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INSILICO PHARMACOPHORE ANALYSIS OF POTENTIAL INHIBITORS TO TREAT THE COMPLICATIONS OF EMERGING INFECTIOUS BOURBON VIRUS

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ABSTRACT

The computational drug designing is the principal streamline to evaluate the affinity of small molecules toward specific targets that unveils a potential to disparage the consumption of time in industries with the combination of computational, biological and chemical knowledge. In-silico approaches in drug development play a key role to reconnoiter molecular aspects of targeting specific proteins through various tools and softwares, and analyzing the bioactivities and inhibitory effects across mechanisms underlying for treatment of several chronic diseases. An enigmatic suffering that killed a farmer in Kansas, US has led to the discovery of a new virus called the Bourbon Virus as reported by Vox media on February 25th, 2015. Because the virus has been recently discovered and tagged with various life threatening symptoms, it is necessary to gain profound understanding and perform in-silico analyses to treat and possibly cure the virus. The current research deals with the Insilico analysis of drugs reported to treat tick-borne diseases as the virus is suspected to be under this category as cited in research works and case studies carried out earlier where treatment with Doxycycline proved to be ineffective to this virus. Comparative analysis of commercial drugs and phytochemicals were carried out using virtual screening and molecular docking approaches to a specific target of Bourbon virus (Matrix Protein), which was modeled using computational modeling. The selected compounds were subjected for visualization to interpret the receptor-ligand interactions and their molecular properties and bioactivities were

reported for comparison. The approach of using phytochemicals as inhibitors may reduce the cost dependent factor and can be widely used as medicinal purposes to treat chronic diseases using CADD.

KEYWORDS: *Bourbon Virus, Modeller, Autodock Vina, Doxycycline, I-GemDock, Discovery Studio 4.1, and Pymol.*

INTRODUCTION

An enigmatic suffering that killed a farmer in Kansas, US has led to the discovery of a new virus called the Bourbon virus as reported by Vox media[2] on February 25th, 2015. Because the virus has been recently discovered and tagged with various life threatening symptoms, it is necessary to gain profound understanding and perform in-silico analyses to treat and possibly cure the virus. It is commonly known as Bourbon Virus, owing to the discovery in Bourbon County, Kansas. It is an RNA virus in the genus Thogotovirus (family *Orthomyxoviridae)*. This virus is an addition to the genus Thogotovirus which already includes at least six different viruses namely Araguari, Aransas Bay, Dhori, Jos, Thogoto, and Upolu viruses[4-5], none of which had caused a fatality until Bourbon came in the picture.[4]

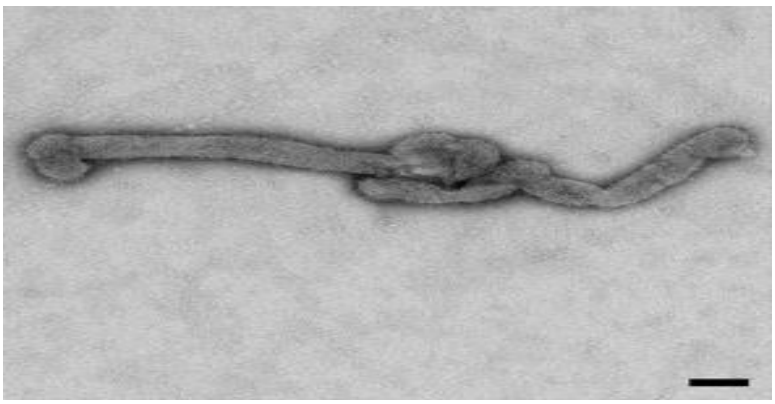

Fig1. Electron micrograph of a filamentous form of Bourbon virus (scale bar: 100 nm)[1]

The previously healthy case patient had several tick bites on his body before he became ill with nausea, weakness and diarrhea. The following day brought fever, headache, fatigue and joint pain. The patient was prescribed Doxycycline because of a history of tick bites, fever and fatigue, but proved ineffective. The patient's body condition did not improve resulting in multi organ failure and ultimately death.[3]

