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Research Article

IN-SILICO DOCKING ANALYSIS OF PHYTOCONSTITUENTSFROM SWERTIA AGAINST DENGUE VIRAL NS3 PROTEASE

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ABSTRACT

Context

Dengue virus (DENV) causes disease globally, with an estimated 25 to 100 million new infections per year. At present, no effective vaccine is available, and treatment is supportive.At present, no effective vaccine is available, and treatment is supportive.Dengue virus, the cause of dengue fever is a mosquito-borne single positive-stranded RNA virus of the family Flaviviridae.

Objective:

The aim of this study is to investigate the NS3 protease inhibitor potential of active constituents of Swertiachirayitaby insilico molecular docking analysis.

Materials and Methods:

The ability of the phytoconstituents to bind with the targets is given in terms of MolDockScore. The MolDockScore is used as the parameter for analysing the docking results. The phytoconstituents are ranked according to their MolDockScore.

Results:

Amarogentin, 1,3,5,8-tetrahydroxyxanthone and Amaroswerin were found to be potentially active dengue viral protease.

Conclusion:

Amarogentin, Amaroswerin and tetrahydroxanthone were found to have potential inhibitory effects against dengue NS3 protease. These effects has to however be validated using wet lab assays so as to develop potential leads through ligand based approaches.

INTRODUCTION

Dengue virus (DENV) causes disease globally, with an estimated 25 to 100 million new infections per year. At present, no effective vaccine is available, and treatment is supportive.Dengue virus, the cause of dengue fever is a mosquito-borne single positive-stranded RNA virus of the family Flaviviridae. The length of the dengue viral genome is about 11000 bases that codes for three structural proteins such as the capsid protein C, membrane protein M, envelope protein E and seven nonstructural proteins namely NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5. The DENV NS3 is a serine protease, as well as an RNA helicase and RTPase/NTPase. Viral proteases are excellent antiviral targets, as evidenced by the nine protease inhibitors of human immunodeficiency virus (HIV) currently in clinical use and the numerous protease inhibitors of hepatitis C virus (HCV) in clinical trials. By analogy with the successes of HIV and HCV protease inhibitors, efforts have been made to design inhibitors against DENV using dengue virus NS2B/NS3 protease as a molecular target. The protease domain consists of six β-strands arranged into two β-barrels formed by residues 1–180 of the protein. The catalytic triad (His-51, Asp-75 and Ser-135), is found between these two β-barrels, and activity is dependent on the presence of the NS2B cofactor. This cofactor wraps around the NS3 protease domain and becomes part of the active site. The remaining NS3 residues (180–618), form the three subdomains of the DENV helicase. A six-stranded parallel β-sheet surrounded by four α-helices make up subdomains I and II, and subdomain III is composed of 4 α-helices surrounded by three shorter α-helices and two antiparallel β-strands.

Swertiachirayita is an annual or biennial herb that is usually 60 – 125 cm tall with robust stem, branching, cylindrical below, four angled upwards with a large pith. Leaves are opposite, decussate, broadly ovate or lanceolate, 3.5 – 10 x 1.5 – 4 cm, glabrous, obtuse or cordate at base, acuminate at apex, margins entire, usually with 3 – 7 prominent lateral veins. Various pharmacognostical and folklore claims have listed the dengue curing potential of the plant. The main active constituents of the plant are xanthone alkaloids. The

aim of this study is to investigate the NS3 protease inhibitory potential of active constituents of *Swertiachirayita*by insilico molecular docking analysis.

MATERIALS AND METHODS

Preparation of Ligand

The major active constituents are identified from the selected medicinal plant namely *swertiachirata and acoruscalamus* which possess anti viral properties according to traditional claims and the 3D structures of the active constituents (Amarogentin, Amaroswerin, Syingaresinol, Mangostin, Taraxerol, Sweroside, Decussatin, Sertan, Ursoilic acid, 1,3,5,8-tetrahydroxyxanthone, Gentianine, Enicoflavine, b-Amyrin, KairatenylPalmitate, Erythrodiol) are retrieved either from PubChemchemical databases [1]or drawn using ChemSketch software [2] and saved in .mol format. The ligands are imported to the workspace and preparation of them is done.

