Quantum Computational Study on Vinflunine with Multiple Targets

By

B.Anitha

(14PPH002)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore - 641 043

In partial fulfilment of the requirements for the degree of

Master of Science in Physics

April, 2016

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CERTIFIED AS A BONAFIDE RESEARCH WORK

29/4/16

Signature of the Head of the Department

29/4/16

Signature of the Guide

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B.ANITHA

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CHAPTER 1

INTRODUCTION

1.1 VINKA ALKALOIDS

Among several plant-derived compounds which have been an important source of many clinically useful anti-cancer agents, Vinca plants are known to exhibit potential anti-tumor properties. The Vinca alkaloids are a class of pharmaceutically relevant binary indole-indoline alkaloids based on and including natural extracts of the periwinkle plant, Catharanthus rosea. Two natural products, Vinblastine and Vincristine, have been in clinical use as important chemotherapy agents for over four decades. Two semi-synthetic Vinca alkaloids, Vindesine and Vinorelbine, are currently in investigational chemotherapy programs, and a third semi-synthetic, Vinflunine, is in advanced clinical trials such as to treat leukemia, lymphomas and childhood cancers, as well as several other types of cancer and some non-cancerous conditions [1].

1.2 VINFLUNINE

Vinflunine or 20', 20’-difluoro-3', 4’-dihydrovinorelbine, is a novel Vinca alkaloid selectively fluorinated by superacid chemistry in a rarely exploited region of the velbanamine moiety [2]. Vinflunine is the second Vinorelbine a new type of semi-synthetic vinca alkaloids. The structure of Vinflunine is very similar to that of Vinorelbine, from which it differs only by the presence of a group gem-difluorinated in C20, and by the absence of the double bond C2'-C4' that is Vinflunine differs from that of vinorelbine in C-19 'position by two fluorine atoms replace two hydrogen atoms, in the 15 'and 20' position of the carbon-carbon double bond is reduced to a single bond is shown in the figure1.1.

Vinflunine has a marketing authorisation for use as 'monotherapy for the treatment of adult patients with advanced or metastatic transitional cell carcinoma of the urothelial tract after failure of a prior platinum-containing regimen. It demonstrates shorter relaxation times compared with Vinorelbine, and the data are consistent with spiral formation occurring by the addition of liganded heterodimers and the annealing of oligomers. And it may demonstrate reduced neurotoxicity relative to the other three Vinca alkaloi

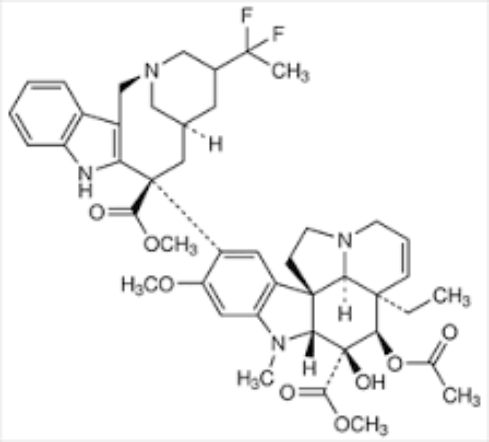

FIGURE: 1.1 CHEMICAL STRUCTURE OF VINFLUNINE

1.3 SERUM ALBUMIN

Serum albumin is the most plentiful protein in blood plasma. Each protein molecule can carry seven fatty acid molecules. Serum albumin binds to many drug molecules. Serum albumin produced by the liver, dissolved in blood plasma and most abundant blood protein in mammals. Serum albumin is an albumin found in vertebrate blood. Albumin is essential for maintaining the oncotic pressure needed for proper distribution of body fluids between blood vessels and body tissues, without albumin, the high pressure in the blood vessels would force more fluids out into the tissues. The diagram of Serum albumin is shown in FIGURE: 1.2

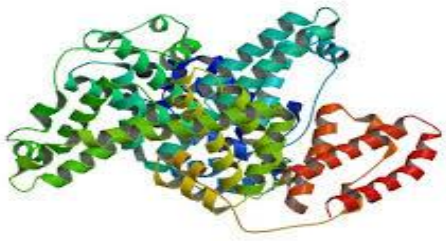

FIGURE: 1.2SERUM ALBUMIN

1.4 BOVINE SERUM ALBUMIN

Serum albumin is the most abundant protein in the circularity system. The most important physiological function of serum albumin is to maintain the osmotic pressure, pH of blood and transport a wide variety of endogenous and exogenous compounds including fatty acids, metal, amino acids, steroids and drugs [3]. The 3-D structure of BSA is very similar to HSA, because the two proteins share 76% sequence identity [4]. The heart shaped BSA molecule is made up of three homologous α-helical domains (I. II, III). Each domain contains two subdomains A and B. each domain can be divided into 10 helical segments. Bovine serum albumin structure is α-helical (67%) with the remaining polypeptide occurring in turns and extended or flexible regions between subdomains, and contains no β-sheets [5]. The subdomain is indicated along with the amino acid sequences of BSA and HSA.

The full length BSA precursor protein is 607 amino acids. The initial protein product contains 589 amino acid residues. An additional 4 amino acids are cleaved to yield the BSA protein contains 583 amino acids. The diagram of Bovine Serum Albumin is shown in FIGURE: 1.2

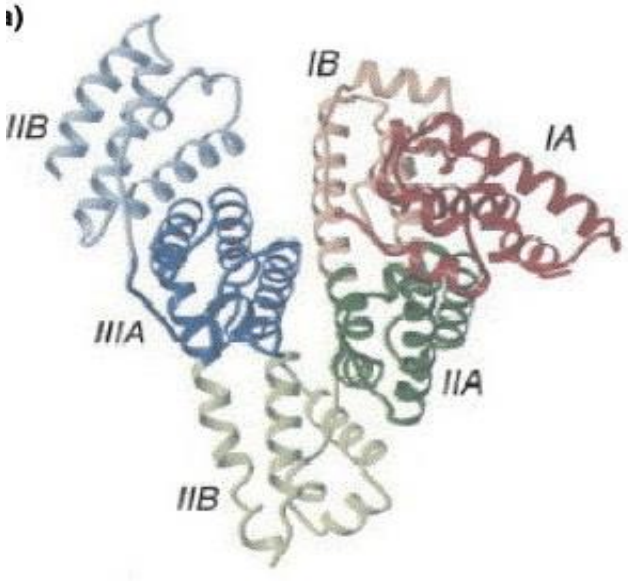

FIGURE: 1.3BOVINE SERUM ALBUMIN

1.5 TUBULIN ALPHA- BETA DIMER

The α-β tubulin dimer is the structural sub unit of microtubules, which are cytoskeletal elements that are essential for intracellular transport and cell division in all eukaryotes. Each tubulin monomer binds a guanine nucleotide, which is nonexchangeable. When it is bound in the α subunit, or N site, and exchangeable, when bound in the β subunit, or E site. The α and β -tubulins share 40% amino-acid sequence identity, both exist in several isotype forms, and both undergo a variety of posttranslational modifications. Limited sequence homology has been found with the proteins FtsZ and Misato, which are involved in cell division in bacteria and Drosophila, respectively. The structures of α and β –tubulin are basically identical: each monomer is formed by a core of two β-sheets surrounded by α-helices. The monomer structure is very compact, but can be divided into three functional domains: The amino terminal domain containing the nucleotide-binding region, an intermediate domain containing the Taxol-binding site, and the carboxy-terminal domain, which probably constitutes the binding surface for motor proteins. The figure 1.4 shows the structure of alpha-beta tubulin dimer [6].

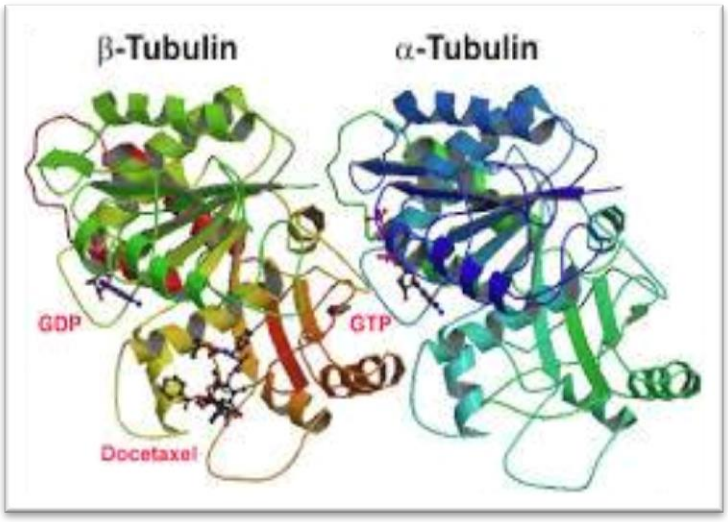

FIGURE: 1.4ALPHA-BETA TUBULIN

1.6 DEOXYRIBO NUCLEIC ACID (DNA)

DNA is the prime genetic molecule, carrying all the hereditary information within chromosomes, immediately focused attention on its structure. It consists of two polynucleotide chains running in opposite directions and twined round one another. The two chains are held together by hydrogen bonds between the bases, each base being joined to a companion base on the other chain. This pairing of bases is specific, odenine going with thymine, and guanine with cytosine. But is also present in intact biological material such as sperm heads and bacteriophage.

The basic unit of the DNA molecule is the nucleotide. Nucleotides are found in the cell either as components of nucleic acids or as individual molecules. Nucleotides have several different roles and are not just used to make DNA. For example, some nucleotides are important in the cell as carriers of energy used to power enzymatic reactions. The nucleotide is itself quite a complex molecule, being made up of three distinct components. The figure 1.5 shows the structure of a DNA.

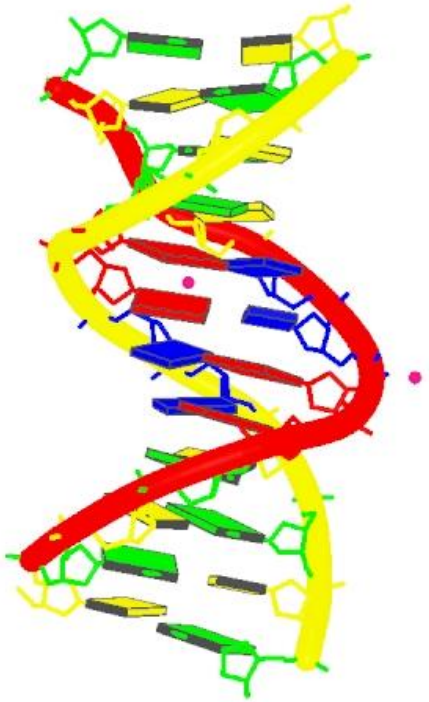

FIGURE: 1.5 STRUCTURE OF DNA

1.7 AMINO ACIDS:

Amino acids are biologically important organic compounds containing two functional groups, amino and carboxyl. The amino group (-NH_2) and carboxyl group (COOH). Along with a side-chain specific to each amino acid [7][8][9]. Amino acids are the building blocks of protein. An ionized amino acid that has positive and negative charge is a dipolar ion called a zwitterion. The general structure of amino acids is shown in the FIGURE 1.6.

