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In silico Molecular Docking and Dynamic Study of MDM2-p53 Inhibitor Alkaloids Extracted from *Withania somnifera* Against Tumor Growth

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Abstract

In today's world, human tumor growth has become a critical health issue. Many patients experience ineffective chemotherapeutic treatments, prompting the need for alternative methods. Our recent research has explored active alkaloid extracts from *Withania somnifera* as potential inhibitors of the MDM2-p53 interaction, a key factor in tumor growth. Using *in silico* molecular docking and dynamic approaches, we identified eight effective alkaloids with high docking score are WithanolideD (–10.0Kcal/mol), Withanolide (–10.0Kcal/mol), WithaferinA (−9.9Kcal/mol), WithanolideB (−9.5Kcal/mol), WithalongolideA (–9.4Kcal/mol), WithanolideE (− 9.4Kcal/mol), WithanolideA (−9.0Kcal/mol), Withanone (−8.9Kcal/mol). Among them, WithaferinA, WithanolideA, WithanolideD, WithanolideE, and Withanone demonstrated the most stable hydrogen bonding, stable RMSD, and RMSF. Lipinski's rule of five, which assesses drug-like qualities, confirmed their potential through canonical SMILES verification. Molecular dynamics of the protein-ligand complex revealed that these docked complexes remain thermally stable, with consistent bond energy, dihedral angles, and stereochemical conformation over a 100 ns time frame. Our findings suggest that these alkaloids from *Withania somnifera* hold promise as effective treatments against tumor growth. Further investigation and exploration are necessary to confirm their efficacy in clinical settings.

Keywords: Withania somnifera, alkaloids, tumor, drug design

Introduction

The application of the computational approach in drug design and discovery has changed the scenario in the last few decades (Ahmed et al., 2022). Molecular docking expands the dimension to understand protein-protein or protein-ligand interaction (Meng et al., 2011). The method of docking is carried out through server-based (Docking, Modelling, Prediction, Binding, Protein structure quality assessment, Molecular dynamic, Remote access or analysis) and programme-based (Docking, Dynamics, Visualization, Analysis, and interpretation) (Pagadala et al., 2017). Molecular docking is the ultimate tool for designing and simulation of drugs (Alonso et al., 2006). In the present research work, we have designed a noble and promising drug to inhibit MDM2-p53 interaction against tumor growth in *Homo sapiens*.

The p53 is an oncogene that has a significant role in cell cycle regulation. It is a tumor suppressor gene also known as the 'guardian of the human genome'. Its activity shows numerous cellular signals including DNA repairing, cell cycle arrest, and maybe apoptosis (Efeyan & Serrano, 2007). Phosphorylation of p53 and MDM2-p53 linkage disturbance causes p53 abnormalities and then becomes wild-type (Brooks & Gu, 2003). Induced p53, possibly involved in cell cycle arrest for DNA repair to maintain and restart the cell cycle in a usual way, otherwise, apoptosis may cause death and damage the cell (Hanel & Moll, 2012). Such biological phenomenon conspicuously occurs in cells for genetic stability (Chen, 2016). Transcription in p53 is induced through radiation, oncogene abnormalities, DNA damage, nutrition, stress, and aging factors (Rufini et al., 2013). Defective p53 oncogene interacts with MDM2 and causes uncontrolled growth of the cell as the initiation of apoptosis (Ashcroft & Vousden, 1999). In the majority of clinical diagnoses, the p53 oncogene was found surprisingly mutant (Song et al., 2007). Normal cell cycle works with unstable p53 activity, whereas in DNA damage, its activity increases accordingly as a negative regulator (Hafner et al., 2019). While running the repair mechanism, deformities from DNA to the new replication trend stop (Torgovnick & Schumacher, 2015). The mechanism of p53 activity is maintained by stabilizing the binding affinity of MDM2 with p53 interaction at a normal autoregulatory function that suppresses the abnormal activity of p53 in transcription. Similarly, phosphorylation of p53 activity occurs as a usual oncogene phenomenon and MDM2 is solely responsible for the degradation of p53(Michael & Oren, 2003). The p53 activity is controlled by MDM2 protein working as E3 ligase enhancing the degradation of p53 in proteasome degradation (Mihara et al., 2003). The p53 activity is persisted in the cell through the inhibitors for MDM2-p53 (Ahmed et al., 2022). The mechanism associated with p53 oncogene implies that its tumor suppressive function can be restored by inhibiting MDM2-

p53 interaction. In this present research work, we have selected *Withania somnifera* extract as a potential inhibitor in MDM2-p53 interaction through molecular docking.

Withania somnifera, also known as 'Ashwagandha,' a medicinal shrub, belongs to the Solanaceae family and has been in practice since ancient times in Nepal, India, and South Asia (Ven Murthy et al., 2010). In Nepal, it is distributed in the lower Himalayan range below 1500 meters of subtropical zone. In Ayurved practice, the plant extract is used to make brain tonic, stimulant, anti-rheumatic, peristaltic disorder, malignant growth, cardiovascular obstruction, atrophy, power buster, etc. (Duke, 2002). Clinical and pharmaceutical success has revealed that *Withania somnifera* extract can be used in a wide range of medicines effectively as an antidiabetic, neurogenic, immunoregulator, cancer, and parkinson (Kumar et al., 2015). Bioactive natural product contains a high amount of polyphenolic compounds as an antioxidant, metabolites, bioflavonoids, terpenes, sesquiterpenes, diterpene esters, triterpenes, etc (Sovrlić & Manojlović, 2017). Traditional medicinal practice has shown that its roots and leaves contain sufficient active alkaloids to show anticancer activity. The major alkaloids found in *Withania somnifera* are Withanolide, WithaferinA, Withanone, Anaferine, Withasomnine, Beta-sitosterol, Chlorogenic acid, Withanolides A-Y, Withanine, Withananine, Tropanol, Anahygrine, Somniferiene, Somniferinine, Cuscohygrine, Pseudotropine etc. (Bone, 1996; Elsakka et al., 1990). Withanoferin, Withanolides, and metabolites like Withanone and Withnosides are found effective against cancer growth (Rai et al., 2016). The prominent activity of the bioactive alkaloid Withaferin is highly used in cytotoxic activity as an anti-carcinogenic compound. The inhibitory activity of Withaferin is so remarkable that it is widely used against different types of cancer in the brain, lungs, colon, prostate, breast ovaries, etc. (Dutta et al., 2019). The biological activity of WithaferinaA with different concentrations of the main protease was found effective against breast cancer cells (Szarc vel Szic et al., 2014). The activity of Withanolide and Withaferin is found effective against prostate cancer cells in early treatment (Roy et al., 2013). The tumor growth in human beings can be suppressed by the biological activity of Withanolides (Jayaprakasam et al., 2003).

