Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 388-396 | ISSN:2251-6727

Exploring Potential Antiviral Agents Against Dengue Virus: A Comprehensive Analysis of Drug Candidates and Natural Compounds

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(Received: 04 February 2024 Revised: 11 March 2024 Accepted: 08 April 2024)

KEYWORDS
Dengue Virus,
NS1 Protein,
NS3 Protein,
E Protein,
Capsid,
Alkaloids,
Macrolide-Antibiotics,
Antiviral- Agents.

ABSTRACT:

Introduction: Dengue fever, caused by the Dengue Virus (DENV), has become a significant global concern due to its widespread prevalence in tropical regions. Understanding the viral proteins and exploring potential antiviral agents are crucial to combating this disease.

Objectives: The study aims to investigate potential antiviral agents against DENV by analysing drug candidates and natural compounds for their interactions with viral proteins.

Methods: Ligands' 3D structures were obtained from PubChem, energy was minimized with PyRx AutoDock Vina, and interactions were assessed using Discovery Studio. Protein structures from RCSB PDB were prepared, active sites were identified with PLIP, and docking was executed with Auto Dock Vina. PyMol visualized results, while Biovia Discovery Studio documented interactions.

Results: Among the compounds analysed, Avermectins, especially Doramectin, demonstrated significant binding affinity with DENV proteins, suggesting their potential as antiviral agents. Additionally, compounds from Carica papaya leaf extract showed promising interactions with viral proteins. Macrolide antibiotics, particularly Clarithromycin, also exhibited a notable affinity for DENV target proteins.

Conclusions: Our findings highlight the potential of Avermectins, Carica papaya leaf extract alkaloids, and Macrolide antibiotics as prospective antiviral agents against DENV. Further research, particularly in vivo studies, is warranted to validate these findings and explore their therapeutic efficacy in combating dengue fever.

1. Introduction

Dengue, colloquially known as "break-bone fever," is characterized by symptoms such as fever, headache, and myalgia. The Dengue virus poses a global threat, infecting humans in over 100 countries annually, putting approximately 3.6 billion people at risk. Most infections go unnoticed or result in mild symptoms like fever, joint pain, or rashes lasting for a few days. However, in severe cases, the consequences can be far more serious, leading to conditions like dengue haemorrhagic fever and dengue shock syndrome, responsible for around 20,000 deaths each year. Epidemics resembling dengue-like illness have been documented for over two centuries and were once prevalent in Asia, the Gulf coasts of the United States, and the Caribbean until the first half of the 20th century [1]. The Dengue virus is an RNA virus belonging to the Flaviviridae family. Dengue virus exhibits four distinct serotypes: DENV-1, DENV-2, DENV-3, and

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DENV-4. Aedes aegypti, the primary mosquito vector, plays a crucial role in the transmission of dengue as well as urban yellow fever. The proposed etiologies for dengue fever are Viral replication, primarily in macrophages and direct skin infection by virus.

Dengue virus (DENV) is an enveloped positive-strand RNA virus, and its genetic material encodes three structural proteins: Capsid protein (C), which consists of 100 amino acids, Membrane protein (M) with 75 amino acids, and Envelope protein (E) with 495 amino acids. Electron micrographs reveal that dengue virions have a relatively smooth surface, a diameter of about 500 Å, and an electron-dense core surrounded by a lipid bilayer [2]. Additionally, DENV possesses seven Non-Structural (NS) proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. With the assistance of host proteins, these NS proteins play pivotal roles in reshaping the inner organization of host cells, maturing the polyprotein, replicating viral RNA, and aiding the virus in evading the immune system [3]. The precise functions of NS1, NS2A, and NS4A are still not fully understood [4]. NS1 is associated with the endoplasmic reticulum vesicle membrane on the lumen side and plays a role in anchoring the viral replication complex. It can also be secreted and is believed to assist the virus in evading the immune system [5]. NS3 acts as a protease-helicase, working in tandem with NS2B as a cofactor [6].

A thorough literature review has guided the selection of drug candidates from various classes, including Avermectin macrocycles, Macrolide antibiotics, Indolizidine Alkaloids, and Alkaloids extracted from the Carica papaya leaf. These selections were made based on their potential antiviral properties. Avermectins, traditionally used as antiparasitic agents, have demonstrated inhibitory effects on flavivirus helicases in multiple studies [7, 8]. Macrolide antibiotics, due to their macro lactone ring structure, have also shown promise in inhibiting viruses, including Zika virus and other flaviviruses [9, 10]. Indolizidine Alkaloids, with a special mention of Castanospermine, are recognized as potent antivirals [11].