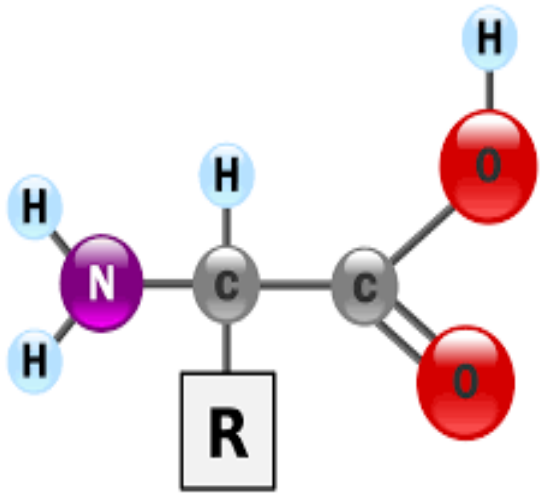

FIGURE: 1.6 GENERAL STRUCTURE OF AMINO ACIDS

1.8 CLASSIFICATION OF AMINO ACIDS:

The amino acids are classified in five groups based on their chemical structure:

1.8.1 Aliphatic amino acid

1.8.2 Aromatic amino acid

1.8.3 Neutral polar amino acid

1.8.4 Acidic amino acid

1.8.5 Basic amino acid

1.8.1 ALIPHATIC AMINO ACID

Alanine, Valine, Leucine, and isoleucine are referred to as aliphatic amino acids, have saturated hydrocarbons as side chains. Glycine which has only a hydrogen side chain is also included in this group. All of these amino acids are hydrophobic in nature.

1.8.2 AROMATIC AMINO ACID

Phenylalanine and tyrosine have aromatic side chains. The nonpolar aliphatic and aromatic amino acids are buried in the protein core and involved in hydrophobic interactions with one other. Tyrosine has a weakly acidic hydroxyl group and located on the surface of proteins.

1.8.3 NEUTRAL POLAR AMINO ACID

Neutral polar amino acids contain hydroxyl or amide side chain groups. Serine and threonine contain hydroxyl groups. These amino acids are sometimes found at active sites of catalytic protein enzymes. Asparagine and glutamine have amide bearing side chains. These are polar but uncharged under physiological conditions.

1.8.4 ACIDIC AMINO ACID

Aspartic and glutamic acids contain carboxylic acids on their side chains and ionized at pH 7.0 and it has negative charges on their β and γ carboxyl groups respectively. In the ionized state, these amino acids are referred to as aspartate and glutamate respectively.

1.8.5 BASIC AMINO ACID

Three amino acids that have basic side chains at neutral pH, which are Arginine, Lysine and Histidine. Their side chains contain nitrogen and resemble ammonia which is a base.

1.9 PROTEIN

Proteins are large biomolecules or macromolecules consisting of one or more long chains of amino acid residues, and have highly complex organic compound found in living

cells. Most proteins consist of linear polymers built from series of up to 20 different L-α amino acids [10]. Amino acids are commonly called proteins building blocks.

1.10 PROTEIN STRUCTURE

Protein structure is the three dimensional arrangement of atoms in a protein molecule. Proteins fold into more specific spatial conformations driven by a number of non-covalent interactions such as hydrogen bonding, ionic interactions and hydrophobic packing. The protein structure is divided into four levels of organisms. They are

A) Primary structure

B) Secondary structure

C) Tertiary structure

D) Quaternary structure

A) PRIMARY STRUCTURE

The primary structure of protein refers to the linear sequence of amino acids in the polypeptide chain. The primary structure held together by covalent bonds such as peptide bonds, which are made during the process of protein biosynthesis. The two ends of the polypeptide chains are referred to as the carboxyl terminus and amino terminus based on the nature of the free group on each extremity. The primary structure also requires specifying the cross linking atom. The primary structure of protein sequence is shown in the fig 1.7.

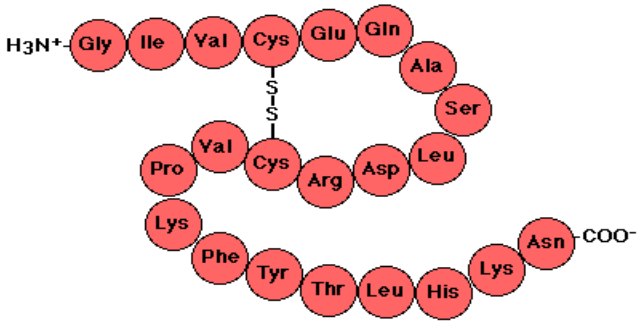

FIGURE: 1.7 PRIMARY STRUCTURE

B) SECONDARY STRUCTURE

The secondary structure of protein refers to the local structure of polypeptide chain. The structure is determined by hydrogen bond interactions between the carbonyl oxygen group of one peptide bond and the amide hydrogen of another nearby peptide bond. They are two type of secondary structure:

(i) α-Helix

(ii) β-Pleatedsheet

(i) α-HELIX

The α-helix is a rod like structure with the peptide chain tightly coiled with the side chains of amino acid residues. Expending outward from the axis of the spiral. Each amide carbonyl group is hydrogen bonded to the amide hydrogen of a peptide bond that is four residues away along the same chain. An average of 3.6 amino acid residues present in per turn of the helix and the helix winds in a right handed manner in almost all natural proteins. The fig 1.8 shows the secondary structure of α-HELIX.

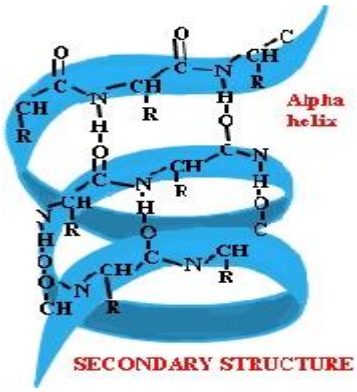

FIGURE: 1.8α-HELIX

ii) β-PLEATED SHEET

Hydrogen bonds are formed between peptide bonds in different chains, the chains become arrayed parallel or antiparallel to another called a β-pleated sheet. The figure shows

in 1.9. The β-pleated sheet is an extended structure as opposed to the coiled α-helix, because the carbon-carbon(C-C) bonds are tetrahedral and cannot exist in a planar configuration. If the polypeptide chain runs in the same direction, it forms a parallel β-sheet. But in the opposite direction, it forms an antiparallel structure. The β-turn refers to the segment in which the polypeptide in reverse direction. Glycine (Gly) and proline (Pro) residues often occur in β-turns in the surface of globular protein.

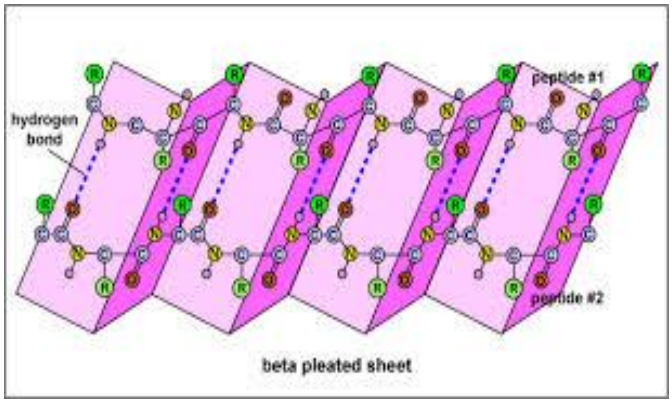

FIGURE: 1.9 β-PLEATED SHEET

C) QUATERNARY STRUCTURE

Many proteins are made up of multiple polypeptide chains often referred to as protein subunits. These subunits may be the same or different. The quaternary structure refers to the protein subunits interact with each other and arrange themselves to form a larger aggregate protein complex. The final shape of the protein complex is stabilized by various interactions including hydrogen bonding, disulfide bridges and salt bridges [11]. The figure 1.10 shows Quaternary structure of protein.

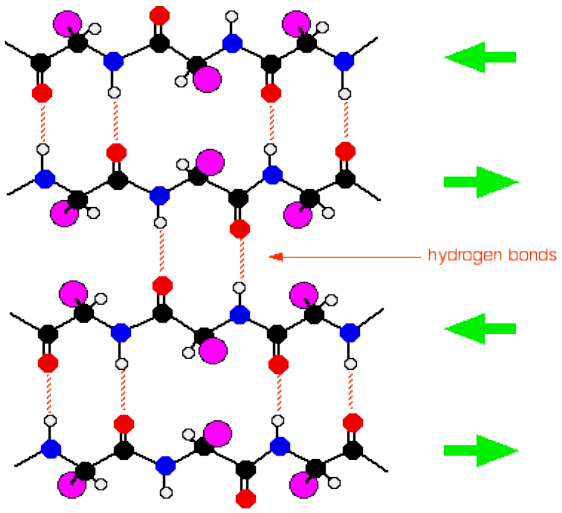

FIGURE: 1.10 QUATERNARY STRUCTURE

D) TERTIARY STRUCTURE

The overall three dimensional shape of an entire protein molecule is the tertiary structure. The protein molecule will band and twist in such a way as to achieve maximum stability or lowest energy state fig 1.11 shows the three dimensional shape of protein may seem irregular and random, it is fashioned by many stabilizing forces due to bonding interactions between the side chain groups of the amino acids [11].

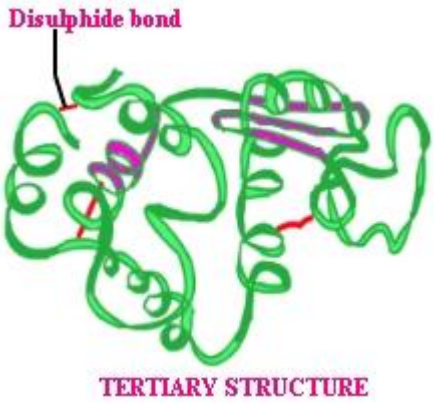

FIGURE: 1.11 TERITARY STRUCTURE

1.11 TYPES OF MOLECULAR BOND

i).Covalent bond

ii).Ionic bond

iii) Hydrogen bond

i) COVALEND BOND

A covalent bond is a chemical bond that involves the sharing of electron pairs between atoms. These electron pairs are known as shared pairs or bonding pairs. The stable balance of attractive and repulsive forces between atoms are due to sharing of electrons is known as covalent bonding [12]. The figure 1.12 shows the covalent bond between two oxygen atoms. Covalent bonding occurs when pairs of electrons are shared by atom will covalently bond with other atoms in order to gain more stability, which is gained by forming a full electron shell. Covalent bonding is the sharing of electrons between atoms. This type of bonding occurs between two of the same element or elements close to each other in the periodic table. This bonding occurs primarily between nonmetals; however, it can also be observed between nonmetals and metals as well. In these cases the electron pair and the formed electron-cloud is shared equally and the formed bond is called as non-polar covalent bond. The non-polar bond is formed with H_2, O_2, N_2, Cl_2 etc.

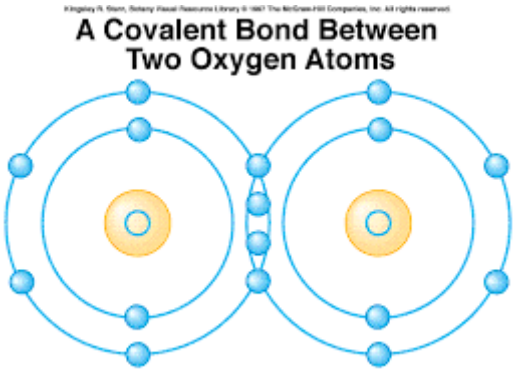

FIGURE: 1.12COVALENT BOND

ii) IONIC BOND

Ionic bond also called electrovalent bond, formed from the electrostatic attraction between oppositely charged ions in a chemical compound. Such a bond forms when the valence electrons of one atom are transferred permanently to another atom. The atom that loses the electrons becomes a positively charged ion (cation), while gains them becomes a negatively charged ion (anion). Ionic bonds occur between metallic atom and non-metallic atom in binary atomic system [figure 1.13].