Oncogene p53 and MDM2 interaction inhibition is a prime concern to stop cancerous activity in the human body. Radiotherapy and Chemotherapeutic drugs fail to revive the cell in many cases and make the scenario more critical (Gewirtz et al., 2008). Studies from ancient therapeutic trials have shown that some specified *Withania somnifera* extracts are effective in avoiding tumor growth (Yin et al., 2013). These bioactive compounds have a strong tendency to bind MDM2 protein and p53 becomes free to perform usual cellular function. Strong and stable hydrogen bonding of these alkolides may be the possible candidates against rampant tumor growth

in cells (De Mejía & Prisecaru, 2005). This research work focuses mainly on the interaction between MDM2 with different bioactive compounds present in *Withania somnifera* based on molecular docking and dynamics. The selection of bioactive alkaloids-drug-like features is based on Lipinski's rule of five (Lipinski, 2004) selection or rejection method.

Methods and Procedures

Docking and Dynamics Software

In-silico molecular docking and dynamic approach were applied in the present work. The experimental part of molecular docking is strictly based on computer-based and server-based programs. The server-based online programs were carried out by modern technological methods including Swiss dock (Swiss drug design, https://www.swissdock.ch/, based on attracting cavities with auto dock Vina docking engine), Homology modeling, ADMET prediction, Binding site prediction, protein structure quality assessment, and molecular dynamics. The computer-based programs involve docking, dynamics, visualization, and interpretation. The major software used in the research work is listed below. These software are modern in-silico, versatile, and precise tools widely used in molecular modeling, docking, and dynamic study.

Table 1

Different Softwares Used in the Experiment

Name of the Software	Version	Script	Operating System	Distributor/ Developer	Applications	Software link
PyMol	3.0	C, C^{++}, Python	Cross-platform, Linux, Windows, MacOS, Unix	Schrödinger, Inc.	3D-Molecular visualization	https://pymol.org/2/#download
BIOVIA Discovery Studio Visualizer	21.1	Perl script	Linux, Windows, MacOS, Unix	Dassault Systemes BIOVIA	Interactive 2D and 3D visualization	https://discover.3ds.com/discovery-studio-visualizer-download
Avogadro	1.0	C^{++} (Qt)	Linux, Windows, MacOS, Unix	Wikimedia Foundation and contributors	Molecule editor	https://avogadro.cc/

VMD (Visual Molecular Dynamics)	1.9.4a53	C	Linux, Windows, MacOS, Unix	University of Illinois at Urbana–Champaign	Molecular modelling	https://www.ks.uiuc.edu/Development/Download/download.cgi?PackageName=VMD
ADFR	V2.7	C, C^{++}	Linux, Windows, MacOS, Unix	Avada	Molecular docking	https://ccsb.scripps.edu/adfr/

Homology Modelling: Protein Preparation

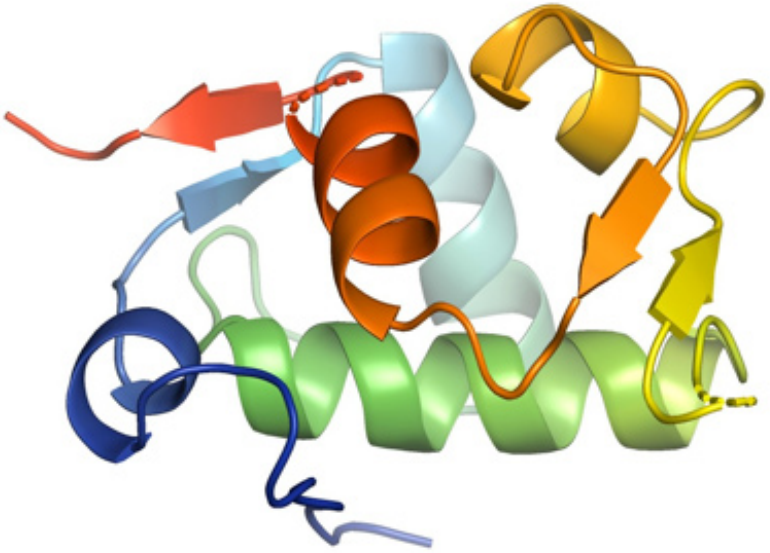
The Swiss modeler technique was used in this research study to curtail the gap between protein sequence and experimental structure (Waterhouse et al., 2018). Comparative modeling gives the nearest matching 3D geometry of the protein in SBDD format. Target identification, sequence alignment, model building, and model refinements are the principal features of this study. The backbone of the protein, loop, side chain creation of templet, and defect-free protein preparation are carried out through modern computational tools. Amino acid sequence (FASTA) and pbd format were downloaded from the Research Collaborator for Structural Bioinformatics (RCSB.org) as (PBD ID: 5TRF). Protein residue was observed in PyMol (TM) 2.5.5. The sequence identity was carried out by QMEANDisCO, GMQE. While preparation of protein, all the water molecules are removed first. The process is followed by Removing ligands, ions, or any other molecules and then adding polar hydrogens to the protein. The formed protein was represented by "5TRF_clean.pbd". The 'pbd' file is further converted into 'pddqt' by issuing a command in Power Shell in the working directory. Following power shell command was issued.

prepare_receptor -r .\5TRF_clean.pdb -o .\5TRF_clean.pdbqt

X-ray diffraction technique has shown its resolution of 2.10Å, R-value free 0.208, R-value work 0.185, and observed R-value 0.186. The molecular weight of the protein is 11,140.088g/mol, dipole moment 648.433, number of atoms 960, bonds 974, and number of residues 98 in residue segment A. The energy was found 25850 KJ/Mol by Avogadro software.

Figure 1

3D Structure of 5TRF Protease from Homo Sapiens



Reference: *RCSB, PDB, (Wang et al., 2014)*

Calculation Procedure: Avogadro Software

The ".sdf" file format was opened and hydrogen atoms and bonds were checked. Avogadro Software was used here as a cross-plate form molecular editor 3D-visualization design. Which gave flexible, high-quality rendering and strong plugin architecture (Hanwell et al., 2012). Water molecules were removed from the protein, ligands were removed, and ions or other molecules were eliminated from the structure. The pH was adjusted to 7.4. The software examined hydrogen atoms and bonds in protein structure. The energy of the protein and ligand was minimized through molecular mechanics DFT (Fonseca & Fleming, 1998). The target was prepared then the clean protein was exported as "5TRF_Clean.pbd". The file was further saved as "5TRF_Clean.pbdqt".

