



Withaferin A (WIT) Interaction with beta-Tubulin to Promote Tubulin Degradation: In Silico Study

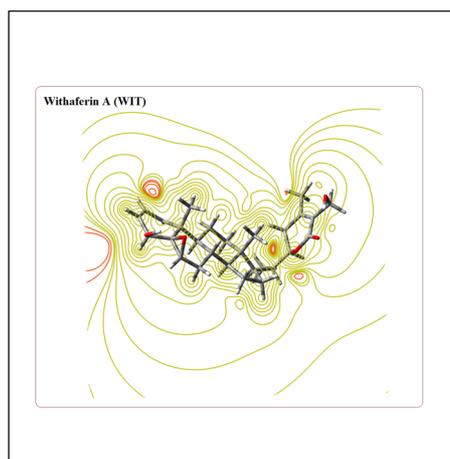
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ABSTRACT

The main purpose of present study is evaluation of structural and medicinal properties for Withaferin A (WIT) using density functional theory (DFT) method. All studies are done via computational chemistry methods using Gaussian 03 and Molegro Virtual Docker (MVD) software packages and SwissADME web-based tool. Molecular structure of WIT was optimized at the B3LYP/6-311++G(d,p) theoretical level of DFT. The reactivity and stability properties of the optimized molecule were explored via global reactivity indices. Calculating the reactivity indices using energies of frontier molecular orbitals (FMOs) showed that WIT is stable against the oxidizing agents in the cell and has low reactivity against the biomolecules. On the other hand, the docking analysis data indicated the steric interactions play important role in WIT binding to beta-Tubulin via the residues Tyr224, Cys12, Gln11, Asn101, Gly143, Gln15, Gly144, Asn206, Gly142, and Asp179.



Keywords: In silico; Molecular docking; Molecular simulation; Tubulin; Withaferin A.

Introduction

Withaferin A (WIT), an important natural compound, is extracted from the Indian medicinal plant *Withania somnifera*. This plant has been used for over 3000 years in Unani and Indian medical systems. Also, its extracted medicinal

compound has been used in ayurvedic medicine with multiple pharmacological activities containing immune-modulatory, anti-diabetic, cardioprotective, anti-inflammatory, anti-metastasis, anti-angiogenesis and anti-carcinogenic properties [1-6]. Several active

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groups, such as hydroxyl, epoxide ring, carbonyl, double bond and lactone ring are in the molecular structure of WIT. These active groups cause strong interactions of the molecule with amino acid residues of different targets, such as Vimentin, I κ B kinase β (IKK β), Annexin II and N-terminus of heat shock protein 90 (HSP90) [7-12]. Recently, the researchers have found out the anti-carcinogenic properties of the title medicinal compound is due to its interaction with beta Tubulin. In 2014, Antony and co-workers [13] reported that WIT inhibits beta-Tubulin (the major component of the eukaryotic cytoskeleton) activity by predicting the ligand-receptor complex formation of WIT binding to Cys303 of beta-Tubulin. In 2019, Yang and co-workers [14] conducted an in-depth study of the

interactions between WIT and Tubulin. Their studies showed that the molecule under study forms covalent bonds with residues Cys303 and Cys239 of the protein. Unfortunately, they did not provide much information about the exact structural and molecular drug-receptor interactions, such as hydrogen bond, ionic and steric interactions. Analysis of the exact molecular mechanisms involved in interaction of WIT with beta-Tubulin proteins is the main aim of the present research work. These studies are done using computational chemistry and molecular docking methods. Moreover, the pharmacokinetic behavior and biological attributes of the titled medicinal molecular structure are determined using SwissADME web tool.

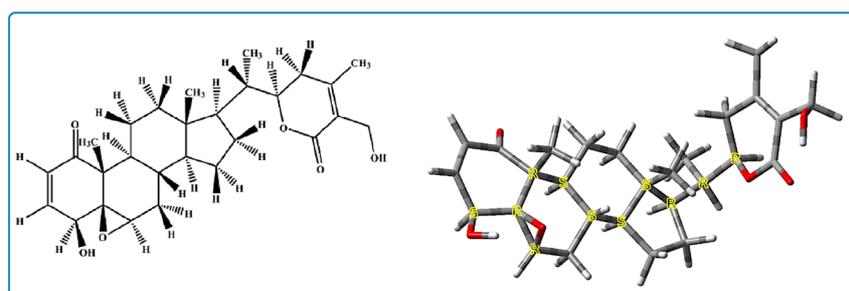


Fig. 1. The optimized molecular structure of Withaferin A.

