https://doi.org/10.1016/j.cbi.2020.109030.
14. Pandey, A.; Upadhyaya, A.; Kumar, S.; Yadav, A.K. Dynamics and Stability Analysis of Some Minor Groove Binders with B-DNA Dodecamer 5 ' (CGCAAATTTGCG) -3 '. *Drug Des.* **2020**, *10*, 7–12.
15. Pandey, A.; Mishra, R.; Shukla, A.; Yadav, A.K.; Kumar, D. In-silico docking studies of 2 , 5-bis (4-amidinophenyl) furan and its derivatives. *Proceeding of ISAFBM-2019* **2019**, *1*, 11–18.
16. Atrahimovich, D.; Avni, D.; Khatib, S. Flavonoids-Macromolecules Interactions in Human Diseases with Focus on Alzheimer, Atherosclerosis and Cancer. *Antioxidants* **2021**, *10*, 423, https://doi.org/10.3390/antiox10030423.
17. Hussain, T.; Tan, B.; Murtaza, G.; Liu, G.; Rahu, N.; Saleem Kalhoro, M.; Hussain Kalhoro, D.; Adebowale, T.O.; Usman Mazhar, M.; Rehman, Z. ur; et al. Flavonoids and type 2 diabetes: Evidence of efficacy in clinical and animal studies and delivery strategies to enhance their therapeutic efficacy. *Pharmacol. Res.* **2020**, *152*, https://doi.org/10.1016/j.phrs.2020.104629.
18. Shukla, A.; Mishra, R.; Pandey, A.; Dwivedi, A.K.; Kumar, D. Interaction of Flavonols with DNA : Molecular Docking Studies. *Proceeding of ISAFBM-2019* **2019**, 4–10.
19. Moulishankar, A.; Lakshmanan, K. Data on molecular docking of naturally occurring flavonoids with biologically important targets. *Data Br.* **2020**, *29*, 105243, https://doi.org/10.1016/j.dib.2020.105243.
20. Singh, R.K.; Dwivedi, A.K.; Pandey, J.P.; Yadav, R.; Awasthi, N.; Shukla, A. A Review on the interaction of various types of Flavonoids with DNA. *J. Sci. Comput.* **2020**, *9*, 39–51.
21. Tajmir-riahi, S.D.Æ.H.A. An Overview of DNA and RNA Bindings to Antioxidant Flavonoids. **2007**, 29–36, https://doi.org/10.1007/s12013-007-0037-2.
22. Zhang, T.; Tian, T.; Zhou, R.; Li, S.; Ma, W.; Zhang, Y.; Liu, N.; Shi, S.; Li, Q.; Xie, X.; et al. Design, fabrication and applications of tetrahedral DNA nanostructure-based multifunctional complexes in drug delivery and biomedical treatment. *Nat. Protoc.* **2020**, *15*, 2728–2757, https://doi.org/10.1038/s41596-020-0355-z.
23. Sirajuddin, M.; Ali, S.; Badshah, A. DNA interactions and their study by UV – Visible , fluorescence spectroscopies and cyclic voltametry. *J. Photochem. Photobiol. B Biol.* **2013**, *124*, 1–19, https://doi.org/10.1016/j.jphotobiol.2013.03.013.
24. Rupasinghe, H.P.V. Special Issue "Flavonoids and Their Disease Prevention and Treatment Potential": Recent Advances and Future Perspectives. *molecules* **2020**, *25*, 1–7.
25. Bi, S.; Qiao, C.; Song, D.; Tian, Y.; Gao, D.; Sun, Y.; Zhang, H. Study of interactions of flavonoids with DNA using acridine orange as a fluorescence probe. *Sensors Actuators B* **2006**, *119*, 199–208, https://doi.org/10.1016/j.snb.2005.12.014.
26. Kanakis, C.D.; Tarantilis, P.A.; Polissiou, M.G.; Diamantoglou, S.; Tajmir-Riahi, H.A. DNA Interaction with Naturally Occurring Antioxidant Flavonoids Quercetin , Kaempferol , and Delphinidin DNA Interaction with Naturally. *J. Biomol. Struct. Dyn.* **2005**, *22*, 719–724, https://doi.org/10.1080/07391102.2005.10507038.
27. Janjua, N.K.; Siddiq, A.; Yaqub, A.; Sabahat, S.; Qureshi, R.; Ul Haque, S. Spectrophotometric analysis of flavonoid-DNA binding interactions at physiological conditions. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* **2009**, *74*, 1135–1137, https://doi.org/10.1016/j.saa.2009.09.022.
28. Kocharyan, G.H.; Minasyan, S.H.; Tavadyan, L.A. Interaction Of Flavonoids: Morin, Quercetin And Rutin, with DNA. *Chem. Biol. 2016, № 1,* **2016**, *1*, 49–54.
29. Septama, A.W.; Simbak, N.; Rahmi, E.P. Prospect of plant-based flavonoids to overcome antibacterial resistance: A mini-review. *Walailak J. Sci. Technol.* **2020**, *17*, 503–513, https://doi.org/10.48048/wjst.2020.5583.
30. Divakaran, S.A.; Hema, P.S.; Nair, M.S.; Nair, C.K.K. Antioxidant capacity and radioprotective properties of the flavonoids galangin and kaempferide isolated from Alpinia galanga L . (Zingiberaceae) against radiation induced cellular DNA damage. *Int. J. Radiat. Res.* **2013**, *11*, 81–89.
31. Payán-Gómez, S.A.; Flores-Holguín, N.; Pérez-Hernández, A.; Piñón-Miramontes, M.; Glossman-Mitnik, D. Computational molecular characterization of the flavonoid rutin. *Chem. Cent. J.* **2010**, *4*, 12, https://doi.org/10.1186/1752-153X-4-12.
32. Roy Dennington, Todd A. Keith, and John M. Millam, Semichem Inc., S.M. GaussView, Version 6.1. *KS* **2016**, 2016.
33. M. J. Frisch, G. W. Trucks, E. a. *Gaussian 09, Revision A.02*;

