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RESEARCH ARTICLE

Identification of Natural Terpenoid Compounds as Potential Inhibitors of Nucleoprotein of Influenza A Virus using in silico Approach: ADMET, Molecular Docking, and Molecular Dynamic Simulation

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Abstract: ***Background:*** We continue to struggle with the prevention and treatment of the influenza virus. The 2009 swine flu pandemic, caused by the H1N1 strain of influenza A, resulted in numerous fatalities. The threat of influenza remains a significant concern for global health, and the development of novel drugs targeting these viruses is highly desirable.

Objective: The objective of this study is to explore the inhibitory potential of terpenoid compounds against the Nucleoprotein (NP) of influenza A virus, which is a highly effective drug target due to its ability to facilitate the transcription and replication of viral RNA.

Method: *In silico* research was performed to identify potential inhibitors of NP. Molecular docking studies were conducted to assess the binding of terpenoid compounds to the active site residues of the target protein. The most promising hits were then subjected to molecular dynamics simulations to examine the stability of the protein-ligand complexes. Additionally, ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) studies and Lipinski's rule of five were employed to evaluate the drug safety and druglikeness of the compounds.

Result: Docking studies revealed that the terpenoid compounds bind strongly to the active site residues of the NP protein. Molecular dynamics simulations demonstrated the stability of the protein-ligand complexes for the best-hit compounds. ADMET studies and Lipinski's filter indicated that the compounds exhibit desirable drug safety and drug-likeness profiles.

Conclusion: This work may contribute significantly to drug discovery and the development of therapeutic agents against the influenza A virus. The identification of terpenoid compounds that bind strongly to the NP protein and exhibit favorable drug-like properties through in silico studies provides a promising foundation for further research and the development of potential inhibitors targeting this critical viral protein.

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1. INTRODUCTION

The influenza A virus is one of the major causes of respiratory illness. Globally, every year, this infection causes 3 to 5 million severe cases and about 290,000 to 650,000 deaths (https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal)). The burden is much higher among vulnerable hosts, like children <5 years and >60 years aged population. The virus was also responsible for pandemics in 1918, 1957, 1968, and 2009 caused by H1N1 (Spanish flu), H2N2 (Asian flu), H3N2 (Hong Kong flu), and H1N1pdm09, respectively [1]. This virus remains a potential threat to global health due to its high antigenic evolution rate, ability to escape pre-existing immunity, and zoonotic

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nature that allows exchange or reassortment of RNA segments between viruses [2]. The vaccine is available to combat the virus, but efficacy is a concern, which warrants an update of the vaccine every year separately for the Northern and Southern Hemispheres [3]. Therefore, several countries, especially LMICs, have not included influenza vaccines in national immunization programs and clinically managed the diseases.

The influenza A virus genome contains eight negative-sense RNA segments, which encode proteins. The viral membrane comprises hemagglutinin (HA), neuraminidase (NA), and M2 proton channel proteins. Other proteins, such as matrix protein M1, nucleoproteins (NP), and nuclear export proteins, are found within the virus [4-5]. Drugs against the influenza virus are developed as neuraminidase and M2 channel blockers [6]. The first and second-generation M2 channel blocker drugs were amantadine and rimantadine, which are not currently in use due to resistance [6-7]. Viral neuraminidase inhibitor drugs zanamivir and oseltamivir are now used for the clinical management of diseases [8-9]. Due to frequent use, resistance has emerged against neuraminidase inhibitor drugs [10-12]. Therefore, we immediately need to develop the next generation of antivirals for influenza [13].

Many compounds are in the preclinical and clinical stages of development against the influenza virus [9, 14-15]. As a structural protein with no inherent enzymatic function, NP is the predominant viral protein in infected cells. It also plays an important function in the transcription and replication of viral genomes [16-17]. Therefore, NP inhibitors could be the most valuable target for a broad spectrum of antivirals with multiple drug-binding sites [18-19]. Due to the conserved genetic nature, NP inhibitors could be less prone to developing resistance [6]. Plants and their derived natural products show enormous chemical composition and have been used in drug development, design, and research [20]. Terpenoids phytochemicals are low-molecular lipophilic compounds that can easily cross the blood-brain barrier [21-22]. These compounds have numerous medicinal properties, including anticancer, antioxidant, anti-inflammatory, antiviral, and antibacterial properties [23-26]. Computer-aided drug design and virtual screening have recently been used to filter out potential drug candidates from large databases [27]. Furthermore, molecular docking and recognition can play significant roles in understanding the complex relationships in the biological system.

In this study, we intend to identify potential phytochemicals that can act as NP inhibitors against the influenza virus and assess the drug-likeness properties of the identified phytochemicals using a computational approach. Molecular docking and other in silico methods for studying molecular interactions may be used to analyze promising ligands and receptor complexes in natural medicines. These methods have the aptitude to provide precise predictions that may be confirmed by later experimental and clinical studies, as well as to reveal information on previously undiscovered molecular structures, such as those of enzymes and their potential ligands. The in silico techniques allow proteins to be categorized based on their structure and function, and they may be useful for creating platforms for novel molecules utilizing machine learning [28]. Computational methods have been used to save time, cost, and labor since developing a drug costs $985 million and takes 10–15 years [29]. So, this study has been designed for terpenoids which was previously reported as a wide variety of pharmacological effects such as anti-inflammatory, antioxidant, anti-viral, anti-atopic dermatitis, *etc*. [30].

2. MATERIALS

2.1. Ligand and Protein Source

In this study, terpenoid derivatives were selected for in silico approach. The structure of terpenoid compounds was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). An amino acid sequence of nucleoprotein of the influenza A virus was collected from the UniPort (https://www.uniprot.org/) database (Accession# p15682). Using this sequence data, similar protein templates were retrieved from the NCBI data base, and we used PDB IDs to retrieve the similar protein data from RCSB protein data bank (https://www.rcsb.org/) (PDB id# 5TJW, 3ZDP, 2IQH, 4DYT, 7NT8, 2Q06, 6H9G, 7DKG, 4DYS, 6I85). Based on previous studies and reports, 65 terpenoid derivatives were selected for this study [31-32].

2.2. Active Site Finding

Sequence data from section 2.1 was used for pairwise alignment and to generate 3D protein structure by using MODELLER 4.0 [33]. The best protein model was identified by PROCHECK, which used the Ramachandran plot to verify the quality of the protein structure [34]. The active site of modeled NP was predicted by CASTp version 3.0 web-based server [35]. The CASTp 3.0 server identifies topographic features and calculates area and volume using the alpha shape method pioneered in computational geometry. In protein-ligand binding investigations, an alpha shape is utilized to simulate the protein surface, whereas discrete triangle flow is utilized for pocket or active site discovery.