Testing of the specimen with Plaque Reduction Neutralization techniques that are originally used to test for Heartland virus antibodies, resulted in presence of another virus. Sequencing

and Phylogenetic analyses proved crucial to identify and classify the virus as 'Bourbon Virus' as the member of Thogotovirus.[6-7] Ticks, the vectors of Heartland virus and Lyme disease, are also strongly suspected to be associated with the Bourbon virus. Although the ticks vary by geographic climate and areas, they tend to be generally more active during warmer months. Commonly experienced symptoms are fever, body aches, fatigue or rashes. The strategy employed for this analysis was based on prediction of structure, combinatorial library preparation, virtual screening, molecular docking and property, bioactivity analysis of compounds.[18] CADD approaches play a significant role in drug designing and computational methods and are believed to offer means of improved efficiency for the pharmaceutical industries. They are expected to limit and focus chemical synthesis and biological testing and thereby greatly decreasing traditional resource requirements.

MATERIALS AND METHODS

Sequence Retrieval

The 270 amino acid sequence for M protein [Bourbon Virus] was considered for study of the virus with accession no. AJP32540.1. The FASTA sequence acquired from the NCBI protein database was submitted to BLASTp[8] with default parameters, for comparing primary biological sequence. The BLAST algorithm result showed 74% identity match with two sequences and E-values 0.63 and 0.71 respectively. The Accession (PDB) IDs are 2W3N_A and 2W3Q_A. Both the PDB structure file data were obtained through the PDB database.

Modelling of Protein and Validation

The basic tool for homology or comparative modeling of a three-dimensional protein structure was used to model the Bourbon Virus. The two PDB data files were used as the foundation to build the Bourbon Virus structure. Multiple template method was used to develop and build 20 protein structures using Modeller 9.15.[9] After studying the values of DOPE score and molpdf, one structure, which fit the requirements, was chosen and tagged as final model. The Ramachandran plot analysis was performed on the modeled structure-using RAMPAGE.[10]

Active site Prediction

To identify binding sites and active sites of a protein, CASTp Server[11] was used. This server analysis showed us the structural pockets and cavities in the submitted protein structure. The measurement of surface accessible pockets was predicted with the help of this server.

Virtual Screening Using I-GemDock

The set of eight phytochemical compounds and eight approved antibiotics compounds have been subjected to I-GemDock[12] for drug screening using default GEMDOCK scoring function with 200 population size, 70 generation, 3 solutions with default cutoff scoring values. Further the compounds screened were directed for post screening analysis for generating clusters and interacting residues plot to analyze the residues interacted with the selected compounds. Docking convention used was "Drug Screening" strategy to filter out the drugs suitable for molecular docking approach. The scoring was evaluated based on the fitness score.

Molecular Docking

In the field of Computer Aided Drug Designing, docking is a method, which envisages the desired orientation of molecules to a specified target when bound to each other to form a stable complex to study the inhibitory and binding efficiency with the residues, which participate in the interaction. Auto Dock Vina[13], a command based tool is widely used genetic algorithm method for docking study. The protein and ligand preparation were done using PMV (Python Molecular Viewer) from MGL tools in which hydrogen atoms were added and merged with the non-polar ones. Kolmann Charges were added to the protein and subjected to find any missing residues and close contacts. GRID box preparation at specified binding site that was predicted from CASTp server was carried out by defining the box centers at x, y, z centers with a spacing Armstrong of 1.000. In a similar fashion adding hydrogen atoms and merging with non-polar hydrogen atoms, ligand preparation was achieved. Torsional rotations TORSDOF for the ligands were set for 8 rotational bonds defining the flexibility of ligands and Gasteiger charges were added accordingly

RESULTS AND DISCUSSION

Structure Prediction

The FASTA sequence of Bourbon virus M-protein was subjected to Multiple Template Modeling using Modeller9.15 and 20 structures were generated using model-multiple script, which then evaluated and selected the best structure, based on DOPE score and molpdf score of 6923.08496 (Fig 2a) visualized using Chimera.[19] The least molpdf model was selected and assessed for stereo chemical properties. The RAMPAGE analysis revealed that only 2 residues (0.7%) fell under the category of outlier region and 95.9 % fell under favoured region (Fig 2b).