34. Berman, H.M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T.N.; Weissig, H.; Shindyalov, I.N.; Bourne, P.E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28*, 235–242.
35. Wang, Y.; Schnetz-boutaud, N.C.; Kroth, H.; Yagi, H.; Jane, M.; Kumar, S.; Jerina, D.M.; Stone, M.P. 3′-Intercalation of a N2 -dG 1R-trans-anti-Benzo[c]phenanthrene DNA Adduct in an Iterated (CG)3 Repeat. *Chem Res Toxicol.* **2009**, *21*, 1348–1358, https://doi.org/10.1021/tx7004103.3.
36. Pettersen, E.F.; Goddard, T.D.; Huang, C.C.; Couch, G.S.; Greenblatt, D.M.; Meng, E.C.; Ferrin, T.E. UCSF Chimera — A Visualization System for Exploratory Research and Analysis. *J. Comput. Chem.* **2004**, *25*, 1605–1612, https://doi.org/10.1002/jcc.20084.
37. Morris, G.M.; Huey, R.; Lindstrom, W.; Sanner, M.F.; Belew, R.K.; Goodsell, D.S.; Olson, A.J. NIH Public Access. *J Comput Chem.* **2010**, *30*, 2785–2791, https://doi.org/10.1002/jcc.21256.AutoDock4.
38. Morris, G.M.; Huey, R.; Lindstrom, W.; Sanner, M.F.; Belew, R.K.; Goodsell, D.S.; Olson, A.J. Software News and Updates AutoDock4 and AutoDockTools4 : Automated Docking with Selective Receptor Flexibility. *J. Comput. Chem.* **2009**, https://doi.org/10.1002/jcc.
39. Case, D.A.; Cheatham, T.E.; Darden, T.O.M.; Gohlke, H.; Luo, R.A.Y.; Merz, K.M.; Onufriev, A.; Simmerling, C.; Wang, B.; Woods, R.J. The Amber Biomolecular Simulation Programs. *J. Comput. Chem.* **2005**, *26*, 1668–1688, https://doi.org/10.1002/jcc.20290.
40. Srivastava, H.K.; Sastry, G.N. Efficient estimation of MMGBSA-based BEs for DNA and aromatic furan amidino derivatives. *J. Biomol. Struct. Dyn.* **2013**, *31*, 522–537, https://doi.org/10.1080/07391102.2012.703071.
41. Kumari, R.; Kumar, R.; Lynn, A. G-mmpbsa -A GROMACS tool for high-throughput MM-PBSA calculations. *J. Chem. Inf. Model.* **2014**, *54*, 1951–1962, https://doi.org/10.1021/ci500020m.