2. Objectives

The primary objective of this investigation is to conduct a thorough exploration of potential antiviral agents targeting Dengue Virus (DENV). Through an integrated approach, diverse drug candidates and natural compounds were analysed, to elucidate their interactions with key viral proteins. Leveraging sophisticated computational methodologies, we aim to identify promising candidates with robust binding affinities to DENV proteins, thus offering insights into their potential as therapeutic interventions for Dengue fever. Additionally, we seek to assess the feasibility of repurposing existing drugs and harnessing natural compounds as alternative therapeutic modalities for combating Dengue virus infection. By providing comprehensive insights, this research endeavours to contribute to the development of effective antiviral strategies against Dengue fever, addressing the significant global health burden posed by this infectious disease.

3. Methods

Ligand preparation:

Following a thorough Literature review, we identified 19 ligands as potential candidates with potential anti-dengue properties. To validate their effectiveness, we employed a molecular docking process. We obtained the necessary 3D structures from the PubChem database, using 2D structures when 3D conformations were unavailable.

Subsequently, we conducted energy minimization for all the selected ligands using PyRx AutoDock Vina software [12]. This crucial step aimed to optimize the energy levels associated with these ligands.

To delve deeper into the docked poses of all the ligands and to investigate and evaluate the hydrogen bonding and hydrophobic interactions between the ligands and the target proteins, we employed Discovery Studio [13].

Preparation of protein:

The proteins utilized in our molecular docking study were sourced from the RCSB PDB (RCSB.org) and carried the following PDB IDs: 6VG5, 2VBC, 4O6B, 1OKE, and 3C5X. To prepare these protein targets for further analysis, all non-amino acid residues were removed using the Swiss-PDB software.

For pinpointing the active sites within the protein structures, we made use of the PLIP database, which effectively highlighted these crucial regions. Subsequently, the Auto Dock Vina software [12] was employed to select the active residues within the protein and complete the process of docking the ligands.

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To visualize the results obtained from PyRx, we turned to PyMol [14]. Furthermore, Biovia Discovery Studio [13] was used in documenting the 2D and 3D interactions between the protein targets and the ligands.

4. Results

Table 1: Binding Affinities of the ligands with each of the selected proteins.

CAPSID → 6VG5

The Capsid protein exhibited its highest binding affinity with Doramectin at -9.1. Notably, the ligand's binding site was situated within a pocket bounded by both chains A and B of the protein. The primary interactions observed in this context were predominantly Van Der Waals interactions, in addition to Pi-alkyl bonding. A significant interaction was observed between the ligand's ring system and chain B, involving Valine (B51), Arginine (B55), Proline (B61), Leucine (B66), and Tryptophan (B69). A salt bridge was also seen between the Arginine (A90) residue and the carboxylate group of the ligand at a distance of 4.17 Å.

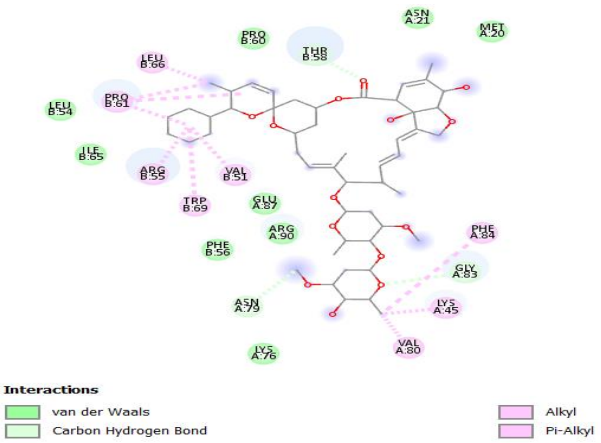

Figure 1: 2D interaction of Doramectin with Capsid protein

The ligand, Moxidectin, displayed a binding affinity of -8.4. Interestingly, this interaction was observed within the same binding pocket as Doramectin. Hydrogen bonding was noted between the Hydroxyl group of the Macrocyclic Lactone and Arginine (A22). Additionally, Pi-alkyl interactions were also observed, involving Valine (B51), Leucine (B54), Arginine (B55), Proline (B61), Isoleucine (B65), Leucine (B66), and Tryptophan (B69).