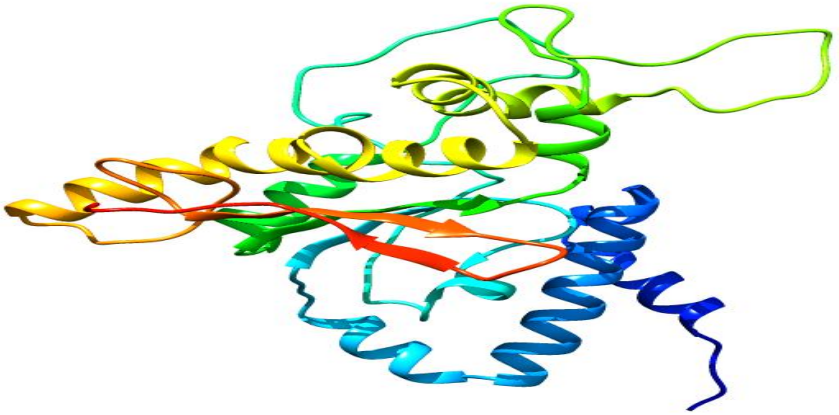

Fig 2a. M-protein of Bourbon Virus modeled by Modeller9v15.

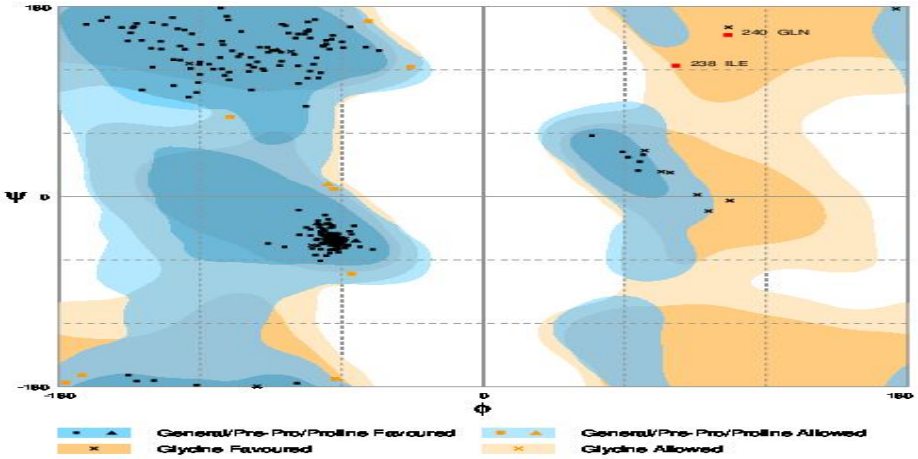

Fig 2b.Ramachandran Plot assessment

CASTp Analysis

The binding site / active site residues were predicted using CASTp server that serves as a basis of protein GRID preparation in Molecular Docking approach using Autodock Vina. CASTp analysis resulted in the active site Area of 2136.3 and Volume of 4974.6 (Fig 3).

