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Conformational, Toxic, Physicochemical and Molecular Docking Analysis of the Anticancer Acalabrutinib Molecule

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Abstract - Acalabrutinib is an inhibitor of Bruton's tyrosine kinase (BTK) activity and prevents the activation of the B-cell antigen receptor (BCR) signaling pathway. For having these properties acalabrutinib recently was approved for medical use as an anticancer drug. Determining the conformational properties of a bioactive molecule is necessary to reveal its bioactivity. For this reason, the conformational states of the acalabrutinib were examined first. The AM1, a semi-experimental method, was used to examine the stable conformations of the acalabrutinib molecule. Nine lowest energy conformers of the acalabrutinib molecule were determined and their relative energies were calculated. Afterwards, the interactions of the most stable conformer of acalabrutinib with DNA and integrin were examined by docking simulations, and the most active interaction sites and binding affinities were determined.

Keywords: Acalabrutinib, Conformational analysis, Anticancer, Molecular Docking Analysis, Physicochemical Features

1. Introduction

The chemical formula of acalabrutinib (ACP-196) is $C_{26}H_{23}N_7O_2$ and its total weight is 465.5 g / mol. Acalabrutinib is a small molecule and Bruton tyrosine kinase (BTK) inhibitor. BTK is a component of B cell receptor and myeloid cell signaling pathways, and plays multiple roles in the production of autoantibodies. [1]. Therefore, proper control of BTK activity is important for B cell homeostasis. The BTK signal promotes malignant B cell growth and proliferation, as well as the development of malignant cells [2]. BTK inhibitors are of interest for the treatment of cancer. Acalabrutinib binds to BTK in an irreversible manner, blocking the enzyme's cancer-causing activity. In the United States, acalabrutinib was licensed in 2017 as a treatment for refractory mantle cell lymphoma, and in 2019 as a therapy for CLL and small lymphocytic lymphoma. Waldenstrom macroglobulinemia is under evaluation in other malignancies such as pancreatic and non-small cell lung cancer [3].

Although there is currently no definitive treatment for chronic lymphocytic leukemia (CLL), various studies are being conducted to find solutions to long-term response times in chemotherapy treatment and to provide clinical benefit to patients with increasing treatment options. [4-6] Bruton

Thinozine Ibrutinib, the first inhibitor of the kinase (BTK) class, has been used clinically in the treatment of Chronic Lymphocytic Leukemia (CLL), mantle cell lymphoma, and Waldenstrom macroglobulinemia, but since the ibrutinib molecule has various adverse effects on targets other than BTK, researchers are investigating more selective BTK inhibitors [7,8]. Acalabrutinib (ACP-196) is one of the oral inhibitors of Bruton's Tyrosine Kinase (BTK) used in the treatment of B cell malignancies including resistant mantle cell Lymphoma and Chronic Lymphocytic Leukemia.

Chronic Lymphocytic Leukemia (CLL) is the most common type of chronic leukemia that occurs in the elderly people, especially in Europe and America. In this type of leukemia, which progresses rapidly and fatally, the traditional chemotherapy method does not provide a definite improvement [9]. Therefore, researchers, who want to increase the healing process and rate, are working on a number of treatment methods, aimed at the development, proliferation and survival of B cells, which play an important role in the pathogenesis of Chronic Lymphocytic Leukemia (CLL) treatment. We can divide the treatment methods that emerged as a result of the research, into two main groups; the first of these methods is the B-cell receptor (BCR) pathway activated by the stimulation of the micro-environment, the second is the treatment methods targeting the nuclear factor kappa-B (NF-κB) pathway and the cell surface receptor [10]. Ibrutinib, a sub-class of these treatments, is the first orally administered, non-reversible, and selective Bruton Thinozine Kinase inhibitor. It inhibits B cell viability, proliferation and growth and prevents the influence of the tumor microenvironment. The most common side effects during use of ibrutinib are bleeding, rash, and atrial fibrillation [6, 11-14]. After observing these side effects, scientists carried out studies on more selective Bruton Tyrosine Kinase (BTK) inhibitors and focused on Acalabrutinib (ACP-196), one of the more selective second generation Bruton Tyrosine Kinase (BTK) inhibitors.