Preparation of ligands: PubChem

The literature review has shown that more than ten bioactive compounds from *Withania somnifera* have anti-carcinogenic properties. PubChem database

shows that the active compound in (Sdf) with their ID as WithaferinA (PubChem ID: 155887202), WithanolideA (PubChem ID: 11294368), Withasomnine (PubChem ID: 442877), WithanosideIV (PubChem ID: 71312551), WithanolideD (PubChem ID: 161671), WithalongolideA (PubChem ID: 56649343), WithanosideIV (PubChem ID: 91827019), Withanolide (PubChem ID: 53477765), Anaferine (PubChem ID: 443143), Tropanol (PubChem ID: 4824), Anahygrin (PubChem ID: 36762693), WithanolideE (PubChem ID: 301751), WithanolideB (PubChem ID: 14236711), Cuscohygrin (PubChem ID:1201543). A power shell command was issued to convert 'pbd' into 'pddqt' file format. In working directory following command was issued for the conversion of 'pdb' file format into 'pddqt'.

prepare_ligand -l .\Ligand.pdb -o .\Ligand.pdbqt

prepare_ligand -l .\WithaferinA.pdb -o .\WithaferinA.pdbqt

prepare_ligand -l .\WithanolideA.pdb -o .\WithanolideA.pdbqt

prepare_ligand -l .\Withasomnine.pdb -o .\Withasomnine.pdbqt

prepare_ligand -l .\WithanosideIV.pdb -o .\WithanosideIV.pdbqt

prepare_ligand -l .\Withanolide.pdb -o .\Withanolide.pdbqt

prepare_ligand -l .\WithanolideE.pdb -o .\WithanolideE.pdbqt

prepare_ligand -l .\WithanolideB.pdb -o .\WithanolideB.pdbqt

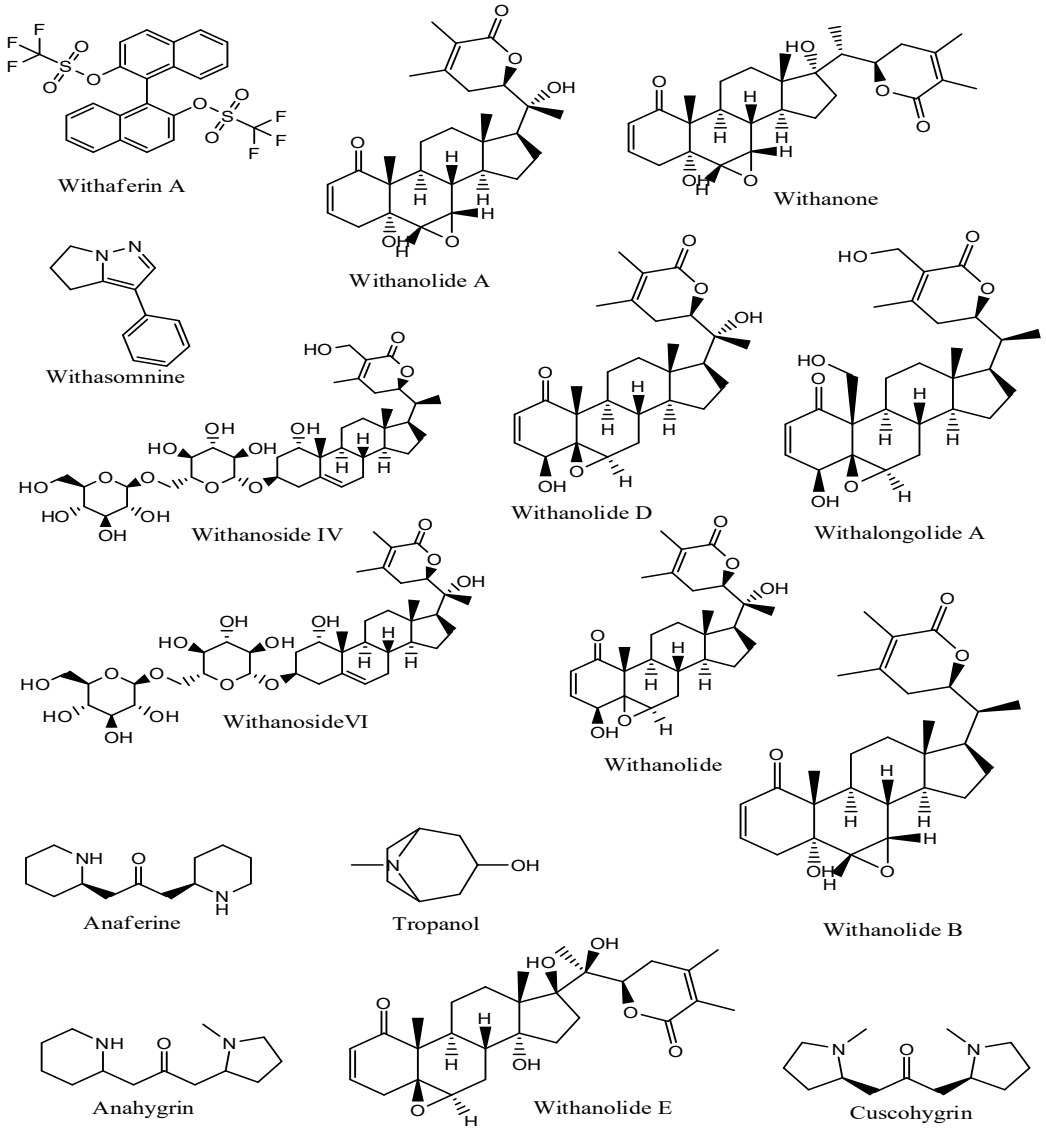
prepare_ligand -l .\Anaferin.pdb -o .\Anaferin.pdb

prepare_ligand -l .\Cuscohygrin.pdb -o .\Cuscohygrin.pdbqt

Withania somnifera is a vital traditional herbal plant. It contains many metabolites, phenols, and antioxidants. Reports have shown that *Withania somnifera* mainly contains bioflavonoids, terpenes, sesquiterpenes, diterpenes esters, triterpenes, steroids, and lignans. The ligands for the docking with 5TRF were chosen through their important anticancerous property. The selected ligands are: WithaferinA, WithanolideA, Withanone, Withasomnine, WithanosideIV, WithanolideD, WithalongolideA, WithanosideVI, WithanolideD, WithalongolideA, WithanosideVI, Withanolide, Anaferine, Tropanol, WithanolideB, Anahygrin, WithanolideE, Cuscohygrin etc.

Figure 2

Chemical Structure of Different Alkaloids and Compounds Found in Withania Somnifera



(Source: RCSB PDB, modified on ChemDraw Ultra 12.0)

ADMET lab 2.0

The toxicity and pharmacokinetics nature of bioactive compounds resists a possible candidacy of the drug. The major criteria like absorption, distribution, metabolism, excretion, and toxicity (ADMET) should be examined before docking

(Xiong et al., 2021). Lipinski's rule of five was tested by inserting canonical SMILES for each ligand and the BBB barrier was observed for all ligands.