Preparation of Enzyme

The target for docking studies is selected as dengue virus protease enzyme. Docking analysis is done by initially selecting the target for the disease and followed by obtaining the 3D structure of dengue virus protease enzyme (PDB ID: 3U1I) from protein data bank in .pdb format [3, 4]. It is well known that PDB files often have poor or missing assignments of explicit hydrogens, and the PDB file format cannot accommodate bond order information. Therefore, proper bonds, bond orders, hybridization and charges were assigned using the MVD. The potential binding sites of both the targets were calculated using the built-in cavity detection algorithm implemented in MVD. The search space of the simulation exploited in the docking studies was studied as a subset region of 25.0 Angstroms around the active side cleft. The water molecules are also taken in to consideration and the replaceable water molecules were given a score of 0.50.

MolegroVirtual Docker's docking search algorithms and scoring functions

Ligand docking studies were performed by Molegro Virtual Docker(MVD), which has recently been introduced and gained attention among medicinal chemists. MVD is a fast and flexible docking program that gives the most likely conformation of ligand binding to a macromolecule. MolDock software is based on a new heuristic search algorithm that combines differential evolution with a cavity prediction algorithm [5]. It has an interactive optimization technique inspired by Darwinian Evolution Theory (Evolutionary Algorithms - EA), in which a population of individuals is exposed to competitive selection that weeds out poor solutions. Recombination and mutation are used to generate new solutions. The scoring function of MolDock is based on the Piecewise Linear Potential (PLP), which is a simplified potential whose parameters are fit to protein-ligand structures and a binding data scoring function [6, 7] that is further extended in GEMDOCK (Generic Evolutionary Method for molecular DOCK) [8] with a new hydrogen bonding term and charge schemes.

MolDock Optimizer

In MVD, selected parameters were used for the guided differential evolution algorithm: number of runs =5 by checking constrain poses to cavity option), population size=50, maximum interactions =2000, cross over rate=0.9, and scaling factor=0.5. A_o variance-based termination scheme was selected rather than root mean square deviation (RMSD).To ensure the most suitable binding mode in the binding cavity, Pose clustering was employed, which lead to multiple binding modes.

Parameters for scoring functions

MolDock score

They ignore-distant-atoms option was used to ignore atoms far away from the binding site. Additionally, hydrogen bond directionality was said to check whether hydrogen bonding between

potential donors and acceptors can occur. The binding site on the protein was defined as extending in X, Y & Z directions around the selected cavity with a radius of 25 Angstroms.

Rerank Score

The reranking scoring functions are used to create and predict models for estimation of chemical properties (e.g. QSAR).The reranking score function is computationally more expensive than the scoring function used during the docking simulation but it is generally better than the docking score function at determining the best pose among several poses originating from the same ligand.While the rerank-score in MVD provides an estimate of the strength of the interaction, it is not calibrated in chemical units and it does not take complex contributions (such as entropy) into account. Even though the rerank score might be successful in ranking different poses of the same ligand, it might be less successful in ranking poses of different ligands. It is therefore recommend ranking the results of a virtual screening run using the rerank score. The binding affinity measure may then be used subsequently to get a rough estimate of the highest ranked poses

RESULTS & DISCUSSIONS

In-silico docking results

The ability of the phytoconstituents to bind with the targets is given in terms of MolDockScore. The MolDockScore is used as the parameter for analysing the docking results. The phytoconstituents are ranked according to their MolDockScore. The ligand possessing the highest mol dock score shows a strong affinity towards its target. In-silico docking analysis of phytoconstituents from *swertiachirata and acoruscalamus*on dengue virus protease enzyme (PDB ID: 3U1I) ranking based on MolDockScore is represented in table 1, Rerank score in table 2 and H-Bond is represented in table 3. The figure 1-3

corresponds to the docking pose evaluated and captured by the ligand energy inspector tool in the Molegro virtual docker.The descriptor calculation for the ligands are represented in separate excel file.