2.3. ADMET Prediction

ADMET stands for Absorption, Distribution, Metabolism, Excretion, and Toxicity. It's a crucial aspect of drug discovery, as it refers to the processes a drug goes through in the body. Predicting these properties with ADMET prediction helps assess a drug candidate's potential for success. These pharmacological properties were used to evaluate the drug-likeliness of selected compounds [36]. ADMET predictions can guide medicinal chemists in modifying a molecule's structure to improve its drug-like characteristics. By predicting potential toxicity, ADMET studies can help identify molecules that might cause harmful effects. It also allows for the early elimination of problematic candidates and avoids potential safety issues later in development. For this purpose, we utilized two different tools: swiss-ADME (http://www.swissadme.ch/index.php) and pkCSM (https://biosig.lab.uq.edu.au/pkcsm/prediction) web servers.

2.4. Molecular Docking

The main goal of the molecular docking technique is to use computational methods to accurately predict the structure

of the ligand-receptor (NP) complex. Molecular docking involves examining the interactions between ligands and receptors, unraveling predictions of their interactions, and assisting in drug discovery and design efforts [37]. The three-dimensional protein structures were imported into the BIOVIA Discovery Studio Visualizer version 4.5 software to remove water molecules and unwanted heteroatoms in Figs. (**1** and **2**). Water molecules typically do not influence the substrate's binding to the receptor [38]. Therefore, they were removed to speed up computations and clear the binding site. Swiss PDB Viewer v.4.10 was used for energy minimization [39]. The PyRx version 0.8 virtual screening tools were employed to accomplish molecular docking [40]. Molecular docking and ligand optimization were carried out by Auto-Dock Vina using a universal force field (UFF) to minimize ligand energy [41]. BIOVIA Discovery Studio visualizer version 4.5 was used to analyze target protein-ligand interaction, the docking results, complex structure, binding affinity, non-binding interactions, and binding pocket between terpenoid (ligand) and NP (receptor).

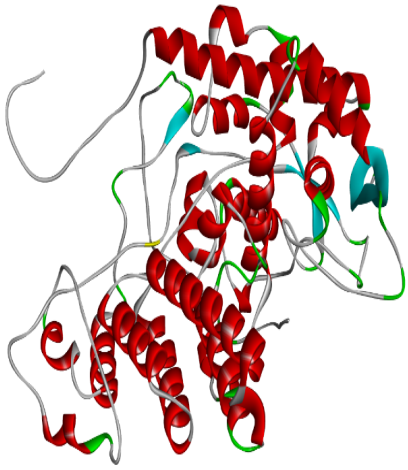


Fig. (1). Crystal structure of target Nucleoprotein (NP), these structure is created by homology modelling. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

2.5. Molecular Dynamic (MD) Simulations

Molecular dynamics simulations were conducted with the GROMACS package on the docked protein-ligand complex to assess structural stability and protein characteristics. It is also an essential tool for evaluating the stability of intra- or interatomic interactions within the protein-ligand complex over a user-defined timeframe. The highest-scoring docking models of the most promising inonotusol E with NP from the influenza A virus were selected as the initial configurations for a 100-ns all-atom molecular dynamics simulation.

Finally, 100ns MD simulation was performed in water on GROMACS simulation software package [42] with CHARMM36 force field[43]. For MD simulation, a cubical box with a 10Å distance was created and filled with water where the docked complex was immersed. Then Na^{+} and Cl^{-} were added in solvent to neutralize the system. For energy minimization, we used GROMACS system and created an environment with 1 atm pressure and 300k temperature. Lastly, a 100ns MD simulation was done to study the stability and changes in shape of the complex [44]. Root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), and hydrogen bond graphs are all calculated and xmgrace software [45] tools were used to make the graphs.

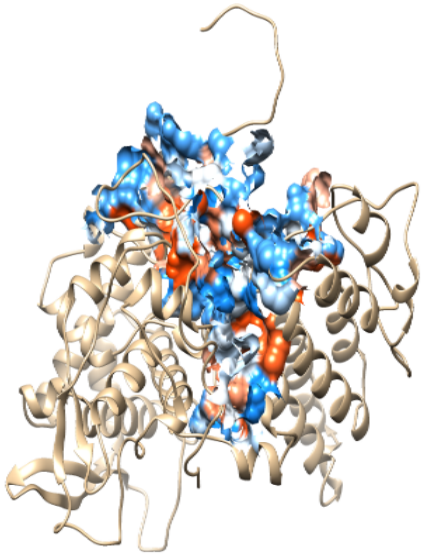


Fig. (2). Active residue of nucleoprotein (NP), the active sites are determined by the CASTp v3.0. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3. RESULTS AND DISCUSSION

3.1. Homology Modelling

Homology modeling is usually used for creating 3-D structure of protein by amino acid sequence of protein. During homology modeling phase, we targeted to construct experimentally protein structure with other nucleoproteins of influenza A virus that has a very high “sequence identity” [46]. For building a model by homology modeling we follow well defined known accepted steps: (1) sequence alignment between the target and template; (2) building an initial model; (3) refining the model and (4) evaluating the model.

For target, we used nucleoprotein of the influenza A virus sequence and for template, PDB ID: 5TJW, 2IQH, 4DYT, 6H9G, 4DKG protein sequence aligned. The template sequences are selected in BLAST based on the highest similarity sequence and lowest E-value. We determine the nucleoprotein crystal structure of chain A by using template and then modified the missing loop. The overall view of 3-D homology modeled protein is shown in Fig. (**1**). We validated our target protein by psi and phi angles of the Ramachandran plot which was used to assure the accuracy and reliability of the target protein structure. Many other protein validation tools, including PROCHECK, PROVE, ERRAT2, and VARIFY 3D were used to validate best 3-D modeled integrity. The best Ramachandran plots of protein shown in Fig. (**3**), 91.3% (399aa) of total residues are in most favored regions, and 7.6% (33aa) residues are in additional allowed regions and 0.5% (2aa), 0.7% (3aa) residues are in generously allowed regions and disallowed regions respectively, indicating very high quality of model in Figs. (**3** and **4**).

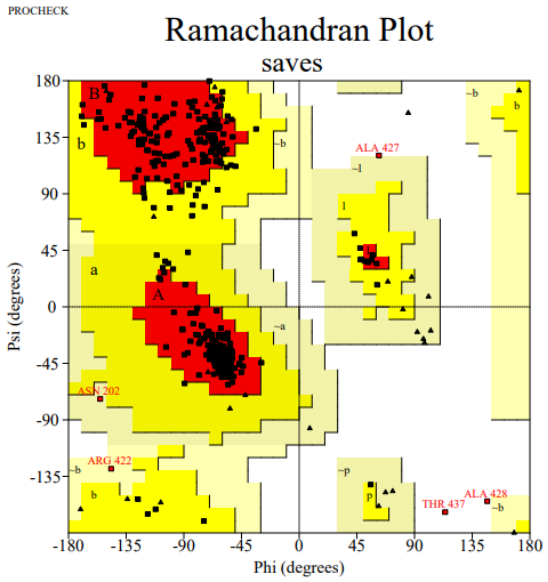


Fig. (3). Ramachandran plot. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The active site of our modeled protein is shown in Fig. (**2**), and it's crystal structure of amino acid residue is shown in Fig. (**1**). These predicted model proteins are used as a receptor in the following study docking and molecular dynamic simulation.