Molecular Docking: AGFR 1.2

AGFR is a molecular docking program with flexible receptors and desolvation. Receptor and ligand were loaded on the software in pdbqt format and software was run for all atom types, the pocket was computed (Auto site 1.1). After identifying the active site with AS score, ligand position, and catalytic site were verified by CASTp. A grid box with proper dimension and centering was created by adjusting padding (2.0) and the water map was set with weight and entropy. The target file was generated by taking at least 30 flexible residues. After issuing a command in the power shell, flexible receptor molecular docking began in a hydrated environment. Binding affinity was calculated for each ligand-protein interaction. The command issue for the docking was operated as:

adfr -l Ligand.pdbqt -t Protein.trg --jobName flexRes --nbRuns 4 --maxEvals 5000 -O --seed 1

adfr -l Ligand.pdbqt -t Protein.trg --jobName flexRes --nbRuns 4 --maxEvals 50000 -O --seed 1

adfr -l WithferinA.pdbqt -t 5TRF_clean.trg --jobName flexRes --nbRuns 4 --maxEvals 50000 -O --seed 1

adfr -l WithanolideA.pdbqt -t 5TRF_clean.trg --jobName flexRes --nbRuns 4 --maxEvals 50000 -O --seed 1

adfr -l Withasomnine.pdbqt -t 5TRF_clean.trg --jobName flexRes --nbRuns 4 --maxEvals 50000 -O --seed 1

adfr -l WithanosideIV.pdbqt -t 5TRF_clean.trg --jobName flexRes --nbRuns 4 --maxEvals 50000 -O --seed 1

adfr -l Withanolide.pdbqt -t 5TRF_clean.trg --jobName flexRes --nbRuns 4 --maxEvals 50000 -O --seed 1

adfr -l WithanolideE.pdbqt -t 5TRF_clean.trg --jobName flexRes --nbRuns 4 --maxEvals 50000 -O --seed 1

adfr -l WithanolideB.pdbqt -t 5TRF_clean.trg --jobName flexRes --nbRuns 4 --maxEvals 50000 -O --seed 1

adfr -l Anaferin.pdbqt -t 5TRF_clean.trg --jobName flexRes --nbRuns 4 --maxEvals 50000 -O --seed 1

adfr -l Cuscohygrin.pdbqt -t 5TRF_clean.trg --jobName flexRes --nbRuns 4 --maxEvals 50000 -O --seed 1

The lowest possible binding affinities and clusters (DLG file) were chosen for the experiment. The file obtained as Poses "_flexRes_out.pdbqt''. Which was followed by opening PyMOL, the receptor 5TRF_clean.pdbqt and *flexRes_out.pdbqt together loaded, and the best pose as ".pdb" file was saved.

Biovia Discovery Studio 2021

The 2D and 3D protein-ligand interaction was observed in Biovia Discovery studio. The display had shown macromolecule setup, simulation, interaction, pharmacophores, X-ray, etc. The bond distance in each conventional hydrogen bonding and hydrophobic interaction including alkyl and pi-alkyl bonds were analyzed.

CABS-flex 2.0

Protein structure fluctuation was observed in CABS-flex 2.0. online server for 10ns. Larger and multimeric proteins 5TRF and heavy ligands distance restrain and simulation including contact maps were analyzed (Kuriata, 2018).

Results and Discussion

Active Site Prediction of Protein: CASTp 3.0 Server

The CASTp 3.0 server was used to predict active sites in the protein. It was found that after removing water molecules, ions, metals, and unnecessary residue proteins become free for docking with active alkaloids. The protein volume was found 1220.906 $Å^3$ and its surface area was found to be 1785.909 $Å^2$ with a resolution of 2.10 Å. After removing B, C, and D residue from the main protease, chain A contains 109 residues and the predicted active site in protein was found to contain AA residue 22.

Table 2

Active Site Prediction for Targeted Protein 5TRF with CASTp 3.0 Server

Protein	Volume (SA) $Å^3$	Area (SA) $Å^2$	Resolution Å	Total AA residue in Chain A	AA residue at the predicted active site
5TRF	1220.906	1785.909	2.10	109	22

Lipinski's Rule of Five: Screening of drug-like properties in ADMET Lab 2.0

Physiochemical properties of ligands were studied through the selection and screening of active alkaloids in ADMET Lab 2.0. Only eleven bioactive compounds were selected. The properties of ligands like molecular weight, volume, and density are determined from the SMILES string. The number of Hydrogen bond acceptors (nHA), number of Hydrogen bond donors (nHD), number of rotatable bonds (nRot), Number of rings (nRing), MaxRing, number of heteroatom (nHet), fChar, Topological polar surface(TPSA), Logarithm of aqueous solubility value (LogS), Water distribution coefficient at 7.4 pH and Caco-2-permeability were determined before testing Lipinski's Rule of five.

Molecular Weight(MW) ≤ 500
LogP (n-Octanol/ water distribution coefficient)≤ 5

Hacc ≤ 10 and Hdon ≤ 5

No. of Violation $< 2 \rightarrow Green(Acceptable)$, No. of Violation $\geq 2 \rightarrow$ Red(Rejectable)

(Lipinski 2004)

Table 3

Physiochemical Properties of Bioactive Compounds from Withania Somnifera

Compound Name	M.Wt. < 500	nHD <5	nHA < 10	Log P < 5	Max Ring	HIA	LogS > -5	Caco-2 (cm/m)	Carcino gencity	Lipinski Rule	Violation
Withaferin A	470.27	2	6	3.190	18	- - -	4.204	-5.023	+	Accepted	No
Withanolide A	470.27	2	6	3.445	18	- - -	-4.475	-4.745	++	Accepted	No
Withasomnine	184.10	0	2	2.955	8	- - -	-2.965	-4.517	++	Accepted	No
Withanoside IV	782.41	9	15	1.345	17	+++	-3.361	-6.291	+	Rejected	Yes
Withanolide D	470.27	3	6	3.524	18	- - -	-4.440	-4.826	++	Accepted	No
Withalongolide A	470.27	2	6	3.198	18	-	-4.204	-	+	Accepted	No
Withanolide	470.27	2	6	3.620	18	- - -	-4.469	-4.846	++	Accepted	No
Anaferine	224.19	2	3	0.300	6	- -	-0.491	-5.425	--	Accepted	No
Withanolide E	486.26	3	7	2.140	18	- - -	-4.479	-5.254	+++	Accepted	No
Withanolide B	454.27	1	5	5.100	18	-	4.642	-	-	Accepted	No
Cuscohygrin	508.04	0	3	-2.329	5	-	0.341	-5.818	++	Accepted	No

Lipinski's rule of five was applied to each alkaloid from *Withania somnifera*. For the analysis of drug-like properties, ligands should not violate more than five categories. The molecular weight of Cuscohygrin was 508.05 which is the highest of all alkaloids taken and a minimum molecular weight was found for Withasomnine. All the alkaloids were suitable for docking though they have high molecular weight and were found in the acceptable range between 100∼600. The Number of Hydrogen bond acceptors should be in the range of 0∼7 and all the chosen ligands have nHD

from 0∼7 except WithanosideIV. The maximum ring for each alkaloid found in the range 5∼18 that obeys the soft rule to be drunk-like properties. The logarithm of the aqueous solubility Log(S) value should be > -5. For the considerable dissolution of drugs in the solvent its value should be in the range of -4 to 0.5Log(Mol/L). All the selected alkaloids except WithaferinA and WithanolideD do not show this property but the remaining criteria are sufficient for them to be considered as drug-like ligands. Out of eleven ligands screened from ADMET lab 2.0, eight ligands are selected for docking with the 5TRF database protein (Lipinski, 2004).