Element Count

Counts the number of atoms for a given element.By default H, C, N, O, P, and S are counted. All other elements are counted as 'other'.

Simple Descriptors

A set of common descriptors including molecular weight, hydrogen donor / acceptor count, and other simple descriptors.The available descriptors are:

- MW - Molecular Weight
- Atoms - Atom count (including hydrogens)
- HeavyAtoms - Atom count (excluding hydrogens)
- Rot - The number of rotatable bonds
- Rot2 - The number of rotatable bonds excl. bonds rotating terminal atoms
- HD - The number of hydrogen donors
- HA - The number of hydrogen acceptors
- Rings - The number of rings
- Aro - The number of aromatic rings

Andrews Affinity Terms

Andrews Affinity together with the terms needed for the calculation. See 'Functional goup contributions to drug-receptor interactions' PR Andrews, DJ Craik, JL Martin Journal of medicinal chemistry 27:1212, 1648-1657, American Chemical Society, 1984.

Chemical Feature Distance Matrix

The CFDM descriptors are obtained by calculating the minimum, maximum, and mean topological distance between all pairs of chemical features. The topological distance is defined as the smallest number of covalent bonds between the two features.

The following chemical features are investigated: hydrogen acceptors, hydrogen donors, positively and negatively charged atoms, and ring systems. Notice that a minimum charge of ±0.2 is required for an atom to be considered charged (this threshold may be changed in the settings dialog).

Wiener Index

The Wiener Index is the sum of the topological distance between all heavy atom pairs.

TABLES & FIGURES

Table 1: In-silico docking analysis of phytoconstituentsfrom *swertiachirata and acoruscalamus*on**dengue virus protease enzyme (PDB ID: 3U1I) ranking based on MolDock Score**

Table 2: In-silico docking analysis of phytoconstituentsfrom *swertiachirata and acoruscalamus*on**dengue virus protease enzyme (PDB ID: 3U1I) ranking based on Rereank Score**

Table 3: In-silico docking analysis of phytoconstituents from *swertiachirata and acoruscalamus*on**dengue virus protease enzyme (PDB ID: 3U1I) ranking based on HBond interaction**

Figure 1: Molecular Docking Analysis of Amarogentin

Figure 2: Molecular Docking Analysis of Amaroswerin

Figure 3:Molecular Docking Analysis of tetrahydroxanthone

CONCLUSION

Amarogentin, Amaroswerin and tetrahydroxanthone were found to have potential inhibitory effects against dengue NS3 protease. This effect has to however be validated using wet lab assays so as to develop potential leads through ligand based approaches.

Table 1: In-silico docking analysis of phytoconstituentsfrom *swertiachirata and acoruscalamus*on**dengue virus protease enzyme (PDB ID: 3U1I) ranking based on MolDock Score**

Name	Ligand	MolDock Score	Rerank Score	HBond
[00]Amarogentin	Amarogentin	-149.178	-98.7099	-13.2794
[00]Amaroswerin	Amaroswerin	-142.072	-95.7593	-6.37837
[00]Syingaresinol	Syingaresinol	-95.1782	-53.6998	-1.24172
[00]Mangostin	Mangostin	-91.1814	-81.9104	0
[00]Taraxerol	Taraxerol	-83.0653	-42.5246	-2.14029
[00]Sweroside	Sweroside	-77.8549	-61.0819	-2.95934
[00]Decussatin	Decussatin	-71.8302	-63.4479	-1.84302
[00]Sertan	Sertan	-65.2349	-55.3856	0
[00]Ursoilic acid	Ursoilic acid	-61.4175	-45.2122	-2.1329
[00]1,3,5,8-tetrahydroxyxanthone	1,3,5,8-tetrahydroxyxanthone	-59.0987	-60.864	-6.12108
[00]Gentianine	Gentianine	-55.7715	-46.784	-3.25591
[00]Enicoflavine	Enicoflavine	-54.3304	-51.6498	-3.10469
[00]b-Amyrin	b-Amyrin	-51.1084	43.6802	0
[00]KairatenylPalmitate	KairatenylPalmitate	922.474	38.7129	-2.37014
[00]Erythrodiol	Erythrodiol	1019	120.289	-2.5

