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Design and In-Silico Studies of Abacavir Analogues as Anti-DENV-2 NS5 Methyltransferase

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Abstract: Researchers have demonstrated substantial biological activity of the FDA-approved anti-HIV drug "abacavir" against the cancer cell line and SARS-CoV-2. Using computational analysis, the most potent abacavir analogs were chosen in this investigation to find an anti-DENV inhibitor against the DENV-2 NS MTase. Twenty-four (24) compounds were collected through the SwissSimilarity program, and four of them were submitted to molecular docking research based on the similarity score (1.000 to 0.400). According to the studies, DB02947 has been identified as a lead molecule against DENV-2 NS5 MTase with a binding affinity of -7.4 kcal/mol and high druggability ratings. To find and discover a novel medicine to treat DENV, in vitro and in vivo experiments on DB02947 are advised to be conducted to ascertain its antiviral efficacy.

Keywords: anti-dengue; methyltransferase; in-silico; abacavir; Swiss similarity.

1. Introduction

Dengue fever (DF) is a mosquito-transmitted illness caused by the dengue virus (DENV) that affects over 3.9 billion people worldwide and results in over 20,000 fatalities each year [1]. Today, it is recognized as a worldwide human health issue threatening 50% of the world's population. *Aedes aegypti*, *A. albopictus*, and *A. polynesiensis* are the main vectors of DENV transmission to humans [2]. The four genetically interrelated DENV-1, DENV-2, DENV-3, and DENV-4 serotypes comprise the genus DENV, a subfamily of the flavivirus family [3]. Even though more than 80% of illnesses are often moderate [4], few individuals may experience acute DF, bringing with it potentially fatal consequences such as plasma leakage and coagulopathy that can impair organ function and cause circulatory shock [5]. Considering that there is now no antiviral drug approved by science to treat DENV, developing a new anti-dengue medication that may inhibit viral replication may lead to a more reliable treatment for severe DENV infections.

DENV may cause a wide range of mild to severe illnesses [6], and DENV-2 is known to cause more severe dengue infections than other serotypes [7]. The 11-kb-long dengue genome has a single open reading frame (ORF) that codes for three structural proteins as well as seven other non-structural proteins. Capsid protein (C), membrane precursor protein (prM), and envelope protein (E) are the three structural proteins of the DENV, while NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 are the seven non-structural (NS) proteins [8]. Most

dengue infections are self-limiting; however, there are a small number of severe cases that manifest as dengue shock syndrome (DSS) and dengue hemorrhagic fever (DHF) [9]. Furthermore, a key therapeutic target is the non-structural protein NS5, which has methyltransferase (MTase) activity at its N-terminal and RNA-dependent RNA polymerase (RdRp) activity at its C-terminal [10]. The emergence of antiviral inhibitors and their design should be improved with the help of knowledge of NS5 structural dynamics. The ideal target for creating inhibitors for flavivirus infections is NS5 protease due to its significant involvement in viral replication [3].

Abacavir is a potent nucleoside analog reverse transcriptase inhibitor (NRTI) used to treat the viral diseases HIV and AIDS [11]. Additionally, it has anti-cancer action in both PC3 and LNCaP prostate cancer cell lines [12]. An infectious condition called DF is spread from person to person by certain mosquitoes. Moreover, abacavir has a binding affinity of -6.91 kcal/mol to SARS-CoV-2 [13]. Although the FDA has authorized abacavir as an antiviral medication for HIV, we hypothesized that abacavir analogs would inhibit DENV-2 NS5 MTase.

A broad-spectrum antiviral ribavirin, an analog of guanosine, was first isolated approximately 30 years ago and was used as a reference compound in this investigation. Among the few nucleoside analogs now used in clinics to treat RNA virus transmission is ribavirin, but its molecular basis of effectiveness is still not fully understood [14]. Important human infections, including West Nile, dengue, and yellow fever viruses, are members of the genus Flavivirus and are susceptible to ribavirin [15].

