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MOLECULAR DOCKING AND MOLECULAR DYNAMICS STUDIES OF QUASSINOIDS AS HIV-1 TAT INHIBITORS

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ABSTRACT: TAT (Trans-activator-Transcription Protein) is a viral protein encoded by the TAT gene in HIV-1-which is a lethal subtype of HIV (Human immunodeficiency Virus). It is vital for transcription of the viral genome. Previous studies show that in Human, TAT is a toxin-producing protein allowing cell death in obtained from QSAR studies, best quassinoid compounds were used to find the highest binding affinity compound by performing normal T-cells. Thereafter allows for progression towards AIDS (Acquired immunodeficiency syndrome). Traditionally, herbal medicines have played a vital role in the treatment of many diseases and ailments. Likewise, Quassinoids from the plant family Simaroubacaea, possess insecticidal, antimalarial and anticancer properties. Although studies have been conducted to find anti-HIV activities against other HIV-1 proteins, there are no traces of studies against Trans-activator-Transcription protein (1JFW). The main objective of this study is to find an efficacious inhibitor against a synthetic HIV TAT protein (PDB- IJFW). After a thorough literature survey, the molecular and biological activity of quassinoid phytoconstituents has been recorded for QSAR (Quantitative structure-activity relationship) studies. Based on the results obtained from QSAR studies, best quassinoid compounds were used to find the highest binding affinity compound by performing docking analysis. Finally, the best compound with the highest binding affinity along with its measurement has been viewed in PYMOL. Furthermore, the aforementioned results from the docking studies were used to perform molecular dynamics simulation to confirm the efficiency of the compound against HIV-1.

INTRODUCTION: Acquired Immunodeficiency Syndrome is caused by the lethal Human Immunodeficiency Virus (HIV), [1, 2] which spreads through the vital cells in the human immune system specifically, the CD^{4+} T cells and dendritic cells [3, 4]. HIV is classified into two types, namely HIV-1 and HIV-2 that differ in their virulence.

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HIV-1 is more fatal compared to HIV-2 [5]. Statistics reveal that till date alone, approximately 34 million people are living with HIV worldwide, and 8 million are surviving on anti-retroviral drugs. HIV-1 infection can be targeted at various stages, for example, viral entry, viral replication or assembly of viral components. Unfortunately, there is no cure for HIV (Human Immunodeficiency Virus) [2, 4, 6].

HIV-I TAT (Trans-activator-Transcription Protein) is a viral protein encoded by the TAT gene. It is essential for transcription of the viral genome. The primary function of the protein is reproduction of the virus but it also plays a pivotal role in HIV-based immunodeficiency.

TAT is a short protein encoded by two exons and its size varies from 86 to 106 residues [7]. TAT contains six different regions with distinct functions. Region I (residues 1–21) is a proline-rich region and has the conserved Trp 11. Region II (residues 22-31) has seven conserved cysteines at positions 22, 25, 27, 30, 31, 34 and 37. No other cysteines are found in the sequence and there is no evidence of disulphide bridges required for TAT function. Region III (residues 38-48) has the Phe 38 and the conserved sequence LGISYG from residues 43-48. Region IV (residues 49-59) is rich in basic residues and has the conserved sequence RKKRRQRRRPP. Region V (60-72) is a glutamine-rich region. Region VI constitutes the C-terminus of TAT encoded by the second exon but its size can be variable depending on the HIV isolate. The C-terminus shows similarities with the N-terminus in a high percentage of prolines. Resolution of this structure was a determining factor in drug designing. The chemical synthesis of the drugs allowed the specific binding and the inhibition of TAT to be verified [8].