Acalabrutinib is rationally designed to be more selective and potent than Ibrutinib, with the most important differences showing improved pharmacological properties, including favorable plasma exposure, rapid oral absorption, short half-life, and the absence of irreversible targeting to alternative kinases [9]. Due to these properties, it is named and accepted as a second generation irreversible Bruton Thinozine Kinase (BTK) inhibitor. In theoretical studies on Acalabrutinib, comparation to Ibrutinib was made and this Bruton Thinosine Kinase (BTK) inhibitor showed much less adverse effects on other targets. Acalabrutinib, known as ACP-196, is an FDA-approved therapy and received the title of Orphan Drug to promote treatment of rare diseases. Today, clinical studies are conducted on more than 2500 patients in 40 countries in order to increase the usage areas of Acalabrutinib and to better understand its effects [15]. The reason why a lot of emphasis on side effects are considered are that patients discontinue the treatment due to the adverse effects observed during Ibrutinib treatment. The most common reason for not continuing treatment for chronic lymphocytic leukemia is the emerging side effects [16, 17]. It was predicted that acalabrutinib (ACP-196) could be used twice a day without increasing toxic effects due to its low side effects. Based on this prediction, Phase 1 and Phase 2 studies of Acalabrutinib were conducted at Ohio State University, and as a result of the studies, low rates of side effects such as headache, diarrhea, weight gain, pyrexia, and upper respiratory tract infection were observed in patients. Although the findings of Phase 3 studies on acalabrutinib (ACP-196), a second-generation inhibitor of the Bruton Tyrosine Kinase (BTK) family, are yet unknown, it appears to be promising in the treatment of Chronic Lymphocytic Leukemia (CLL) [18].

In order to reveal the structure-function relationships of the Acalabrutinib molecule due to all these described properties, we performed the conformation analysis in this study and determined the possible conformers and the lowest energy conformation. Molecular docking simulations was performed to determine the interaction of the most stable conformer of Acalabrutinib with DNA and integrin as well as binding affinities and the binding sites. The toxicological and physicochemical properties of Acalabrutinib were also investigated.

2. Materials and Methods

The conformational analysis of the Acalabrutinib molecule was conducted with the help of the Spartan06 software [19] and the AM1 semiempirical quantum mechanical method [20]. The CAVER software [21] was used to predict potential binding sites on the surface of the receptors. Molecular docking investigations were done using AutoDock-Vina software on the identified active sites [22]. A semi-flexible docking protocol, where the ligand (Acalabrutinib) is flexible and target DNA or target protein is rigid, was applied.

The predicted values of the toxicity risks of Acalabrutinib and certain essential physicochemical features are determined using the OSIRIS Property Explorer software [23], which offers the total drug score to estimate the risks.

3. Results and Discussions

3.1. Structure

The conformational analysis of Acalabrutinib revealed nine lowest energy conformers. The relative energies of these nine conformers are tabulated in Table 1. In Figure 1, the molecular modes of these nine most stable conformers of Acalabrutinib are shown.

Table 1. The relative energies of the nine most stable conformation obtained by conformational analysis.

Conformers	Relative energy (kj/mol)
(I)	0
(II)	0.01
(III)	0.01
(IV)	1.03
(V)	1.04
(VI)	2.8
(VII)	4.77
(VIII)	4.79
(IX)	4.93