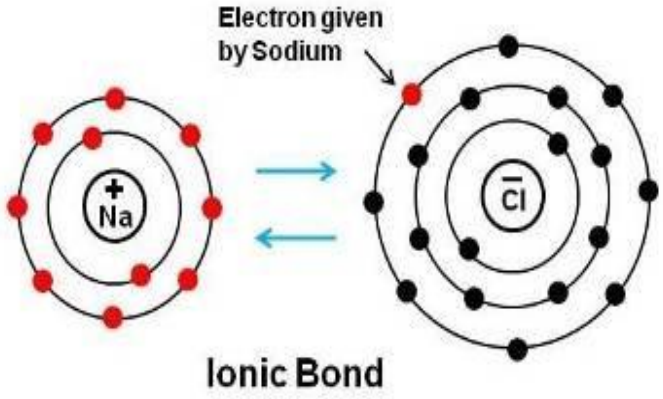

FIGURE: 1.13IONIC BOND

iii) HYDROGEN BOND

Hydrogen bond is covalently bounded to a strong electronegative and small sized atom (O_2, N), the shared electron pair between the hydrogen atom and strongly electronegative atom lies much more nearer to the electronegative atom. This results in the development of partial ionic character in the covalent bond, with a fractional positive charge on the hydrogen atom and a fractional negative charge on the electronegative atom. The attractive force that binds hydrogen atom of one molecule with electronegative atom of another molecule of the same substance, called hydrogen bond or hydrogen bridge because hydrogen bond is usually denoted by a dotted line as shown in fig 1.14 [13].

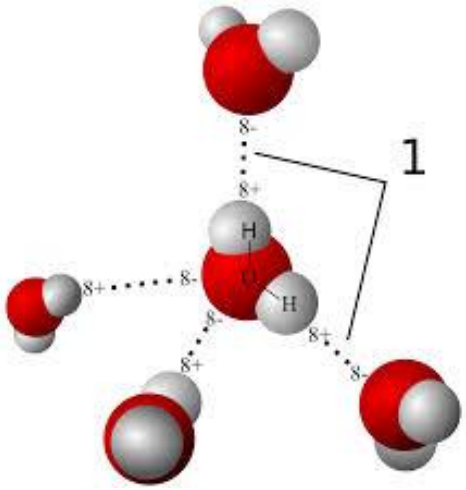

FIGURE: 1.14 HYDROGEN BOND

1.12 OBJECTIVE OF THE PRESENT STUDY

The main objective of the present study is

- To optimize the structure of Vinflunine by using Jaguar software.
- The electron properties were studied.
- IR and Raman spectrum were analysed.
- To dock the ligand Vinflunine with BSA, tubulin and DNA using maestro/Schrodinger.

1.13 REFERENCES

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REVIEW OF LITERATURE

CHAPTER II

LITERATURE OF REVIEW

2.1 INTRODUCTION

Review of literature is an important part of project. Purpose of this chapter carried out on topic to the Quantum computational study on Vinflunine with multiple targets was briefly recorded in this chapter. In these literatures, geometrical parameters and its interaction are studied by using theoretical method.

[1] Evan B. Kelly et al (2011) have done docking studied on Vinca alkaloids to tubulin. The Vinca alkaloids are a class of pharmaceutically relevant binary indole-indoline alkaloids based on and including natural extracts of the periwinkle plant, Catharanthus rosea. Two natural products, Vinblastine and Vincristine, have been in clinical use as important chemotherapy agents for over four decades. Two semi-synthetic Vinca alkaloids, Vindesine and Vinorelbine, are currently in investigational chemotherapy programs, and a third semi-synthetic, Vinflunine, is in advanced clinical trials. The Vinca alkaloids are anti-mitotic agents that affect the cellular protein tubulin and bind to a specific site known as the Vinca domain located on β-tubulin. While the Vinca domain is well established, the specific binding mode of each drugis not. However, there is much insight into the binding mode and this has provided a strong base of information to begin simulations and to make comparisons against. Complicating the issue, however, is the large size of the Vinca alkaloids and their complex molecular structure, including a rotatable single bond joining the indole and indoline portions of each compound. The differential geometric and tubulin binding properties of the drugs are not fully known. In the present work, the projection of the potential energy surface on the major torsional angle was calculated at the semi-empirical AM1 level, through in vacuo geometry optimizations. QM/MD simulations were performed, with the drugs at the AM1 level, of each Vinca alkaloid free in TIP3P water, and also bound to β-tubulin. A single equilibrium structure, resembling a known crystallographic vinblastine structure, for the free drugs was found. Further, the 1Z2B crystal structure of vinblastine bound to tubulin appears to be a valid starting point for simulations of all five Vinca alkaloids mentioned above.

[2] JayasreeGanugapati et al (2015) have used Tubulin Alpha-Beta for docking studies with the Vinca alkaloids. There are four major Vinca alkaloids in clinical use: Vinblastine (VBL), Vinorelbine (VRL), vincristine (VCR) and Vindesine (VDS). Vinca alkaloids are a subset of drugs obtained from the Madagascar periwinkle plant, Catharanthus roseus and have hypoglycemic as well as cytotoxic effects. They are used for treating diabetes, high blood pressure and have been used as disinfectants. The Vinca alkaloids are important for being cancer fighters, as they work by inhibiting the ability of cancer cells to divide. Upon acting on tubulin, they prevent the formation of microtubules, an essential component for cellular division. Vinblastine is most often used to treat breast cancer and germ cell tumors. Vinorelbine has exhibited significant antitumor activity in patients with brest cancer and anti-proliferation effects on osteosarcom (bone tumor cells). Vincristine is FDA approved to treat Wilm'stumor, acute leukaemia and other lymphomas. Vindesine is used in the treatment of melanoma, lung cancers and uterine cancers. Vinflunine is used for the treatment of second-line transitional cell carcinoma of the urothelium. Auto dock binding energies and binding interactions of the Vinca alkaloids indicate that Vinblastine is the most probable inhibitor of Tubulin Alpha-Beta.

[3] A Kruczynski et al (2002) have defined themolecular mechanisms of cell killing in both parental sensitive and Vinflunine-resistant P388 leukaemia cells. Vinflunine is the most recent Vinca alkaloid in clinical development, demonstrated superior antitumor activity to other Vincas inpreclinical tumour models. Vinflunine treatment of these cells resulted in apoptosis characterized by DNA fragmentation and proteolysis cleavage of poly-(ADP-ribose) polymerase. Apoptosis-inducing concentrations of Vinflunine caused c-jun N-terminal kinase 1 stimulation, as well as caspases-3/7 activation. This activation of caspases and the induction of apoptosis could be inhibited by the caspase inhibitor acetyl-Asp-Glu-Val-Asp-aldehyde. Interestingly, the apoptosis signal triggered by Vinflunine in these P388 cells was not mediated through Bcl-2 phosphorylation. In addition, when Vinflunine resistance was developed in P388 cells, it was associated with resistance to Vinflunine-induced apoptosis, as reflected by a loss of capacity to induce DNA fragmentation and PARP degradation, and characterized by increased levels of Bcl-2 and Bfl-1/A1. Therefore, these data indirectly implicate Bcl-2 and Bfl-1/A1 in Vinflunine-induced cell death mechanisms.

[4] **GIREESH KAMATH H et al (2015)** have evaluated in-silico docking exercise of different herbal based ligands with anti-tumor properties. The studies revealed that Vinorelbine got docked onto breast cancer kinase protein with the lowest calculated interaction energy. Since the molecule was present in its unrefined form, it was further refined using geometrical optimization technique as implemented in GAUSSIAN software package. There was no substantial difference in the calculated interaction energy between unrefined and refined Vinorelbine structure when docked onto the kinase protein; however, the protein complexed with the drug molecule, tamoxifen shared few residues that were interacting with refined Vinorelbine structure, which was not seen in unrefined one.

[5] Alexander A. Makarov et al (2007) have studied the interaction of Vinflunine with the Microtubule-Associated Protein STOP. Vinca alkaloids Vinblastine and Vincristine and some of their derivatives such as Vinorelbine are widely used in therapy of leukemia and several solid tumors. Their action is associated with alterations of the mitotic spindle functions that prevent the cell cycle progression and lead to mitotic block. A number of studies show that some Vinca alkaloids inhibit CaM-target interaction. The newest microtubule inhibitor, Vinflunine (Javlor), currently in clinical trials, is remarkably more active than vinblastine against a number of tumors. Moreover, Vinflunine is significantly less toxic than other Vinca alkaloids. The high antitumor activity of this molecule is not well understood since it binds to tubulin with an overall affinity several fold lower than that of Vinblastine or vincristine. In this study the interaction of Ca^{2+}-CaM with Vinflunine, Vinblastine, and stable tubule only polypeptide (STOP) by using a combination of Thermodynamic and mass spectrometric approaches were examined. The influences of Vinca alkaloids on Ca2+-CaM-STOP complex formation were characterized. The results revealed different binding modes to Ca^{2+}-CaM for Vinflunine and Vinblastine, highlighting that adding fluorine atom on the cleavamine moiety of the Vinca alkaloid molecule is critical for the localization of the drug on calmodulin. It was observed that Vinflunine is a better inhibitor for STOP binding to calmodulin than Vinblastine. The Vinflunine action on calmodulin can have an effect on microtubule dynamics. These data may contribute to a better understanding of the superior antitumor efficiency and lower toxicity of Vinflunine.

[6] M. Mousavi et al (2013) have done DFT studies of nano anticancer on Vinblastine and Vincristine molecules. Medicinal chemistry depends on many other disciplines ranging

from organic chemistry and pharmacology to computational chemistry. Typically, medicinal chemists use the most straightforward ways to prepare compounds. The validation of any design project comes from the biological testing. The investigations of Vinblastine and Vincristine have been studied by theoretical methods. It has been established the best structural and functional of Vinblastine and Vincristine. In this study the information of Vinblastine and Vincristine with hybrid density functional theory (B3LYP, BLYP) and hartree-fock (HF) methods by different basis sets, and then total energy, band gap, dipole moment, NMR parameter of VCR and VLB have been studied. Also, the information gathered in this investigation from the atomic structure of tubulin involved dynamic instability of microtubules, gives additional help in determining crucial binding site for the activity of potent antimitotic drugs.

[7] Zahra Varmaghani et al (2013) have optimized Vinblastine in vacuum and then in different solvents by Density Functional Theory (DFT) method. Nuclear Magnetic Resonance (NMR) shift measurements were made in different solvents by various dielectric constants by Continuous Set of Gauge Transformations (CSGT). Vinblastine is antimitotic, anticancer medicine that disturbs normal microtubule formation and favours depolymerisation. Structural study and finding the active site of Vinblastine were the targets of this research. The best structure and function of Vinblastine was established. The conformational preferences may be attributed to stereo electronic effects. The results showed that the structure of Vinblastine is more stable in water rather than the other media. The most active atoms of Vinblastine were realized by various spectra of Vinblastine in different media including vacuum and diverse solvents. Discovery of active site of Vinblastine that could bind to tubulin to perform theantimitosis and anticancer effect in process of cell division was accomplished in this investigation. These data can be applicable to study the binding site of vinblastine-tubulin complex.

[8] SHARON LOBERT et al (1998) presented a comparison of the energetics of spiral formation for two vinca alkaloids: a novel difluorinated Vinorelbine derivative20$^{'}$,20$^{'}$-difluoro-3$^{'}$,4$^{'}$-dihydrovinorelbine (F12158, or Vinflunine)and the parent compound, Vinorelbine. Vinca alkaloids are anti-neoplastic agents that halt cell division at metaphase by Inhibiting microtubule assembly and inducing tubulin self-association into spiral aggregates. The overall affinities for tubulin of Vincristine, Vinblastine, and Vinorelbine

seem to correlate with their clinical doses, where Vincristine with the highest overall affinity is used at the lowest doses. In the physicochemical study sedimentation velocity are used to compare Vinorelbine- and Vinflunine- induced self-association of porcine brain tubulin in the presence of 50 μM GDP or 50 μM GTP. Vinflunine demonstrates 3–16-foldlower overall affinity for tubulin and induces smaller polymers compared with Vinorelbine. Sedimentation velocity provides the only direct evidence to date that Vinflunine is a tubulin-binding drug. Stopped-flow light scattering demonstrates the shortest relaxation times for polymer redistribution for Vinflunine consistent with induction of the shortest spirals. Data collected at 5°,15°, 25°, and 37° show increasing $s_{20,w}$ values with increasing temperature and are consistent with an entropically driven process. These data are entirely consistent with our hypothesis that Vinflunine is likely to result in reduced clinical neuro toxicity relative to Vinorelbine, Vinblastine, and Vincristine.