Interaction of Flavonols with DNA: Molecular Docking Studies

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Abstract

DNA is the most important genetic substance and basic building block of all organisms. It is responsible for all types of hereditary information. It's a nucleic acid that contains all the information necessary for specifying the biological development of all living bodies. The study of interaction of small drugs with DNA is of prime importance for the designing of effective and cost efficient chemotherapeutic agents and better anticancer drugs. This study deals with the computational interaction of some flavonols with DNA sequences. Flavonols are a type of Flavonoids. Flavonoids are found in various part of plant like fruits, seeds, bark etc. They are natural antioxidant. From literature survey it is reported that flavonoids show various biological activities like anticancer, antitumor, antibiotic, antiviral, antiallergic, anti-inflammatory etc. In present study, Molecular Docking was performed between four flavonols (Kaempferide, Karanjachromene, Quercetin and Rutin)and DNA segments with PDB ID 1BNA, 195D, 1CP8, 1D66 and 1RMX. To perform molecular docking AUTODOCK software was used. Best docked poses and binding energies of the drug-DNA complexes were calculated computationally. This study can be helpful in the designing of new and less toxic drugs and enhance their further applications in pharmacology.

Keywords: Flavonoids, flavonols, molecular docking, binding energy, stability, binding constant.

1. Introduction

In the field of drug design and drug discovery, interaction between DNA and drug molecules is a very popular and necessary subject [1]. The study of the interactions of small molecules with DNA is a field of high interest. The number and variety of techniques devoted to evidence drug-DNA interactions is continuously growing [2].

Watson and Crick were the first to give the basic and precise structure of DNA in 1953 [3].According to them, DNA consists of two helical polynucleotide chains each coiled around the same axis. DNA is usually in the form of a right-handed double helix. The backbone of each strand of the helix is composed of alternating sugar and phosphate residues that are joined together via phosphodiester linkages. The bases are on the inside of the helix and the phosphates on the outside. The planes of the bases are perpendicular to the fiber axis. These bases are: adenine and guanine, which are purines and thymine and cytosine, which are pyrimidines.

Adenine pairs up with thymine and guanine pairs up with cytosine via hydrogen binding. The two chains of the double helix have complementary sequences. The complementarity between the bases on the two strands gives DNA its self-coding character.

DNA is also the prime target of many anticancer and antibiotic drugs [4]. Studies are going on in different laboratories to analyze and understand the interaction of various drugs with DNA. These studies and researches help the researchers to design effective chemotherapeutic agents and better anticancer drugs. As DNA is the main building block, a drug for any disease interacts with DNA. Drugs are mainly small molecules which can bind with DNA via two type of binding: Groove binding and intercalation. On the basis of the mode of binding and binding energy between particular drug and DNA, their usefulness as a medicine for any specific disease can be predicted [5].

Here we are dealing with the prediction and investigation of binding properties of flavonols, a class of flavonoid, with DNA using molecular modeling. 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 antioxidant [6-8]. 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-inflammetoryetc [9, 10]. Flavonoids have potential to bind with DNA and show various pharmacological properties. Flavonols 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, flavonolscan 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.

2. Computational Details

Dataset

DNA: The pdb format file of DNA sequences with PDB ID 1BNA, 195D, 1CP8, 1D66 and 1RMX were downloaded from RCSB Protein Data Bank [11]. CHIMERA was used to remove ligands and water molecules from each sequence [12]. PDB ID and sequence of the DNA used are shown in Table 1.

Drugs: The structures of Kaempferide, Karanjachromene, Quercetin and Rutinwere collected from literature [13-16]. Fig 1 shows the chemical structures of these flavonols.The drug molecules were subjected to geometry

optimization using Gaussian 09 at B3LYP/6-31G* level [17].

Table 1: PDB ID and sequence of the DNA used.

S.no.	PDB ID	DNA Sequence
1	1BNA	5'-CGCGAATTCGCG-3'
2	1CP8	5'-TTGGCCAA-3'
3	1D66	5'-CCGGAGGACAGTCCTCCGG-3'
4	1RMX	5'-CGACTAGTCG-3'
5	195D	5'-DCGCGTTAACGCG-3'