3.2. Pharmacophore Development and Ligand Screening

Based on different studies and reports in previous literature, 65 compounds were selected to accomplish this study. These selected terpenoids derivatives were used for further analysis through ADMET and drug likeness pharmacokinetics tools.

3.2.1. ADMET Studies

The studied compounds pharmacokinetic properties are presented in Tables **2-4**. These properties were carried out by using the pkCSM web server. Water solubility is a key contributor to drug absorption; higher water solubility indicates better absorption and bioavailability. The standard label of water solubility log S ranges from insoluble < -10, poorly soluble < -6, moderately < -4, soluble < -2 and highly

Table 1. Docking score and compound information.

Compound No.	CID Number	Chemical Name	Molecular Formula	Binding Affinity kcal/mole
1	139584951	Inonotusol E	C30H48O5	-9.7
2	128563	Salvinorin A	C23H28O8	-8.6
3	9909368	Ginkgolide A	C20H24O9	-8.5
4	139586026	Inonotusic acid	C21H28O2	-8.1
5	275196	Noscapine	C22H23NO7	-8
6	47936	Forskolin	C22H34O7	-7.4
7	442202	Drimenin	C15H22O2	-7.2
8	14433053	3b-hydroxycinnamolide	C15H22O3	-7.2
9. std	60855	zanamivir	C12H20N4O7	-7.1

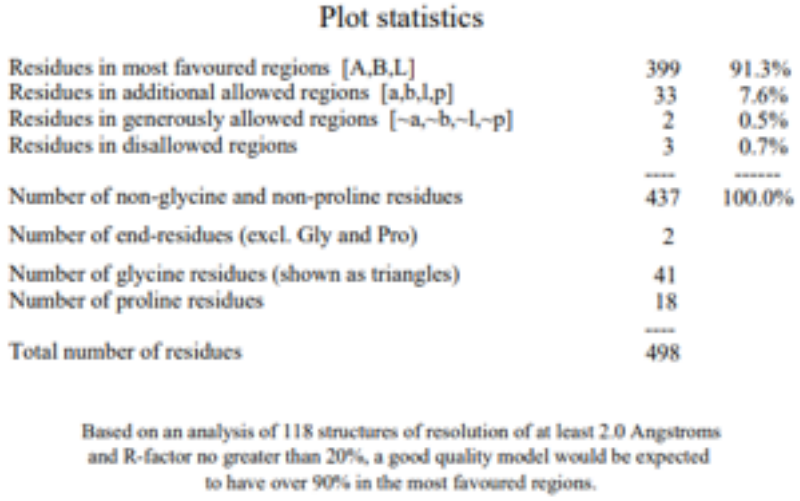


Fig. (4). Ramachandran plot statistics.

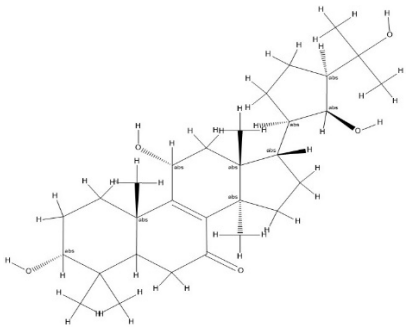
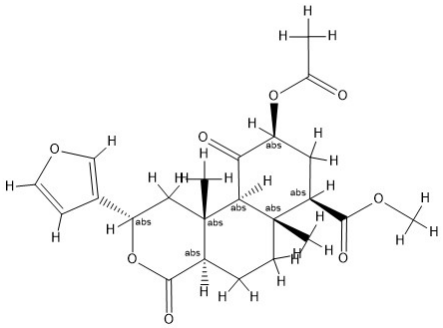
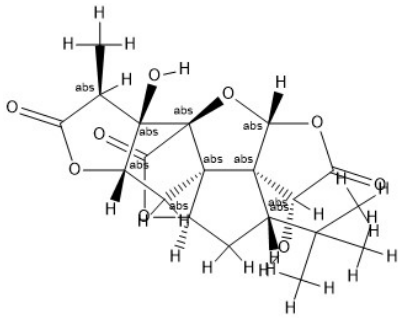
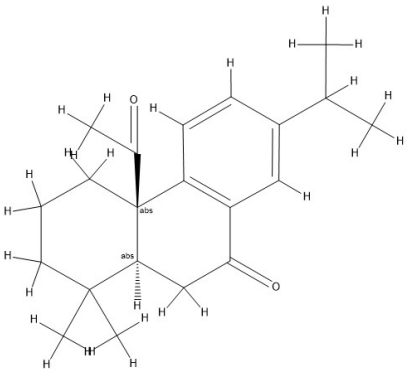
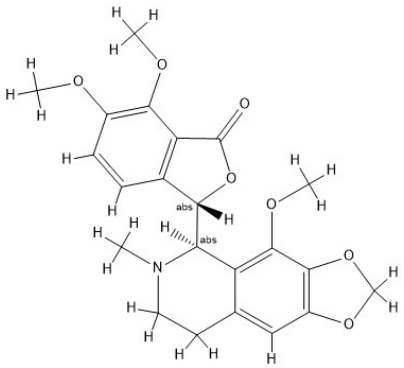
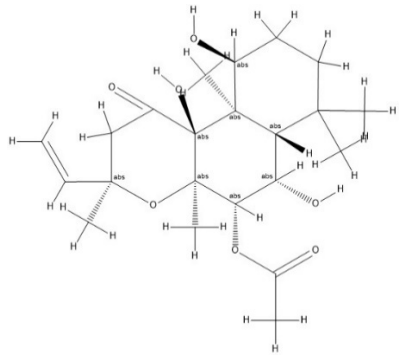
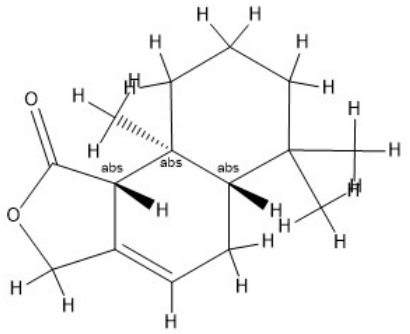
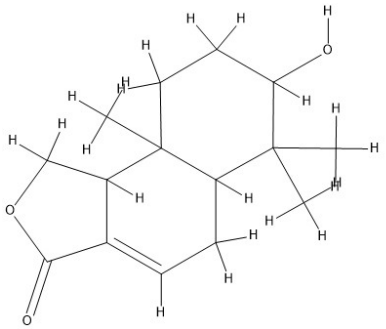
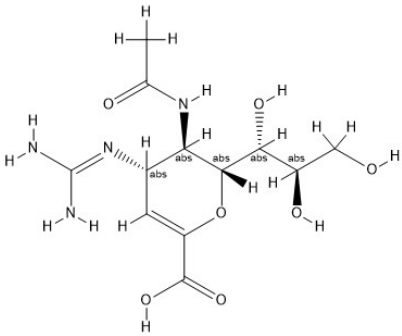
1. Inonotusol E	2. Salvinorin A	3. Ginkgolide A
		
4. Inonotusic acid	5. Noscapine	6. Forskolin
		
7. Drimenin	8. 3b-hydroxycinnamolide	9. zanamivir (std)
		

Fig. (5). 2d structure of reported ligands.