Quassinoids refer to the group of chemically altered triterpenes that has undergone extensive oxidative biodegradation of the Simaroubaceae plant family. The Quassinoids are natural phytochemicals that contribute towards anti-malarial, Insecticidal, anti-inflammatory, and anticancer activities. Pentacyclic derivatives of the quassinoids have shown a remarkable anti-tumor activity. The main active groups of quassinoids are ailanthionone and benzoquinone. Theailanthionone consists of glaucorubinone and halocanthone while, benzoquinone consists of glaucarubine, simarolide, sitosole and melianone [9]. The antimalarial activities of the quassinoids were evaluated earlier by the folate or antiifolates drugs which prevent folic acid production, essential for the folate dependent enzymes. These plant extracts are known widely for their anti-leukemic activity besides showing a host of other medicinal properties [10]. The main aim of this study was to investigate the interaction between quassinoid compounds and the active sites of the synthetic HIV-1 TAT (PDB: IJFW) protein, [8] using Argus Lab software to propose its efficiency as an anti-HIV agent. Furthermore, the quassinoids were subjected to molecular properties prediction to filter and confirm their anti-HIV activity. The dynamics study was executed to understand the stability and flexibility of the protein-ligand complex to identify pharmacological targets.

MATERIALS AND METHODOLOGY:

Compounds Collection: Compounds collected from literature studies were utilized for this study. After thorough literature studies, 36 quassinoid compounds with IC_{50} values and pIC_{50} have been retrieved (See **Table 1**) [9].

Calculation of ADME properties and pIC_{50} Values: Molinspiration server was used to predict the molecular property of the alkaloids that predicts both physiochemical and pharmacological properties such as LogP, hydrogen bonding characteristics, molecular size, and rotatable bonds. Thus, the Quassinoids were subjected to Molinspiration server analysis. Furthermore, the scores were compared with the standard anti-HIV drug [15]. Lipinski's rule of five was used to evaluate the acceptability of the quassinoids. For QSAR studies, the IC_{50} value of each compound is converted into the predicted pIC_{50} values by using Sanjeev's Lab online tools [16].

QSAR: QSAR study was performed for 20 drug-like compounds using BUILDQSAR software. The parameter is separated into two groups of variables *i.e.* dependent variables (pIC_{50}) and independent variables (number of atoms, Molecular weight, hydrogen donors, hydrogen acceptors, LogP, number of rotatable bonds, number of Violations and Total polar surface area (TPSA)). Best correlation activity compound was selected against HIV-1 from multiple linear regression (MLR) graph, generated for dependent variable against the independent variable group [17].

Protein and Ligand Preparation: The 2-Dimensional structures of the compounds downloaded from PUBCHEM in SDF format, were converted to PDB format using Pymol molecular graphic system, version 1.5.0.3 (www.pymol.org). The Three Dimensional structure of the synthesized TAT protein (PDB ID: 1JFW) was retrieved from Protein Data Bank (PDB) [8]. The binding pockets of the synthesized TAT protein were predicted using the Meta pocket online tool [13].

Molecular Docking: The Docking interaction of the synthesized TAT protein with one best compound obtained from QSAR study, was carried

out using Argus lab software. Docking was performed using "Genetic Algorithm (GA) Dock" exhaustive search with a grid resolution of 0.40 Å. Docking precision was set to "Regular precision" and "Flexible" ligand docking mode was employed for each docking run. The stability of each docked pose was evaluated using Argus Lab energy calculations and the number of hydrogen bonds formed.

Molecular Dynamics Simulation: The simulation of the ligand-enzyme complex was performed using the GROMACS5.1 software. Ligand-enzyme complexes with the lowest binding energy were selected for molecular dynamics (MD) simulation. The ligand parameters were analyzed using PRODRG online server in the framework of the GROMOS force-field 43a1. The ligand-enzyme complex was solvated using a simple point charge water box, under periodic boundary conditions using 1.0 nm distance from the protein to the box faces. The system was then neutralized by Cl^- or Na^+ counter ions for TAT-Holacanthone and TAT-Holacanthone complex systems, respectively.

