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# *In-silico* screening for anti-Zika virus phytochemicals

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ABSTRACT

Zika virus (ZIKV) is an arbovirus that has infected hundreds of thousands of people and is a rapidly expanding epidemic across Central and South America. ZIKV infection has caused serious, albeit rare, complications including Guillain–Barré syndrome and congenital microcephaly. There are currently no vaccines or antiviral agents to treat or prevent ZIKV infection, but there are several ZIKV non-structural proteins that may serve as promising antiviral drug targets. In this work, we have carried out an *in-silico* search for potential anti-Zika viral agents from natural sources. We have generated ZIKV protease, methyltransferase, and RNA-dependent RNA polymerase using homology modeling techniques and we have carried out molecular docking analyses of our in-house virtual library of phytochemicals with these protein targets as well as with ZIKV helicase. Overall, 2263 plant-derived secondary metabolites have been docked. Of these, 43 compounds that have drug-like properties have exhibited remarkable docking profiles to one or more of the ZIKV protein targets, and several of these are found in relatively common herbal medicines, suggesting promise for natural and inexpensive antiviral therapy for this emerging tropical disease.

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## 1. Introduction

Zika virus (ZIKV) is an emerging arbovirus that belongs to the genus *Flavivirus* in the family Flaviviridae. Other members of the genus include the West Nile virus, dengue virus, yellow fever virus, and tick-borne encephalitis virus [1]. Zika virus was initially isolated from a rhesus monkey in the Zika forest of Uganda [2]. There had been sporadic human infections in sub-Saharan Africa and Southeast Asia [3]. However, in 2007 there was an outbreak in Yap Island (Micronesia) and subsequent epidemics in French Polynesia, New Caledonia, the Cook Islands, and Easter Island between 2013 and 2014 [4]. In 2015 the number of human infections with ZIKV showed a dramatic increase in the tropical Americas, particularly Brazil [5,6]. By the end of 2015, there have been an estimated 0.5–1.3 million cases of ZIKV infection [7].

The virus is primarily transmitted by *Aedes* spp. mosquitoes, including *Ae. aegypti*, *Ae. africanus*, *Ae. apicoargenteus*, *Ae. furcifer*, *Ae. hensilli*, *Ae. luteocephalus*, and *Ae. vitattus* [3]. However, there is now evidence that the disease can be sexually transmitted; replicative Zika viruses have been found in the semen of an infected man [8]. In addition, there is evidence that the virus can be transmitted perinatally and transplacentally; Zika viral RNA has been detected in amniotic fluid samples from fetuses of infected mothers [9]. Zika virus infections are typically characterized with symptoms of maculopapular rash, fever, headache, arthralgia, myalgia, and conjunctivitis. In addition, however, ZIKV infection has been associated with additional complications such as Guillain–Barré syndrome [10], congenital microcephaly [11], and macular atrophy [12].

There are currently no vaccines or antiviral agents available to treat or prevent Zika virus infection [13]. However, several *Flavivirus* non-structural proteins have been implicated as potential targets for antiviral drug discovery.

### 1.1. Zika virus protease

ZIKV protease (NS2B-NS3pro) is a trypsin-like serine protease that cleaves the ZIKV polyprotein into individual proteins necessary for viral replication. Flaviviral proteases from dengue virus [14] and West Nile virus [15] have been identified as a potential targets for development of antiviral agents [16].

### 1.2. Zika virus helicase

Flaviviral NS3 helicases possess RNA helicase, nucleoside and RNA triphosphatase activities and are involved both in viral RNA

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replication and virus particle formation. They are essential for viral replication and have been identified as potential drug targets [17].

### 1.3. Zika virus methyltransferase

The flavivirus NS5 methyltransferase (MTase) enzyme is responsible for methylating the viral RNA cap structure [18]. Viruses deficient in cap methylation result in attenuated or non-replicative viruses, and therefore, MTase is an attractive target for discovery of antiviral agents [19,20].

### 1.4. Zika virus RNA-dependent RNA polymerase

RNA-dependent RNA polymerases are employed by flaviviruses and are responsible for initiating and catalyzing replication of viral RNA synthesis in the cytoplasm of infected cells. There are no similar enzymes found in the host since host cells do not require RNA replication. This enzyme, then, is considered to be a prime target for antiviral development [21,22].

In this work, we have carried out an *in-silico* search for potential anti-Zika viral agents from natural sources. We have generated ZIKV protease, methyltransferase, and RNA-dependent RNA polymerase using homology modeling techniques and we have carried out molecular docking analyses of our in-house virtual library of phytochemicals with these protein targets and with the X-ray crystal structure of ZIKV helicase.

## 2. Methodology

### 2.1. Homology modeling

Currently there is only one protein structure available (helicase, PDB 5JMT [23]) for Zika virus non-structural proteins. Homology models for each of the other Zika proteins listed in Table 1 were constructed from crystal structure templates found in the Protein Data Bank using FASTA sequences downloaded from the National Center for Biotechnology Information's (NCBI) GenBank. Sequences with high sequence similarity in the PDB were identified with NCBI's BLAST utility using the BLOSUM80 scoring matrix. Sequences with high similarity to the reference sequence, as well as having good coverage for the active sites in the protein, were chosen for single reference homology modeling.