Materials and Methods

In silico study is a good technique to analyze some properties and applications of a chemical compound which are not easily investigated via experimental methods [15-25]. These studies are mainly done by computer software packages [26]. As mentioned above, the important goal of the present study is analysis the interaction of Withaferin A (WIT) with beta-Tubulin protein. To this aim, the molecular structure of the studied compound was optimized using Gaussian 03 software via B3LYP/6-311++G(d,p) level of theory [27]. Its stability, reactivity and electronic properties were investigated via calculating the global reactivity indices. The frontier molecular orbitals (FMOs) energies were used to compute the titled indices [28-31]. In the next step, the

optimized molecular structure was embedded into the active site of the protein. This work was done using Molegro Virtual Docker (MVD) software. From analysis of molecular docking data, the exact types of interactions of the ligand-protein complex were evaluated. Finally, the physicochemical and ADME properties of the WIT natural compound were predicted via www.swissadme.ca web-based tool.

Results and discussion

Structural Properties of Withaferin A

Withaferin A is a 28-C steroidal lactone that its backbone contains three cyclohexane rings and one cyclopentane ring (Fig. 1). The alpha, beta-unsaturated ketone-containing ring, the epoxide-containing ring, and alpha, beta-unsaturated

ketone-containing ring are more reactive parts of the molecule [13]. The said molecular structure was optimized using DFT methods. After each computation, the calculated bond lengths of the molecular structure were compared with the corresponding empirical data. The highest correlation coefficient of the theoretical-empirical bond lengths dependence was seen for computations at the B3LYP/6-311++G(d,p) level of theory. The electronic energy of the optimized molecular structure is -41444 eV. The optimized molecule has high stability with the dipole moment of 5.8638 Debye. Fig. 2 indicates the dependence between the theoretical and experimental bond lengths of the WIT medicinal compound. This dependency is shown by the equation of $y=1.0475x-0.0676$. The higher correlation coefficient ($R^2=0.9921$) for this equation shows a great convergence. Therefore, the B3LYP/6-311++G(d,p) level of theory is a good method to compute the electronic properties of the title compound.

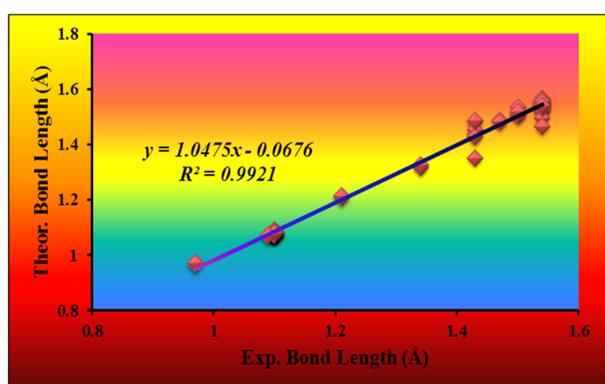


Fig. 2. The experimental and theoretical bond lengths relationship of Withaferin A.

Stability and Reactivity of Withaferin A

Calculating the global reactivity indices is a good way to study the stability and reactivity properties of a chemical compound [28-32]. The global reactivity descriptors like energy gap (E_g), ionization potential (IP), electron affinity (EA), chemical hardness (η), chemical softness (S), electronegativity (χ), electronic chemical potential

(μ) and electrophilicity index (ω) can be obtained from the energies of the frontier orbitals. These reactivity indices are achieved by following formulas [33]:

$$E_g = E_{LUMO} - E_{HOMO}$$

$$IP = -E_{HOMO}$$

$$EA = -E_{LUMO}$$

$$\eta = \frac{(\epsilon_{LUMO} - \epsilon_{HOMO})}{2}$$

$$\chi = \frac{-(\epsilon_{LUMO} + \epsilon_{HOMO})}{2}$$

$$\mu = \frac{(\epsilon_{LUMO} + \epsilon_{HOMO})}{2}$$

$$\omega = \frac{\mu^2}{2\eta}$$

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

Table 1: Global reactivity indices of Withaferin A

Parameter	Energy value (eV)
HOMO	-10.29
LUMO	2.77
Ionization Potential (IP)	10.29
Electron Affinity (EA)	2.77
Energy Gap (E_g)	13.06
Electronegativity (χ)	3.26
Chemical Potential (μ)	-3.26
Chemical Hardness (η)	6.53
Chemical Softness (S)	0.153
Electrophilicity index (ω)	0.814

