Scientific Research Publishing

Journal of Biosciences and Medicines, 2025, 13(2), 268-286
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Favipiravir-RTP as a Potential Therapeutic Agent for Inhibiting Dengue Virus Replication

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How to cite this paper: Tsai, P.-J. (2025) Favipiravir-RTP as a Potential Therapeutic Agent for Inhibiting Dengue Virus Replication. *Journal of Biosciences and Medicines*, **13**, 268-286.
https://doi.org/10.4236/jbm.2025.132021

Received: January 14, 2025
Accepted: February 17, 2025
Published: February 20, 2025

Abstract

Currently, no clinically approved therapeutic drugs specifically target dengue virus infections. This study aims to evaluate the potential of antiviral drugs originally developed for other purposes as viable candidates for combating dengue virus. The RNA-elongating NS5-NS3 complex is a critical molecular structure responsible for dengue virus replication. Using the cryo-electron microscopy (Cryo-EM) structures available in the Protein Data Bank and AlphaFold 3 predictions, this study simulated the replication complexes of dengue virus serotypes 1, 2, 3, and 4. The RNA-dependent RNA polymerase (RdRp) domain of the NS5 protein within the NS5-NS3 complex was selected as the molecular docking template. Molecular docking simulations were conducted using AutoDock4. Seven small molecules—AT-9010, RK-0404678, Oseltamivir, Remdesivir, Favipiravir-RTP, Abacavir, and Ribavirin—were assessed for binding affinity by calculating their binding energies, where lower values indicate stronger molecular interactions. Based on published data, antiviral replication assays were conducted for the four dengue virus serotypes. AT-9010 and RK-0404678 were used as benchmarks for antiviral replication efficacy, while Oseltamivir served as the control group. The Mann-Whitney U test was employed to classify the clinical antiviral candidates—Remdesivir, Favipiravir-RTP, Abacavir, and Ribavirin. Results demonstrated that among the four small molecules, Favipiravir-RTP exhibited the highest binding affinity with the RdRp domain of the NS5-NS3 complex across all four dengue virus serotypes. Statistical classification revealed that in five simulated scenarios—including the four virus serotypes and Cryo-EM structural data—Favipiravir-RTP shared three classifications with the benchmark molecule AT-9010. Based on these findings, Favipiravir-RTP, a broad-spectrum antiviral agent, shows potential as a therapeutic option for inhibiting dengue virus replication. However, further clinical trials are necessary to validate their efficacy in humans.

Keywords

Dengue, Antiviral Drugs, Favipiravir, AlphaFold 3, Molecular Docking

1. Introduction

Dengue virus (DENV) is primarily transmitted to humans through the bites of infected mosquitoes, predominantly in tropical and subtropical regions worldwide, particularly in urban and semi-urban environments. The primary vectors responsible for DENV transmission are *Aedes aegypti* and *Aedes albopictus* mosquitoes, with the latter being more prevalent in regions such as Europe and North America [1]. DENV is classified into four distinct serotypes based on antigenic differences: DENV-1, DENV-2, DENV-3, and DENV-4 [2] [3]. Each serotype contains multiple genotypes, which are phylogenetically categorized based on sequence variations in the envelope (E) gene. These genotypes include DENV-1 (I - VI), DENV-2 (Asia I, Asia II, Asia/America, America, Cosmopolitan, Forest), DENV-3 (I - V), and DENV-4 (Asian I, Asian II, Asian/American, American, Cosmopolitan, Forest) [4]. The different serotypes can elicit distinct immunogenic responses by infecting various target cells, triggering a robust cytokine response that influences the severity of the disease. Moreover, secondary infection with a heterologous serotype may lead to a more rapid immune response due to antibody-dependent enhancement [5]. The World Health Organization (WHO) recognizes dengue fever as a significant global public health threat. Contributing factors such as climate change, rapid population growth, and insufficient medical infrastructure have exacerbated the increasing prevalence of DENV [6].