Molecular Docking

The scoring result has shown that the active alkaloid extracted from *Withania somnifera* has a strong binding affinity with significant scores. Table 3. Shows the different binding energy of protein-ligand complex. The binding affinity of 5TRF with WithanolideD (-10.1Kcal/mol), Withanolide (-10.0 Kcal/mol), WithaferinA (-9.9 Kcal/mol), WithalongolideA (-9.4Kcal/mol), WithanolideB (-9.4 Kcal/mol), WithanolideE (-9.4Kcal/mol), WithanolideA (-9.0 Kcal/mol), Withanone (-8.9 Kcal/mol). Performing search for all the scores were obtained at 12 GA evolutions with 500000 maxEvals each. The 5TRF-Withanolide has box parameter in AGFR1.1 shown for center X: -6.917, Y: 25.094, Z: 17.875 and Size X: 32.350, Y: 36.750, Z: 36.750, spacing 0.375, smoothing 0.500 and AS score point 122. Out of 50 flexible residues only 30 were selected in the docking box: A: VAL14, THR16, SER17, GLN18, ILE19, GLN24, LEU34, VAL41, GLU52, VAL53, LEU54, PHE55, LEU57, GLE59, ILE61, MET62, THR63, ARG65, TYR67, VAL57, PHE91, VAL93, LYS94, HIS96, AR97, ILE99, TYR100, MET102, ILE103. Figure ‘A’ shows the docking box and binding pockets fill points, ‘B’ shows ‘trg map gui’ with receptor surface, and ‘C’ shows receptors in padding 2.

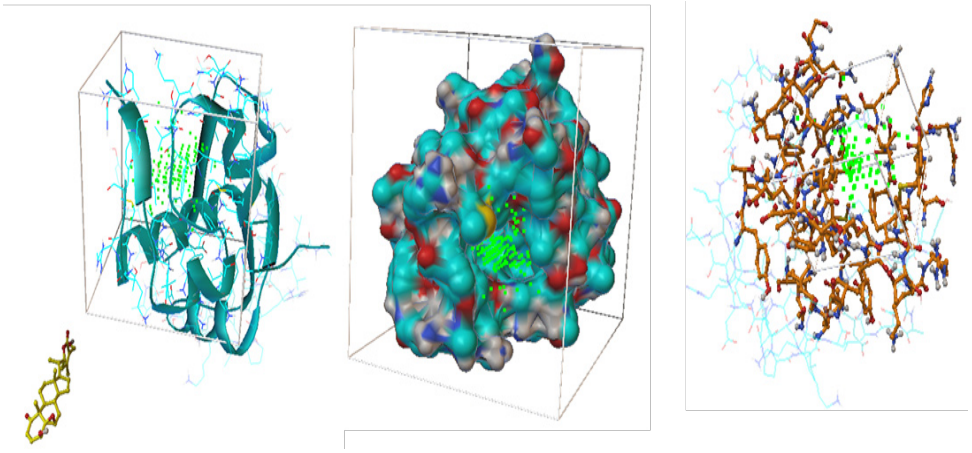
Figure 3

trg for 5TRF-WithanolideA complex in AGFR 1.1

A. *Centre view of docking box showing possible active sites for the interaction with ligand in cartoon model.*

B. *3D box showing receptor surface in docking box*

X. *Selected padding showing ligand binding pocket with ligand around the center of small docking box.*



A B C

The 5TRF-WithaferinA has box parameters shown for center X: -9.917, Y: 17.597, Z: 21.875 and Size X: 32.350, Y: 23.250, Z: 22.500, spacing 0.375, smoothing 0.500 and AS score point 296.38, 72 points exactly. It has RadGyr 5.50 and buriedness 0.85 lies on the box. Out of 50 flexible residues, only 29 were selected in the box: A: THR10, ASP11, VAL14, THR15, THR16, SER17, GLN18, ILE19, GLN24, LEU34, LEU37, LEU38, VAL41, TYR48, THR49, MET50, LYS51, GLU52, VAL53, LEU54, PHE55, TYR56, LEU57, GLN59, TYR60, ILE61, MET62, THR63, LYS64, ARG65, TYR67, GLN72, HIS73, ILE74, VAL75, LEU82, LEU85, PHE86, PHE91, SER92, VAL93, LYS94, HIS96, ARG97, LYS98, ILE99, TYR100, MET102, ILE103, ASN106. The protein-ligand complex has a water map setting in weight 0.60 and entropy -0.20. The computational setup for AGFR 1.1 for all selected ligand-protein was the same.

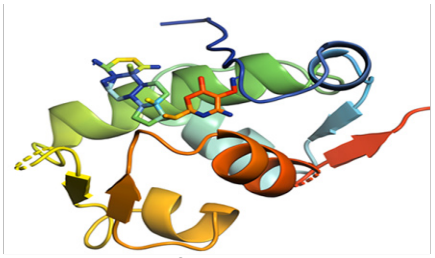
Figure 4

Molecular docking of 5TRF protein with WithaferinA,

A. *3D structure of 5TRF(MDM2) protease from Homo sapiens showing, C and N-terminal docked with WithaferinA.*

B. *2D interaction diagram of WithaferianA with 5TRF showing very strong hydrogen bonding 2.08Å with GLN A:24 and 2.93Åbond distance with VAL A: 14 protein residue.*

X. *Protein-ligand interaction and their distances showing hydrogen bonding.*



2D and 3D representations of 5TRF-WithaferinA represent six conventional hydrogen bonds. Hydrogen bondings are formed in : UNK0:H - A: VAL14:O with bond distance 2.92672Å, DHL 103.08 and HAY 116.792, : UNK0:H - A: GLN24:OE1with bond distance 2.084Å, DHA 130.088 and HAY 150.565, A: HIS96:HE2 - A: VAL93:O interaction forms conventional hydrogen bonding with bond distance 2.207Å, DHA 123.809 and HAY 151.54. Similarly, the A: ILE99:HN - A: HIS96:O interaction has a bond distance of 2.207Å, DHA 117.241 and HAY 90.126. :UNK0:H - A:VAL14:O ineraction gives bond distance 2.926Å with DHA 103.08 and HAY 116.792. : UNK0:H - A: GLN24:OE1 interaction gives conventional hydrogen bonding with bond distance 2.084, DHA 130.088, and HAY 150.565. The hydrophobic alkyl and pi-alkyl bonds are also significantly interacted in A: VAL14 - : UNK0, A: VAL93 - : UNK0, : UNK0 - A: MET62, : UNK0:C - A: VAL93, : UNK0:C - A: ILE99, : UNK0:C - A: VAL14, : UNK0:C - A: LEU54, A: HIS96 - : UNK0:C. Similarly, the pi-Alkyl bond was seen in A: HIS96: UNK0:C with a bond distance of 3.64Å.