The main objective of this study was to investigate the inhibitory ability of abacavir analogs for the non-structural protein NS5 of DENV-2, which is responsible for viral replication. The analogs were initially extracted from the LigandExpo database using the SwissSimilarity software. Figure 1 depicts these analogs. Furthermore, the primary purpose was to design a computational approach for this and to determine if the findings of this study may usher in a new era in the development of innovative, selective dengue inhibitors. The work plan of this research is depicted in Figure 2.

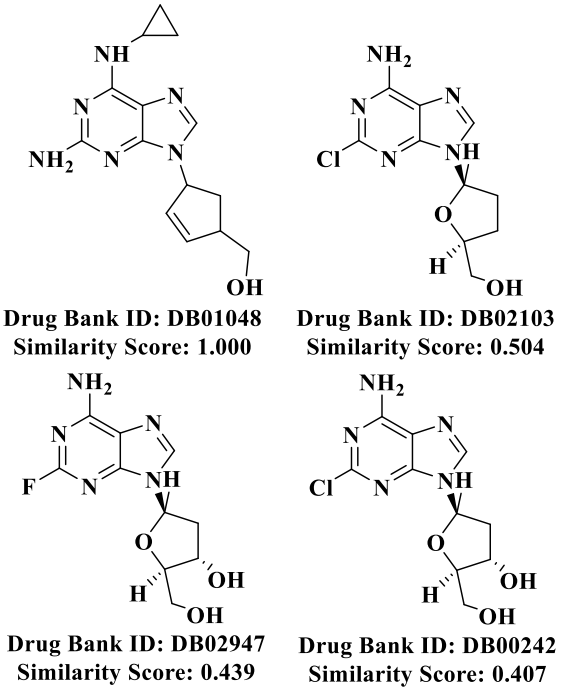

Figure 1. Design of abacavir analogues.

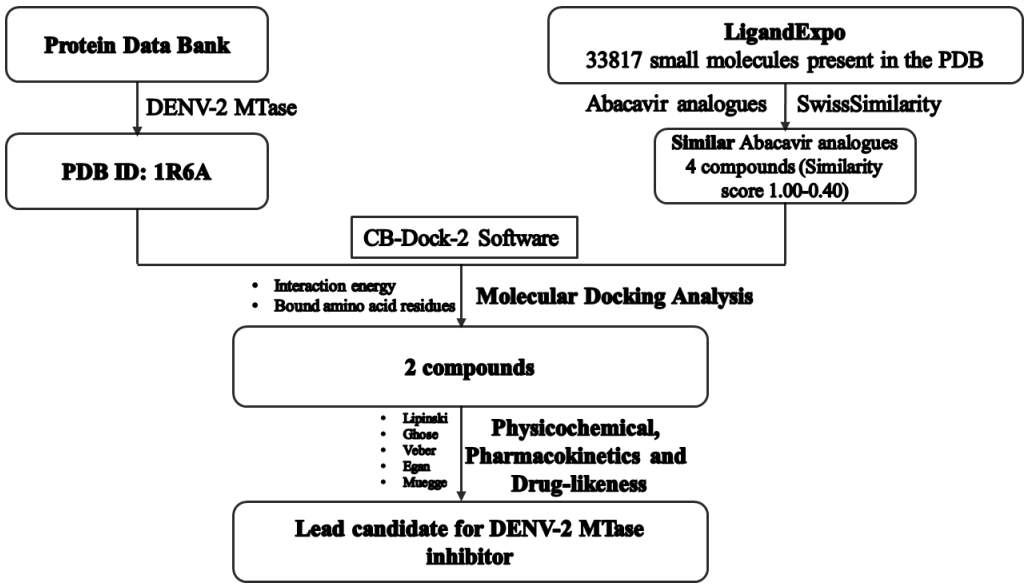

Figure 2. The design of this work is to find out the DENV-2 NS5 MTase inhibitor.

2. Materials and Methods

2.1. Abacavir analogues selection and preparation.

The structure of the abacavir was created with ChemDraw Professional 13, and the SMILES format was produced. SwissSimilarity (http://www.swisssimilarity.ch/) software was used to search for new similar compounds utilizing the SMILES of the abacavir compound [16]. This work used the pharmacophore approach to identify abacavir analogs from the ligandExpo database screening. The compounds with 1.000 to 0.400 similarity scores were chosen for further study (Figure 1). These substances were created with the aid of the ChemSketch program and saved in .mol format for docking with the DENV-2 NS5 MTase target protein.