The DENV genome is a positive-sense (+), single-stranded RNA molecule approximately 10 to 11 kilobases in length, featuring a 5′ cap structure (m7GpppAm) [7]. It encodes a single polyprotein that undergoes post-translational processing to produce three structural proteins (C, prM, and E) and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). These NS proteins localize to the cytoplasmic side of the endoplasmic reticulum membranes [7] [8] and play essential roles in viral genome replication. Among the NS proteins, NS3 and NS5 possess the enzymatic activities crucial for genome replication [9] [10]. NS5 consists of two functional domains: the N-terminal methyltransferase domain, which catalyzes the 5' capping of RNA [11] [12], and the C-terminal RNA-dependent RNA polymerase (RdRp) domain, which is responsible for RNA synthesis [13]. During viral genome replication, a negative-sense (−) RNA strand is synthesized using the genomic positive-sense (+) RNA as a template, resulting in the formation of a double-stranded RNA intermediate. The DENV genome contains conserved structural motifs within its 5' and 3' untranslated regions (UTRs). Cyclization of the viral genome occurs through base pairing between complementary sequences in the 5' and 3' UTRs [13] [14]. This cyclization, along with the promoter stem-loop A (SLA) structure in the 5' UTR, is critical for initiating (−)

RNA synthesis [15]-[17]. The (−) RNA strand in the double-stranded RNA intermediate serves as a template for the synthesis of the genomic (+) RNA. The completion of the genomic (+) RNA involves the addition of a 5' cap. These processes are coordinated by replicase complexes that consist of NS5, NS3, and the viral RNA. The replication process of DENV involves three structural intermediates: SLA-bound NS5 (pre-initiation complex, PC), NS3-bound PC (elongation complex, EC), and an RNA-elongating NS5-NS3 complex. In the PC, the SLA structure connects the methyltransferase and RdRp domains of NS5, while in the EC, the SLA is displaced by the NS3 helicase domain. These structural intermediates represent crucial stages of DENV genome replication, including SLA-dependent initiation, processive RNA elongation, and 5' capping of nascent genomic RNA [18].

Small molecules that specifically target viral proteins and exert antiviral effects are classified as direct-acting antivirals (DAAs) [19]. These molecules typically exhibit promising antiviral activity with lower toxicity profiles compared to host-directed therapies. However, the emergence of resistance remains a significant challenge in the development of DAAs [20]. The successful development of such molecules necessitates a thorough structural understanding of the various proteins encoded by the DENV genome. In the case of DENV, the envelope protein (a structural protein), along with the non-structural proteins NS1, NS3, and NS5, has been extensively investigated as potential therapeutic targets due to their critical functional roles [21]-[23]. This study aims to investigate the potential repurposing of clinically approved antiviral drugs for the treatment of DENV, with a particular focus on the structure of the processive RNA elongation complex.

2. Materials and Methods

2.1. Preparation of the Molecular Docking Template

The RNA-elongating NS5-NS3 complex of dengue virus was analyzed using Cryo-EM structural data (Protein Data Bank [PDB] ID: 8GZR) as the macromolecular template. In subsequent molecular docking simulations, CDP and Zn^{2+} ions were removed from these structures and replaced with seven different small-molecule ligands (drugs) utilized in this study. Additionally, this study specifically focused on the RNA-elongating NS5-NS3 complexes of the four dengue virus serotypes. The molecular structures of NS3 and NS5 proteins were simulated using the artificial intelligence platform AlphaFold Service (AlphaFold 3). The protein sequences for the four DENV serotypes were obtained from the National Center for Biotechnology Information:

DENV-1: https://www.ncbi.nlm.nih.gov/gene/5075725
DENV-2: https://www.ncbi.nlm.nih.gov/gene/1494449
DENV-3: https://www.ncbi.nlm.nih.gov/gene/5075727
DENV-4: https://www.ncbi.nlm.nih.gov/gene/5075729

The RNA sequences for the RNA-elongating NS5-NS3 complex templates were

identical to the RNA sequence within the [PDB] ID: 8GZR structure:

5'-AGUAGUUUGUUUUUUGAACAGGUUCUGAAAAGAACCUGUU-3'.

Molecular structures generated by AlphaFold Service (AlphaFold 3) resulted in four distinct models, which served as templates for the RNA-elongating NS5-NS3 complexes of the four dengue virus serotypes in this study.