Table 2. Drug likeness.

Compound No.	Molecular Weight	Number of Rotatable Bonds	Hydrogen Bond Acceptor	Hydrogen Bond Donor	Molar Refractivity	Partition Coefficient Log P	Lipinski RULE		Bioavailability Score
							Result	Violation	
1	488.7	2	5	4	139.13	3.89	Yes	0	0.55
2	432.46	5	8	0	107.39	2.93	Yes	0	0.55
3	408.4	1	9	2	92.13	1.56	Yes	0	0.55
4	312.45	2	2	0	95.04	3.18	Yes	0	0.55
5	413.42	4	8	0	109.88	3.29	Yes	0	0.55
6	410.5	3	7	3	106.7	2.67	Yes	0	0.55
7	234.33	0	2	0	68.17	2.74	Yes	0	0.55
8	250.33	0	3	1	69.33	2.32	Yes	0	0.55
9. std	332.31	7	8	7	75.65	0.42	No	2	0.17

Table 3. ADMET data.

Compound No.	Absorption			Distribution		Metabolism		Excretion		Toxicity		
	Water Solubility Log S	Caco-2 Permeability x 10-6	Human Intestinal Absorption (%)	VDss (log L/kg)	BBB Permeability	CYP450 1A2 Inhibitor	CYP450 2C9 Substrate	Total Clearance (ml/min/kg)	Renal OCT2 Substrate	Max. Tolerated dose (log mg/kg/day)	Skin Sensitization	Hepato-toxicity
1	-4.991	0.708	High	-0.245	No	No	No	0.178	No	-1.159	No	No
2	-4.577	0.841	High	-0.288	No	No	No	0.497	No	-0.227	No	No
3	-4.21	1.126	High	0.339	No	No	No	0.271	No	-0.654	No	No
4	-5.951	1.71	High	0.993	Yes	No	No	1.018	No	-0.231	No	No
5	-3.89	1.196	High	0.438	No	No	No	0.943	No	-0.127	No	No
6	-4.246	0.501	High	0.232	No	No	No	0.764	No	0.099	No	No
7	-4.249	1.632	High	0.428	Yes	No	No	0.862	Yes	0.043	Yes	No
8	-3.129	1.295	High	0.303	Yes	No	No	0.963	No	0.339	No	No
9 std.	-2.892	-0.62	Low	-0.08	No	No	No	0.347	No	0.454	No	No

Table 4. Short-range Coulombic energies (Coul-SR) and Lennard-Jones energies (LJ-SR)

Energy	Inonotusol E	Zanamivir
Col-SR	-1.247*106	-1.235 * 106
LJ-SR	132172	91301
Total	-1.114*106	-1.143*106

soluble from -2 to zero. Table **4** shows compound 5 is more water-soluble than compound 9, which is marginally more water-soluble. Maximum and minimum Caco-2 permeability from compounds 4 and 6 were 1.71 and 0.501, respectively. The human intestinal absorption (HIA) rate determines how efficiently a drug is absorbed after oral administration. Human intestinal absorption was high fors all ligand compounds except the standard compound.

Blood–brain barrier (BBB) and volume of distribution (VD) are drug distribution characteristics. Compound 2 has a lower VD value (-0.288), which suggests that the distribution of the therapeutic drug in plasma is more uniform than in tissue, whereas compound 4 has a higher VD score (0.993) reflects that the pharmaceutical molecule is more uniformly transported in tissues. Additionally, the Blood brain barrier permeability of all compounds are negative except 4, 7, and 8 ligand compounda. Our ligand cannot be inhibited and substrated by the CYP450 1A2 and CYP450 2C9 enzymes. This enzyme is commonly found in human liver both are important for drug metabolizing enzyme.

Organic cation transporter 2 (OCT2) mediates the initial step in renal secretion of organic cations [47]. The OCT2 substrate plays a vital role in improving renal clearance. Only compound 7 was expected to function as OCT2 substrates. None of our compounds exhibited any toxicity, such as Hepatotoxicity but compound 7 showed Skin Sensitization. Standard drug compound shows maximum tolerated dose 0.454 log mg/kg/day.

Overall, our studied compound shows a promising result to become a next therapeutic agent against the influenza A virus.

3.2.2. Drug Likeness Studies

Drug likeness is a concept used in drug design to assess how similar a molecule's properties are to those of existing drugs. Molecules with good drug likeness are more likely to be successful in the drug development process because they share characteristics that make them easier for the body to absorb, distribute, metabolize, and excrete (these are the processes captured by ADMET). It is a qualitative property of a drug compound that is widely used in drug discovery [48]. Pharmacokinetic properties were approached academically in 1997 when Lipinski and colleagues published the Rule of 5 (Ro5) based on the study of 2245 drug properties in the World Drug Index (WDI) databank accepted for phase 2 clinical trials [49]. The physiochemical properties of drugs like molecular weight, number of rotatable bonds, hydrogen bond donor and acceptor, molar refractivity and partition coefficient are analysis and drug likeness properties as Lipinski rule violation, bioavailability and synthesis accessibility are analyzed in this section. We used the web based tool swiss-ADME(http://www.swissadme.ch/index.php) to obtain the result. Here all the compounds the Lipinski rule except compound zanamivir standard drug, which violated 2 Lipinski rule from five rules shown in Table **2**. The bioavailability score of the first 8 compounds are 0.55 and of the compound zanamivir, the score is 0.17.

3.3. Molecular Docking Analysis

Because of the simple access to databases of small and large molecules, as well as the advancements in computer power and availability, performing molecular docking has become increasingly common. Molecular docking software is used to comprehend and forecast structure recognition, energetic binding affinity prediction, and binding mode prediction. Molecular docking tools provide information about how a ligand interacts with the target through the three-dimensional representation of the interaction. However, this is not enough to finalize a compound as a potent drug, hence, an essential tool has to solve real world problems like molecular dynamic simulations. Terpenoid derivatives were prepared for molecular docking against the target receptor protein of the influenza A virus. Molecular docking was carried out to get elaborate information about protein ligand complex interactions and binding affinity. The compound was screened based on the binding affinity score and the interaction in Table **1** shows a minimum binding affinity score. Docking studies play a very important role in computational based drug design because of analyze ligand configuration with the active site of receptor protein and also analyze nonbond interaction. The present study shows very promising binding energy from -9.7 to 7.1 kcal/mole in Table **1**. Herein, the standard drug zanamivir has -7.1 kcal/mole binding energy. The FDA-approved influenza A and influenza B treatment drug zanamivir has been include in these studies, which has much lower binding affinity than the terpenoid derivatives of our studied compound.