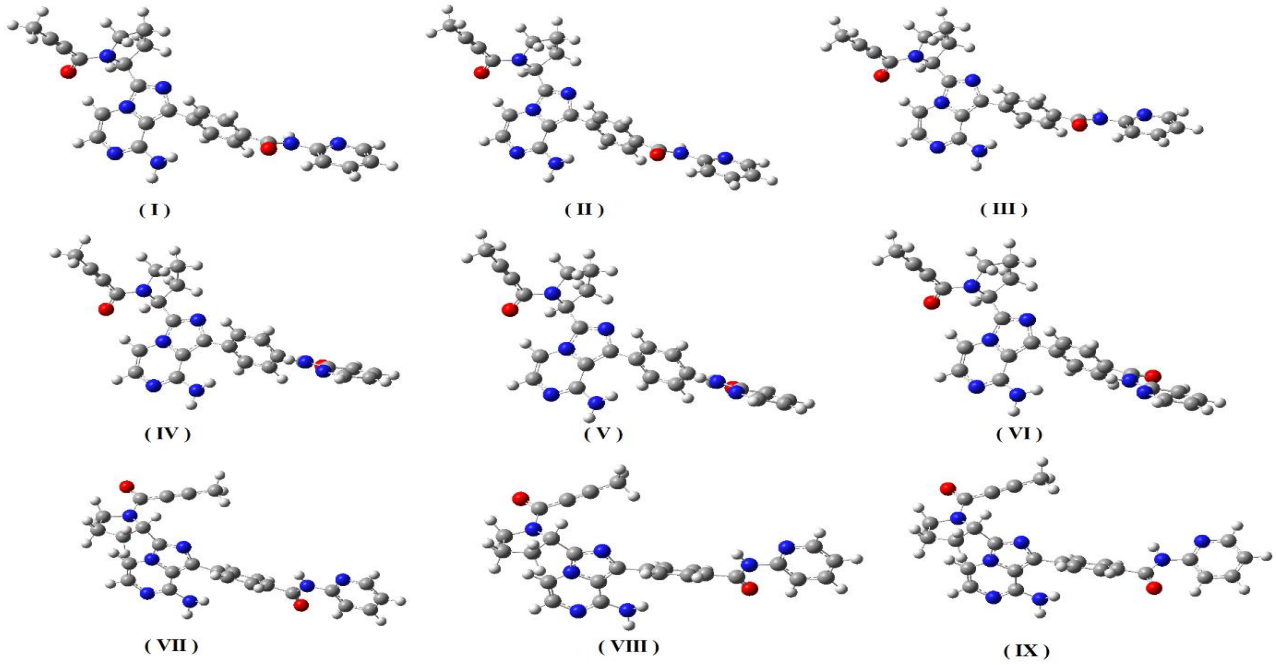

Figure 1. The nine conformers with the lowest energy, obtained by conformational analysis of the Acalabrutinib molecule.

3.2. Molecular Docking

To reveal the interaction mechanism and interaction modes of the anticancer drug Acalabrutinib with DNA and and $\alpha_5\beta_1$ integrin, molecular docking simulations were performed.

The crystal structure of DNA (PDB ID: 1BNA) was acquired with reference to the protein database [24] and the docking studies of the Acalabrutinib molecule was carried out using AutoDockVina [22]. DNA was prepared for the docking study by removing water molecules and adding polar hydrogens, and the DNA charges of Kollman were calculated before the docking study. The Geistenger technique was used to identify the partial charges of the Acalabrutinib molecule, and the active region of DNA was designated as 40Å × 40Å × 40Å grid.

The 3D molecular structure of Acalabrutinib molecule docked in DNA is shown in Figure 2. The most stable conformer of the Acalabrutinib molecule, obtained in gas phase calculations was found to form hydrogen bonds with the nucleic acids DG10 and DG16 (See Figure2) of DNA. The binding affinity (ΔG_{bind}) of Acalabrutinib to DNA is found to be -8.7 kcal/mol, as a result of the calculations. The following are the interactions between the compound Acalabrutinib and nucleic acids:

DG10 and Acalabrutinib molecule: hydrogen bond interactions with lengths of 2.11 and 2.14 Å;

DG16 and Acalabrutinib molecule: hydrogen bond interactions with lengths of 2.06 and 2.47 Å.

In the molecular docking study on cyclo(Ala-His)-DNA by Celik et al., it was found that the peptide interacted with nucleic acids DC9, DG10, DC11, DG16 and DA17 of DNA by hydrogen bonding interactions with 3.1, 2.39, 2.96, 2.53, 2.39 and 2.99 Å lengths, respectively [25]. In another study on molecular docking between 5-chlorouracil (5-FU) and DNA, 5-FU was found to interact with DG10, DC15 and DG16 nucleic acids, through hydrogen bond interactions [26]. Our results are compatible with the previous findings [25-27].