2.2. DENV-2 NS5 MTase protein retrieved and prepared.

The DENV-2 NS5 MTase crystal structure was chosen from published works [17] and downloaded using the PDB ID 1R6A [14] with a resolution of 2.60 Å. The chosen protein was stored in .pdb format to perform the dock with the chosen ligands.

2.3. Molecular docking of abacavir analogs with DENV-2 MTase protein.

Using the online docking program CB-Dock (http://clab.labshare.cn/cb-dock/php/blinddock.php), the analysis was estimated using a previously published method [18]. A PDB file for the protein and a .mol file for the ligands were entered into the CB-Dock program prior to docking. This technique automatically chose several top cavities and utilized them for docking analysis using the AutoDock Vina [19].

2.4. Physicochemical, pharmacokinetics, drug-likeness, and medicinal chemistry studies.

SwissADME (http://www.swissadme.ch) was used to conduct investigations on physicochemical, pharmacokinetics, drug-likeness, and medicinal chemistry properties [20]. This program required the SMILES of the compounds; it was not essential to understand the active site or the binding mechanism.

3. Results and Discussion

3.1. Abacavir analogues selection.

The LigandExpo database provides the structural and chemical information for small molecules found in protein data bank structure entries. The LigandExpo database (http://ligand-expo.rcsb.org/) demonstrates that 33,817 small molecules are included in at least one experimental structure retained in the PDB. SwissSimilarity software was used to perform a pharmacophore method-based screening on this database. The results revealed 24 compounds with similarity scores ranging from 1.000 to 0.350. After that, four compounds (Figure 1) with similarity scores >0.400 from this group were put through molecular docking to find out how well they bonded with the target protein DENV-2 NS5 MTase.

3.2. Molecular docking of Abacavir analogs with DENV-2 MTase protein.

In drug development and molecular interaction research, small compounds are frequently docked into the binding sites of therapeutic target proteins [21]. The inhibitory action of small molecules in cell line tests does not provide complete details about the binding orientations of ligands to the target protein. However, in-silico molecular docking studies provide a clear explanation of how chemicals interact in the active region of proteins [22].

Therefore, to investigate the possible mechanism of action of the abacavir analogs, computational docking experiments were conducted using the CB-Dock [18] between the DENV-2 NS5 MTase and compounds chosen from the SwissSimilarity program with a similarity score >0.400. CB-Dock is a protein-ligand blind docking program that uses AutoDock Vina to execute molecular docking after automatically determining the binding sites, calculating the center and size, and customizing the docking box size for the query ligands [19]. The method built into AutoDock Vina was used to determine the binding energy automatically. Following docking experiments, abacavir analogs were rated compared to the reference molecule ribavirin (DB00811) based on their binding energies and bound amino acid residues into the target protein's active site, based on docking results, compounds DB01048 and DB02947 exhibited the best binding energies (-7.6 and -7.4 kcal/mol, respectively) against the DENV-2 NS5 MTase protein (Table 1).

Table 1. Docking results of Abacavir analogs and the reference (Ribavirin; DB00811) compound towards the DENV-2 MTase (PDB ID: 1R6A).

Compound ID	Cavity size	Vina score	Bound amino acids
DB00811 (Ribavirin)	300	-6.6	Gly83, Cys82, Ser56, Asp146, Gly58, Tyr219, Glu217 (H-B), Lys181, Trp87, Val55 (C-H), Lys61, Lys181 (ionic)
DB01048	300	-7.6	Val130, Gly83, Thr104, Cys81, Glu111, Cys82 (H-B), Val132, Ile147, Thr104, Lys105, Trp87, Val55 (C-H), Arg84 (ionic)
DB02103	300	-6.5	Thr104, Gly83, Gly81, Cys82, Ser56, Glu111 (H-B), Val55 (C-H), Arg84 (ionic)
DB02947	300	-7.4	Gly83, Glu111, Cys82, Asp146, Ser56, Glu217, Lys181, Tyr219, Gly58 (H-B), Lys181, Trp87, Lys61, Arg57 (C-H), Arg84, Lys161, Lys61 (ionic)
DB00242	300	-6.6	Gly83, Thr104, Asp146, Gly148, Cys82, Ser56, Gly58 (H-B), Val55, Trp87 (C-H), Arg84, Lys181 (ionic)