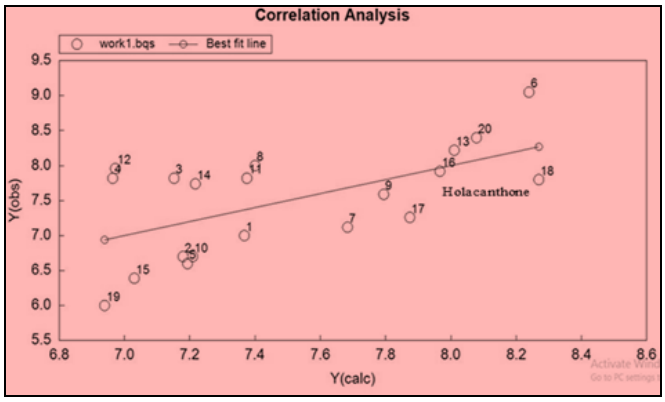
Energy minimization was done for 1,000 steps by the steepest descent method. For the sake of flowing energy minimization, the systems were equilibrated under a constant number of particles, volume, and temperature conditions for 100 ps at 300K, followed by 100 ps under a constant number of particles, pressure, and temperature conditions. All the covalent bonds with hydrogen atoms were constrained using the Linear Constraint Solver algorithm. Finally, MD simulation was performed for 25 ns to check the stability of the ligand-enzyme complexes. The potential of each trajectory produced after MD simulations were analyzed using Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation(RMSF), and Hydrogen-bond(H-bond) using the GROMACS Utilities. The graphs were produced using the grace tool.

RESULTS AND DISCUSSION: Total of 37 quassinoid compounds were collected from literature studies 9 Only 20 of the 37 compounds, abided to the Lipinski`s rule of five. The following quassinoid compounds classified by their plant species, were obtained using literary search: 4 compounds collected from the species *Quassia indica*; 2 compounds from the species *Brucea javanica*; 1 compound from *S. cedron*; 1 compound from *S. guianensis*; 2 compounds from *C. taxena*; 4 compounds from *A. altissima*; 1 compound from *E. longifolia*and 1 compound from *S. amera*. Remaining 3 compounds were not mentioned in the literature.

TABLE 1: SHOWS QUASSINOID COMPOUNDS DERIVED FROM VARIOUS PLANT SPECIES

Q. indica	*B. javanica*	*S. cedron*	*S. guianensis*	*C. taxena*	*A. altissima*	*E. longifolia*	*S. tometasa*	*S. amera*
SamederineY	Brucein D	Cedronin	Simalikalactone D	Glaucarubolone	Ailanthone	eurycamanone	Soularibinone	Ailanthinone
Samederine Z	Bruceolide			Holacanthone	Chapparin			
SamederineE					Glaucarabol			
Indiquassin C					Glaucarubin			

QSAR Studies: 37 compounds were used to perform ADME studies (using Molinspiration tool) to see which compounds bide the Lipinski rule of five. 20 compounds that abided the Lipinski rules were used for QSAR studies in order to find a potential compound against HIV TAT activity. The Molecular properties obtained from molinspiration tool has been tabulated for reference **Table 2**. The 20 compounds that supports Lipinski`s Rule of 5 were obtained and a correlation analysis was performed using Build QSAR tool. An MLR (Multiple linear regressions) graph was plotted and the best compound-Holacanthone was obtained. As per previous studies Holacanthone is one of the active groups amongst the quassinoid compounds [12]. This quassinoid compound has been used previously for antimalarial studies.



GRAPH 1: SHOWS THE MULTIPLE LINEAR REGRESSION GRAPH THE X-AXIS SHOWS THE DESCRIPTORS AND Y-AXIS CONTAINS THE pIC_{50}. HOLACANTHONE (LIGAND 16) WAS PREDICTED TO BE THE BEST COMPOUND

The best compound obtained from BUILDQSAR was used for molecular docking using Argus Lab.