The protein sequences were first aligned to their respective template sequences using the BLOSUM62 substitution matrix and a protein backbone constructed and superposed to the reference structure using the protein alignment tool in MOE 2014.0901. The homology modeling interface in MOE was used to generate a set of putative protein structures by aligning atomic coordinates of the amino acid sequence to those of the template sequence backbone and minimizing permutations of side chain orientations using the AMBER10:EHT force field [24–26] with reaction field solvation. The candidate structure with the lowest RMSD deviation from the template backbone was selected and optimized using a constrained minimization.

The homology models for the NS3 protease was generated for the stretch of the whole Zika protein (accession number Q32ZE1) that correspond to the incomplete 257 amino acid NSP3 sequence, AHL16750.1, found in the NCBI GenBank. The closest matching template in the PDB was the combined NSP3 Protease-Helicase from Dengue Virus (2WHX) [27]. The protease binding site in this template is poorly defined in the crystal structure with the highest similarity (2WV9) [28], so a second template, from the homologous protease in West Nile Virus (2FP7; exp. value $4 \times 10^{-7}$) [29] with a bound ligand (Bz-Nle-Lys-Arg-Arg-H; (*N*-benzoyl-L-norleucyl-6-ammonio-L-norleucyl-*N*-5-[amino(imino)methyl]-*N*-[(2*S*)-5-carbamimidamido-1-hydroxypentan-2-yl]-L-ornithinamide)) was used to generate a homology model specifically for the protease. Since the sequence similarity with the NS3 protease is much lower for this template, a constrained minimization of the peptide-receptor complex was carried out using the AMBER10:EHT force field and the bound peptide removed prior to docking. The RMSD between 2FP7 and the homology model is 0.753 Å for all common atoms. The binding interactions for both are shown in Fig. 1. The GenBank protein sequence A0A0B4ZYV2 was used for the NS5 methyltransferase and RNA-dependent RNA polymerase homology models using the relevant portion of the complete NS5 protein from Japanese Encephalitis Virus (4K6M) [30]. A second homology model for NS5 RdRp was generated using the template structure 2HFZ from West Nile Virus [31]. Protein sequence alignments and Ramachandran plots of the template proteins and the homology modeled ZIKV proteins are available as Supplementary material.

### 2.2. Molecular docking

Protein-ligand docking studies were carried out based on homology-modeled structures of Zika virus (ZIKV) NSPB/NS3 protease, based on the crystal structure of Murray Valley encephalitis virus protease, PDB 2WV9 [28]; ZIKV NS5 methyltransferase (MTase), based on the crystal structure of Japanese encephalitis virus methyltransferase, PDB 4K6M [30]; and ZIKV NS5 RNA-dependent RNA polymerase (RdRp), based on crystal structures of West Nile virus RdRp, PDB 2HFZ [31] and Japanese encephalitis virus RdRp, PDB 4K6M [30]. Molecular docking calculations for all compounds with each of the proteins were undertaken using Molegro Virtual Docker (version 6.0, Molegro ApS, Aarhus, Denmark) [32], with a sphere large enough to accommodate the cavity centered on the binding sites of each protein structure in order to allow each ligand to search. Standard protonation states of the proteins based on neutral pH were used in the docking studies. Each protein was used as a rigid model structure; no relaxation of the protein was performed. Assignments of charges on each protein were based on standard templates as part of the Molegro Virtual Docker program; no other charges were necessary to be set. Overall, 2263 plant-derived secondary metabolites have been docked. This molecule set was comprised of 384 alkaloids (158 indole alkaloids, 143 isoquinoline alkaloids, 5 quinoline alkaloids, 18 piperidine alkaloids, 14 steroidal alkaloids, and 46 miscellaneous alkaloids), 670 terpenoids (35 monoterpenoids, 153 sesquiterpenoids, 212 diterpenoids, 83 steroids, 51 limonoids, 42 withanolides, and 97 triterpenoids), 1043 polyphenolic compounds (22 aurones, 72 chalcones, 9 chromones, 111 coumarins, 297 flavonoids, 113 isoflavonoids, 69 lignans, 69 stilbenoids, 29 xanthones, and 172 miscellaneous phenolics), and 166 miscellaneous phytochemicals. This in-house virtual library represents a practical selection of phytochemical classes and structural types. Each ligand structure was built using Spartan '14 for Windows (version 1.1.8, Wavefunction Inc., Irvine, California). For each ligand, a conformational search and geometry optimization was carried out using the MMFF force field [33]. Flexible ligand models were used in the docking and subsequent optimization scheme. Variable orientations of each of the ligands were searched and ranked based on their re-rank score. For each docking calculation the maximum number of iterations for the docking algorithm was set to 1500, with a maximum population size of 50, and 30 runs per ligand. The RMSD threshold for multiple poses was set to 1.00 Å. The generated poses from each ligand were sorted by the calculated re-rank score. In analyzing the docking scores, we have attempted to account for the recognized bias toward high molecular weight compounds [34–37]. We have used two different schemes to re-evaluate the Molegro docking scores ($E_{dock}$): (1) $E' = 6.96 \times E_{dock}/MW^{1/3}$, which accounts for biasing by dividing by a molecular weight function (MW), where 6.96 is a scaling con-

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