Table 2: In-silico docking analysis of phytoconstituentsfrom *swertiachirata and acoruscalamus*on**dengue virus protease enzyme (PDB ID: 3U1I) ranking based on Rereank Score**

Name	Ligand	MolDock Score	Rerank Score	HBond
[00]Amarogentin	Amarogentin	-149.178	-98.7099	-13.2794
[00]Amaroswerin	Amaroswerin	-142.072	-95.7593	-6.37837
[00]Mangostin	Mangostin	-91.1814	-81.9104	0
[00]Decussatin	Decussatin	-71.8302	-63.4479	-1.84302
[01]Sweroside	Sweroside	-71.8547	-61.1088	-2.53432
[00]1,3,5,8-tetrahydroxyxanthone	1,3,5,8-tetrahydroxyxanthone	-59.0987	-60.864	-6.12108
[00]Sertan	Sertan	-65.2349	-55.3856	0
[00]Syingaresinol	Syingaresinol	-95.1782	-53.6998	-1.24172
[00]Enicoflavine	Enicoflavine	-54.3304	-51.6498	-3.10469
[01]Ursoilic acid	Ursoilic acid	-56.8172	-50.4418	-1.74532
[00]Gentianine	Gentianine	-55.7715	-46.784	-3.25591
[00]Taraxerol	Taraxerol	-83.0653	-42.5246	-2.14029
[01]b-Amyrin	b-Amyrin	-49.6295	25.1659	0
[00]KairatenylPalmitate	KairatenylPalmitate	922.474	38.7129	-2.37014
[00]Erythrodiol	Erythrodiol	1019	120.289	-2.5

Table 3: In-silico docking analysis of phytoconstituents from *swertiachirata and acoruscalamus*on**dengue virus protease enzyme (PDB ID: 3U1I) ranking based on HBond interaction**

Name	Ligand	MolDock Score	Rerank Score	HBond
[00]Amarogentin	Amarogentin	-149.178	-98.7099	-13.2794
[01]1,3,5,8-tetrahydroxyxanthone	1,3,5,8-tetrahydroxyxanthone	-53.2103	-56.345	-6.62655
[00]Amaroswerin	Amaroswerin	-142.072	-95.7593	-6.37837
[01]Erythrodiol	Erythrodiol	1024.29	128.134	-4.41058
[00]Gentianine	Gentianine	-55.7715	-46.784	-3.25591
[00]Enicoflavine	Enicoflavine	-54.3304	-51.6498	-3.10469
[00]Sweroside	Sweroside	-77.8549	-61.0819	-2.95934
[00]KairatenylPalmitate	KairatenylPalmitate	922.474	38.7129	-2.37014
[00]Taraxerol	Taraxerol	-83.0653	-42.5246	-2.14029
[00]Ursoilic acid	Ursoilic acid	-61.4175	-45.2122	-2.1329
[01]Sertan	Sertan	-54.2146	-34.8819	-2.07255

[00]Decussatin	Decussatin	-71.8302	-63.4479	-1.84302
[01]Syingaresinol	Syingaresinol	-87.1722	-33.308	-1.6663
[01]b-Amyrin	b-Amyrin	-49.6295	25.1659	0
[00]Mangostin	Mangostin	-91.1814	-81.9104	0