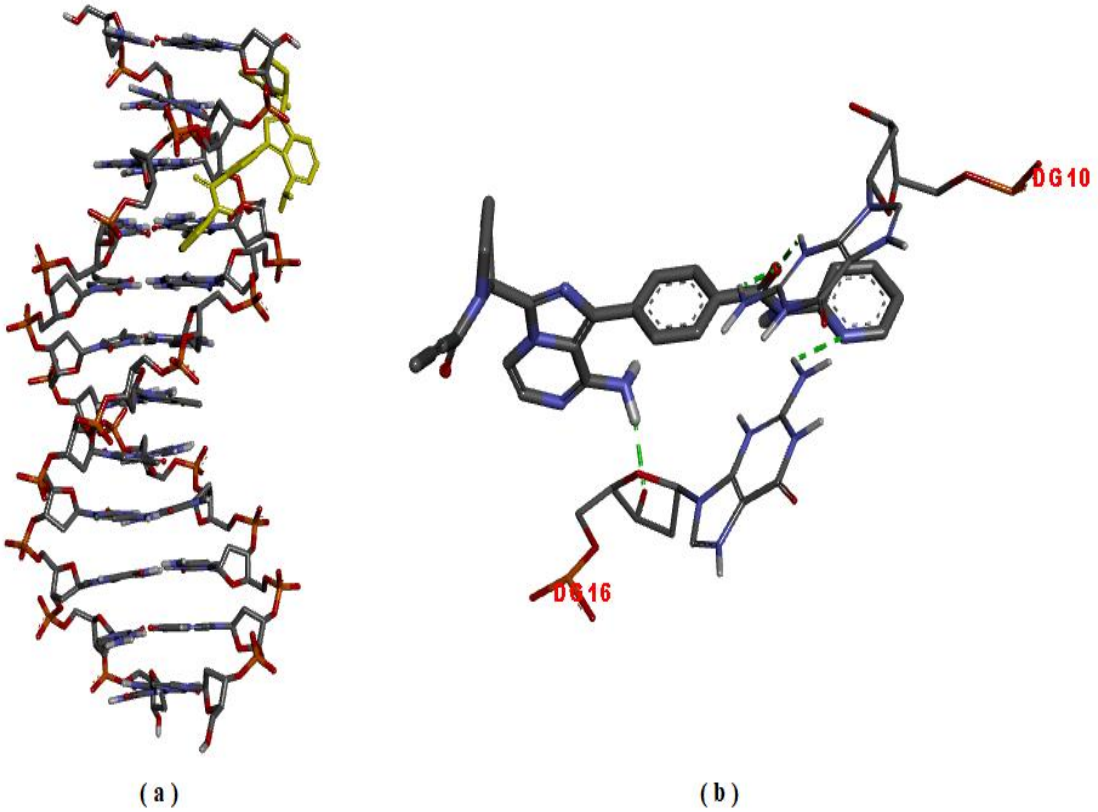

Figure 2. Acalabrutinib docked in DNA (a), The interactions between the ligand and target DNA are labeled using colored dashed lines (b) (ΔG_{bind} = -8.7 kcal/mol).

Molecular docking analyses in the target protein $\alpha_5\beta_1$ integrin were conducted to explore the anti-proliferative impact of Acalabrutinib for anticancer function.

The docking simulations of Acalabrutinib to the $\alpha_5\beta_1$ integrin (PDB ID: 4WK0) were done for the most active site after the $\alpha_5\beta_1$ integrin (PDB ID: 4WK0) was prepared for molecular docking [28]. The most efficient binding was discovered in the active site of the $\alpha_5\beta_1$ integrin, with a binding affinity of -10.7 kcal/mol. Figure 3 depicts the 3D view of Acalabrutinib docked in $\alpha_5\beta_1$ integrin and the amino acid residues involved in the interactions with the ligands are shown.