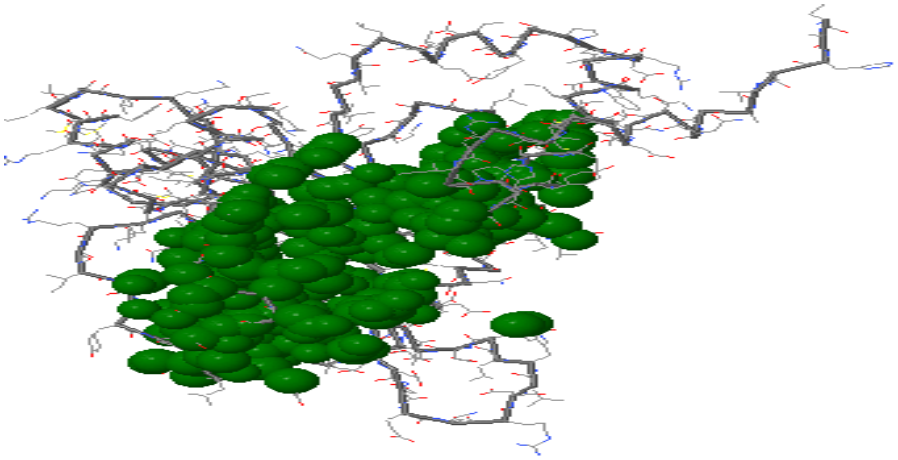

Fig 3. Active site as predicted by CASTp server

Library Preparation

FDA approved antibiotics available to treat the tick-borne diseases were retrieved from PubChem Database[14], a public repository of small chemical compounds that are classified under approved antibiotics. The phytochemical compounds bearing antibiotic properties were also categorized under Phytochemicals. Eight approved antibiotics and eight phytochemical compounds were considered for this study for the specified target mentioned below.

Approved Antibiotics

Doxycycline is a synthetic tetracycline derivative with similar antimicrobial activity (PubChem CID 54671203), Tetracycline is a broad-spectrum naphthacene antibiotic having (PubChem CID 54675776), Amoxicillin is a broad-spectrum semisynthetic antibiotic similar to AMPICILLIN (PubChem CID 33613), Cefuroxime is a Cephalosporin Antibacterial, Broad-spectrum cephalosporin antibiotic resistant to beta-lactamase (PubChem CID 5479529), Clavulanic acid is a beta Lactamase Inhibitor (PubChem CID 5280980), Naproxen is a Non-steroidal Anti-inflammatory Drug (PubChem CID 156391), Ibuprofen is a propionic acid derivate and non-steroidal anti-inflammatory drug (NSAID) with anti-inflammatory, analgesic, and antipyretic effects(PubChem CID 3672), Ceftriaxone is a Cephalosporin Antibacterial (PubChem CID 5479530) have been used in this research against Bourbon M-Protein viral receptor.

Phytochemicals

Curcumin is a phytopolylphenol pigment isolated from the plant Curcuma longa, commonly known as turmeric, with a variety of pharmacologic properties (PubChem CID 969516), Stigmasterol[15] is a steroid derivative characterized by the hydroxyl group (PubChem CID 5280794), Mangrove derived phytochemicals like Tricin (PubChem CID 5281702),

Pyrethrum (PubChem CID 45270414), Triterpenoid (PubChem CID 451674). Orthoxicol, Tulsi Plant (PubChem CID 12305042), Sulfadiazine (PubChem CID 5215), Azadirachtin, Neem plant (PubChem CID 6437066) were taken as phytochemical compounds.

Virtual Screening using I-GEM-DOCK

The computational screening of small compounds is a vital aspect in CADD approach, which is characterized as Lead Identification methodology in CADD workflow as proposed by many structural bioinformaticians. Virtual screening reveals the aspect of binding affinity towards a specific target using I-GemDock for instance. The selected antibiotics and phytochemicals were screened using I-GemDock using GEMDOCK scoring function, which unveils the binding affinities in Kcal/mol energy based on summation of H-Bond energy, VdW energy, Electrostatic energy as shown in (Table 1). Based upon the binding energy the selected compounds were subjected to molecular docking to know the binding efficacy of the compounds to the protein receptor.

Table 1: Virtual screening of the approved antibiotics and phytochemical compounds.

Receptor Used	Drug Category	No. of Ligands Used	No of Ligands Selected	Name of the Selected Ligands	Total Energy Values
M-Protein Bourbon Virus	Approved Antibiotics	8	8	Tetracycline Amoxicillin Cefuroxime Clavulanic acid Naproxen Ibuprofen Ceftriaxone Doxycycline	-102.7 -80.6 -78.7 -73.5 -72.2 -63.8 29.7 145.0
	Phytochemicals	8	8	Curcumin Stigmasterol Tricin Pyrethrum Orthoxicol Sulfadiazine Triterpenoid Azadriactin	-105.7 -83.1 -81.8 -76.2 -61.4 -54.6 111.3 196.1

Molecular Docking using Autodock Vina

Molecular Docking strategies employed in our research work reveal the molecular aspects of ligand – receptor interactions, which emphasizes on binding efficacy based on energy values and binding residues. Different modes were obtained for each ligand of which the optimal mode was selected based on the binding affinity (kcal/mol) value. The details of the results obtained are listed in the (Table 2).