TABLE 2: MOLECULAR PROPERTIES OBTAINED FROM MOLINSPIRATION TOOL

S. no.	Name	IC_{50}	pIC_{50}	miLogP	TPSA	natm	MW	nON	nOHNH	nviol	nrtb	vol
1	Samederine Y	100	7	0.22	113.29	27	378.4	7	3	0	0	333.2
2	Samederine Z	200	6.7	-0.39	133.52	28	394.4	8	4	0	0	341.2
3	Samederine E	15	7.82	-0.73	133.52	28	394.4	8	4	0	0	341.2
4	Brucein D	15	7.82	-1.33	153.75	29	410.4	9	5	0	0	348.9
5	Cedronin	31	7.51	-1.15	113.29	26	364.3	7	3	0	0	316.4
6	Simalikalactone D	250	6.6	1.73	139.6	34	478.5	9	3	0	4	427.9
7	Bruceolide	2	8.7	-0.32	159.83	31	438.4	10	4	0	2	369.2
8	Chaparrinone	0.9	9.05	0.59	113.23	27	378.42	7	3	0	0	333.2
9	Mefloquine	8.5	8.07	4.29	45.15	26	378.32	3	2	0	4	296.9
10	Glaucarubolone	75.2	7.12	-0.02	133.52	28	394.42	8	4	0	0	341.2
11	Ailanthone	8.2	8.09	0.42	113.29	27	376.4	7	3	0	0	327.5
12	eurycamanone	0.8	9.1	-1.13	153.75	29	408.4	9	5	0	0	343.3
13	Soularubinone	3	8.52	0.61	159.83	35	494.5	10	4	0	4	435.6
14	Chaparrin	2	8.7	0.77	116.45	27	380.4	7	4	0	0	339
15	Glaucarabol	11	7.96	0.17	136.68	28	396.4	8	5	0	0	347.1
16	Halocanthone	5	8.3	0.68	139.6	31	436.4	9	3	0	2	377.7
17	Glaucarubin	46	7.34	1.34	162.98	35	496.5	10	5	0	4	441.5
18	ailanthinone	10	8	2.1	139.6	34	478.5	9	3	0	4	427.9
19	Indaquassin C	26	7.59	-1.64	153.75	29	410.4	9	5	0	0	348.9
20	Gutolactone	200	6.7	1.78	139.6	34	476.5	9	5	0	3	421.7

[Note: TPSA- Total Polar surface area, natm- number of atoms, MW- Molecular weight, nON- No. of H-bond acceptors, nOHNH- No. of H-bond donors, nviol- number of violation, nrtb –number of rotatable bond and Vol- Volume].

Molecular Docking Results: Holacanthone was further used for docking studies against HIV-1 TAT proteins using Argus Lab and was found to have a binding energy of -8.87072 kcal/mol. Holacanthone interacted with the protein forming Hydrogen bonds. Hydrogen bond interactions occur between active residues -Ser-68, Arg-7 and GLN-54 and the ligand (see **Table 3**).

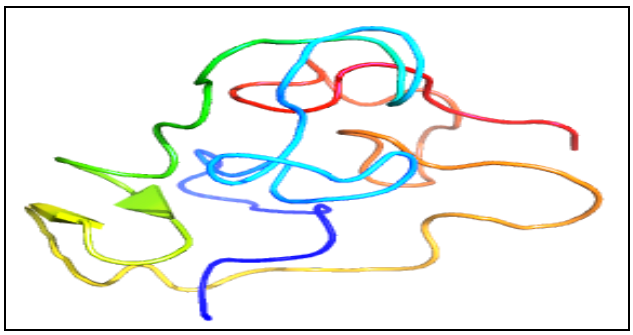


FIG. 1: 3D IMAGE OF SYNTHETIC HIV-1 TAT PROTEIN, OBTAINED FROM PROTEIN OBTAINED FROM PROTEIN DATA BANK (PDB ID: 1JFW) AND VISUALIZED IN PYMOL

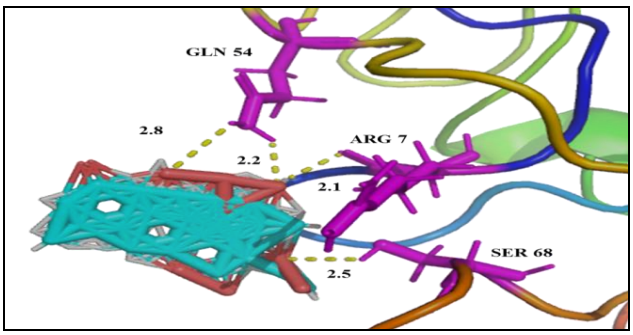


FIG. 2: PREDICTED BEST SCORE COMPOUND INTERACTION WITH ACTIVE SITE RESIDUES

TABLE 3: SHOWING THE BINDING ENERGY AND INTERACTING SITES OF HALOCANTHONE WITH SYNTHESISED TAT PROTEIN

S. no.	Compounds	Position (QSAR)	Interacting sites	Binding energy Kcal/mol	Distance (Å)
1	Holacanthone	16	SER-68---H---O	-8.57	2.5
			ARG-7---O---H		2.1
			GLN-54---H---O		2.2
			GLN-54---H---O		2.8