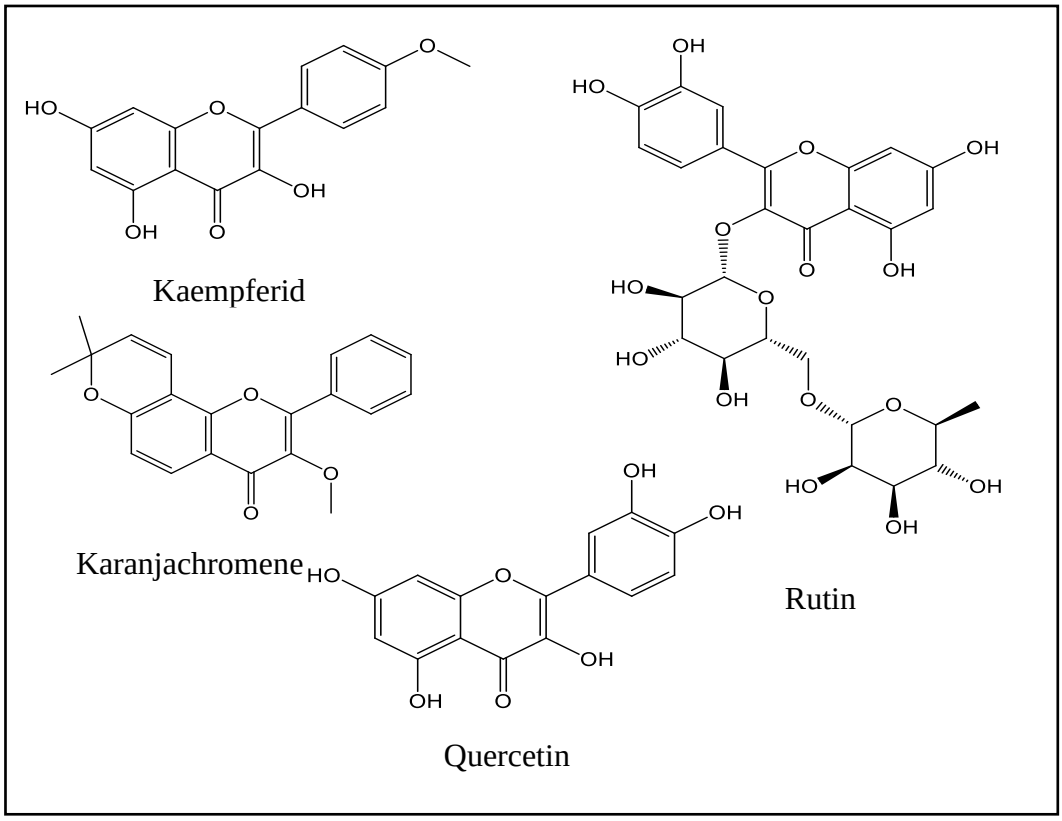

Fig 1. Structure of used flavonols.

Molecular Docking

AutoDock4.2was used for molecular docking simulations using Lamarckian Genetic

Algorithm (LGA) [18].The docking was performed using DNA sequences as rigid receptor molecule, whereas flavonols were treated as a flexible ligand. The receptor and ligand files were prepared for docking using Auto Dock Tools (ADT) [19].Grid boxes of various dimensions were used to prepare grid maps using Auto-Grid for each DNA sequence. The Gasteiger charges were added to the complex by Auto Dock Tools (ADT) before performing docking calculations. Lamarckian genetic algorithms, as implemented in AutoDock, were employed to perform blind docking calculations. For each of the docking cases, the lowest energy docked conformation, according to the Auto Dock scoring function, was selected as the binding mode.

3. Results

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 Fig 2. 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.

The complex of Karanjachromene with 195D have lowest binding energy -9.23 kcal/Mol, this shows that the interaction between DNA duplex of sequence 5'-DCGCGTTAACGCG-3' and karajachromene is more stable in comparison to other complexes. So it can prove to be an effective drug against cancer and other diseases. For each of the DNA sequences, non-covalent interaction for most stable complex is shown in Fig 3.