Table: 2. Binding efficacy on the basis of binding affinity score of the screened compounds against Bourbon M-protein.

Drugs	Energy (kcal/mol)
Approved Antibiotics	
Tetracycline	-7.6
Amoxicillin	-6.6
Cefuroxime	-6.9
Clavulanic acid	-4.8
Naproxen	-6.5
Ibuprofen	-5.6
Ceftriaxone	-7.7
Doxycycline	-6.9
Phytochemicals	
Curcumin	-7.7
Stigmasterol	-8.1
Tricin	-7.2
Pyrethrum	-6.0
Orthoxicol	-5.1
Sulfadiazine	-4.4
Triterpenoid	-8.2
Azadriactin	-7.5

Interaction Analysis

The most important factor in protein ligand interaction is the hydrogen bonding as it plays a vital role in predicting the binding efficacy of small compounds to specific targets and their orientations. The observation thus depicted from docking analysis implied the vital aspect of phytochemicals in inhibitory effect in comparison to commercial antibiotics. Triterpenoid, Stigmasterol and Curcumin proved to be more effective as the binding affinity toward the M-protein of Bourbon virus is higher than antibiotics used in this study. Curcumin binds to ALA67, Triterpenoid to ARG 186, ASP 154, and Stigmasterol to ASP 190 that falls under the active site residues. Doxycycline, Ceftriaxone and Tetracycline also competes with the phytochemicals and shown better binding affinities but Doxycycline proved to be ineffective in terms of binding energy in comparison to phytochemicals used for our research. The H-

Bond interactions were visualized using 2D interaction plot of Discovery Studio Visualizer 4.1[19] and using Pymol[21] the structural representation was shown in (Fig 4.1-4.6).

Table 3: Hydrogen bonded Interacting Residues of docked compounds against M-protein of Bourbon virus receptor.

Receptor Used	Ligand	Binding Affinity	Interacting Residues
M-Protein Bourbon Virus	Curcumin	-7.7	ALA 67
	Triterpenoid	-8.2	ARG 186 ASP 154
	Stigmasterol	-8.1	ASP 190
	Doxycycline	-6.9	SER 75 LYS 248 GLY 259
	Ceftriaxone	-7.7	ARG 37 GLY 80 GLN 86
	Tetracycline	-7.6	HIS 30 LYS 79