[9] JaafarBennouna et al (2008) have studied about Vinflunine. Vinflunine (Javlor) is the first fluorinated microtubule inhibitor belonging to the Vinca alkaloids family. Vinflunine is obtained by semisynthesis using super acidic chemistry to selectively introduce two fluorine atoms at the $20^{'}$ position of the catharanthine moiety. This compound has been selected for clinical development on the basis of encouraging preclinical activity that warrant study in patients with a wide spectrum of solid tumors. Clinically significant activity has been seen in phase II studies, mainly in the treatment of transitional cell carcinoma of the urothelial tract, non- small cell lung cancer, and carcinoma of the breast. Vinflunine is currently in phase III trial assessment in patients with (second line) transitional cell carcinoma of the urothelium and first line advanced breast cancer. The efficacy of vinflunine in patients with advanced non- small cell lung cancer previously treated with a platinum-containing regimen was confirmed by a large phase III trial.

[10] Claire Coderch et al (2012)have used molecular modeling and simulation techniques to build, refine and perform a comparative analysis of the three-dimensional complexes of Vinblastine, Vincristine, Vinorelbine and Vinflunine with a $\beta_1\alpha_2$-tubulin interface in explicit water to rationalize the binding affinity differences in structural and energetic terms. The Vinca alkaloids are a group of widely used anticancer drugs, originally extracted from the Madagascar periwinkle, that disrupt microtubule dynamics in mammalian cells by interfering with proper assembly of α,β-tubulin heterodimers. They

favor curved tubulin assemblies that destabilize microtubules and induce formation of spiral aggregates. Their binding energy profiles have been characterized by means of sedimentation velocity assays and the binding site of Vinblastine at the interface between two tubulin dimmers ($\alpha_1\beta_1 - \alpha_2\beta_2$) has been ascertained by X-ray crystallographic studies on a complex of tubulin with the stathmin-like domain of protein RB3, albeit at relatively low resolution. The results shed some more light into the binding determinants and the structure-activity relationships of these clinically useful agents.

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METHODOLOGY

CHAPTER III

METHODOLOGY

3.1 INTRODUCTION

The real strength of computational chemistry is the ability to generate data from which a human may gain insight, rationalize the behaviour of large class of molecules. Such insights and rationalizations are much more likely to be useful over a longer period of time than the saw results themselves. Although computational chemistry has evolved to the stage where it often can be competitive with experimental methods for generating a value for a given property of a given molecule, the number of possible molecules and their associated properties is so huge that only a very tiny fraction will ever be amenable to the calculations.

3.2 DOCKING

Molecular docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex[1]. Knowledge of the preferred orientation is used to predict the strength of association or binding affinity between two molecules using scoring functions. The associations between biologically relevant molecules such as proteins, nucleic acids, carbohydrates, and lipids play central role in signal transduction. The relative orientation of the two interacting partners may affect the type of signal produced (e.g.Agonism/ antagonism). Docking is useful for predicting both the strength and type of signal produced. Docking is frequently used to predict the binding orientation of drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs[2].

A binding interaction between a small molecule ligand and an enzyme protein may result in activation or inhibition of the enzyme. If the protein is a receptor, ligand binding may result in agonism or antagonism. Docking is most commonly used in the field of drug design. Most drugs are organic molecules, and docking may be applied for:

Docking combined with a scoring function can be used to quickly screen large databases of potential drugs in silico to identify molecules that are likely to bind to protein target of interest.

Docking can be used to predict in where and in which relative orientation a ligand binds to a protein (i.e. Binding mode or pose). This information may in turn be used to design more potent and selective analogs. Protein ligand docking can also be used to predict pollutants that can be degraded by enzymes [3].

Drug discovery projects having access to high-resolution crystal structures for their targets, high-performance ligand receptor docking is the clear computational strategy of choice to accelerate structure based drug design. The docking process involves the prediction of ligand conformation and orientation within a targeted binding site .In general; there are two aims of docking studies: accurate structural modeling and correct prediction of activity.

The identification of molecular features that is responsible for specific biological recognition, or the prediction of compound modifications that improve potency, are and issues that are difficult to understand. Thus simulation carried out on a computer.

Glide searches for favorable interactions between one or more ligand molecules and a receptor molecule, usually a protein. Each ligand must be single molecule, while the receptor may include more than one molecule, e.g., a protein and a cofactor. Glide can be run in rigid or flexible docking modes; the later automatically generates conformations for each input ligand.

The combinations of position and orientation of a ligand relative to the receptor, along with its conformations in flexible docking, is referred to a ligand pose. The ligand pose's that glide generates, pass through a series of hierarchical filter tests the spatial fit of the ligand to the defined active site, and examines the complementarities of ligand receptor interaction using a grid –based method patterned after the empirical ChemScore function.

Poses that pass these initial screens enter the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the non–bounded ligand

receptor interaction energy. Final scoring is then carried out on the energy minimised poses. By default, Schrödinger's proprietary Glide score multi-ligand scoring is used to score the poses.

Glide score was selected as the scoring function, a composite E model score is then used to rank the poses of each ligand and to select the poses to be reported to the user. Emodel combines Glide score, the non-bounded interaction energy, and for flexible docking the excess internal energy of the generated ligand conformation. Thus, a molecular docking procedure consists of the following

- **Protein preparation,**
- **Ligand preparation,**
- **Receptor grid preparation,**
- **Ligand docking preparation,**

Protein Preparation

The preparation of the protein calls for great care. Important decisions include the choice of the tautomeric forms of Histidine residues, the protonation states of amino-acids and conformations of some residues; their incorrect assignments may lead to docking errors.

Ligand preparation

Ligands can be obtained from various databases like Pubchem or can be sketched using chemsketch. Ligands were subjected to auromatic preparation process, performed with Ligprep tool of the Schrodinger package. It generates all possible protonation and tautomeric states available within the pH range of 7.0+2.0.

Receptor Grid Generation

To perform more efficiently the docking calculations Glide does not work with the structure itself but with a grid representing the properties of the structure (i.e. electrostatic potential generated on each grid points, van der Waals etc...). We will thus generate such a grid from the prepared structure

Docking

Docking is a method which predicts the preferred orientation of one molecule to another molecule when they are bound together to form a stable complex. Molecular docking can be referred as “lock and key” model. Here the protein can be called as a lock and the ligand can be called as key, which describes the best fit orientation of the ligand which it goes and binds to a particular protein. To perform a docking, first one may require a protein molecule

3.3 COMPUTATIONAL CHEMISTRY

Computational chemistry is used in a number of different ways. One particularly important way is to model a molecular system. Although computational models may not be perfect, they are often good enough to rule out 90% of possible compounds as being unsuitable for their intended use. Computational chemistry is very useful because synthesizing a single compound could require months of labor and raw materials, and generate toxic waste. A second use of computational chemistry is in understanding a problem more completely. There are some properties of a molecule that can be obtained computationally more easily than by experimental means. There are also insights into molecular bonding, which can be obtained from the results of computations that cannot be obtained from any experimental method. Thus, many experimental chemists are now using computational modeling to gain additional understanding of the compounds being examined in the laboratory. As computational chemistry has become easier to use, professional computational chemists have shifted their attention to more difficult modeling problems [4].

3.4 THEORETICAL METHODS

A theoretical model or method is a way to model a system using a specific set of approximations. These approximations are combined with a calculation algorithm and are applied to atomic orbitals, defined by the basis set, in order to compute molecular orbitals and energy. In general, the methods can be separated into four main

3.4.1. Ab initio method

3.4.2. Density functional theory

3.4.3. Semi empirical methods

3.4.4. Molecular mechanics

3.4.1. AB INITIO METHOD

The ab initio method term is the first principle for polyatomic molecules. Ab initio calculations uses the true molecular Hamiltonian and not the empirical data in the calculation except for the fundamental constants of nature such as the mass of the electron, plank's constant that are required for the arrival numerical predictions[5]. This type of computation is based only on theoretical principles using no experimental data. In quantum chemistry, the system is described by a wave function which can be found by solving the Schrodinger equation which can be applied for obtaining the energy associated with a wave function describing the position of the nuclei and electrons in the system. The numerous methods have a same basic approach though they differ in the mathematical approximations used. Despite the fact that the computational time is lengthy, these are the most popular ones. This basic type of ab initio electronic structure calculation is the Hatree Fock (HF) scheme.

3.4.2. DENSITY FUNCTIONAL THEORY

The ab initio methods described above all start the Hatree-fock approximation in that the HF equations are first solved to find spin orbitals that can then be used to construct configuration state functions. These methods are widely used by quantum chemistry today. However they do have limitations in particular the computational difficulty of performing accurate calculations with the large basis sets on molecule containing many atoms and many electrons [6].

The approach of Density Functional Theory (DFT) was developed in 1960s by using mathematical functions, called a functional, to describe the electron density. DFT methods are attractive they include the effects of electron correlation in the energy.

Developments in DFT have led of nonlocal (gradient-correlated) functional -BLYP (Becke-Lee-Yang Parr), and to hybrid functional-B3LYP. The main idea of DFT is to describe an interacting system of fermion via its density and not via its many body wave function. The energy is not obtained as Eigen values of a wave function but rather as a functional of the electron density.

In DFT there is a relationship between the total electronic energy and the overall electronic density. The Thomas Fermi model contains some of the basic element and the real breakthrough came with a paper by Hohenburg and Khon in 1964, who showed that the ground state energy and other properties of a system were uniquely defined by the electron density. This is sometimes expressed by testing that the energy, E is a unique functional of ρ(r). A functional enables a function to be mapped to a number and is usually written using square bracket. Thus

$$Q[f(r)] = \int f(r)dr \qquad (1.1)$$

The function f(r) is usually dependent upon other well defined functions. A simple example of a functional would be the area under a curve, which takes a function f(r) depending the curve between two points and returns a number (the area in this case). In the case of DFT, the function depends upon the electro density, which would make Q a function of ρ(r), in the simplest case f(r) would be equivalent to the density, if the function f(r) were to depend upon the gradients of ρ(r), then the functional is referred to as being non-local or gradient corrected. By contrast, a local functional have a simple dependence upon ρ(r).

In DFT, the energy functional is written as a sum of two terms.

$$\mathrm{E}[\rho(r)] = \int V_{ext}(r)\,\rho(r)dr + F[\rho(r)] \qquad (1.2)$$

$$E[\rho(r)] = E_{KE}[\rho(r)] + E_H[\rho(r)] + E_{XC}[\rho(r)] \qquad (1.3)$$

Where E_{KE} [ρ(r)] is the kinetic energy, E_{HE} [ρ(r)] is the electron-electron columbic energy and E_{XC} [ρ(r)] contains contributions from exchange and correlation. It is important to note

that the first term in equation (1.7), E_{KE} [ρ(r)], is defined as the kinetic energy of a system of non-interacting electrons with the same density ρ(r) as the real system.

$$V_{\chi C}[r] = \left(\frac{\delta E_{\chi C}[\rho(r)]}{\delta \rho(r)}\right) \qquad (1.4)$$

To solve the Kohn-Sham equation a self-consistent approach is taken. An initial guess of the density is fed into equation, from which a set of orbitals can be derived, leading to an improved value for the density, which is then used in the second interaction, and so on until convergence is achieved [7].