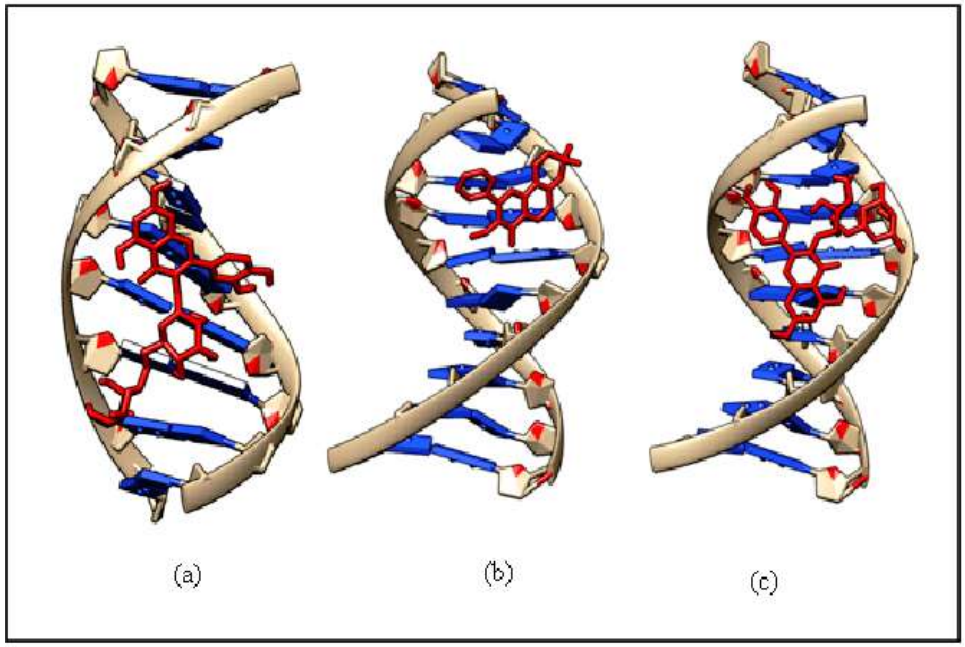

Fig 2. Represent of major groove binding. (a) 1CP8 and rutin (b) 1RMX and karanjachromene (c) 1RMX and rutin

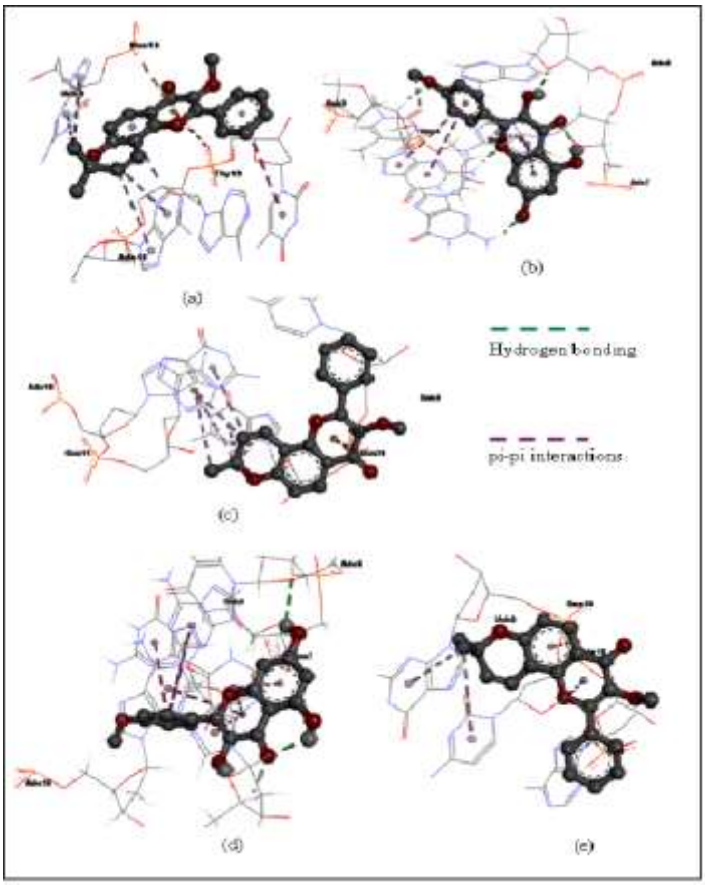

Fig 3. Non-covalent interaction between DNA and drug having lowest binding energy for each DNA sequence. (a) 1BNA and Karanjachromene (b) 1CP8 and Kaempferide (c) 1D66 and Karanjachromene (d) 1RMX and Kaempferide (e) 195D and Karanjachromene

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

4. Conclusion

Flavonols are a class of flavonoids having possible application in pharmacology. The computational study gives details about the interaction of flavonols Kaempferide, Karanjachromene, Quercetin and Rutin with DNA and binding energies of the complexes.

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 study helps in getting a deeper insight regarding the DNA binding mechanism and binding affinity of natural antioxidantflavonols. It can serve as a good database for structure-energy relationship of anticancer drug-DNA complexes.