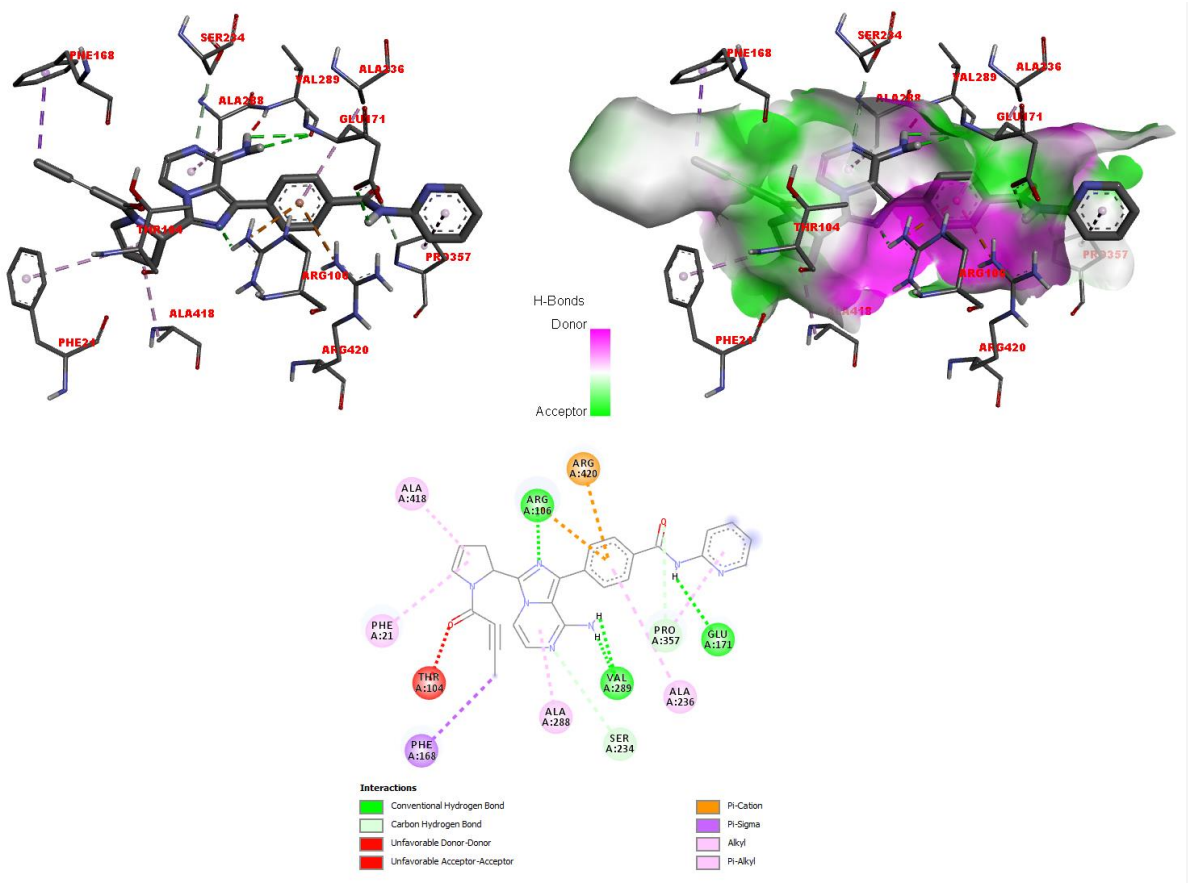

Figure 3. The 3D docked view of the most stable conformer of Acalabrutinib in active site of $\alpha_5\beta_1$ integrin (-10.7 kcal/mol). The interacted amino acid residues with the ligand are shown.