Structural bioinformatics and drug development research depends on characterizing interactions in protein-ligand complexes [23]. It is essential to have a thorough knowledge of the molecular mechanisms underlying biological systems [22]. In an open conformational environment of protein structures, hydrogen bonding and hydrophobic interactions play a significant role in stabilizing energetically favored ligands [24]. Nevertheless, it is still unclear

how the binding properties connected to these interactions help a drug lead recognize a particular target and boost therapeutic effectiveness (Lu et al. 2009). The binding energy of the reference substance DB00811 in the active site of DENV-2 NS5 MTase was -6.6 kcal/mol. In all, seven hydrogen bonds involving the residues of Gly83, Cys82, Ser56, Asp146, Gly58, Tyr219, and Glu217 were shown to be involved in the interaction plot between DENV-2 NS5 MTase and the DB00811 complex. Additionally, it was shown that three amino acid residues—Lys181, Trp87, and Val55—of the DENV-2 NS5 MTase pocket were implicated in the hydrophobic interaction with DB00811 (Figure 3c).

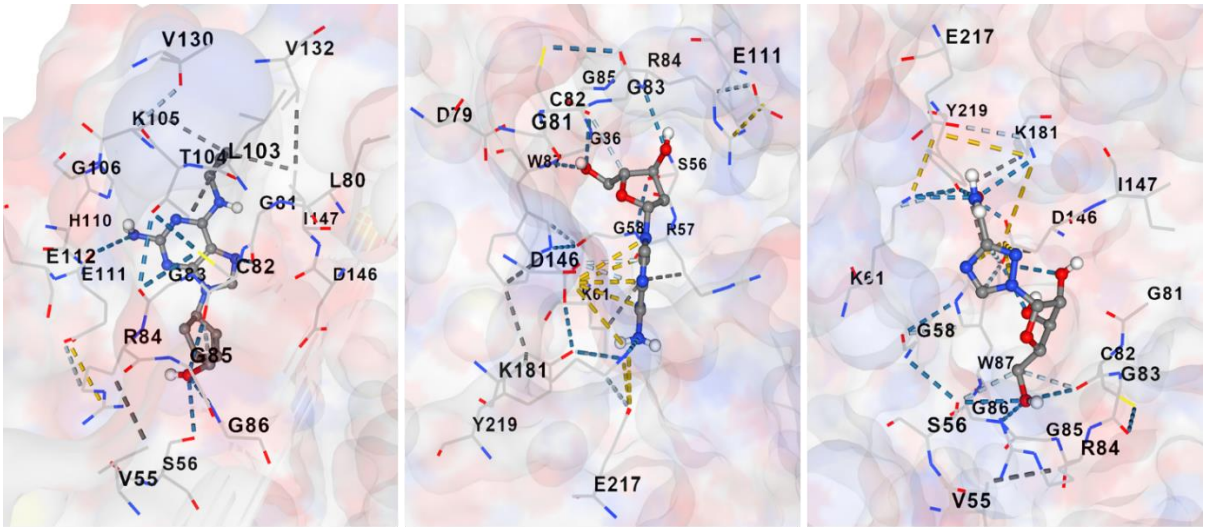

Figure 3. Docking results of Abacavir analogs and the reference (Ribavirin; DB00811) compound towards the DENV-2 MTase (PDB ID: 1R6A).

The top two possibilities (DB01048 and DB02947) were chosen to research how each potential analog interacted with the DENV-2 NS5 MTase. Six residues, Val130, Gly83, Thr104, Cys81, Glu111, and Cys82, are involved in the formation of hydrogen bonds, while six other residues, namely Val132, Ile147, Thr104, Lys105, Trp87, and Val55 residues, are involved in the formation of hydrophobic interactions in the binding region of DENV-2 NS5 MTase (Figure 3a). Furthermore, nine hydrogen bonds with the residues Gly83, Glu111, Cys82, Asp146, Ser56, Glu217, Lys181, Tyr219, and Gly58 in the active site of DENV-2 MTase seemed to have an impact on the excellent binding energy of DB02947. DB02947 also interacted hydrophobically with the residues Lys181, Trp87, Lys61, and Arg57 (Figure 3b).