3.4. Molecular Docking Pose and Interaction Analysis

Molecular docking pose and interaction analysis have been carried out to evaluate the binding region of protein-ligand and how many active sites are present in the binding regions [50]. Inonotusol E established hydrogen bonds with ARG-267 in a usual manner, Alkyl and Pi-Alkyl form covalent bonds with PHE-479, VAL-343, PRO-477, VAL-463, ARG-461, and PHE-458 amino acid residues of proteins (Figs. **5** and **6**). Comparatively, Salvinorin A formed typical hydrogen bonds with ILE-388 as well as alkyl and pi-alkyl bonds with PHE-458 and VAL-343 (Fig. **6**). Ginkgolide A produced alkyl and pi-alkyl bonds with PHE-458 and ARG-461 in addition to two typical hydrogen bonds with ILE-388 and THR-390. Inonotusic acid exhibited a Pi sigma bond with PHE-458 was produced while alkyl and Pi-alkyl bonds were created by VAL-476, PRO-477, and PHE-479 residues, with PHE-458, a pi-pi stacking bond was also created. The standard compound has six conventional hydrogen bonds with TYR-487, PHE-338, SER-165, SER-392, ARG-391 and GLN-168. All docking interactions are shown in Fig. (**6**).

3.5. Molecular Dynamics (MD) Simulation

MD simulation provides a forward-looking understanding of the equilibrium and stability properties of the fully solvated membrane protein (NP) complex [51]. Virtual screening aims to search the hits and leads compounds based on their physiochemical and pharmacokinetic properties. It's very advanced and importance tools for computer aided drug design (CADD), these not only offer insight into natural dynamics to bring the bio-molecular structure to alive but also provide details on stability and conformational changes of complex. MD simulation is used to examine the binding stability of protein-ligands complex that we get from docking. MD simulation also provides information about intermolecular interaction with respect of time [52]. We analyzed MD simulation of protein-ligand complex that shows best docking score (binding affinity) and standard complex here 100ns of simulation carried out for explore stability and intermolecular interactions of complex. We describe simulation results based on RMSD, RMSF, SASA, and Rg in order to find the stability of protein (NP) and ligand complexes.

3.5.1. RMSD Analysis

Root Mean Square Deviation (RMSD) in MD simulation is useful because it measures the average distance generated by displacement of atom (amino acid) during simulation in a specific time frame with respect to the reference time frame. Depending on RMSD result, we can make a decision on whether the simulation has equilibrated or not. Where a much larger value of RMSD graph indicates a more conformational change of the protein and the system is unstable [53]. RMSD of two protein ligand complexes and standard complexes for 100ns are shown in Fig. (**7**). Complex 1 RMSD graph, in beginning to 20ns gradually rises, after that it has stable graph from 20 to 40ns. Certain deviations are observed from 40ns to 55ns and rest of the graph was quite stable. On the other hand, the standard complex has stable RMSD from 35ns to 85ns. The stable RMSD demonstrated that the protein ligand complex was maintained in equilibrium during the course of the simulation and that the binding complex was stable.

Overall, Complex 1 has better stability than standard one.

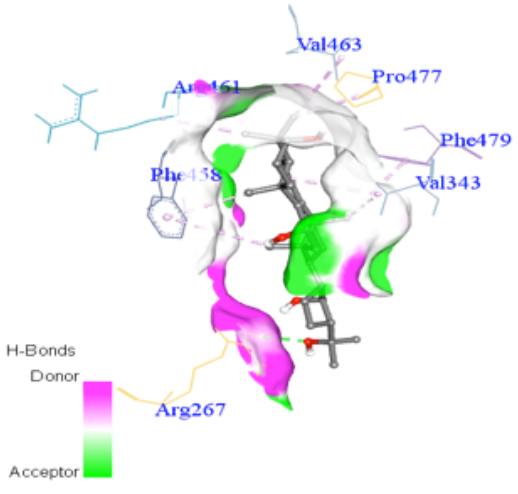
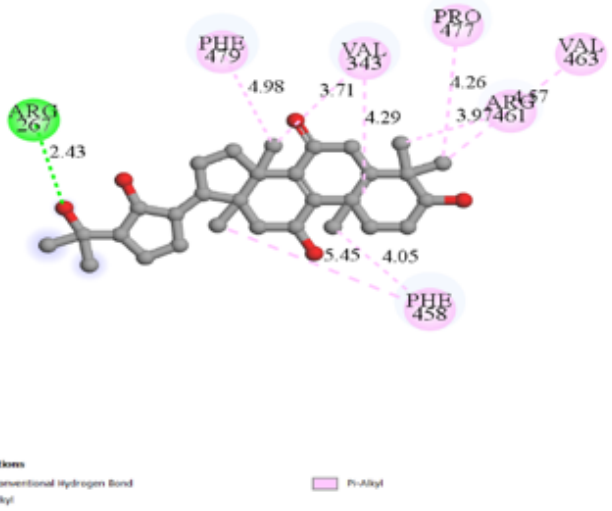
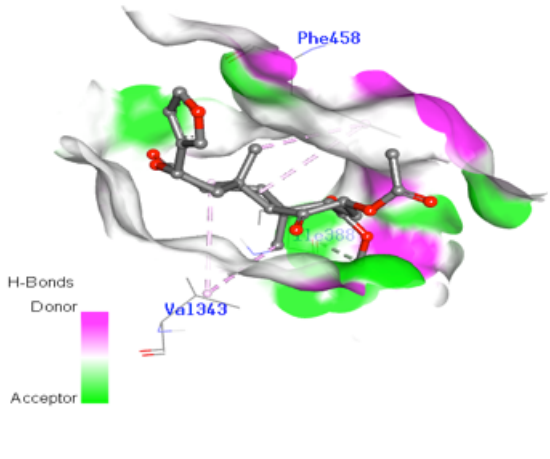
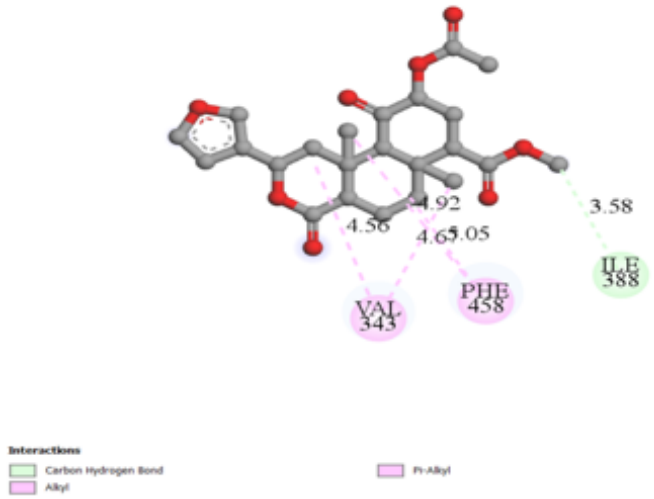
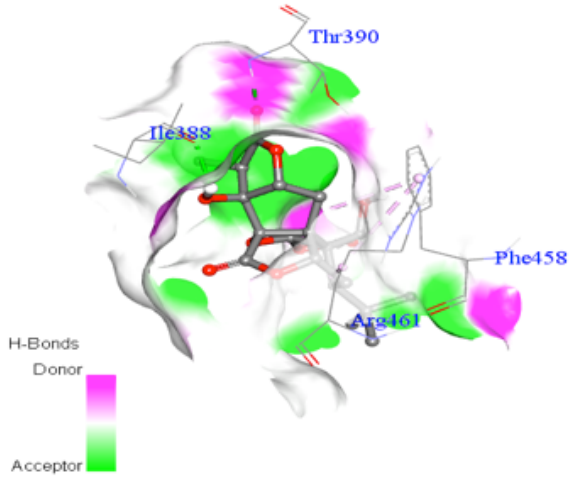
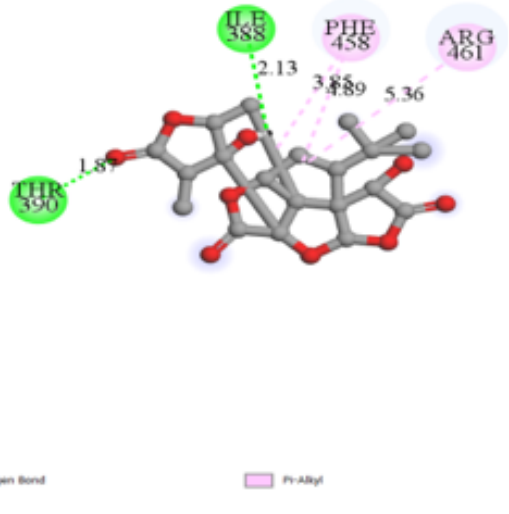
3.4.2. RMSF Analysis