References

1. A. Paul and S. Bhattacharya, Curr. Sci 02 (2012) 212–231.
2. J. Li, S. Shuang and C. Dong, Talanta, 77 (2009) 1043–1049.
3. J. D. Watson and F. H. C. Crick, Nature 171 (1953) 737–738.
4. L.H. Hurley, Nat. Rev. Cancer 2 (2002) 188–200.
5. R. Mishra, A. S. Gaur, R. Chandra and D. Kumar, Int. J. Phar. Chem. Analysis 2(4) (2015) 161-169.
6. P.A. Ragazzon, J. Iley and S. Missailidis, Anticancer Research 29 (2009) 2285-2294.
7. J.E.N. Dolatabadi, Int. J. Biol. Macromol. 48 (2011) 227-233.
8. B. Tu, Z.-F. Chen, Z.-J. Liu, L.-Y. Cheng and Y.-J. Hu, RSC Adv.5 (2015) 33058-33066.
9. E. Middleton, C. Kandaswami and T.C. Theoharides, Pharmacol. Rev. 52 (2000) 673–751.
10. M. J. Hannon, Chem. Soc. Rev. 36 (2007) 280-295**.**
11. The Protein Data Bank, H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I.N. Shindyalov and P.E. Bourne, Nucleic Acids Research 28 (2000) 235-242.
12. E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng andT. E. Ferrin. J. Comput. Chem. 25(13) (2004) 1605-12.
13. S. Nafisi, M. Hashemi, M. Rajabi and H. A. Tajmir-Riahi, Dna and cell biology 27(8) (2008)433–442.
14. S. Balakrishnan and S. Jaldappagari, Journal of Luminescence 142 (2013) 17–22**.**
15. V. Mary, P. Haris, M. K. Varghese, P. Aparna, and C. Sudarsanakumar, J. Chem. Inf. Model. 57 (2017) 2237−2249.
16. N. Arshad, N. Rashid, S. Absar, M.S.A. Abbasi, S. Saleem and B. Mirza, Cent. Eur. J. Chem. 11(12) (2013) 2040-2047.
17. Gaussian 09, Revision E.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M.Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, J. E.Peralta, F.Ogliaro, M.Bearpark, J. J.Heyd, E.Brothers, K. N.Kudin, V. N.Staroverov, R.Kobayashi, J.Normand, K.Raghavachari, A.Rendell, J. C.Burant, S. S.Iyengar, J.Tomasi, M.Cossi, N.Rega, J. M.Millam, M.Klene, J. E.Knox, J. B.Cross, V.Bakken, C.Adamo, J.Jaramillo, R.Gomperts, R. E.Stratmann, O.Yazyev, A. J.Austin, R.Cammi, C.Pomelli, J. W.Ochterski, R. L.Martin, K.Morokuma, V. G.Zakrzewski, G. A.Voth, P.Salvador, J. J.Dannenberg,

S.Dapprich, A. D.Daniels, O.Farkas, J. B.Foresman, J. V.Ortiz, J.Cioslowski andD. J.Fox, Gaussian, Inc., Wallingford CT(2009).

18. G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell and A. J. Olson, J. Comput. Chem. (2009) 302785–2791.
19. G. Morris, D. Goodsell, R. Halliday, R. Huey, W. Hart, R. Belew and A. Olson, J. Comput. Chem. 19 (1998)1639–1662.

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Interplay Between Theory and Experiment: A Future Approach for Biomedical Research

4

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Abstract

Theoretical and computational chemistry have become indispensable in various fields of chemical research as they provide structures, properties, and reactivities of the molecules. Computational enzymology is a rapidly developing area and is testing theories of catalysis, challenging "textbook" mechanisms, and identifying novel catalytic mechanisms. Modeling of enzymes is contributing to the experimental study of enzyme-catalyzed reactions in the field of drug discovery, catalyst design, and interpretation of experimental data. In the present text, we would discuss some controversies and reaction mechanisms which were later resolved with the help of theoretical calculations.

Keywords

Metalloenzyme · Drugs · Substrates · Controversies

4.1 History

In late 1960s with advent of microprocessor technology (Moore 1965; Whitworth 1979; Brinkman et al. 1997), foundation of interdisciplinary scientific field, which is today known as computational chemistry, was laid. This field is an amalgamation of mathematics and computational science with scientific discipline such as physics, chemistry, and biology. A few decades ago, theoretical modeling played a minor role in understanding redox-active reaction of metalloenzymes. The theoretical methods were underdeveloped; due to this, either result were not accurate enough, or processing time was too long.

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Efficient anticarcinogenic activity of α-Fe_2O_3 nanoparticles: In-vitro and computational study on human renal carcinoma cells HEK-293