All interactions observed here occurred within the range of 4.00 Å.

Ivermectin also displayed a binding affinity of -8.4. The interactions observed encompassed conventional hydrogen bonding involving the oxygen atoms in the ligand and Arginine (A55). Additionally, alkyl bonding interactions were observed with residues of the A chain, including Leucine (A66), Tryptophan (A69), Arginine (A90), Isoleucine (A94), and Arginine (A97).

Ligand	Name	3C5X	2VBC	6VG5	1OKE	4O6B
1684	Pleconaril	-7.3	-7.7	-7.1	-8.4	-7.8
12560	Erythromycin	-6.9	-6.6	-6.3	-6.6	-7.1
51683	Swainosoine	-6	-6	-5.4	-5.8	-5.9
54445	Cantonospermine	-5.6	-6.4	-5.5	-6.2	-5.9
60734	Clegosivir	-5.9	-6.2	-6.8	-6.7	-6.4
84029	Clarithromycin	-7.2	-6.9	-6.7	-7.3	-8.1
88363	Slaframine	-5.2	-6	-6	-5.1	-5.3
442630	Carpaine	-8.1	-7.6	-7.7	-8.6	-8.9
447043	Azithromycin	-6.4	-7	-7	-6.9	-8.3
471161	Maribavir	-6.4	-6.7	-7.3	-6.5	-6.7
5280343	Quercetin	-7.3	-7.8	-7.9	-9	-8
6434889	Avermectin	-8.1	-8.8	-8.2	-8.7	-9.8
9832750	Doramectin	-8.4	-8.5	-9.1	-11	-10.8

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9832912	Moxidectin	-8.2	-8.8	-8.4	-8.2	-9.8
11019091	Tashiromine	-5	-5.1	-5.8	-5.1	-5
11498684	Manghasline	-8.7	-8.3	-7.5	-7.5	-9.5
45114068	Ivermectin	-7.9	-8	-8.4	-11.4	-10.1
129720114	p-Coumaroylmalic acid	-6.3	-7.2	-6	-7.2	-6.7
131750991	Dehydrocarpaine	-8.6	-7.8	-7.9	-8.6	-9.1

ENVELOPE PROTEIN →1OKE

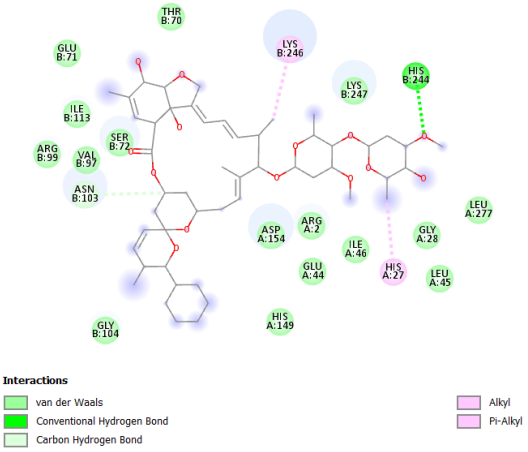

Figure 2: 2D interactions of Doramectin with Envelope protein

The analysis of the viral envelope protein revealed that the drug Doramectin exhibited the highest binding affinity, with a remarkable binding affinity of -11. Quercetin followed closely with a binding affinity of -9.

In both Doramectin and Quercetin, the interactions primarily involved Van der Waal's interactions. Additionally, conventional hydrogen bonding interactions were noted in both cases, with one hydrogen bond in the case of Doramectin and five in the case of Quercetin. Pi-alkyl bonds were also observed in these interactions.

For Doramectin, a salt bridge was formed between a lysine (+) and the carboxylate (-) group on the ligand, with a binding distance of 4.71 Å. The conventional hydrogen bond present was seen between the ligand and glutamate (-) (A269) at a distance of 3.13 Å.

In Quercetin, a salt bridge was formed between a histidine (-) and an aromatic (+) group on the ligand, with a binding distance of 5.71 Å. Interestingly, Quercetin exhibited T-shaped pi-pi interactions between the ligand and histidine (A244), along with pi-cation interactions between the ligand (-) and lysine (+) (A247).