3.4.3 SEMI EMPERICAL METHODS

There are clearly computational limitations to treating molecular systems with large numbers of electrons accurately. Even with increases in computer speed and memory and the development of efficient algorithms, ab initio methods are not applied routinely to molecules with several dozen atoms. On the other hand, semi empirical methods are fast enough to be applied routinely to larger systems and, thus, make electronic structure calculations available for a wider range of molecules. Ab initio methods represent a more theoretically 'pure' approach, and one of the limitations to the accuracy of the semi empirical methods in addition to the approximations inherent in their formulation is the accuracy of experimental data used to obtain the parameters. However, in large part because adjustable parameters are optimized to reproduce a number of important chemical properties, semi empirical methods have become widely popular.

The optimization of parameters is, in general, a difficult task for several reasons. First, accurate experimental data are often not available. Second, the simultaneous optimization of several parameters for a large number of molecules is very time-consuming. The parameters are interconnected in the sense that a significant variation in the value of one parameter in a nearly optimal parameter set must be accompanied by variations in several other parameters too. Successively optimizing each parameter is not feasible. Semi empirical methods were first developed for conjugated π-electron systems and we shall therefore begin our discussion with them and later describe more general methods [8].

3.4.4. MOLECULAR MECHANICS

For very large systems, as in biochemical applications, it is not computationally practicable to use solely quantum mechanical approaches to compute potential energies. For these applications, often a mixture of quantum mechanics and molecular mechanics is employed, the latter using potential functions from classical mechanics to compute the potential energy for a specified arrangement of atoms.

In molecular mechanics (MM), the electrons in the system under study are not considered explicitly but rather each atom (the atomic nucleus and the associated electrons of the atom) is treated as a single particle. Therefore, MM is not very useful for chemical problems that involve bond-breaking or bond forming since electronic effects are critical in such cases. Rather, MM is commonly used in large systems for predicting the potential energy of a particular molecular conformation (that is, arrangement of atoms). The absolute values of the potential energies are not particularly meaningful in such calculations; instead it is energy differences between conformations that are significant [9].

3.5. BASIS SET

A basis set is a set of functions used to create the molecular orbitals, which are expanded as a linear combination with coefficients to be determined [10].

Both the HF and DFT methods use set of mathematical functions to represent the atomic orbitals. These are called the basis set. The molecular orbitals are written as linear combination of atomic orbitals located on each nucleus in the molecule. Instead of using hydrogen like atomic orbitals better orbitals, for those with optimized values of orbital exponents (effective nuclear charge) ξ or either linear combinations were used.such orbitals constitute the basis set.

3.5.1 CLASSIFICATION OF BASIS SET

A. Minimal basis sets

Minimal basis sets contain the minimum number of basic functions that are needed for each atom. Minimal basis sets use fixed size atomic type orbitals. The STO-3G basis set

is a minimal basis set (through it is not the smallest possible basis set). It uses three Gaussian primitives per basis function ("3G"). "STO" stands for "Slater type orbitals", and the STO-3G basis set approximates Slater orbitals with Gaussian functions. STO has the form

$$Nr^{n-1}e^{-\xi r}Y_l^m(\theta,\phi)$$

Where $Y_l^m(\theta,\phi)$ is the spherical harmonics

B. Split valence basis sets:

One way to increase the size of a basis set is to take more basis functions per atom. Split valence basis sets, such as 3-21G and 6-31G basis sets, have two (or more) sizes of basis function for each valence orbital. The double zeta valence basis sets form molecular orbitals from the linear combinations of two sets of functions for each atomic valence orbital. Similarly, triple split valence basis sets such as 6-311G, use three sets of contracted functions for each valence orbital type.

C. Polarized basis sets

Split valence basis sets could be improved by adding orbitals with different shapes. Polarized basis sets add orbitals with angular momentums going beyond of requirement for the proper description of the ground state of each atom at the HF level.

D. Diffused functions

Basis sets with additional diffuse functions are large by size versions of s-and p-type split valence basis sets. Diffuse orbitals occupy a larger region of space. Basis sets with diffuse functions are important for systems where electrons may be far from the nucleus [11].

3.6 CHEMICAL PARAMETERS

3.6.1 CHEMICAL POTENTIAL

Chemical potential can be defined as the partial derivative of internal energy with respect to the amount of substance in the constant entropy and constant volume. It is an important thermodynamic property and have an important application in thermodynamics [12].

$$\mu_i = \left(\frac{\partial G}{\partial n_i}\right)_{T,P,n_i}$$

Where,

μ=chemical potential

ΔG=free energy

n_i =mole of components (i)

Chemical potential is also defined as:

$$\mu = (-I + A)/2$$

Where I is the ionization potential ($-E_{HOMO}$) and A is the electron affinity (E_{LUMO})

HOMO= highest occupied molecular orbital

LUMO=lowest unoccupied molecular orbital

3.6.2 CHEMICAL HARDNESS AND SOFTNESS

The chemical hardness measures the stability of the molecules indicator for the most stable structure of various isomers of a particular system. The chemical hardness is defined as the second derivative of the ground state with respect to the number of electrons (N).

$$\eta = \left[\frac{\partial^2 E}{\partial N^2}\right]_{V_r}$$

$$= \frac{1}{2}[E_{LUMO} - E_{HOMO}]$$

The chemical softness(S) of an atoms or molecule is the reciprocal of the respective chemical hardness (ɳ)

$$S = \frac{1}{2}\eta$$

3.7 COMPUTATIONAL DETAILS OF THE PRESENT STUDY:

- The structure of vinflunine is optimized by using DFT method with B3LYP level using 6-31G basis set.
- The spectroscopic values have been computed with IR and Raman spectrum.
- HOMO-LUMO energies have been calculated.
- Vinflunine was docked with BSA, tubulin and DNA using Glide/maestro of schrodinger software.

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RESULTS AND DISCUSSION

CHAPTER IV

RESULT AND DISCUSSION

4.1 INTRODUCTION

Vinflunine is a novel Vinca alkaloid selectively fluorinated by super acid chemistry in a rarely exploited region of the Velbanamine moiety. Vinca plants are known to exhibit potential antitumor properties. Vinflunine has shown promising and unique result in clinical trials such as to treat several types of cancers [1]. Vinca alkaloids are a subset of drugs obtained from the Madagascar periwinkle plant, Catharanthus roseus and have hypoglycemic as well as cytotoxic effects. Vinflunine(20',20'-difluoro-3',4'-dihydro Vinorelbine), a third semi-synthetic Vinca alkaloid is currently in investigational chemotherapy program and used for the treatment of second-line transitional cell carcinoma of the urothelium. In our present study, structure of the Vinflunine has been optimized and the structural characteristics have been determined by using density functional theory (B3LYP) method with 6-31/G as basis set using Jaguar software. The quantum geometrical parameters namely, energy, molecular vibrations, Chemical reactivity parameters like electron affinity (*A*), ionization potential (*I*), the absolute electronegativity (χ), absolute hardness (η), softness (σ), E_{HOMO} (highest occupied molecular orbital energy), E_{LUMO} (lowest unoccupied molecular orbital energy), the energy difference (ΔE) between E_{HOMO} and E_{LUMO}, were calculated by using same method.

Molecular docking is a fast and reliable tool for the study of inter-molecular interactions in the systems of biological and therapeutic significance. In the present investigation, Tubulin Alpha-Beta, Bovine Serum Albumin (BSA), Deoxyribo Nucleic Acid (DNA) have been used for docking studies with the Vinca alkaloids, namely Vinflunine by using maestro /Schrodinger.

4.2RESULT AND DISCUSSION

4.2.1MOLECULAR GEOMETRY

The structure of Vinflunine is optimized by using Jaguar Software. The optimized structure is shown in the figure 4.1.Energy of the Vinflunine is calculated from the optimized structure and the energy value is -2768.573640264708ev.

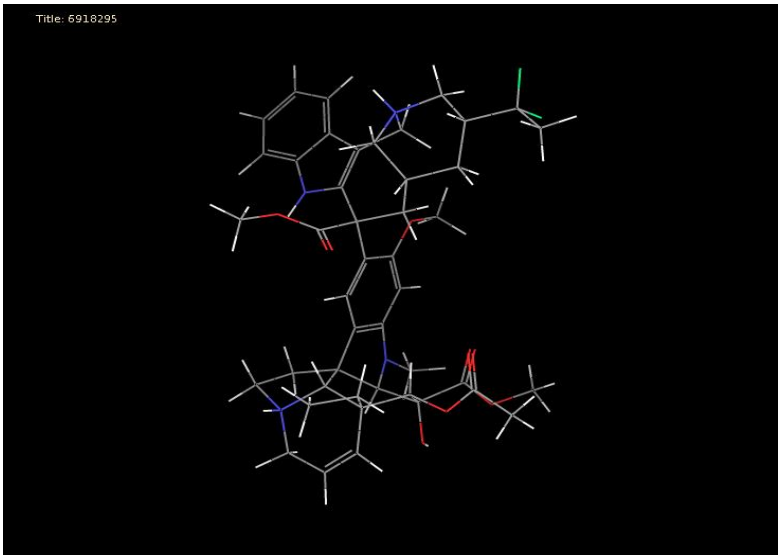


Figure: 4.1 Optimized Structure of Vinflunine With (B3LYP/6-31G)

4.3 ELECTRONIC PROPERTIES HOMO-LUMO ANALYSIS

The frontier molecular orbitals are very much useful for studying the electric and optical properties of organic molecules. The stabilization of the bonding molecular orbital and destabilization of the antibonding can increase, when the overlap of two orbitals increase when the overlap of two orbitals increase. In the molecular interaction, there are two important orbitals that interact with each other. One of the highest energy occupied molecular orbital called HOMO, represents the ability to donate an electron. The other one is the lowest energy unoccupied molecular orbital called LUMO, as an electron acceptor. These orbitals are sometimes called the frontier orbitals [2]. Eight important molecular orbitals are examined for Vinflunine at B3LYP/6-31G using Jaguar.

The total energy and energy gap affect the stability of a molecule. Surfaces for the frontier orbital were drawn to understand the bonding scheme of present compound and it is shown in Fig.4.2.The conjugated molecules are characterized by a highest occupied molecular orbital-lowest unoccupied molecular orbital (HOMO–LUMO) separation, which

is the result of a significant degree of intermolecular charge transfer (ICT) from the end capping electron-donor to the efficient electron acceptor group through p-conjugated path. The HOMO is the orbital that primarily acts as an electron donor and the LUMO is the orbital that largely acts as the electron acceptor, and the gap between HOMO and LUMO characterizes the molecular chemical stability. The energy gap between the HOMO and the LUMO molecular orbitals is a critical parameter in determining molecular electrical transport properties because it is a measure of electron conductivity. The computed energy values of these eight molecular orbitals are shown in the table 4.1. The energy gap value is 0.180957ev for Vinflunine molecule. The energy values of the frontier orbitals are presented in Table 4.1. The energy gap between HOMO and LUMO determines the kinetic stability, chemical reactivity and, optical polarizability and chemical hardness–softness of a molecule.