Figure 5

Molecular docking of 5TRF protein with WithanolideD,

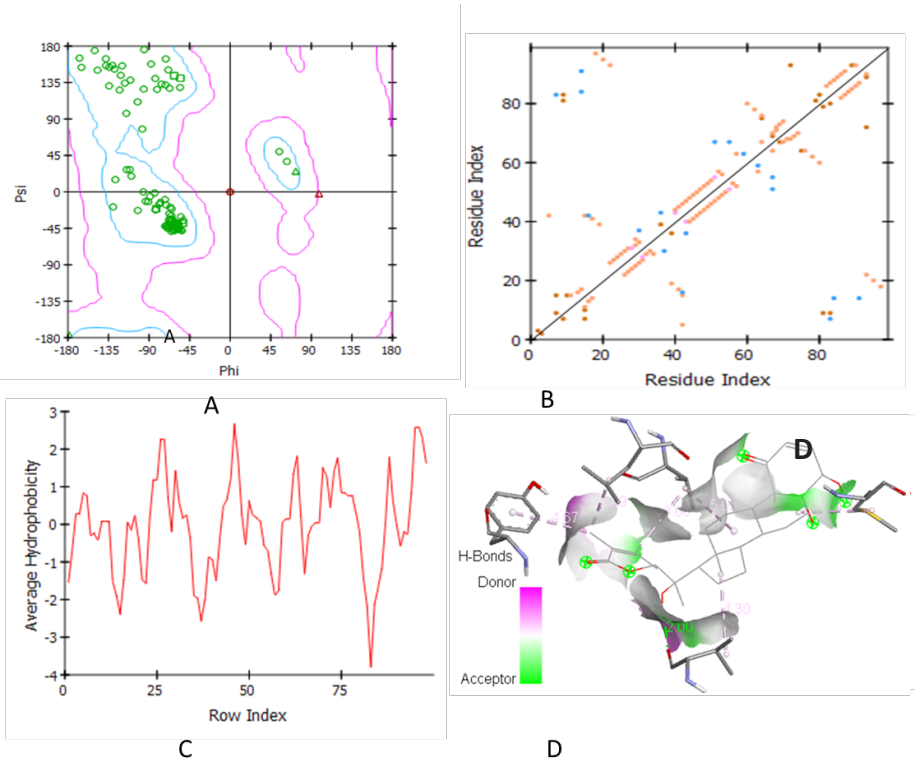
A. *Ramachandran plot showing stereochemical property of 5TRF(MDM2)-*

WithanolideD showing Phi(Ø) vs Psi(ψ) in polypeptide linkage, the highest dot density shows allowed energy region

B. *Hydrogen bonding contact plot.*

X. *Five residue average running hydrophobicity vs residue index.*

Δ. *3D interaction diagram of WithanolideD with 5TRF showing very strong hydrogen bonding 2.0Å bond distance with protein.*



2D diagram for receptor-ligand for 5TRF-WithanolideD has shown single but stable hydrogen bonding UNK0:H - A:VAL93:O. The interaction has a distance of 2.0034Å. It is a conventional hydrogen bond with angle DHA 164.042 and angle HAY 117.08. Alkyl and pi-alkyl bonds are distinctly seen in A:VAL14 - :UNK0 with bond distance 5.02036Å, A:VAL93 - :UNK0 with bond distance 4.30426Å, :UNK0 - A:MET62 with bond distance 4.15207, A:UNK0:C - A:LEU54 with bond distance 3.89433, :UNK0:C - A:VAL14 with bond distance 3.59888Å and A:TYR100 - :UNK0:C with bond distance 4.66782.

5TRF-WithanolideE interaction shows conventional single but very strong and stable hydrogen bonding. The interaction between UNK0:H1 - A: VAL93:O

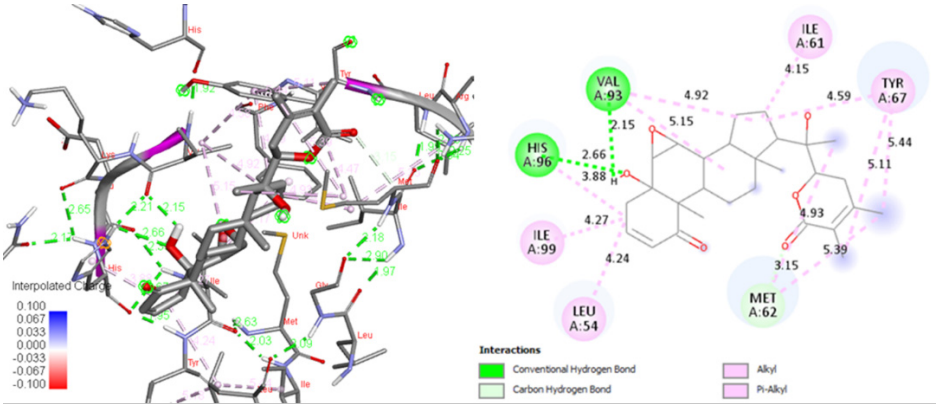
shows a 1.774Å bond distance, DHA 178.456 and HAY 132.7. The hydrophobic interaction between protein and ligand is prominent in the 5TRF-WithanolideE complex. The alky bondings are: UNK0 - A:LEU54, UNK0:C14 - A:ILE61, :UNK0:C25 - A:LEU54, :UNK0:C27 - A:VAL14, and the least possible bond distance has shown by A:VAL14 - :UNK0 with distance 4.207Å. The pi-alkyl interaction was found in A:PHE55 - :UNK0, A:TYR100 - :UNK0:C25, and A:TYR100 - A:LEU54.The stable and strong interaction of protein-ligand interaction reveals the stability in the docked complex.

Figure 6

Molecular Docking of 5TRF Protein with WithalolideA

A. *3D structure of 5TRF (MDM2) protease with WithanolideA, showing interpolated charge in the complex.*

B. *2D interaction diagram of Withanolide with 5TRF showing very strong hydrogen bonding 2.15Å and 2.66Å bond distance with protein, and Protein-ligand interaction and their bond distances with carbon hydrogen bond, alkyl and pi-alkyl bond*



A B

5TRF database protein was docked in AGFR 1.1 revealing the the highest docking score with WithanolideD and the free energy change was found to be -10.1 $KCalMol^{-1}$. The last docking score -6 $KCalMol^{-1}$ was obtained by Withasomnine. Similarly, the docking score for Withanolide (-10.0 $KCalMol^{-1}$), WithaferinA (-9.9 $KCalMol^{-1}$), WithanolideB (-9.5 $KCalMol^{-1}$), wwithalongolideA (-9.4 $KCalMol^{-1}$), WithanolideE (-9.4 $KCalMol^{-1}$), WithanolideA (-9.0 $KCalMol^{-1}$), and Withanone (-8.9 $KCalMol^{-1)}$ with highest docking score and the most stable conventional hydrogen bonding. The WithanolideA, WithaferinA, and WithanolideE are possible inhibitors

in MDM2-p53 interaction against the mutant growth of cancerous tumors in *Homo sapiens*.