3.3. Physicochemical, Pharmacokinetics, drug-likeness, and medicinal chemistry studies.

According to the physicochemical properties analysis of abacavir analogs, DB01048 and DB02947 have hydrogen bond acceptor numbers (HBA) of 4 and 7 and donors (HBD) of 3 and 4, respectively. The HBA and HBD need not exceed ten and five, respectively, of a compound for it to be a promising therapeutic candidate [25]. According to the results, DB01048 and DB02947, which made the shortlist, have the potential to be therapeutic candidates. The topological polar surface area (TPSA) of potential treatment candidates varies from 75 $Å^2$ to 140 $Å^2$ [26]. In this investigation, the TPSA values for DB01048 and DB02947 were 101.88 $Å^2$ and 119.31 Å2, respectively; DB00811 showed a value of 143.72 $Å^2$. Finding a high-standard TPSA value that will function well for authorized pharmaceuticals and future drug candidates is also a contemplation or in-depth study topic. Low molecular weight-based

molecules are more easily absorbed, refracted, and transported than high molecular weight-based compounds. With a few rare exceptions, the bulkiness of the molecules increases along with the increase in molecular weight [21]. The chosen compounds had molecular weights of 286.33 and 269.23, which were less than 500 g/mol. DB01048 and DB02947 have good druggability and small biomolecule characteristics. The molar refractivity of DB01048 and DB02947 proves that they can pass across membranes and maintain their constant state despite strong or weak solute-solvent and solvent-solvent interactions [27]. Table 2 contains a summary of these data.

Table 2. Physicochemical Properties of selected compounds and the reference compounds.

Compound ID	MW	HBA	HBD	MR	TPSA
Reference Value	<500	<10	<5	<120	<140
DB00811 (Ribavirin)	244.20 g/mol	7	4	51.06	143.72 Å²
DB01048	286.33 g/mol	4	3	80.40	101.88 Å²
DB02947	269.23 g/mol	7	3	61.47	119.31 Å²

The study of how medicines enter, move through, and exit the body is known as pharmacokinetics [25]. High intestine absorption in humans suggests a reduced risk of adverse effects, including fatigue or weakening of the central nervous system [28]. The blood-brain barrier (BBB) is determined by comparing the chemical concentration in the blood to that in the brain. Information on drug distribution across the BBB is one of the key factors in maximizing drug discovery [21]. A glycoprotein P (P-gp) substrate was employed to forecast dispersion. The results of this experiment suggested that DB01048 and DB02947 were substantially absorbed in the GI, couldn't be a P-gp substrate, and crossed the BBB. The drug similarity of active compounds employed as therapeutic treatments is frequently assessed using the oral bioavailability criteria [29]. Drug metabolism depends on several CYP enzyme isoforms, including CYP3A4, CYP2D6, CYP2C9, CYP2C19, and CYP1A2. The first and most important isoform of CYP3A4 has an intestine and a kidney and is responsible for 50% of the drug's metabolism. An enzyme's capacity for metabolism may be harmed by inhibitors [21]. According to the data, DB01048 produces a positive value for the CYP1A2 inhibitor, whereas DB02947 gives a negative value for all CYP inhibitors, including the reference molecule (DB00811). Finally, it was shown that the DB02947 mentioned above had a better capacity for human intestinal absorption (Table 3).

Table 3. Pharmacokinetics properties of selected compounds and the reference compounds.