The Root Mean Square Fluctuation (RMSF) is an important statistical tool in MD simulation where we can determine conformational changes and show the flexibility of amino acid residue. RMSF demonstrates the dynamism of the protein-ligand interaction. If the RMSF graph fluctuates very large, it means the amino acid residues are unstable, otherwise, the residues are stable [54]. The protein zanamivir complex shows much higher RMSF graph than the other graphs in Fig. (**7**) between residue 400 to 420 which is the highest RMSF value. Rest of the residue shows an RMSF value of less than 0.5nm. Therefore, we can say the inonotusol E (complex 1) ligand is more stable than the zanamivir (standard) ligand.

The residues 400 to 420 that have high RMSF values are part of a loop that is in opposition to the active site. Since these residues are not involved in the ligand binding side, we believe they have no impact on the stability or the inhibitor of this enzyme. It has been established that loops frequently exhibit conformational changes [55-56].

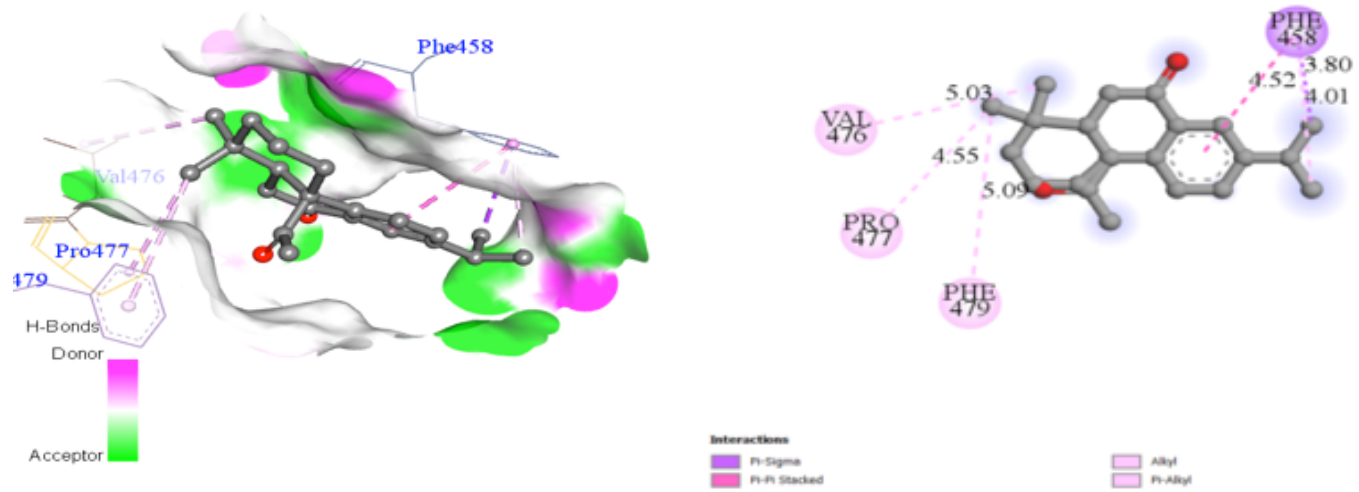
3.4.3. SASA

The surface area of proteins that is available in the water that surrounds them is referred to as the Solvent Accessible Surface Area (SASA). It is one of the crucial parameters that gives insight into the structural folding and unfolding dynamics of a protein while it is in an aqueous solvent, and it is one of the most critical parameters [57]. SASA value of the

Hydrogen bond	2D interections
Nucleoprotein and Inonotusol E	
	
Nucleoprotein and Salvinorin A	
	
Nucleoprotein and Ginkgolide A	
	

(Fig. 6) Contd….