Table 4

List of Bioactive Chemicals with Binding Energy

Ligand	Withanolide D	Withanolide	Withaferin A	Withanolide B	Withalongolide A	Withanolide E	Withanolide A	Withanone	Withasomnine
Free energy (K Cal Mol^{-1})	-10.1	-10.0	-9.9	-9.5	-9.4	-9.4	-9.0	-8.9	-6.0

Targeted protein 5TRF(A) formed very stable and strong conventional hydrogen bonding. WithaferinA interacts with residue GLA A: 24 with a bond distance of 2.08Å and with residue VAL A: 14 with a bond distance of 2.92Å. Similarly, WithanolideA formed two hydrogen bonds with residue Val A: 93 having a bond distance of 2.15Å and HIS A: 96 forming a bond distance of 2.66Å. WithanolideD formed hydrogen bonding with residue VAL A: 93 with a bond distance of 2.15Å, and with HIS A: 96 with a bond distance of 2.66Å. WithanolideE also forms very stable hydrogen bonding with interactin residue VAL A: 93 having a bond distance of 1.77Å. High docking scores and strong hydrogen bonding of WithanolideA, WithanolideD, WithanolideE, and WithaferinA explore the possibility of the drug against the growth of the tumor.

Hydrogen bonding and its stability is a major parameter for the characterization of protein-ligand complex (Panigrahi et al., 2007). It affects the topological polar surface area (TPSA) of the complex, water distribution coefficient, LogS, and LogP (Ali et al., 2012). Their variation in the ligand/protein/complex significantly affects the hydrophobic binding of macromolecules, plasma protein, and enzyme activity. Maintaining hydrogen bonding in the long run of time is challenging and the fluctuation considerably reduces the chance of being selected as a drug. Table 4. Shows the stable hydrogen bonding of 5TRF targeted protein with Withanolide, WithanolideE, WithanolideD, WithanolideA, and WithafrinA.

Table 5

Interacting Amino Acid Residues of Targeted 5TRF Protein with Selected Bioactive Compounds

Targeted Protein	Bioactive compounds	Number of Hydrogen bonding	Interacting residue	Distance (Å)
5TRF(A)	Withaferin A	2	GLN A: 24 VAL A:14	2.08, 2.93
	Withanolide A	2	VAL A: 93, HIS A: 96	2.15, 2.66
	Withasomnine	2	VAL A: 93, HIS A: 96	2.15, 2.66
	Withanolide D	2	VAL A: 93, HIS A: 96	2.15, 2.66
	Withalongolide A	2	MET A: 62, VAL A: 14	2.75, 2.05
	Withanolide	1	VAL A: 93	2.00
	Withanolide E	1	VAL A: 93	1.77
	Withanolide B	3	VAL A: 93 TYR A: 67 LEU A: 66	1.91, 2.34, 2.96
	Withasone	1	GLY A: 58	2.92

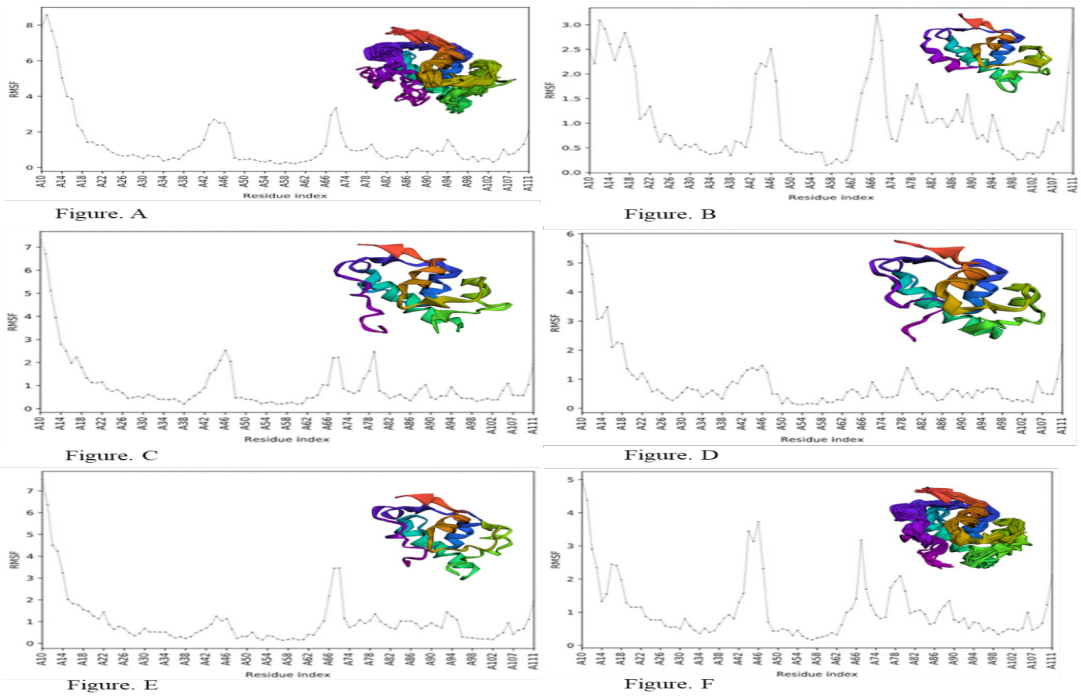
RMSF plots: CABS-flex 2.0

The flexibility of the protein-ligand structure is analyzed by the simulation modeling from the CABS-flex 2.0 online server. Protein dynamics obtained from the curve nearly correlate with high-resolution NMR. The aggregation propensity of protein is determined by the RMSF curve which shows the stable complex for all. Dynamic properties of the donor-acceptor complex were analyzed in RMSF in 10ns. RMSF values for all complexes were < 4Å which indicates the less flexible complex in the given circumstances. The RMSF values for the 5TRF-WithanolideA, 5TRF-Withasomnine, 5TRF-WithanolideA, 5TRF-Withanolide, 5TRF-Withalongolide, 5TRF-WithanolideE are 3.2, 3.1, 2.2, 1.3, 3.5, 3.7nm, respectively. The results reveal

that up to 10ns protein-ligand complex does not change its configuration and remains intact in structure and stability.