Another notable interaction was observed with Avermectin, which displayed a binding affinity of -8.7.

prM- E HETERODIMER → 3C5X

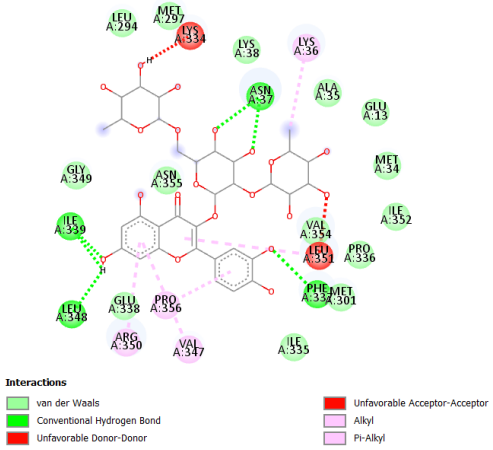

Figure 3: 2D interaction of Manghasline with prM-E Heterodimer

The drug Manghasline demonstrated the highest binding affinity with the Pre-membrane – Envelope Heterodimer. It showed a bonding affinity of -8.7. This was closely followed by Dehydrocarpaine with a score of -8.6.

The compound Manghasline exhibited the highest binding affinity with the Pre-membrane – Envelope Heterodimer, scoring -8.7, followed closely by Dehydrocarpaine with a score of -8.6. Manghasline's binding primarily comprised conventional hydrogen bonding with the A chain of the heterodimer. Notably, Proline (A356) formed pi-alkyl bonds with two ring structures. Additionally, multiple van der Waals interactions occurred with the pocket residues. A salt bridge formed between the Arginine (A350) residue and the carboxylate group of the ligand, measuring 4.59 Å,

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with the protein contributing to the positive group. However, some unfavourable interactions were observed, particularly with Lysine (A334) and Leucine (A351).

Dehyrocarpaine primarily engaged in van der Waals interactions with the protein heterodimer. Alkyl interactions were also noted with the Methionine residue of the A chain (A34) and the Valine residue (A354). An attractive charge interaction occurred with the Glutathione residue (A338), with the ligand providing the positive moiety as cyclic N+. Hydrogen bonding with Leucine (A351) was also observed.

Doramectin and Moxidectin demonstrated notable interactions with the Pre-membrane – Envelope Heterodimer, exhibiting binding affinities of -8.4 and -8.2, respectively.

NS1 → 4O6B

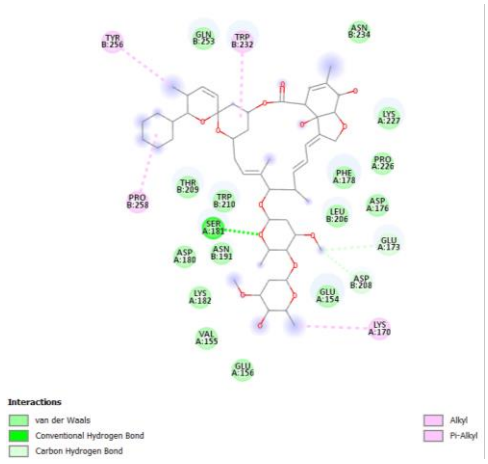

Figure 4: 2D interactions of Doramectin with NS 3 protein

Doramectin exhibited the highest binding affinity with the NS1 protein, scoring -10.8, followed by Ivermectin with a binding affinity of -10.1.

The Doramectin-NS1 complex predominantly featured van der Waals interactions, with the binding pocket spanning both the A and B chains of the protein. A Traditional Hydrogen Bond was identified between the Serine Residue (A181) and an oxygen atom of the ligand. Additionally, the glutamic acid residue (A173) and the Aspartic acid residue (B208) participated in Carbon-Hydrogen bonding with the ligand.

Similarly, Ivermectin displayed numerous van der Waals interactions, along with Alkyl and Pi-Alkyl interactions involving residues from both the A and B chains of the protein. The Lysine residue (B170) engaged in two hydrogen bonds with separate oxygen atoms of the ligand. The binding pocket in this case was also situated between the A and B chains of the protein.

Moxidectin and Avermectin showcased notable interactions with binding affinities of -9.8, followed by Manghasline with a binding affinity of -9.5.