Figure 1: Molecular Docking Analysis of Amarogentin

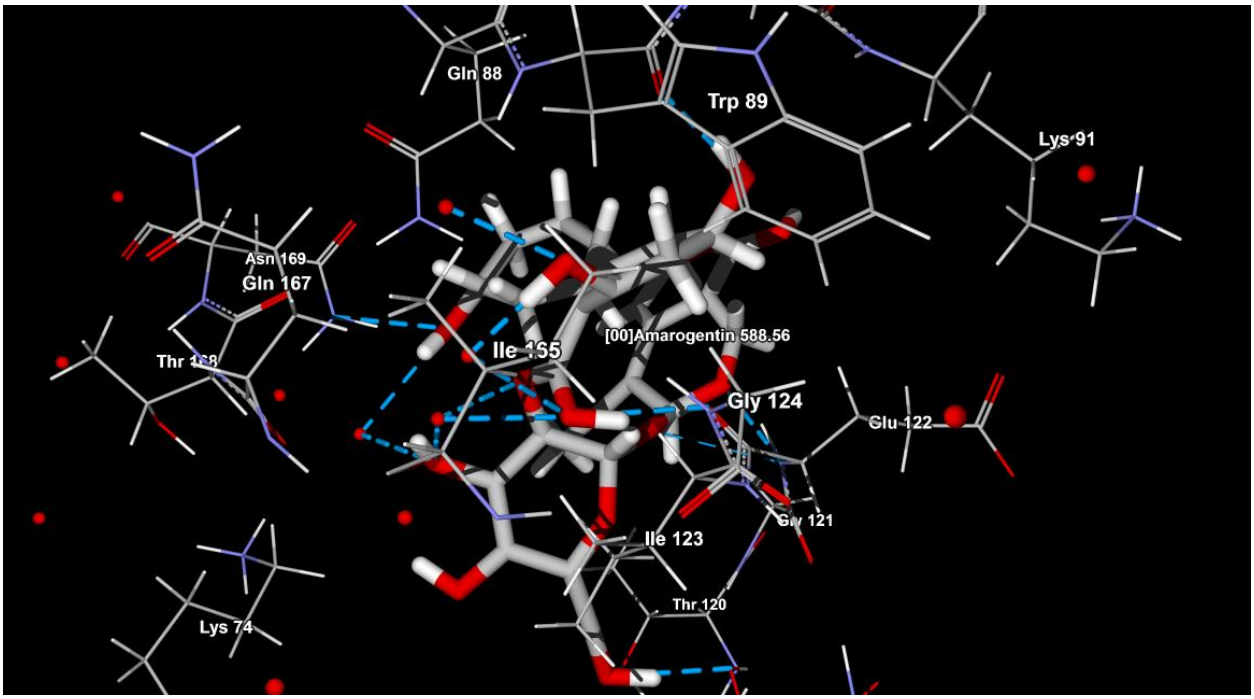


Figure 2: Molecular Docking Analysis of Amaroswerin

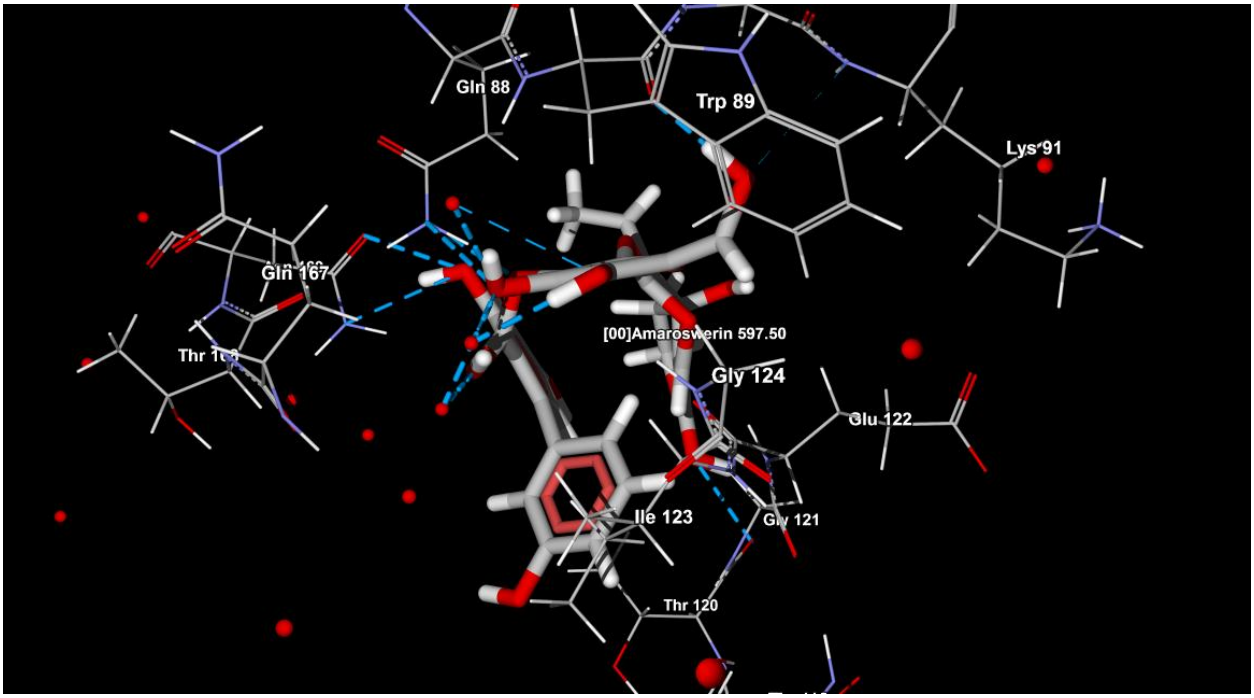