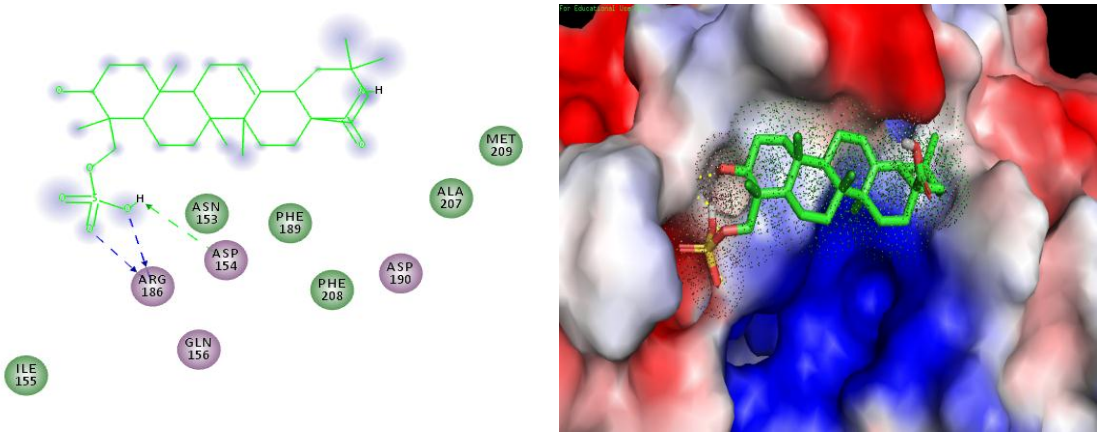

Fig4.1-Triterpenoid

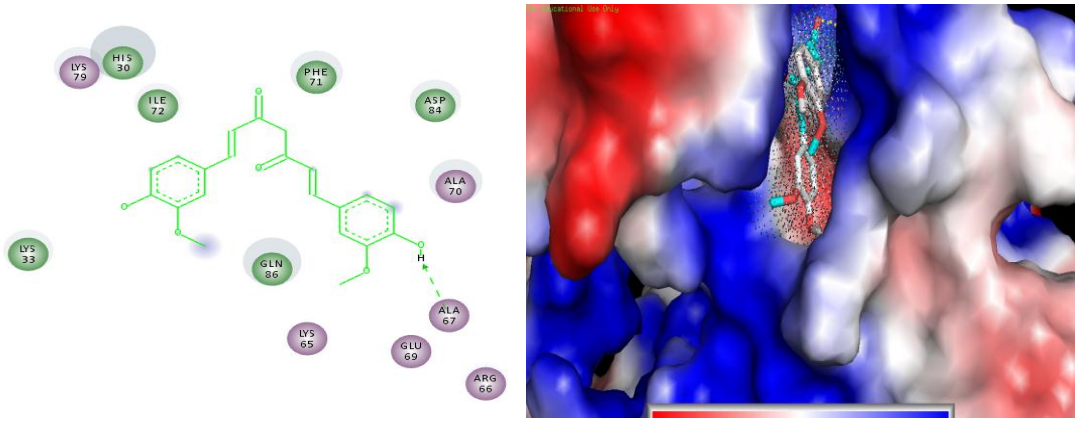

Fig 4.2-Curcumin

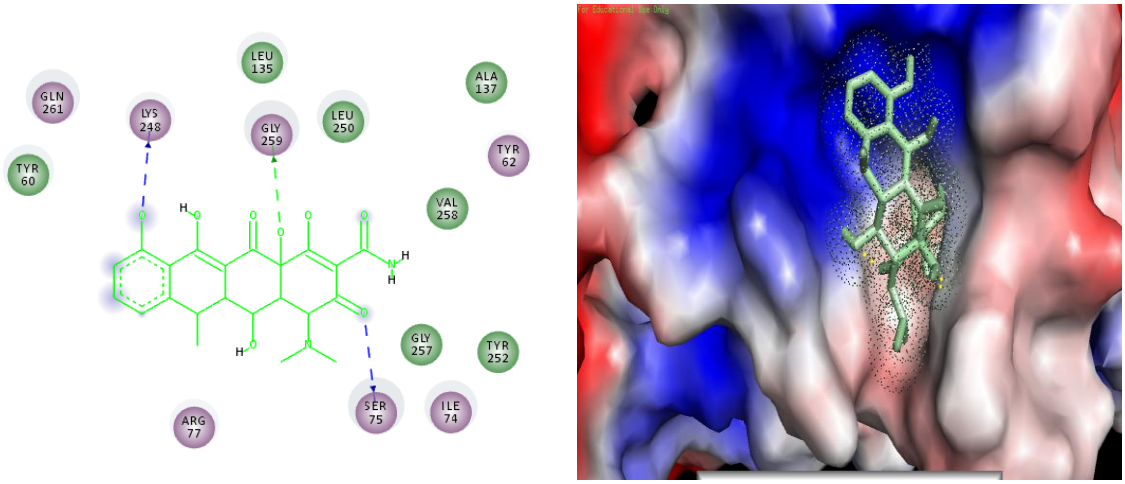

Fig4.3-Doxycycline

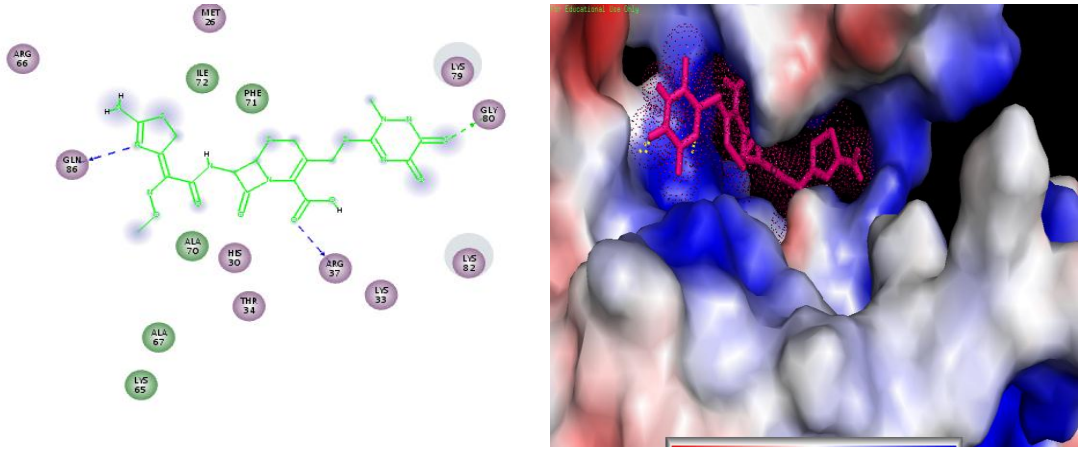

Fig4.4-Ceftriaxone