NS 3 (PROTEASE AND HELICASE) → 2VBC

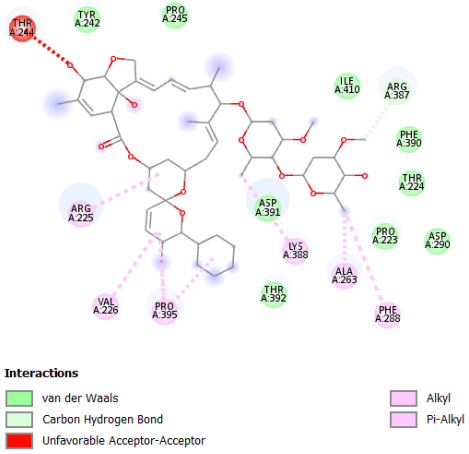

Figure 5: 2D interactions of Doramectin with NS 3 protein

In the analysis of the NS protease and helicase, Abamectin and Moxidectin exhibited the highest binding affinity, with a binding affinity of -8.8 for both drugs. The primary interactions for both drugs were of the pi-alkyl type, although other interactions involved hydrophobic and hydrogen bonding interactions.

In the case of Moxidectin, a salt bridge was formed between a histidine (+) and the carboxylate (-) group on the ligand, at a binding distance of 4.15 Å. Moxidectin exhibited attractive charge interactions between the ligand (+) and glutamic acid (-) at atom A:173, a conventional hydrogen bond was also seen between the ligand and asparagine (A329), carbon and hydrogen bond interactions between the ligand and serine (A328), and pi-alkyl interactions between the ligand and histidine (-) (A194).

In the case of Abamectin, there was a conventional hydrogen bond between the ligand and proline (+) at

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atom (A319), an unfavourable donor-donor interaction between the ligand and glutamine (A:327), pi-alkyl interactions between the ligand and isoleucine (A190), and a hydrogen bond present between the ligand and glutamine at a distance of 2.15. A salt bridge was also formed between a histidine (+) and the carboxylate (-) group on the ligand but at a slightly greater binding distance of 5.17 Å.

Additionally, Doramectin and Manghasline showed notable interactions with affinities of -8.5 and -8.3, respectively.

5. Discussion

Dengue fever has emerged as a major global concern in recent years. By the last decade of the 20th century, the dengue viruses had spread to nearly every country in the tropical regions [15].

The range of the disease manifestations varies from a flu-like illness known as dengue fever (DF) to a severe and sometimes fatal condition characterized by bleeding and shock, referred to as dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) [16].

Dengue Virus (DENV), a member of the Flaviviridae family, falls within the flavivirus genus, which includes other pathogenic viruses such as West Nile virus (WNV), Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBEV), and yellow fever virus (YFV) [17].

The viral genome consists of positive-sense RNA, totalling 11 kilobases in length. This RNA encodes three structural proteins: Capsid (C), Membrane (prM), and Envelope (E), as well as seven non-structural proteins (NS) - NS1, NS2A/B, NS3, NS4A/B, and NS5 [18]. Initially, the viral RNA is translated into a polyprotein, which is then directed to the Endoplasmic Reticulum (ER). Signal sequences facilitate the translocation of NS1, the ectodomains of prM and E, into the lumen of the ER. In contrast, the C, NS3, and NS5 proteins remain localized in the cytoplasm, while NS2A/B and NS4A/B predominantly function as transmembrane proteins [18].

The NS1 protein, a 45 kDa glycoprotein, is typically secreted from infected cells and plays crucial roles related to the viral RNA replication complex. Unlike other non-structural proteins, NS1 is secreted [19], and elevated levels (up to 50 μg/ml) have been detected in the serum of DEN-infected patients [20] correlating with the severity of the disease. Furthermore, NS1 becomes associated with cell surface membranes through a mechanism that remains unknown [21].

The NS2A/B proteins, despite being well-conserved among the four DENV serotypes [22], are less explored. NS2B serves as a co-factor to NS3, especially in its role as a protease [23]. NS3, a multifunctional protein comprising 618 amino acids, acts as both a chymotrypsin-like serine protease and an RNA helicase and RTP ase / NTP ase. Residues 49–66 from NS2B are linked to the N-terminus of the full-length NS3 by a Gly-Ser linker [24]. The helicase domain of NS3 interacts with the polymerase, NS5 [25].