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ABSTRACT

The present study has witnessed the synthesis of α-Fe_2O_3 NPs using polyethylene glycol (PEG) as a surfactant and L-ascorbic acid (LAA) as a stabilizer. The product has been characterized by UV–vis absorption spectroscopy, FTIR, Dynamic Light Scattering, particle size distribution analysis, X-ray diffraction analysis, TEM, FESEM, EDX and BET, which show formation of variable size and shape, mesoporous, PEG-coated α-Fe_2O_3 NPs (LAA@IONP-PEG) with β-FeOOH as an impurity. The present work emphasizes upon an anti-cancer study of LAA@IONP-PEG against renal carcinoma HEK-293 human embryonic kidney cell lines. The study suggests that LAA@IONP-PEG is a promising material against renal carcinoma HEK-293 human embryonic kidney cell lines. The docking study has confirmed anti-proliferative action of NPs through binding affinity with renal carcinoma molecular targets. The synthesized NPs show the synergistic effect with Axitinib as an anti-cancer drug effective against renal carcinoma cell lines. ROS and 1,1-diphenyl-2-picrylhydrazine (DPPH) free radical scavenging assay have shown the antioxidant capability of synthesized NPs. The efficient biocatalytic activity of LAA@IONP-PEG prescribes its use as one of the best suited drugs for the future perspectives against fatal renal carcinoma and thus it is reckoned that synthesized NPs will have persistent utilization in different field of medical applications.

1. Introduction

Renal carcinoma is one of the most frequently diagnosed malignancies in men, which causes mobility and mortality globally [1]. To improve the efficacy of cancer therapy and to minimize its hazardous impacts on healthy tissues and organs, new treatment methods are urgently needed. Since recent decades, nanotechnology and its interdisciplinary fields are emerging as the best tool to diagnose several diseases fatal to mankind and causing problems to the environment. Synthesis of nanoparticles has gained attention in the field of a wide variety of biomedical applications like hyperthermia, gene or drug delivery, magnetic resonance imaging (MRI) and *in vivo* cell tracking [2,3]. It has been reported that cytotoxicity depends on the nanoparticle size, shape, its structure and morphology [4,5]. The small size of such particles means that they are able to more easily evade the immune system of the body and penetrate cellular membranes to come into close proximity to sensitive biological components [6] Generally, the cytotoxicity of nanoparticles results due to cations on the surface of the metal oxides, which generate harmful free radical species catalytically within the cytoplasm [7] and are then able to chemically attack sensitive cellular components. Thus increased free radical concentrations can lead to apoptosis [8] or can responsible for potential carcinogenesis [9].

Due to structural imperfection and uncompensated spins, magnetic nanomaterials exhibit induced permanent magnetic moments on the surface of the particles [10]. The surface uncompensated exchange couplings at the surface modify the magnetic properties, [11]. Although several associated issues like aggregation, toxic states of the nanoparticles, surface functionalization, poor durability etc. restrain the use of bare magnetic nanoparticles, however, these problems can be addressed by coating magnetic nanoparticles with non-magnetic materials like polymers, organic monolayers, enzymes etc. [12–14]. Such coated magnetic nanoparticles have added advantage in the biomedical field due to easy separation and sensitivity to a magnetic field [15].

Iron oxide nanoparticles exhibited exceptional features in catalysis by reducing unwanted reactions, good selectivity, the requirement of the mild environment in various examples like hydrolysis, esterification,

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Interplay between two degenerate spin state determines the hydroxylation of 4-nitrophenol catalyzed via Cytochrome P450

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ABSTRACT

4-Nitrophenol is formed during the synthesis of paracetamol. It is used in various xenobiotic metabolism processes and other essential biochemical processes. It is metabolized via cytochrome P450 enzyme. The present work reported the hydroxylations of 4-nitrophenol at the ortho position by Cytochrome P450 metalloenzyme. Truncated model of putative active oxidant i.e. ferryl oxo porphyrin cation radical [Fe^{IV}(O)(heme$^{+\bullet}$)], referred as Cpd I in cytochrome P450 enzymes has been used to mimic the behavior of enzyme. In the current investigations, 4-nitrophenol (Substrate) is modeled with Cpd I and reaction mechanism for two degenerate spin states named as doublet (LS) and quartet (HS) is performed to dwell the overall potential energy landscape, along with electronic structures and properties of reactant complex (RC), transition states (TS), intermediates (IM) and product complex (PC). The reaction is stepwise with electrophillic addition as the rate determining step, spin selectivity product formation is observed only at high spin (HS) surface. So, the present reaction pathway is single state reactivity (SSR) by forming the suicidal complex at low spin state (LS).