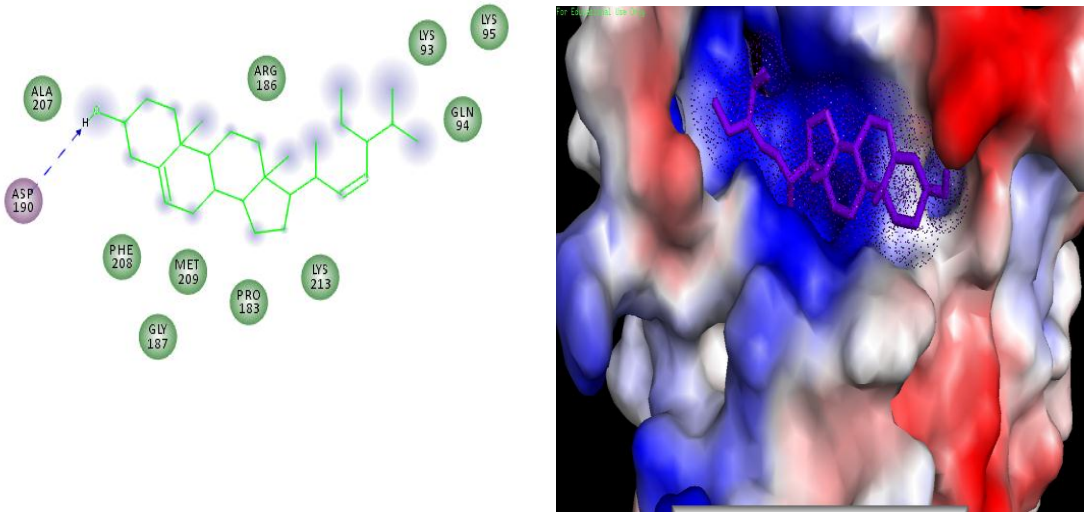

Fig4.5-Stigmasterol

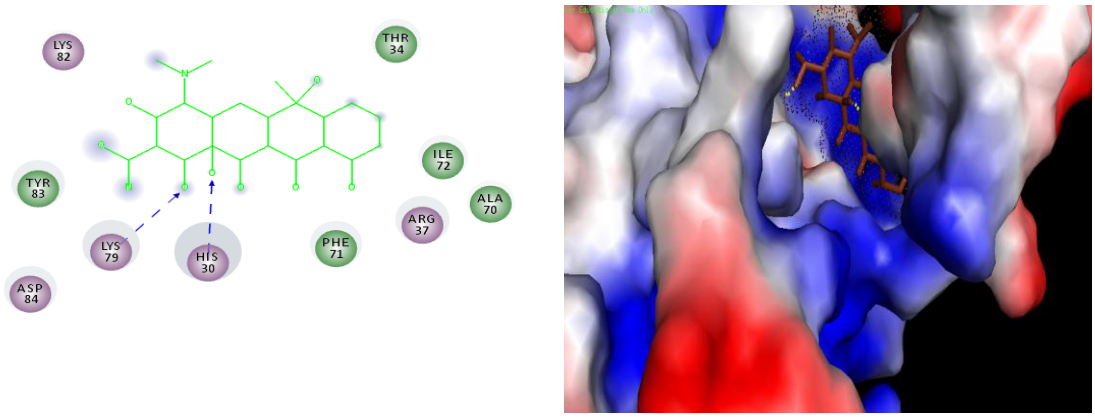

Fig4.6-Tetracycline

Fig (4.1-4.6)-The hydrogen bonding binding site interaction plot as visualized by Discovery Studio 4.1[19] and structural representation using Pymol [21]