Nucleoprotein and Inonotusic acid



Nucleoprotein and Noscapine

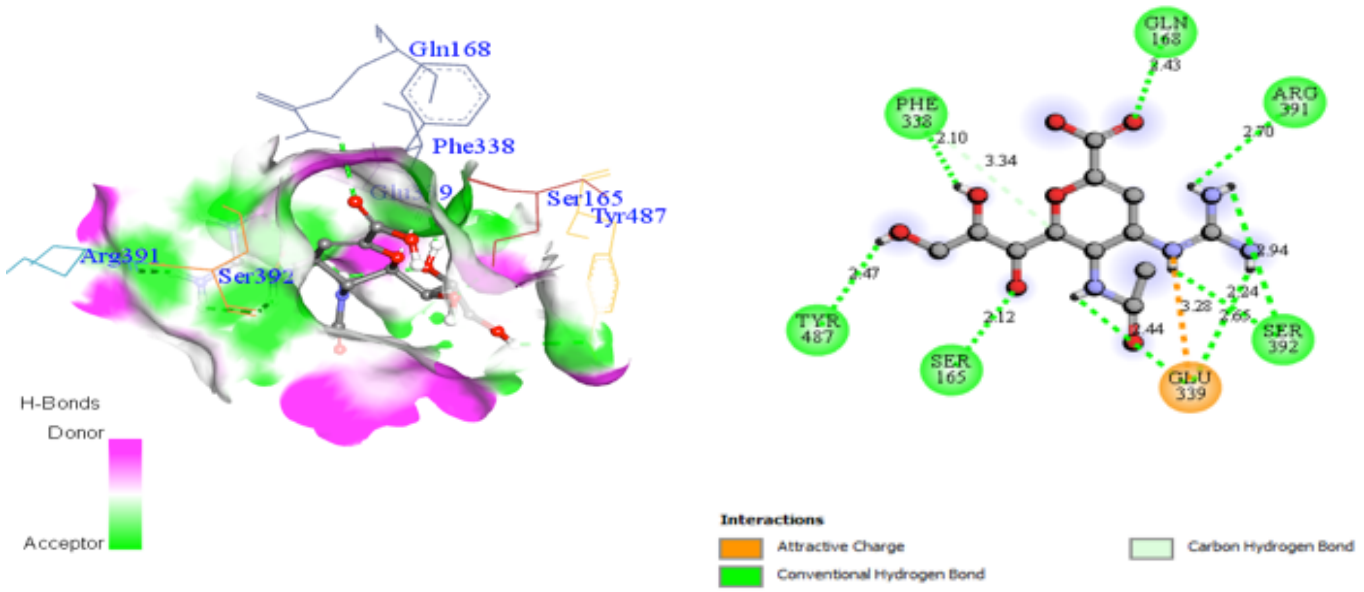


Fig. (6). Molecular docking picture. Right: Hydrogen bond doner and acceptor intensity Left: 2D figure of amino acid and terpenoids interaction with bond type and distance (*Å*). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

protein zanamivir standard complex has a higher surface area than the designed protein ligand complex 1.

3.4.4. Radius of Gyration

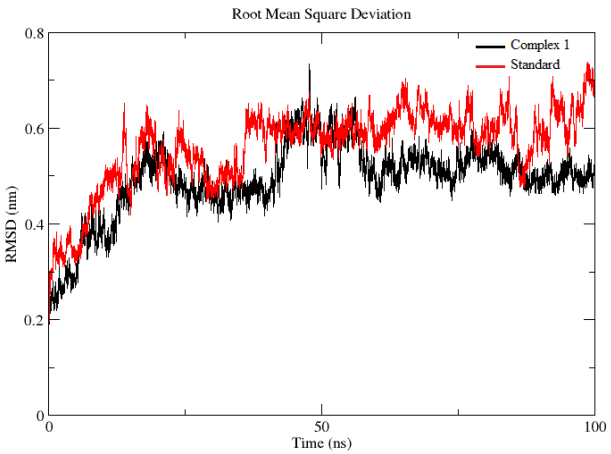
The radius of gyration (Rg), an important part of MD simulation analysis, is related to the protein's tertiary structure volume and provides information about the biological system's structural compactness and folding behavior [58]. High Rg values indicate less structural compactness and folding behavior in proteins. A constant radius of gyration value indicates that ligand compounds do not affect protein folding. In our present studies, complex 1 shows very stable constant radius of gyration value than the zanamivir standard. Increased Rg values signify a sloppy arrangement of amino acid residues inside proteins. The mean of Rg values for complex 1 and standard complex are 2.49nm and 2.51nm, respectively, in Fig. (**4D**).

3.4.5. H-bond Analysis

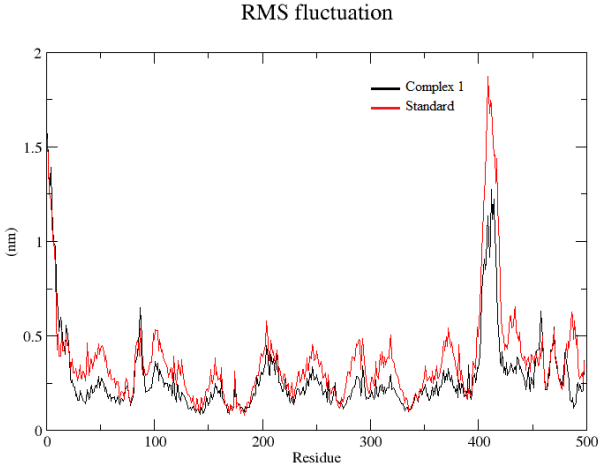
The three-dimensional structure and conformation of a protein are largely determined by the intramolecular hydrogen bonding network of the protein.

The intermolecular hydrogen bonds have been checked to confirm the binding affinity, Affinity and strong binding increase as the number of hydrogen bonds in the complex increases [59]. In Fig. (**4E** and **F**), protein-zanamivir MD

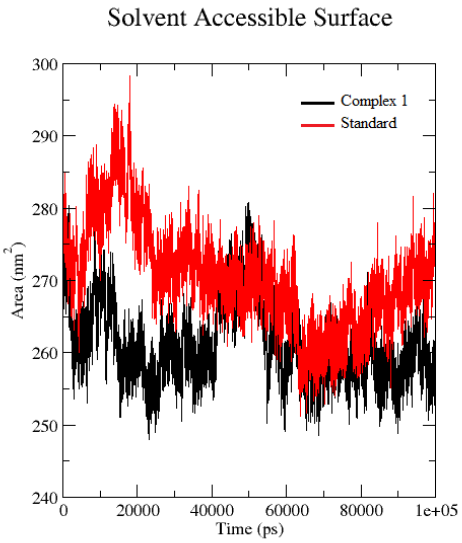
RMSD



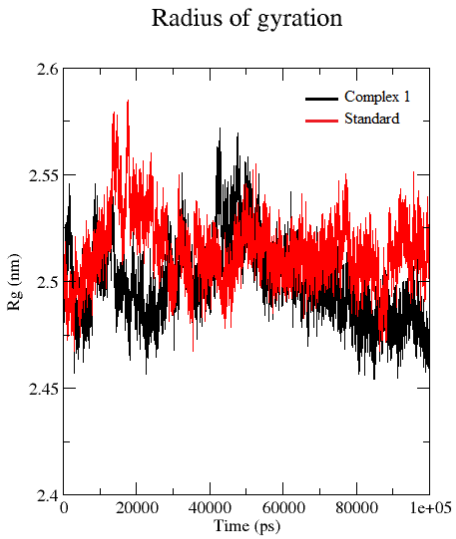
RMSF



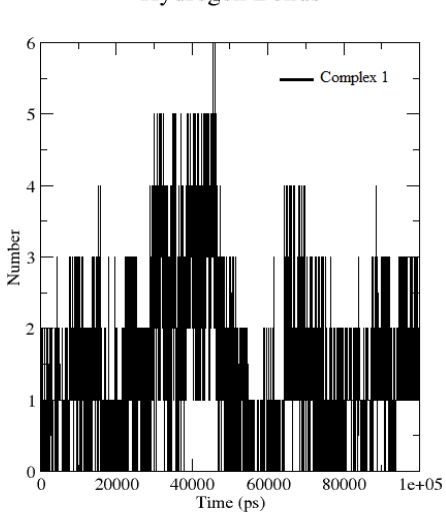
SASA



Radius of gyration (Rg)