AlphaFold is a web-based service designed to predict high-accuracy biomolecular structures, encompassing proteins, DNA, RNA, ligands, ions, and chemical modifications, all within a unified platform utilizing the latest AlphaFold 3 model [24]. The platform adopts an ensemble learning approach to enhance prediction accuracy by generating multiple models under slightly altered conditions or parameters, thereby accounting for potential structural variability and uncertainty. Each CIF file corresponds to one of these predicted models, with a 'seed' employed for internal random number generation. By default, this seed is automatically sampled and resampled during job cloning. However, running multiple models with different seeds can potentially improve prediction accuracy. The seed can be any integer within the range of 0 to 4,294,967,295, and when cloning a job with a specific seed, the selection will revert to automatic sampling by default (AlphaFold Service, https://alphafoldserver.com/about). The predicted template modeling (pTM) score and the interface predicted template modeling (ipTM) score, both derived from the template modeling (TM) score, serve as metrics for evaluating the accuracy of predicted structures [25] [26]. A pTM score exceeding 0.5 suggests that the predicted fold of the complex is likely to approximate the actual structure. The ipTM score, which assesses the accuracy of predicted relative positions of subunits within the complex, provides a high-confidence estimate when greater than 0.8. Scores below 0.6 indicate a likely failure in prediction, while scores ranging from 0.6 to 0.8 fall within a gray zone, indicating that predictions may be either correct or incorrect (AlphaFold Service, https://alphafoldserver.com/about). For a tutorial on AlphaFold 3, please refer to the following video: https://www.youtube.com/watch?v=iGGby6zavAY.

2.2. Preparation of Small Molecule Ligands

The molecular structures of the small-molecule ligands used in this study were sourced from the Protein Data Bank. Extraction was performed using PyMOL (https://www.pymol.org/). To prepare the ligands for docking, hydrogen atoms were added, and the molecular structures were converted to the mol2 format using Open Babel (https://openbabel.org/docs/Installation/install.html).

The following small molecules were utilized: AT-9010 was obtained from X-ray crystallographic data (PDB ID: 8BCR) with a resolution of 1.90 Å (PubChem CID: 162642756). RK-0404678 was sourced from X-ray crystallographic data (PDB ID: 6IZX) with a resolution of 2.43 Å (PubChem CID: 539049). Oseltamivir was obtained from X-ray crystallographic data (PDB ID: 3CL0) with a resolution of 2.20 Å (PubChem CID: 65028). Remdesivir was derived from X-ray crystallographic data (PDB ID: 7BF6) with a resolution of 2.15 Å (PubChem CID: 121304016).

Favipiravir-RTP was modeled based on Cryo-EM data (PDB ID: 7AAP) with a resolution of 2.50 Å (PubChem CID: 492405). Abacavir was sourced from X-ray crystallographic data (PDB ID: 3UPR) with a resolution of 2.00 Å (PubChem CID: 441300). Ribavirin was sourced from X-ray crystallographic data (PDB ID: 4PB1) with a resolution of 2.80 Å (PubChem CID: 37542).

Comparative EC_{50} (μM) values for AT-9010 and RK-0404678 in inhibiting dengue virus replication, based on a cell-based assay, are presented in Table 1: AT-9010 is metabolized intracellularly from AT-281 (the free base of AT-752) and acts as an RNA chain terminator, inhibiting RNA synthesis. EC_{50} values for AT-9010 are: DENV-1 (0.57 μM), DENV-2 (0.3 μM), DENV-3 (0.75 μM), DENV-4 (0.57 μM) [27]. RK-0404678: EC_{50} values are: DENV-1 (29.5 μM), DENV-2 (6 μM), DENV-3 (29.4 μM), DENV-4 (31.9 μM) [28].

This study designates AT-9010 and RK-0404678 as reference molecules for inhibiting DENV replication, with Oseltamivir (a neuraminidase inhibitor) used as the control group. Additional antiviral drugs, including Remdesivir (anti-SARS-CoV-2), Favipiravir-RTP (broad-spectrum anti-RNA virus), Abacavir (anti-HIV), and Ribavirin (antiviral), are also compared in this analysis.