The flavivirus E protein, a multifunctional protein, is involved in cell receptor binding and virus entry via fusion with the host cell membrane. Its functional activities are regulated by interaction with a second viral protein, prM [26]. In essence, prM acts as a chaperone for the E-protein to ensure its conformational changes during various steps of the secretory pathway and to produce infectious viral particles.

The Capsid (C) protein (12 kDa) of mature DENV is highly basic and has an affinity for both the viral genome and lipid membrane [27]. This protein, located in the cytosol, is the first protein translated by the viral genome, followed by prM. The C protein functions akin to RNA-chaperonins, responsible for packaging viral RNA in its properly folded conformation. Its RNA chaperone domain is flexible and rich in basic amino acid residues [28].

Our analysis highlighted that among the drugs, those belonging to the Avermectin class exhibit the most substantial interactions with the target proteins. Avermectins are a group of compounds derived from a 16-membered lactone ring, and they are naturally produced as a fermentation product by Streptomyces avermitilis, a type of actinomycete found in the soil [29]. One, in particular, stands out for its strong interactions with the target proteins. Doramectin, a drug widely used for treating animal parasitic infections, exhibits notable binding affinity. Impressively, Doramectin demonstrated an average binding affinity of -9.56, the greatest of all the ligands analysed. Notably, Doramectin exhibited the strongest interaction with the Viral envelope protein. Studies have additionally uncovered that Doramectin

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exhibits antiviral properties, particularly against Flaviviruses like the Zika virus (ZIKV), without inducing potential cytotoxic effects [30]. This implies that Doramectin carries substantial potential as a prospective antiviral agent. While primarily utilized in veterinary treatments, Doramectin is currently under investigation for potential human applications, particularly in cases of scabies [31]. Ivermectin closely followed suit, with an average score of -9.16. Ivermectin, which is already widely used in humans, demonstrated significant affinity with all the proteins considered.

It is widely acknowledged in traditional knowledge that extracts derived from the Carica papaya leaf possess antiviral properties [32]. In our investigation, we found that among all the considered Carica papaya leaf extract alkaloids, the compound Dehydrocarpaine exhibited the highest affinity for the target proteins. It demonstrated an average binding affinity of -8.4 toward target proteins. Manghasline and Carpaine demonstrated a significant affinity for the targets. Particularly interesting is the finding that Manghasline exhibited the highest binding affinity among all the considered ligands towards the prM-E heterodimer.

When comparing Indolizidine Alkaloids to Macrolide antibiotics, the Antibiotics demonstrated a higher binding affinity. Notably, Clarithromycin emerged as the standout among the antibiotics, with an average score of -7.24. Specifically, Clarithromycin exhibited the highest affinity to the NS1 Protein, scoring -8.1.

Further analysis will be required to determine the most potent antiviral for DENV in vivo. However, based on the in-silico data, we recommend exploring the potential of Avermectins, such as Doramectin and Ivermectin. In the case of the Papaya leaf extract alkaloids, compounds such as Dehydrocarpaine and Manghasline showed the most potential as anti-DENV candidates.

6. Conclusion

Our analysis underscores the significant interactions between Avermectins and target proteins, particularly Doramectin, which demonstrated remarkable binding affinity, notably with the Viral envelope protein. Doramectin not only exhibits antiviral properties against Flaviviruses like the Zika virus but also holds promise for potential human applications, including treating conditions like scabies. Ivermectin, another Avermectin, also showed substantial affinity with all considered proteins, highlighting its potential as an antiviral agent.

Moreover, Carica papaya leaf extract alkaloids, especially Dehydrocarpaine, exhibited a high affinity for target proteins, suggesting their potential as anti-DENV candidates. Manghasline, another compound from Carica papaya leaf extract, showed significant binding affinity, particularly towards the prM-E heterodimer.

Comparatively, Macrolide antibiotics, notably Clarithromycin, demonstrated notable interactions, especially with the NS1 Protein, indicating their potential as antiviral agents against DENV.

While further in vivo studies are needed to confirm the most potent antiviral for DENV, the in-silico data suggest exploring Avermectins like Doramectin and Ivermectin, as well as Carica papaya leaf extract alkaloids such as Dehydrocarpaine and Manghasline, as promising candidates.

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Journal of Chemical Health Risks

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JCHR (2024) 14(3), 388-396 | ISSN:2251-6727

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