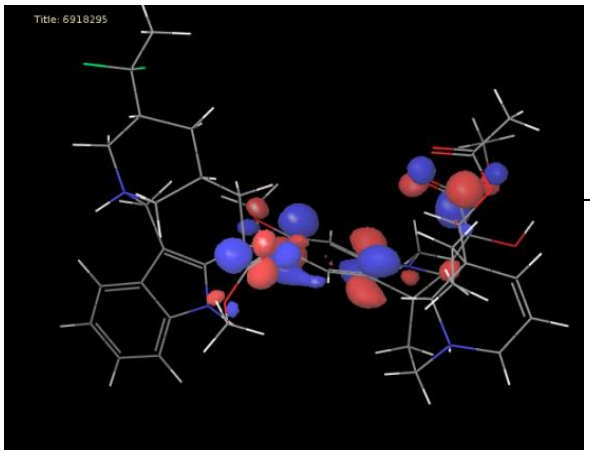
4.3.1 Ionization potential

By using HOMO and LUMO energy values of Vinflunine, the ionization potential (*I)* and chemical hardness (η) , Softness (σ) and Electronegativity (χ) of the molecule were calculated using Koopmans' theorem. The ionization potential of Vinfluine is 0.356290eV. An electron affinity are calculated for same molecule in is 0.175333eV. Considering the chemical hardness, large HOMO–LUMO gap means a hard molecule and small HOMO–LUMO gap means a soft molecule. One can also relate the stability of molecule to hardness, which means that the molecule with least HOMO–LUMO gap means it is more reactive.

TABLE 4.1: Calculated energy value of Vinflunine its ground state

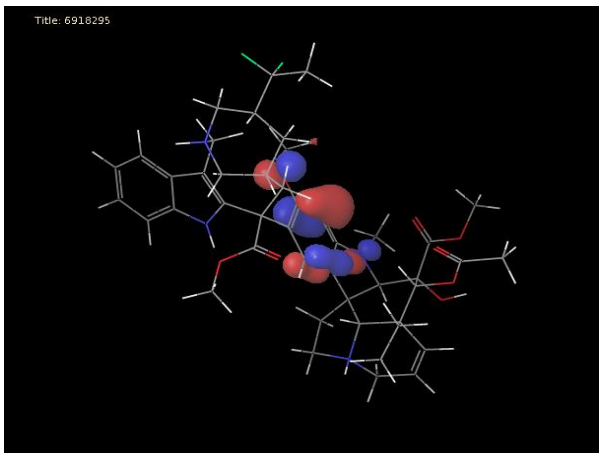
Chemical parameters of Vinflunine at DFT/B3LYP/6-31G	**Energy values**
E_{Total} (Hartree)	-2768.5736
E_{LUMO+1} (eV)	-0.173701
E_{LUMO} (eV)	-0.175333
E_{HOMO} (eV)	-0.356290
E_{HOMO-1} (eV)	-0.357906

$\Delta E_{HOMO-1–LUMO+1}$ (eV)	0.184205
$\Delta E_{HOMO-2–LUMO+2}$(eV)	0.198071
$\Delta E_{HOMO-3–LUMO+3}$(eV)	0.230804
$\Delta E_{HOMO–LUMO}$ (eV)	0.180957
electron affinity (E_A),	0.175333
ionization potential (*I*)	0.356290
Chemical hardness(η)	0.088678
Softness (σ)	0.044339
Electronegativity (χ)	0.090478
Chemical potential (μ)	0.088678
Electrophilicity index (ω)	0.044338

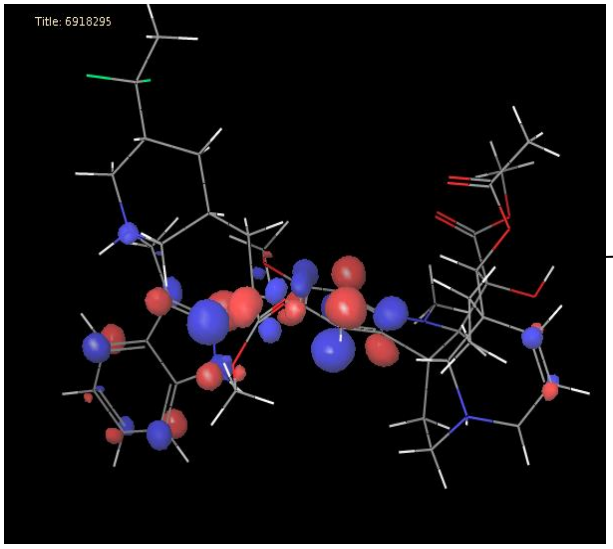


LUMO+3 = -0.161935ev

ΔE=0.230804ev

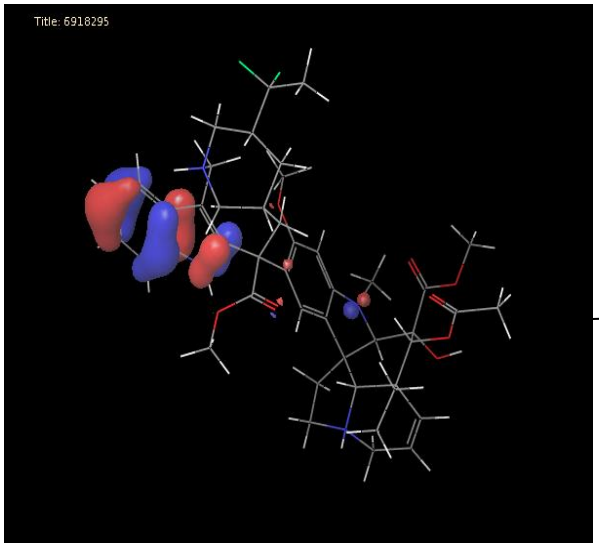


HOMO-3 = -0.392739ev

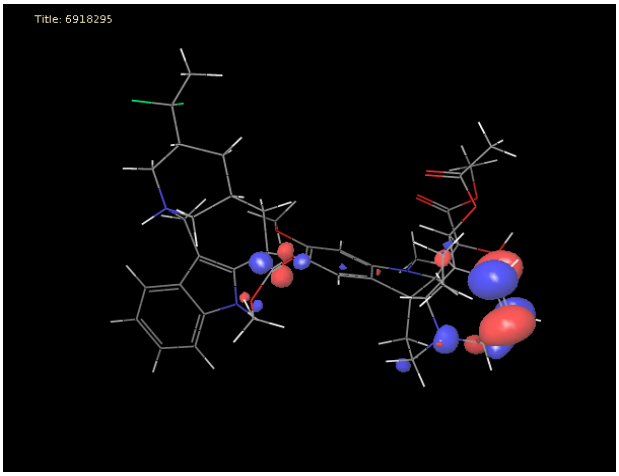


LUMO+2 = -0.171893ev

ΔE= 0.198071ev

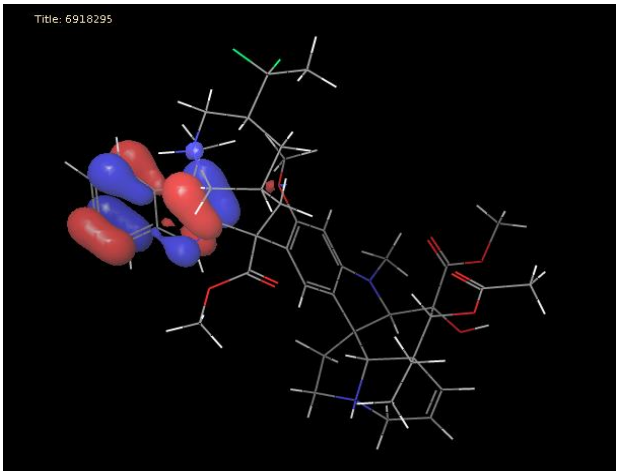


HOMO-2 = -0.369964ev

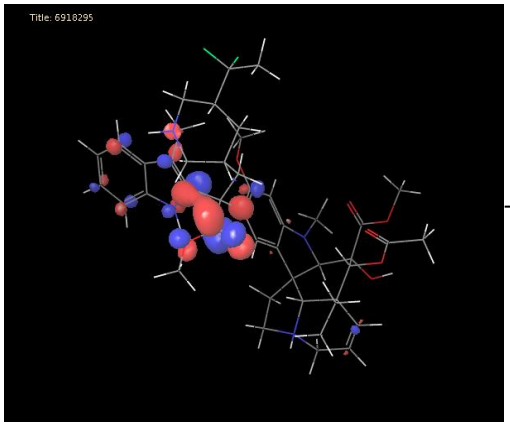


LUMO+1 = -0.173701ev

ΔE= 0.184205ev

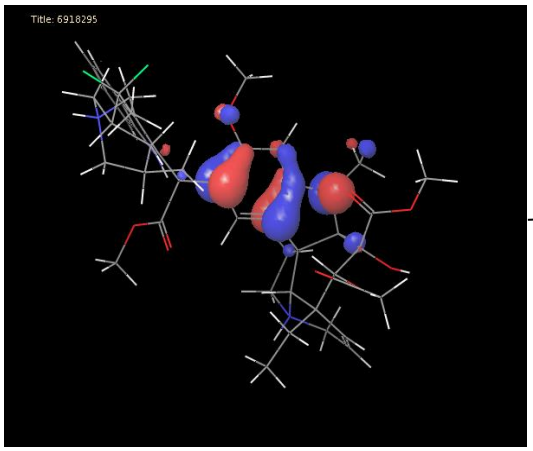


HOMO-1 = -0.357906ev



LUMO = -0.356290ev

ΔE=0.0886785ev



HOMO = -0.175333ev

Figure4.2: Frontier molecular orbitals of Vinflunine

4.4 VIBRATIONS

The vibration of modes is two types:

4.41. Stretching vibration

4.4.2. Bending vibration

4.4.1 STRETCHING VIBRATION

In this types of vibration, the atoms move essentially along the bond axis. So that the bond length increases or decreases at higher energy and occur at higher frequency. Stretching vibration is of two types.

SYMMETRIC VIBRATION

In this type of stretching with respect to a particular atom, other two atoms in a molecule move in the same.

ASYMMETRIC VIBRATION

In this type of stretching one atom moves away from the central atom, while the other atom moves towards the central atom.

4.4.2 BENDING VIBRATION

this vibrations may consists of a change in bond angle between bonds with a common atom the movement of a group of atoms with respect to the remainder of the molecule without movement of a group with respect to one another.

There are four types:

a) **Scissoring**: In Scissoring, the two atoms concerned to a atom move towards and away from each other with deformation of the valency angle (in plane bending).
b) **Rocking**: In rocking the, structural units swings back and forth in the plane of molecule (in plane bending).

c) **Wagging**: In wagging, the structural unit swings back and forth out the plane of molecule (out of plane bending).

4.5 SPECTROSCOPIC ANALYSIS

Spectroscopy is the study of interaction between matter and electromagnetic radiation. The aim of vibration analysis is to find the vibrational modes connected with specific molecular structures of calculated compounds.

An infrared spectrum of substance shows the frequencies of infrared radiations, absorbed by the substance. It is a plot of the radiation absorbed versus frequency of the radiation. The frequency absorbed radiation reveals which bond the molecule contains. Each dip in the spectra is called a bond or peak represents absorption of IR at that frequency by the sample.

Vinflunine is a nonlinear molecule and it consist of 115 atoms, therefore it have 339 vibrational modes. Stretching vibrations and bending vibrations are included in the vibrations of the molecule.

The IR and Raman intensities are calculated at B3LYP level using basis set 6-31G for Vinflunine and their spectra are shown in the fig 4.2 and 4.3. The strongest peak for IR vibrational spectrum is at 1229.88 cm^{-1} and for Raman spectrum is at 1666.7259 cm^{-1}. The second highest peak of IR spectrum is 1264.93 cm^{-1}, and for the Raman spectrum is 1225.7996 cm^{-1}. The TABLE 4.2 shows the Frequency mode of vibration of Vinflunine at DFT/B3LYP/6-31G Levels of theory.