Hydrogen bond of complex



Hydrogen bond of standard

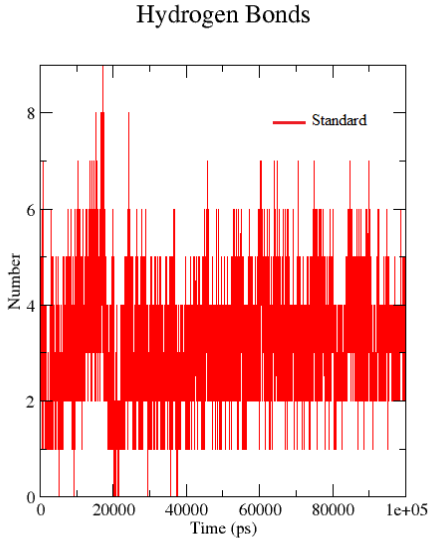


Fig. (7). MD simulation of viral nucleoprotein (NP) with Inonotusol E in black and NP-zanamivir complex in red. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

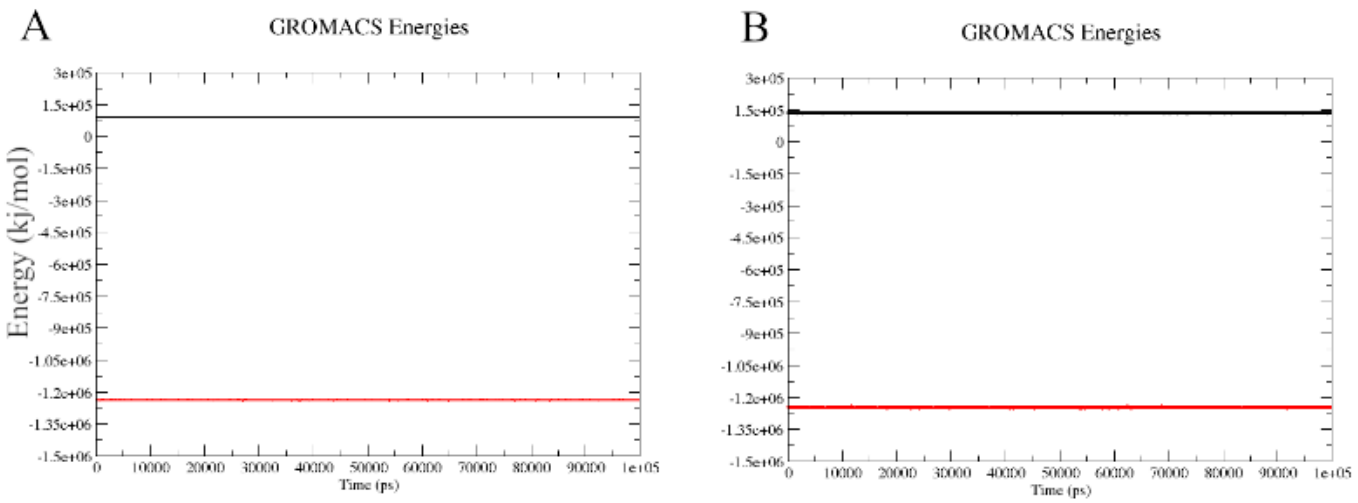


Fig. (8). GROMACS simulation energy of viral nucleoprotein (NP) with Inonotusol E (**A**) and NP with zanamivir complex (**B**). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

simulation shows a maximum number of hydrogen bonds than protein ligand complex. In 100ns simulation trajectory protein ligand complex 1 and zanamivir standard complex formed 4558 and 1731 hydrogen bonds, respectively.

3.4.6. The Energy of Protein–ligand Interaction

Short-range Coulombic energies (Coul-SR) and Lennard-Jones energies (LJ-SR) were calculated for two ligands with the nucleoprotein of the influenza virus. The Coul-SR energy accounts for the electrostatic forces between nearby positively and negatively charged atoms simply electrostatic energy, whereas the LJ-SR energy describes the interaction between nearby neutral atoms or Van Dar Waals energy [60]. The total energy, which reflects the stability of the protein-ligand interaction, is obtained by combining these energies.

Zanamivir shows the most negative value expressed a stronger interaction with the nucleoprotein compared to the Inonotusol E complex Table **4**. Inonotusol E ligand has higher Van Dar Waals energy than Zanamivir ligand (Fig. **8**). The results obtained can offer valuable insights for developing new pharmaceuticals or understanding the mechanisms of potentially effective natural products against influenza [61].

CONCLUSION

The nucleoprotein (NP) of influenza A has been identified and characterized through the use of sophisticated computational strategies and combined drug design approaches. A total of 66 terpenoids have been utilized in these proposed studies as compounds with high potency against nucleoprotein (NP) of influenza A. In order to investigate the compounds' stability and binding affinities against targeted receptors, 100 ns the molecular dynamics simulation and molecular docking, methods were used. Compound 1 showed the best binding affinity whereas, the MD simulation result including RMSD, RMSF, Rg, SASA, and H-bond analysis showed that the complex had very good stability. Finally, our investigation found that the terpenoid derivatives should be suggested as a novel compound to inhibit the target NP of the influenza A virus.

Limitations of the Study

Although this study produced significant findings and promising results, several limitations must be acknowledged. Firstly, it is important to note that this research is entirely theoretical, relying on computational methods and simulations. To confirm these findings and develop safer and more innovative medications, preclinical and clinical trials must be conducted following computational (*in vitro* and *in vivo*) studies to determine the practical utility of these derivatives. Additionally, while the study primarily focused on the binding affinities and drug-like properties of the identified lead compounds, it is crucial to thoroughly investigate factors such as pharmacokinetics, bioavailability, and potential side effects to ensure the development of new and safer drugs from natural sources. Comprehensive experimental research, including computational, preclinical, and clinical trials, is necessary to fully evaluate the investigation's findings and explore potential therapeutic applications.

AUTHORS' CONTRIBUTION

Conceptualization, original draft writing, reviewing, and editing: Md. Saddam Hossain, Md Mosahaq Ali, Prithbay Raj. Formal analysis, investigations, funding acquisition, reviewing, and editing: Md. Parvez Khandocar, S M Jahurul Haque, Yousef A. Bin Jardan. Resources, data validation, data curation, and supervision: Samir Ibenmoussa, Mohammed Bourhia.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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