C/N	P-gp substrate	HIA (% Absorbed)	BBB (Log BB)	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Skin Permeation
Reference Value	No	High	No	No	No	No	No	No	---
DB00811 (Ribavirin)	No	Low	No	No	No	No	No	No	-9.10 cm/s
DB01048	Yes	High	No	Yes	No	No	No	No	-7.43 cm/s
DB02947	No	High	No	No	No	No	No	No	-7.76 cm/s

The study of drug-like properties hastens the drug research and development process. The principles of Lipinski's rule of five are used as a criterion for identifying potential medication candidates. However, it is unacceptable to offer prospective medication if one rule is breached [25]. After molecules are exposed to Lipinski's rule of five, the Ghose filter rule, the Veber rule, the Egan rule, and the Muegge (LGVEM) rule, the results are shown in Table 4. To ascertain if a bioactive function is a powerful drug, these criteria have their own set of

regulations. All expected compounds have a bioavailability (BA) of around 0.55, which is comparable. These factors ensure the acceptance of lead compounds in drug discovery techniques. The chemicals chosen for this investigation strictly adhered to the LGVEM rules, not deviating from any of them. This displays the medications' potential for the discussed compounds. This also raises the necessity for a unique, thorough investigation to establish a strong correlation between drug-likeliness rules and the parameters of authorized drugs to increase the efficacy of drug-likeliness rules as a computational tool in computer-aided drug design.

Table 4. Drug-likeness and medicinal chemistry analysis of selected compounds and the reference compounds.

	Drug-Likeness						Medicinal Chemistry			
Compound ID	**Lipinski**	**Ghose**	**Veber**	**Egan**	**Muegge**	**Bioavailability Score**	**PAINS**	**Brenk**	**Lead-likeness**	**Synthetic accessibility**
Reference Value	Yes	Yes	Yes	Yes	Yes	---	0	0	Yes	1 (very easy) to 10 (very difficult)
DB00811 (Ribavirin)	Yes	No	No	No	Yes	0.55	0	0	No	3.89
DB01048	Yes	Yes	Yes	Yes	Yes	0.55	0	1	Yes	3.77
DB02947	Yes	Yes	Yes	Yes	Yes	0.55	0	0	Yes	3.75

The synthetic accessibility score is primarily based on the supposition that the frequency of molecular fragments in 'really' attainable compounds corresponds with the ease of synthesis regarding medicinal chemistry parameters. The scale score is normalized to range from 1 (extremely simple) to 10 (very difficult to synthesize) [30]. DB01048 and DB02947, as well as DB00811, displayed indices under 4, supporting the presence of chemical groups that are not uncommon in the chosen molecules' structures and indicating the simplicity of novel syntheses. Studies in medicinal chemistry, pharmacokinetics, physical-chemical chemistry, and drug-likeness showed that DB02947 satisfactorily satisfied all the expectations made of it as a potential lead material with good druggability.

DENV infection has also been associated with suppression of HIV replication during the acute phase of dengue illness. The mechanism behind this is not fully understood, but it may involve the inhibition of HIV replication by DENV proteins like NS5 [31]. However, the impact of HIV/DENV coinfection on disease progression appears to depend on the patient's HIV status. In one study, DENV/HIV coinfection was associated with an increased risk of severe dengue in a cohort primarily comprised of AIDS patients [32]. Based on this evidence, we found the Abacavir analogs from the LigandExpo database using the SwissSimilarity software, and selected compounds were docked into the active site of DENV-2 NS MTase protein to find the lead compounds. These compounds were then subjected to physicochemical, pharmacokinetics, drug-likeness, and medicinal chemistry studies to discover druggable anti-dengue inhibitors.

4. Conclusions

The computational studies of compound similarity analysis, docking, physicochemicals, pharmacokinetics, drug-likeness, and medicinal chemistry studies of abacavir analogs were carried out to identify antiviral drugs against DENV-2 NS5 MTase. Comparing DB01048 and DB02947 compounds with DB00811, the results showed they have the best binding affinities towards the DENV-2 NS5 MTase. Furthermore, the DB02947 compound demonstrated beneficial features related to physicochemicals, pharmacokinetics,

drug-like properties, and medicinal chemistry analysis. This finding implies that in-silico analysis is a useful method for drug design, cutting down on the time needed to accept logical concepts for developing anti-DENV medications. However, more in-vitro and in-vivo studies are needed to assess its safety and efficacy in greater detail.

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No.

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Conflicts of Interest

No.

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