TABLE: 4.2 Frequency mode of vibration of Vinflunine at DFT/B3LYP/6-31G Levels of theory

PARAMETERS	DFT/B3LYP/6-31G (cm^{-1})	ASSIGNMENTS
CH	3251.5460	Stretching
C-C-C	1106.6661	Bending
OH	1337.3052	Bending
	1388.7897	
	1426.5778	
NH	3459.1324	Stretching
C-O-H	1393.0029	Bending
	1399.0386	
	1438.9955	
NH	662.4576	Wagging
	758.4814	
	841.3845	
	887.1801	
CH_3	3008.8700	Stretching

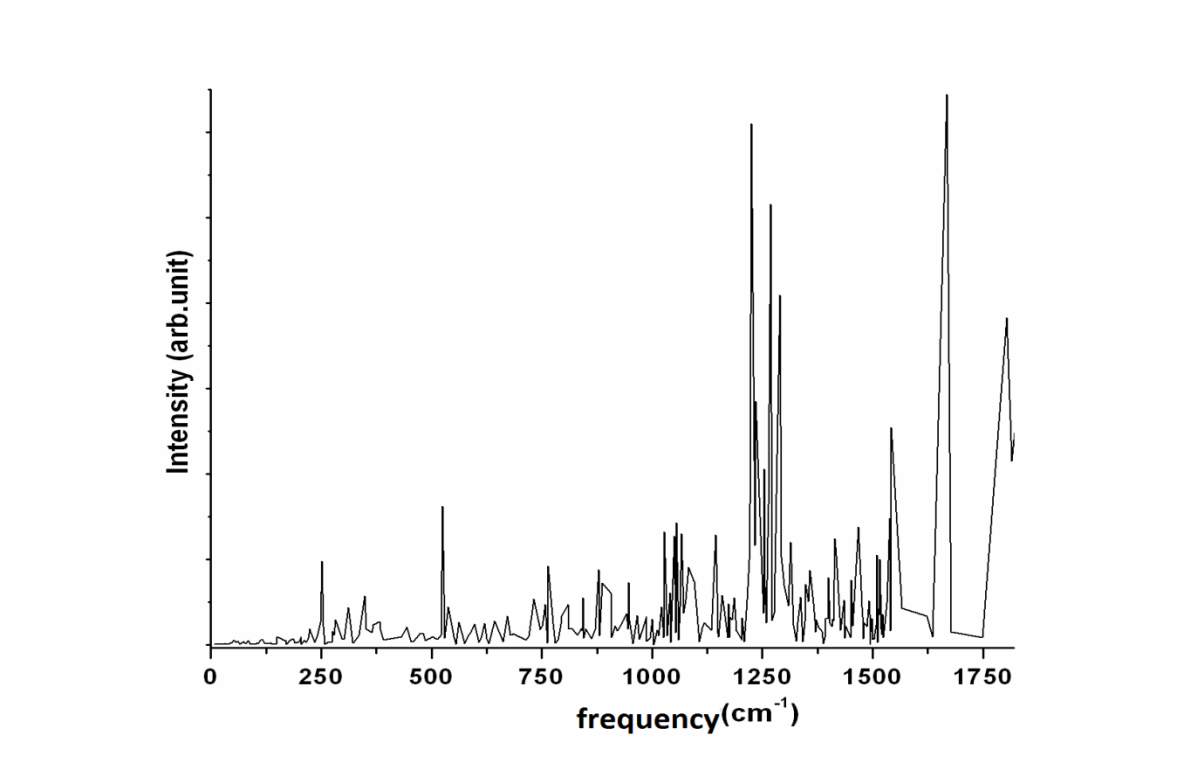


Figure 4.3: A graph between frequency (vs) Raman intensities of Vinflunine

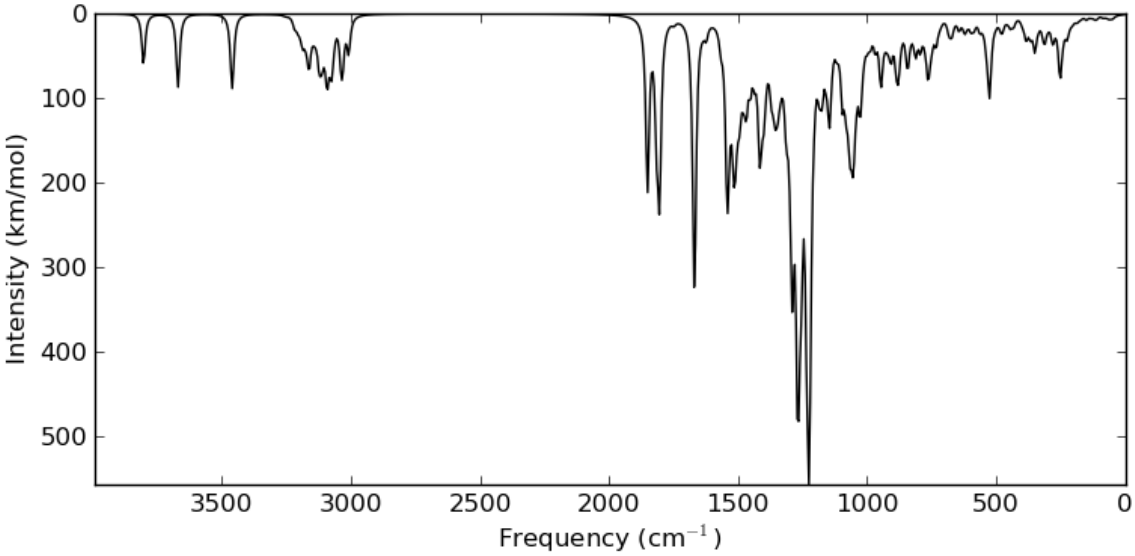


Figure 4.4: A graph between frequency (vs) IR intensities of Vinflunine

.4.6 DOCKING

Molecular docking is a fast and reliable tool for the study of inter-molecular interactions in the systems of biological and therapeutic significance. Although, the QM–MM approach, that combines the accuracy of quantum mechanics and speed of molecular mechanics, was introduced as early as 1970s by Warshel and Levitt to obtain more realistic information about biomolecular interactions, most of the early stages of drug discovery process still involve the use of the molecular mechanics based docking programs to obtain dependable prediction toward the efficacy of the pharmaceutically important molecules. The nature of binding with biomolecules, especially with proteins, nucleic acids, etc. is essential in order to understand the mechanism of their interactions and to identify similar possible ligands of potential medicinal importance. In our present study, we have docked the Vinflunine molecule with Bovine Serum Albumin (BSA), Tubulin Alpha-Beta and Deoxyribo Nucleic Acid (DNA) using Schrodinger maestro software and their binding characteristics computed with each other.

4.6.1 DOCKING OF VINFLUNINE WITH BOVINE SERUM ALBUMIN:

The amino acid sequence of Bovine Serum Albumin had been taken from NCBI database. PDB file of BSA is taken from PDB ID: 3VO3. Vinflunine(VFL) was docked with BSA protein molecule using the Glide in Maestro of Schrodinger software. Site Map, Schrödinger's program was used for identifying, evaluating, and visualizing ligand binding sites. A receptor grid was generated around the active site. The ligand-midpoint box was given a side of 10A˚. The site score is 1.133. The further exploration was carried out with smaller grid map of 10x10x10 points centered at 18.64, 18.38 and 42.49. Ligands were subjected to automatic preparation process, performed with Lig Prep tool of the Schrodinger package. It generates all possible protonation and tautomeric states available within a pH range of 7.0±2.0. The prepared and optimized ligands were flexibly docked in the grid box of the protein. Glide Score (G Score) was used to rank the ligands on the basis of their relative binding affinities. FIGURE 4.5 shows the Ligand interaction diagram of Vinflunine with BSA.

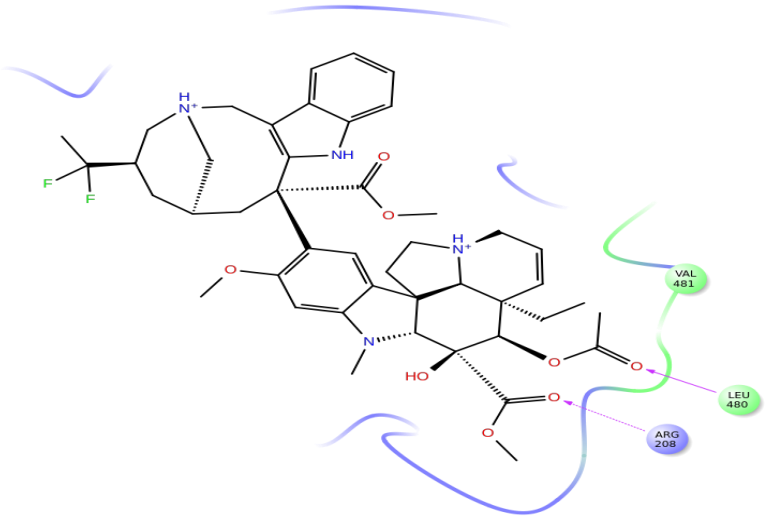


FIGURE 4.5: Ligand interaction diagram of Vinflunine with BSA

The modeled and refined protein was docked against Vinflunine. Table: 4.3 shows the Docking score, glide score, glide energy, glide ligand efficiency, grid box values, site score and potential energy values of binding site of BSA with Vinflunine. Higher the negative value of the docking score, better the binding affinity of the ligand and receptor. The minimum energy for binding and higher number of hydrogen bonds also indicates good binding affinity of ligands towards the receptor. The complex indicates that amino acids Arg208, Val481 and Leu480 are involved in hydrogen bonding. Figure 4.6 shows the binding of Vinflunine near the cofactor making contact with amino acid residues. The C=O molecule of the carbonyl group of Vinflunine was hydrogen bonded to NH-molecule of the Arg206 at a distance of 1.96 Å. Another hydrogen bonding was seen between the NH_2- molecule of Leu480 and the carbonyl group C=O of Vinflunine at a distance of 1.56Å.

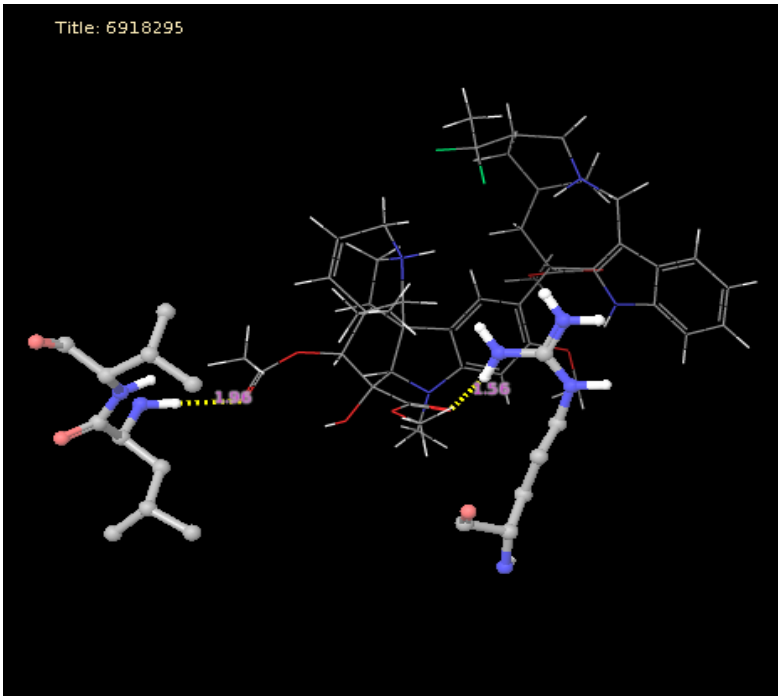


FIGURE 4.6 : Hydrogen interaction diagram of Vinflunine with BSA

4.6.2 DOCKING OF VINFLUNINE WITH TUBULIN α - β DIMER

Protein is primarily present in microtubules two different forms: α-tubulin and β-tubulin these forms almost always exist in a stable non covalent α-β heterodimer [4]. α - β tubulin structure is taken from PDB ID: 1TUB. Vinflunine was docked with α - β tubulin protein molecule using the Glide in Maestro of Schrodinger software. Schrödinger's program was used for identifying, evaluating, and visualizing ligand binding sites. Visualization of the Vinflunine–tubulin complex in the form of an interaction diagram (Fig.7) clearly shows that Vinflunine comfortably rests in the binding pocket on tubulin without much strain.