The interactions between Acalabrutinib and residues marked in Figure 3 are given below:

5.2Å long Pi-Alkyl interaction with **Phe21;** 2.87 Å long unfavorable acceptor-acceptor interaction with Thr104; 2.09 Å long hydrogen bond and 4.07 Å long Pi-cation interaction with **Arg106;** 3.83 Å long Pi-Sigma interaction with **Phe168**; 2.45 Å long hydrogen bond with **Glu171**; 3.62 Å long carbon hydrogen bond with **Ser234**; 5.32 Å long Pi-Alkyl interaction with Ala236; 4.51 Å long Pi-Alkyl interaction with Ala288; 1.46 Å long unfavorable donor-donor interaction with Val289 and 2.97, 2.99 Å long hydrogen bonds; 3.44 Å long carbon hydrogen bond and 4.81 Å long Pi-Alkyl interaction with Pro357; Interaction of Ala418 with 4.56 Å long Alkyl; Pi-cation interaction with Arg420 at a length of 4.1 Å.

In the previous study on the interaction of cationic pentapeptide Glu-Gln-Arg-Pro-Arg with $\alpha_5\beta_1$ integrin it was reported that pentapeptide interacted with the Phe21, Ser22, Val23, Arg106, Phe168, Glu171, Ser234, Val235, Lys269, Tyr287, Leu355, Ser417, Arg420 amino acids of integrin by salt bridge, attractive charge, hydrogen bond, carbon hydrogen bond, unfavorable positive-positive,

unfavorable donor-donor interactions [29]. The common amino acids indicate that Acalabrutinib molecule docked into the same active site of integrin where cationic pentapeptide docked.

The Molecular Mechanics Poisson-Boltzmann Surface Area (MM/PBSA) and the molecular mechanics generalized Born surface area (MM/GBSA) approaches are generally used to estimate the binding free energy of small ligands to biological macromolecules [30-35]. Due to the importance of both MM/PBSA and MM/GBSA approaches, Wang [30] developed a program which combined both methods as MM/PB(GB)SA approach. In this study the binding free energies of Acalabrutinib with DNA and $\alpha_5\beta_1$ integrin, were calculated using the program developed by Wang [30], based on MM/PB(GB)SA approach.

The predicted binding free energy of Acalabrutinib with DNA and with $\alpha_5\beta_1$ integrin were obtained as -12.73 and -11.46 kcal/mol, respectively by using the MM/PB(GB)SA approaches with the GAFF2 and ff14SB force field combination and the GB6 procedure [30].

3.3. Analysis of toxicological and physicochemical properties of Acalabrutinib

Acalabrutinib's drug-likeness properties were computed using OSIRIS (2010), and the findings are given in Table 2. The toxicity risk predictor indicates that there are no hazards of mutagenicity, tumorigenicity, irritant, or reproductive problems with this compound.

CLogP is a critical parameter in drug development and environmental toxicity studies, and it must not exceed 5.0 [23]. The absorption and distribution characteristics of a drug in aqueous solution are influenced by its solubility (logS) qualities. The logS-based OSIRIS method is used to evaluate a compound's solubility. More than -4 [23] is the recommended value. The molecular features of the promoted drugs are used to characterize drug-likeness. The total of the molecular score values of the fragments present determines the drug-likeness.

Table 2. Osiris's estimation of title compounds toxicity hazards and physicochemical properties.

Compound	Toxicity risks			
	Mutagenic	Tumorigenic	Irritant	Reproductive Effect
Acalabrutinib	no indication	no indication	no indication	no indication
	Physicochemical properties			
	cLogP	Solubility	Druglikeness	Drug-score
	2.68	-7.19	-2.38	0.23

4. Conclusions

In this work, conformational analysis of Acalabrutinib, an inhibitor of Bruton's tyrosine kinase (BTK) activity and an approved for medical use as an anticancer drug, was performed by a semi-experimental AM1 conformational study, to determine its most stable conformer. Since the protein-ligand interactions are significant in drug design, docking simulations were used to evaluate the biological activity of the most stable conformer of Acalabrutinib. As a result of molecular docking estimates, the binding affinities of Acalabrutinib to DNA and $\alpha_5\beta_1$ integrin were obtained as -8.7 and -10.7 kcal/mol, respectively. The presence of inhibitory activity of the Acalabrutinib ligand indicates that this ligand has good anti-tumor properties. In addition, the physicochemical properties of Acalabrutinib also show that the molecule has good pharmacokinetic profiles.

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