Fig. 3 indicates the frontier molecular orbitals (FMOs) of the molecule under study. FMOs are called to the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) [33]. The HOMO was made of atomic orbitals of lactone ring. In contrast, the atomic orbitals of the carbonyl containing ring. So, these rings prefer to make complex with the residues of the protein via nucleophilic and electrophilic interactions, respectively. On the other hand, the red, green and blue colors of the molecular electrostatic potential (MEP) graph relate to the negative, zero and positive charge

densities, respectively. We can see the electronegative atoms have more charge density than other atoms. So, these atoms are more susceptible to interact with the electron deficient residues of the protein. The density of states (DOS) graph shows high energy gap (13.06 eV) between HOMO (-10.29 eV) and LUMO (2.77 eV). This high energy gap of the FMOs indicates the high stability of the compound. It can be deduced that the

electronic transition can't happen in valence layer of the molecular orbitals. So, the molecule is stable against the oxidizing agents in the cell. The global reactivity indices have been listed in Table 1. The obtained data show that the compound has high chemical hardness (6.53 eV) and low chemical softness (0.153 eV) indices. So, it could be expected that the molecule has low reactivity against the biomolecules.

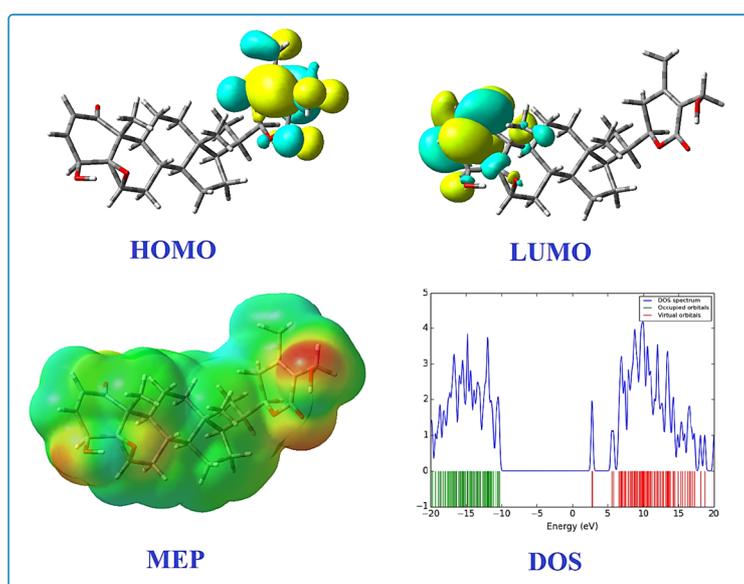


Fig.3. The FMOs, MEP and DOS of Withaferin A.

Physicochemical Descriptors and ADME Parameters for Withaferin A

In medicinal chemistry, ADME is the abbreviation for the absorption, distribution, metabolism and excretion properties. These properties are the key factors to predict the pharmaceutical activity [34]. Here, the ADME properties of the WIT compound was done using SwissADME web-based tool.

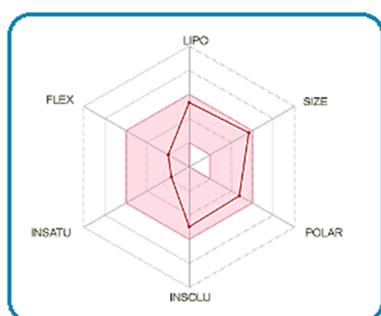


Fig. 4. Physicochemical properties graph of the compound Withaferin A.

Fig. 4 shows the predicted physicochemical graph of the molecule under study. The oral bioavailability of a medicinal compound is identified via six key parameters: lipophilicity ($-0.7 < XLOGP3 < +5.0$), size ($150 \text{ g/mol} < M < 500 \text{ g/mol}$), polarity ($20 \text{ \AA}^2 < \text{TPSA} < 130 \text{ \AA}^2$), insolubility ($0 < \text{ESOL} < 6$), insaturation ($0.25 < \text{Fraction Csp3} < 1$) and flexibility ($0 < \text{number of rotatable bonds} < 9$). The colored zone in this graph shows the suitable physicochemical space for oral bioavailability of a compound [35]. We can see the Fig. 6 indicates WIT has oral bioavailability. The evaluation of the compound's properties showed the lipophilicity ($\text{LogP}_{O/W}$) of 3.83, molecular weight of 470.6 g/mol, TPSA of 96.36 \AA^2 , ESOL of 5.01, fraction Csp3 of 0.79 and 3 rotatable bonds. The molecule under study shows the

bioavailability score of 0.55. On the other hand, the said molecule hasn't any activity as an inhibitor of the cytochrome P450 subunits. Fig. 5 shows the boiled egg graph of the WIT compound. The yellow-colored zone of the graph shows the permeation of the molecules through the blood-brain barrier (BBB). In contrast, the white-colored zone of the boiled egg graph relates to the absorbed molecules by the gastrointestinal tract. The boiled egg graph indicates that WIT is absorbed by gastrointestinal tract. Also, the blue color of the molecule in this graph shows that it is effluated from the central nervous system (CNS) by the P-glycoprotein.