ADMET properties

Compounds having highest binding efficacy and inhibitory properties were employed for ADMET screening using MOLINSPIRATION [16] and specific properties were also studied from PubChem. The property, which comes under Lipinski rule of five, was predicted using Molinspiration. The bioactivity prediction was also done for its inhibitory effect (Table 4).

Table 4: MOLINSPIRATION Properties of selected compounds

Properties	Curcumin	Triterpenoid	Stigmasterol	Doxycycline	Ceftriaxone	Tetracycline
Molecular Weight	368.3799 g/mol	552.76292 g/mol	412.69082 g/mol	444.43456 g/mol	554.57992 g/mol	444.43456 g/mol
Molecular Formula	C21H20O6	C30H48O7S	C29H48O	C22H24O8	C18H18N8O7S3	C22H24N2O8
XLogP3	3.2	5.9	8.6	-0.7	-1.3	-2
Hydrogen Bond Donor	2	3	1	6	4	6
Hydrogen Bond Acceptor	6	7	1	9	13	9
Rotatable Bond	8	4	5	2	8	2
Log P	3.29	3.22	9.43	-0.02	-1.7	-1.37
GPCR ligand	-0.06	0.40	0.12	-0.047	-0.44	-0.38
Ion channel modulator	-0.20	-0.19	-0.08	-0.43	-0.95	-0.34
Kinase inhibitor	-0.26	-0.36	-0.49	-0.68	-0.81	-0.66
Nuclear receptor ligand	0.12	0.63	0.74	-0.38	-1.07	-0.30
Protease inhibitor	-0.14	0.45	-0.02	-0.07	0.32	-0.17
Enzyme inhibitor	-0.08	0.94	0.53	0.59	0.10	0.60

DISCUSSION

The emerging lethal Bourbon Virus, an enigmatic suffering is strongly suspected for mortality. As per the literature review the patient was prescribed Doxycycline and other antibiotics but proved ineffective, which resulted in his death. The information about the mysterious virus is limited and might be attributable to this novel pathogen considering it as a potential infectious etiology that might cause fatality. This led us to gain insightful knowledge to reconnoiter the molecular aspects through CADD approach. The computer aided Pharmacophore study deals with the comparative analysis of commercial antibiotics and phytochemicals in which phytochemicals encumbrances the importance in treatment that has been deduced from in-silico analysis in our current work. Phytochemicals though having several violations in drug likeliness, the refinement of structures may have an impact in treatment of Bourbon viral infection. From docking studies it has been justified that the phytochemical category has greater binding efficacy than commercial approved antibiotics as shown in Table 2. Stigmasterol, Triterpenoid, Curcumin have shown greater binding efficacy and least energy values in AutoDock Vina -8.1, -8.2 and -7.7 respectively in comparison to commercial approved antibiotics like Doxycycline (-6.9). Though Doxycycline has been widely used to treat several tick-borne diseases e.g. Lyme disease but in case of Bourbon virus the treatment with Doxycycline may have negative effect or no effect that leads to mortality as reported earlier. In previous findings also Doxycycline has been proved as an efficient antibiotic/drug in Lyme disease that showed greater affinity to the specific target of Lyme disease receptor.[17] But here in this case phytochemicals stand out to be more effective computationally than Doxycycline. Tetracycline and other antibiotics also showed better binding affinity than Doxycycline. Thus it can be concluded that nature provides us the solutions for the diseases and traditional methods like phytochemical treatment can now also be implemented in this modern scientific era as they are effective and give more significant results to treat chronic and dreadful diseases.

Conflict of Interest: We declare that we have no conflict of interest.

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