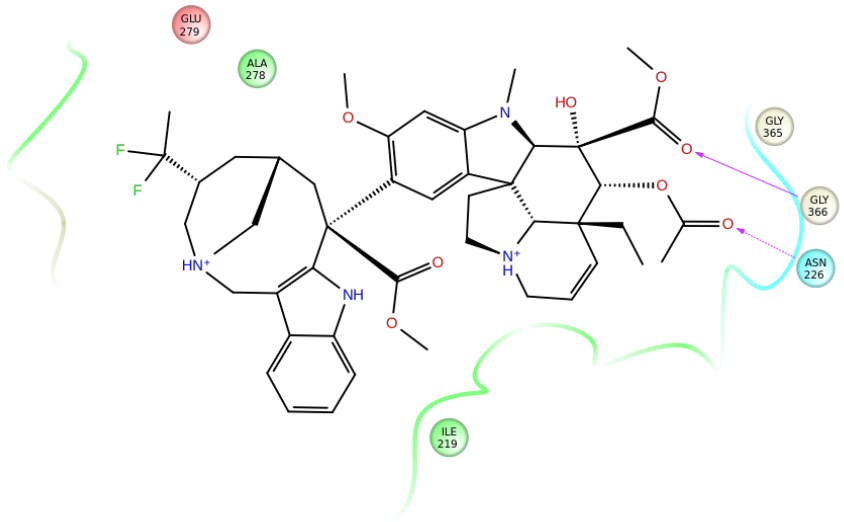


FIGURE 4.7: Ligand interaction diagram of Vinflunine with Tubulin

The site score is found to be 1.062. Table: 4.3 shows the Docking score, glide score, glide energy, glide ligand efficiency, grid box values, site score and potential energy values of binding site of Tubulin with Vinflunine. FIGURE 4.7 shows the Ligand interaction diagram of Vinflunine with tubulin. Tubulin has high binding affinity for vinca alkaloid. The minimum energy for binding and higher number of hydrogen bonds also indicates good binding affinity of ligands towards the receptor. The complex indicates that amino acids Glu279, Ala278, Ile219, Gly365, Gly366, and Asn226 are involved in hydrogen bonding. Figure: 4.8 shows the complex structure which indicates the amino acids involved in hydrogen bonding. Figure 4.8 shows the binding of Vinflunine near the cofactor making contact with amino acid residues. The C=O molecule of the carbonyl group of Vinflunine was hydrogen bonded to NH_2- molecule of the Gly366 at a distance of 2.47Å. Another hydrogen bonding was seen between the NH_2- molecule of Asn226 and the C=O molecule of Vinflunine at a distance of 2.02 Å.

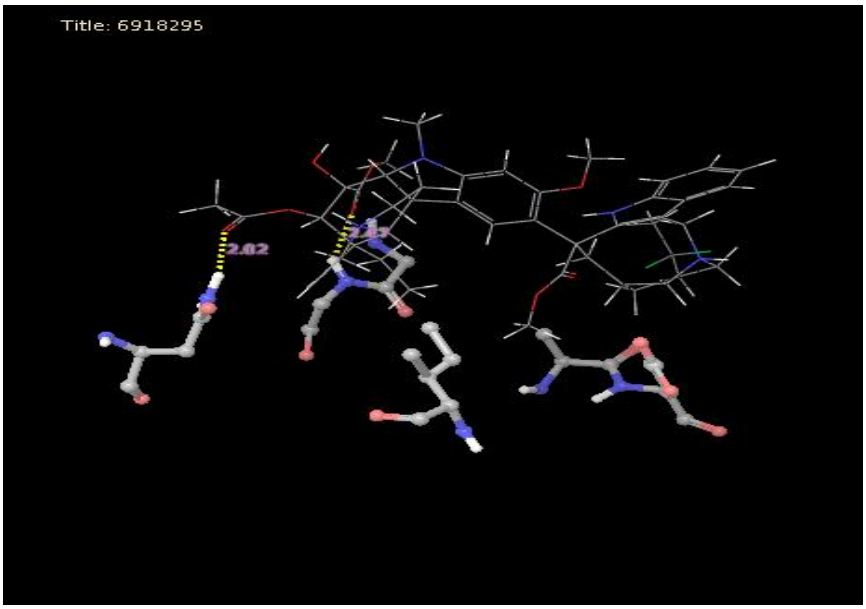


FIGURE 4.8: Hydrogen interaction diagram of Vinflunine with tubulin.

4.6.3 DOCKING OF VINFLUNINE WITH DNA

The basic unit of the DNA molecule is the nucleotide. Nucleotides are found in the cell either as components of nucleic acids or as individual molecules. Vinca alkaloids are different types of known to interact with nucleic acids. Vinca alkaloids, obtained from Catharanthus roseus, are an important class of alkaloids that possess extensive therapeutic potential. The Vinca alkaloids possess features like several H-bond acceptor/donor atoms, planar ring systems, and a large aromatic skeleton that are essential for the DNA binding activity. Vinca alkaloids exhibit fluorescence, which is quenched as a result of drug binding to DNA oligomers. This prompted us to investigate the binding of a third semi-synthetic Vinca alkaloid namely Vinflunine with double helical DNA oligomers. The DNA structure is taken from NCBI data PDB ID: 1BNA and docked with Vinflunine. The site score is 0.959. Figure 4.9 shows the Ligand interaction diagram of Vinflunine with DNA [5]. The Docking score, glide score, glide energy, glide ligand efficiency, grid box values, site score and potential energy were calculated and are shown in the table 4.3. DNA is nucleic acid hence there is no hydrogen bond between DNA and Vinflunine.

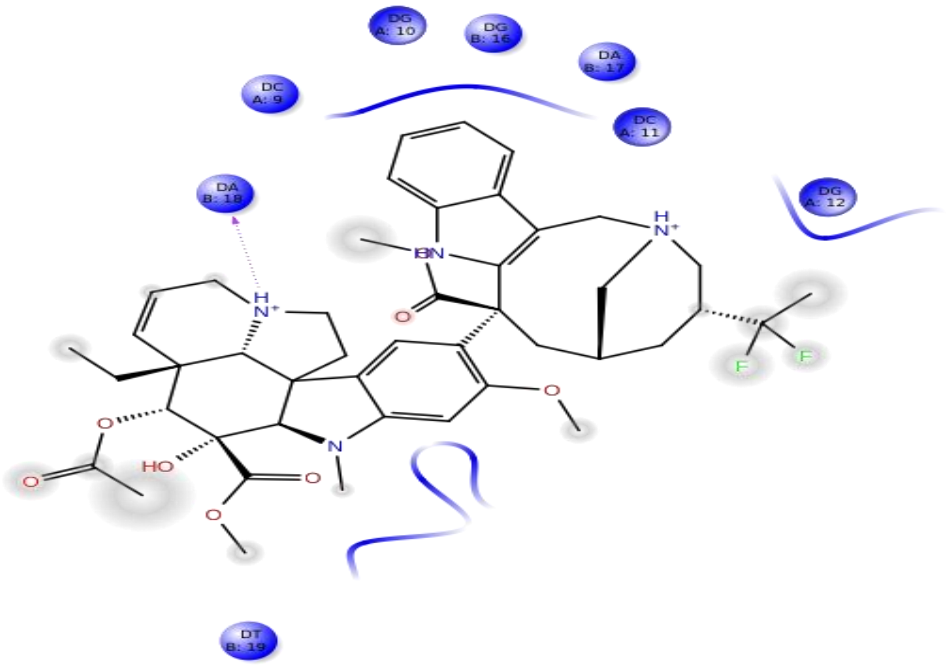


Figure 4.9: Ligand interaction diagram of Vinflunine with DNA

TABLE: 4.3 The Docking score, glide score, binding energy, glide ligand efficiency, grid box values, site score and potential energy of Vinflunine with BSA, Tubulin and DNA

S.N	PARAMETERS	VINFLUNINE WITH BSA	VINFLUNINE WITH TUBULIN	VINFLUNINE WITH DNA
1.	Docking score	-4.777	-2.590	-5.578
2.	Glide score	-5.627	-2.840	-5.582
3.	Glide ligand efficiency	-0.081	-0.044	-0.095
4.	Binding energy	-37.455 Kcal/mol	-28.867 Kcal/mol	-46.282 Kcal/mol
5.	Grid box X cent	18.64	-42.82	13.3
6.	Grid box Y cent	18.38	27.71	22.8
7.	Grid box Z cent	42.79	-29.9	4.03
8.	Potential energy	577.211 kJ/mole	501.458 kj/mole	495.650 kj/mole
9.	Site score	1.133	1.062	0.959

Docking results shows that ligand Vinflunine had good binding affinity in Bovine Serum Albumin, α - β Tubulin and DNA. Higher the negative value of the docking score, better the binding affinity of the ligand and receptor. From the above results, it shows that Vinflunine with DNA had the highest negative score of -5.578 and had good binding affinity with DNA compared to two proteins namely BSA and tubulin. The minimum energy for binding and higher hydrogen bonds also indicates good binding affinity of ligands towards the receptor. Though, DNA is a nucleotide and it does not show hydrogen bond when docked with Vinflunine. But, the docked site of α - β Tubulin with Vinflunine(VFL) shows high hydrogen bonds compared to other receptor BSA. Thus, α - β Tubulin also had good binding affinity. Vinflunine was found to be the best compound interacting with the tubulin protein and inhibiting microtubule formation and can be considered for the treatment of cancer. By analyzing the binding mode of three receptors

with Vinflunine (VFL), DNA had minimum binding energy and shows good binding affinity with the ligand. The trend of binding free energy values of VFL–tubulin, VFL–BSA and VFL–DNA complexes, further suggest that the increasing solvent exposure of VFL, directly affects the hydrophobic contacts between bound pose of VFL and the receptor molecules and consequently reduces the overall binding affinity.

4.7 REFERENCES:

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SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

The minimum energy structure of Vinflunine is obtained by using DFT method with B3LYP/6-31G using Jaguar software. The quantum geometrical parameters namely energy, molecular vibrations, Chemical reactivity parameters like electron affinity (*A*), ionization potential (*I*), the absolute electronegativity (χ), absolute hardness (η), softness (σ), E_{HOMO} (highest occupied molecular orbital energy), E_{LUMO} (lowest unoccupied molecular orbital energy), the energy difference (ΔE) between E_{HOMO} and E_{LUMO}, were calculated by using same method. The vibrational assignments for frequencies of Vinflunine were calculated. The IR and Raman intensities are also calculated for Vinflunine for the same basis set and their spectra have been ploted. The strongest peak for IR vibrational spectrum is observed at 1229.88 cm^{-1} and for Raman spectrum is at 1666.7259 cm^{-1}. Also Vinflunine is docked with Bovine Serum Albumin (BSA), Tubulin Alpha-Beta and Deoxyribo Nucleic Acid (DNA) using Schrodinger maestro software and their binding characteristics computed with each other. The active site resides, binding modes and other parameter are analyzed from the docking results of BSA, Tubulin Alpha-Beta and DNA with VFL. The result shows that Vinflunine had good binding affinity in Bovine Serum Albumin, α - β Tubulin and DNA. It is found that Vinflunine with DNA had the highest negative glide score (-5.578) and had good binding affinity compared to two proteins. The hydrogen bond interactions of vinflunine with BSA and tubulin were also studied.