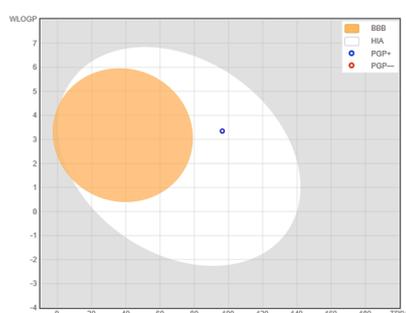


Fig. 5. Boiled egg graph of the compound Withaferin A.

Molecular Docking Analysis of Ticagrelor-P2Y12 Complex

Recent studies show that WIT inhibits beta-Tubulin activity via its binding to Cys303 of the said protein [13]. Here, complex formation between WIT and beta-Tubulin has been studied via molecular docking analysis. The Molegro Virtual Docker (MVD) program was used to perform the docking analysis of the ligand-receptor complex.

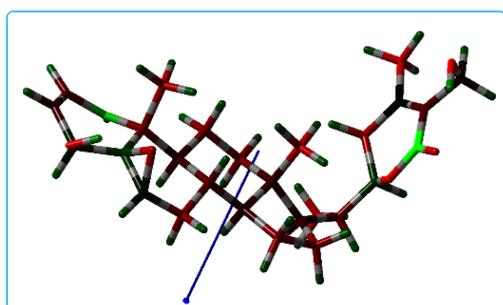


Fig. 6. Charge distribution and dipole moment vector of Withaferin A.

Fig. 6 indicates the charge distribution and dipole moment vector of WIT ($X=1.7191$, $Y=1.3545$ and $Z=5.5821$). The molecule under study showed the dipole moment of 5.8638 Debye. We can see all oxygen and carbon atoms except carbon atoms of the carbonyl functional groups have negative charges. So, the molecule prefers to interact with the electron poor residues.

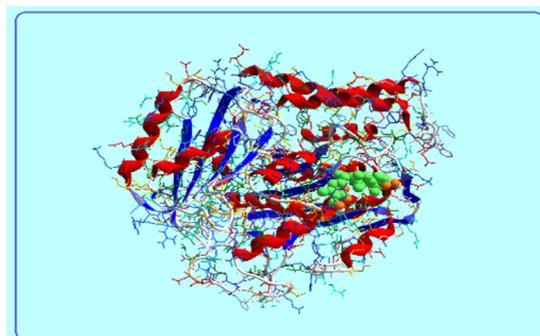


Fig. 7. Ligand Withaferin A embedded in the active site of the beta-Tubulin.

Fig. 7 indicates that the ligand WIT is embedded in the active site of the beta-Tubulin. WIT made complex with the protein structure by MolDock score of -135.589. So, WIT binding to beta-Tubulin is strong and is mainly done via the steric interactions (MolDock score of -152.219). The MolDock score of the hydrogen bonds in ligand-receptor complex was -4.505. The internal ligand interactions weaken the binding between ligand and receptor. The internal torsional strain and steric interactions showed the MolDock scores of 4.203 and 27.859, respectively. The beta-Tubulin residues Gln15, Leu227, Asn228, Ile16, Gly142, Thr180, Asn101, Val171, Asn206, Asp179, Ser140, Tyr224, Cys12, Gly144, Gly143, Gly146, Thr145, Gln11, Asp69, Ala99, Gly100, Glu71, and Gly10 participated in steric interactions. In contrast, only the residues Gln15, Gly146 and Ala99 can make interaction with the molecule using hydrogen bond formation. Our studies showed the strongest interactions of WIT with beta-Tubulin relating to residues Tyr224, Cys12, Gln11, Asn101, Gly143, Gln15, Gly144, Asn206, Gly142, and Asp179.

Conclusion

Investigating physicochemical, structural and electronic properties of Withaferin A (WIT) was the main objective of the present research work. Electronic properties prediction of the molecule was carried out using the computational chemistry methods. The molecular structure was optimized at the B3LYP/6-311++G(d,p) level of theory. The energies of frontier molecular orbitals (HOMO and LUMO) were used to calculate the global reactivity indices. WIT is stable against the oxidizing agents in the cell with low reactivity against the biomolecules. The molecule-receptor Interactions

indicate the main role of the beta-Tubulin residues containing Tyr224, Cys12, Gln11, Asn101, Gly143, Gln15, Gly144, Asn206, Gly142, and Asp179 in the ligand-receptor complex formation. The molecular analysis data showed the steric interactions in formation of the ligand-receptor complex. Finally, the ADME study showed that the said compound is non-toxic and has oral bioavailability.

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