Figure 7

RMSF plot showing RMSF vs Residue index for 10ns obtained from CABS-flex 2.0 5TRF-WithanolideA B. 5TRF-Withasomnine C. 5TRF-WithanolideA D. 5TRF-WithanolideD E. 5TRF-Withalongolide F. 5TRF-WithanolideE

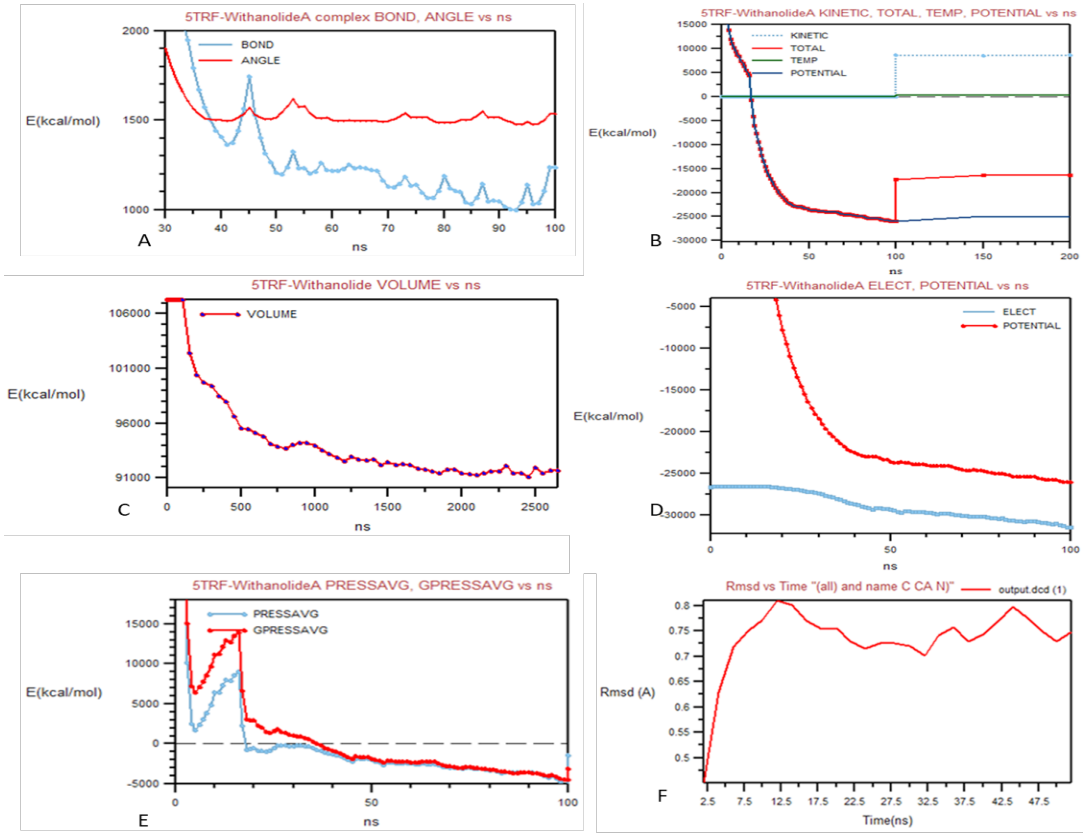


Molecular Dynamics: VMD 1.9.4a53

Figure 8(A) shows bond energy and bond angle vs TS(ns). At high temperatures, bonds are thermally stable which implies that the hydrogen bonds are not broken and possess energy around 1500Kcal/mol up to 100ns. Bond angles are stable with energy 1500Kcal/mol in the applied parameters. In 40ns, bond energy is abruptly increased but its effect is not seen in angle conformation. Bond angle calculation has shown that the 5TRF-WithanolideA complex possesses a bond distance of 2.149Å, angle DHA 105.202, and angle HAY 125.69 in UNK0 H-A: VAL93:O and interaction in A: HIS96:HE2-UNK0:O forms bond distance of 2.66Å, DHA 94.463 and HAY 92.783. Bond energy and bond length are unchanged which shows the formation of a stable complex conformation in a given time frame (Chang et al., 2007).

Figure 8

Molecular Dynamics for the 5TRF-WithanolideA complex(VMD 1.9.4a53, NAMD plots) generated by Dell i5-11th gen 16GB DDR4RAM, 512 NVME SSD, 2.90GHz, GSST, MU.



The thermal stability of the protein-ligand complex is analyzed from its kinetic, potential, and total energy variation in the time frame shown in Figure 8(B). The Kinetic, Potential energy, and total energy vs TS(ns) for 5TRF-WithanolideA complex shows high kinetic energy around 75000 -10000Kcal/mol at 100ns. The potential energy of the complex was obtained at around -375000 to - 42500Kcal/mol. The low potential energy minimizes total energy in the system as a result average or total energy for the complex becomes -18500Kcal/mol while operating the molecular dynamics up to 200ns. The molecular dynamics result reveals that kinetic energy increases in the complex for a given time frame but strong interaction stabilizes its energy as a result complex becomes intact.

Volume vs TS(ns) for 5TRF-WithanoideA, the entropy of the complex increased with increased energy continuously up to 2500TS frame, Figure 8(C).

Though the entropy of the docked complex was kept constant at around 0.2 in AGFR1.1, the volume of the complex decreased constantly within the given time frame with decreased temperature. Electrostatic, Potential vs TS(ns) shows that electrostatic and field potential dropped sharply up to 100 TS(ns), in Pressure, Gpressavg vs TS(ns), in Figure 8(E) Pressure increases abnormally at 10ns but continuously decreases in the shown time frame, gpressavg shows high fluctuation energy below zero at the given circumstances up to 100TS(ns). Figure 8(F) shows RMSD vs Time(ns), RMSD was found in the range 0.7-0.8Å

Conclusion

In the present research work, *in silico* approach is applied to search for potential inhibitors against the MDM2-P53 interaction. Bioactive alkaloids from *Withania somnifera* are screened from ADMET prediction following Lipinki's rule of five gave a successful result. Lipinski's rule of five confirms the nature of the promising drug-like quality of alkaloids through canonical SMILES verification for each ligand. Only drug-like candidates are selected for docking with the protein that gave the highest docking score. Molecular docking has shown that eight different active alkaloids are effective with the highest docking score, WithanolideD (–10.0 Kcal/mol), Withanolide (–10.0 Kcal/mol), WithaferinA (−9.9Kcal/mol), WithanolideB (−9.5 Kcal/mol), WithalongolideA (−9.4 Kcal/mol), WithanolideE (− 9.4 Kcal/mol), WithanolideA (− 9.0 Kcal/mol), Withanone (−8.9 Kcal/mol). The most stable hydrogen bonding is shown by WithalongolideA with VAL A:14 (2.05 Å bond distance), WithaferinA with GLN A:24(2.05 Å) and WithanolideE with VAL A:93 (1.77 Å). The strong and stable hydrogen bonding with bond distance < 2Å protein-ligand interaction is considered a potential inhibitor which is shown by Withaferin, WithanolideA, WithanolideD, WithanolideE, and Withanone. So, these alkaloids become the possible candidates to inhibit MDM2-P53 interaction. Molecular Dynamics of protein-ligand complex reveals that docked complexes are thermally stable. Their structural conformation has not changed up to 100ns time frame. 5TRF and WithanolideA complex shows stable bonds below 1500Kcal/mol and distinct bond angle below 1000Kcal/mol up to 100ns. RMSD, RMSF plot is used to analyze the stability of complex which shows that WithanolideA forms a very strong and stable hydrogen bonding without fluctuation in the given time frame. Molecular docking, simulation, and dynamic of different alkaloids from *Withania somnifera* are found to be potential inhibitors against MDM2-p53 interaction, which profounds the possibility of drug-like properties of the complex to expand further investigation in the direction against tumor growth.

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