1. Introduction

The Cytochrome P450 is an essential enzyme found in nature [1]. It consists of number of enzymes with varying substrate selectivity, such as CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A etc. These enzymes are responsible for the metabolism of about 70% of therapeutic drugs [2]. In human, P450 is found in liver [3]. It contribute in the catalysis of variety of stereo and regio-selective mono-oxygenation reaction processes and also in detoxification processes [4]. Due to their wide range versatility in activation of several substrate, CYP is very important enzyme, in biology, biotechnological and pharmaceutical applications for investigations of new drugs [5]. Moreover, its involvement in drug metabolism make this enzyme a target for research in drug industry and biomedical field [6–9]. Despite of various member of P450 enzyme, CYP2E1 has been paid a considerable attention, because of its toxicological behavior. It is contributed in the metabolism of various organic solvents and environmental pollutants like, acetone, aniline, ethanol, or nitrosamines etc [10,11]. Metabolism by CYP2E1 might result in the formation of more reactive products, such as the toxic metabolite of acetaminophen (paracetamol) [11–13]. Formation of toxic product of acetaminophen (paracetamol) via CYP2E1, is its crucial behaviour. 4-nitrophenol hydroxylation may responsible for inhibition of toxic product of acetaminophen (paracetamol) via CYP2E1 enzyme.

4-nitrophenol is formed during the synthesis of paracetamol. Initially, it reduced to 4-aminophenol, after that acetylated via acetic anhydride [14]. It is also product of essential enzymatic reactions of several substrate like- 4-nitrophenyl phosphate, 4-nitrophenyl acetate, 4-nitrophenyl-β-D-glucopyranoside and more other derivatives. It plays a crucial role in xenobiotic metabolism process in both, human and mouse. In field of medicinal chemistry, it is used in manufacturing of drugs, insecticides, fungicides. Its structure has phenolic compound, in which nitro group is attached at the opposite of the hydroxyl group on benzene ring. It is also known as *p*-nitrophenol or 4-hydroxynitrobenzene due to presence phenol group. It is mostly used in detection of the presence of alkaline phosphatase activity [15].

The P450 enzyme metabolizes various compound using high-valent iron (IV) oxo species complex, generally known as Cpd I. This complex is formed during the catalytic cycle of Cytochrome P450. Initially, in catalytic cycle, P450 is in the resting state and a water molecule is ligated with it. Whenever, substrate enters, it tightly binds with porphyrin by expelling the water molecule. After the oxidation step a ferric peroxide species is formed, known as compound 0 (Cpd 0), and

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In-silico docking studies of 2,5-bis(4-amidinophenyl) furan and its derivatives

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Abstract

Computational docking is a very popular and widely used methodology in bioinformatics and computational biology, in the current scenario. The potential drugs can only be identified and addressed by having the knowledge of their relative DNA binding energies and binding modes. In the present work,2,5-bis(4-amidinophenyl) furan and its derivatives were studied computationally to investigate their DNA binding abilities using molecular docking technique. Computational docking revealed that DNA binding energies of 2,5-bis(4-amidinophenyl) furan and its derivatives followedthe same trend as set up by theirexperimentally obtained thermal melting values.

Keywords: Binding Energies, Bioinformatics, Computational Docking, DNA.

1. Introduction

Deoxyribonucleic acid (DNA) is a twisted, double helical strand that serves as the main ingredient by acting as the carrier of all the genetic information [1]. Almost all the anti-cancer therapies involve the interaction of drugs with DNA. Drug-DNA interactions can be broadly classified into two major categories viz. intercalation and groove binding. Groove binding in DNA takes place via two modes, major groove binding and minor groove binding [2].In the present study, we prefer to analyse the minor groove binding tendency of the DNA. The ability to predict the conformation as well as the associated energy of binding of ligands/drugs to DNA has played a major role in bioinformatics and pharmacology. The design of new drugs and test of their potency has been very easy since past few years due to the presence and wide use of various computational tools. Molecular docking technique has proven itself to be of great importance in such a field of research. Since, almost all the anti-cancer and anti-microbial drugs preferentially bind itself to the DNA for their therapeutic action over DNA to begin. Minor groove binders are crescent shaped and therefore they complement the shape of the minor groove of DNA [3-6]. The binding mechanism of ligand/drug to the minor groove of the DNA can be mainly described via following two steps: in the first step, the ligand gets itself transferred to the minor groove of the DNA via electrostatic interactions and hydrophobic interactions whereas in the second step, numerous covalent interactions are formed in between ligand/drug and DNA base pairs. These interactions include van-der Waal's contacts, hydrogen bonds, electrostatic interactions and hydrophobic interactions. It has been observed that most of the minor groove binders bind themselves at AT-rich regions of the DNA [7-10].Various experimental studies have been performed worldwide in order to explain and understand the binding mechanism of anti-cancer, anti-bacterial and various other diseases. The results obtained from these experiments serve as an excellent database for theoretical scientists to carry out and test the authenticity of their simulations [11-20].

In the present study,2,5-bis(4-amidinophenyl) furan and its derivatives,