Molecular Dynamic Simulations: Molecular Dynamics simulations are carried out to determine the structural stability within a nanosecond time scale for TAT-Holacanthone complex. This complex is selected based on its least binding energy and is subjected to 25 ns MD simulations, and the result is analyzed as follows:

Root Mean Square Deviation (RMSD): RSMD, a crucial parameter to analyze the equilibration of MD trajectories, is estimated for backbone atoms of the complex. Measurements of the backbone RMSD for the complex provided insights into the conformational stability.

Holacanthone-TAT complex was analyzed for RMSD and the RMSD graph depicts that the residues show slight fluctuations at 10 ns and at around 22 ns. From 11 ns to 20 ns the complex remains stable.

H-bond: The intermolecular H-bonding between the protein and ligand plays a vital role in stabilizing the protein-ligand complexes. The stability of the hydrogen bond network formed between Holacanthone and synthesized TAT-protein is calculated throughout the 25 ns simulation period. The Hydrogen bond interaction for the aforementioned complex is stable from 11ns to 25 ns.

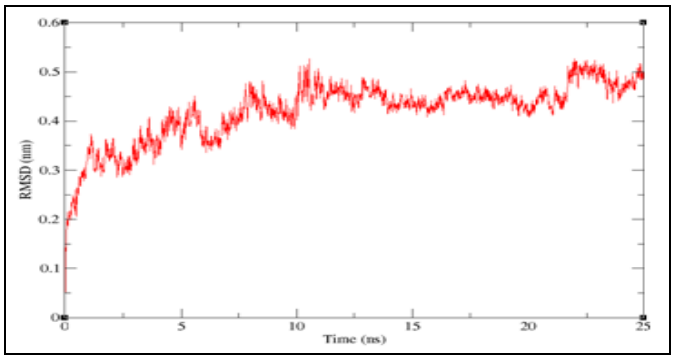


FIG. 3: RMSD GRAPH HOLACANTHONE WITH SYNTHESIZED TAT PROTEIN

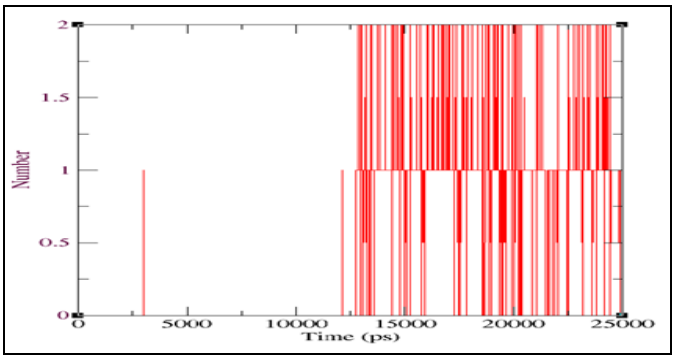


FIG. 4: THE MOLECULAR H-BOND GRAPH SHOWING THE STABILITY FOR HOLACANTHONE WITH SYNTHESISED TAT PROTEIN

CONCLUSION: In the present study, the molecular docking and MD simulations are performed to interpret the possible binding affinity of synthesized TAT protein with the best predicted Quassinoids compound-Holacanthone. The best ligand conformation is chosen based on binding free energy value, hydrogen bonding, and hydrophobic interaction. The conclusion drawn from the docking analysis is that Holacanthone interacts well with synthesized TAT protein (PDB: IJFW). Furthermore, MD simulation is performed to analyze the binding stability of synthesized TAT and Holacanthone. RMSD and H-bond results reveal that TAT-Holacanthone complex is stable. Though there are several reports on the medicinal use of Quassinoids for other diseases, there is no *in-silico* study predicting the anti-HIV activity of Quassinoids against TAT activity. Our study is perhaps the first to attempt to predict that Holacanthone is a potential anti-HIV drug molecule. Hopefully, the proposed molecule can be put forward for a constructive concept of designing HIV inhibitors. Thus, it might be a useful candidate for HIV therapy.

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CONFLICT OF INTEREST: Nil

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