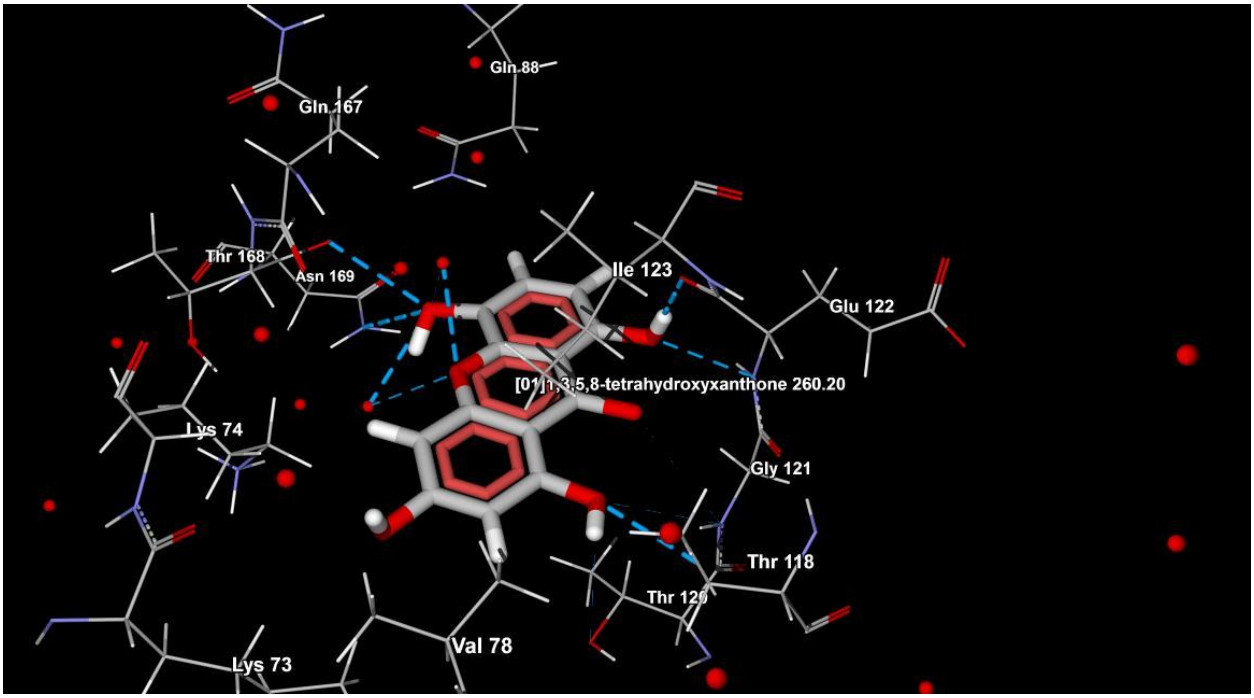


Figure 3: Molecular Docking Analysis of tetrahydroxanthone



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