Table 1. Inhibition of Dengue Virus Replication (DENV-1, DENV-2, DENV-3, DENV-4) by AT-9010 and RK-0404678

Cell-based assay EC_{50} (μM) as shown the average only	DENV-1	DENV-2	DENV-3	DENV-4
AT-9010	0.57	0.3	0.75	0.57
RK-0404678	29.5	6	29.4	31.9

2.3. Molecular Docking Simulations

Molecular docking simulations were performed using AutoDockTools (https://autodocksuite.scripps.edu/adt/) in conjunction with AutoDock 4 (https://autodock.scripps.edu/download-autodock4/). For each RNA-elongating NS5-NS3 template of the dengue virus, the RNA-dependent RNA polymerase (RdRp) domain was selected as the core region for molecular docking with seven distinct small molecules. The five RNA-elongating NS5-NS3 templates were derived from Cryo-EM structural data and AI-generated models of the four dengue virus serotypes. The grid boxes for docking were defined based on the designed grid center values in AutoDock 4, as follows:

RdRp domain {[PDB] ID: 8GZR}: (x center: 101.761, y center: 113.23, z center: 79.041)

DENV-1: (x center: −0.663, y center: −1.809, z center: −21.239)

DENV-2: (x center: −8.447, y center: −22.582, z center: −2.777)

DENV-3: (x center: 9.7, y center: 5.165, z center: −14.433)

DENV-4: (x center: 8.248, y center: −13.091, z center: 7.3)

Consistent parameters were applied across all five templates, including a grid spacing of 0.375 Å and a grid size of 126 × 126 × 126 points. Ligand parameters

were set to their default randomized values. Regarding genetic algorithm (GA) parameters, 50 GA runs were performed for each docking simulation, while all other settings were kept at their default values. The Lamarckian genetic algorithm (4.2) was employed for the output format. For each ligand-template pair, 50 binding energy samples were generated, and the average binding energies along with their confidence intervals were calculated for subsequent comparative analysis. For a tutorial on AutoDock 4, please refer to the following video: https://www.youtube.com/watch?v=ZVKKsK5DsCY.

2.4. Statistical Methods

2.4.1. Shapiro-Wilk Test

Normality is a crucial assumption in various statistical analyses, and the Shapiro-Wilk test, introduced by Samuel Shapiro and Martin Wilk in 1965 [29], is one of the most widely utilized methods for assessing this assumption, particularly in small sample sizes.

Sample Ordering: The first step in the Shapiro-Wilk test involves ordering the sample data in ascending order. This ordered sequence serves as the foundation for evaluating the data's adherence to normality.

Calculation of Expected Values: The test then computes the theoretical expected values derived from the standard normal distribution. These expected values correspond to the quantiles of the normal distribution and act as reference points for comparing the observed sample data.

Test Statistic Calculation: The test statistic, denoted as W, is calculated to quantify the degree of deviation from normality. The formula for W is given by:

$$W = \frac{\left(\sum_{i=1}^{n} a_i x_{(i)}\right)^2}{\sum_{i=1}^{n}\left(x_i - \overline{x}\right)^2}$$

where $x_{(i)}$ represents the ordered sample values, $\overline{x}$ is the sample mean, and a_i are constants derived from the expected values of the normal distribution. In hypothesis testing with the Shapiro-Wilk test, the null hypothesis (H_0) posits that the sample data follows a normal distribution, while the alternative hypothesis (H_1) suggests that the data does not conform to a normal distribution. The W statistic, calculated from the sample data, is compared against critical values to determine the corresponding p-value. If the W value is lower than the critical value at a specified significance level, typically set at $\alpha = 0.05$, the null hypothesis is rejected, indicating that the sample may not be normally distributed. Conversely, if the W value exceeds the critical value, there is insufficient evidence to reject the null hypothesis, suggesting that the sample may be normally distributed. For this study, with a sample size of 50, the critical value for the Shapiro-Wilk test at a significance level of $\alpha = 0.05$ is set at 0.954. The Shapiro-Wilk test can be efficiently calculated using the following resource: https://scistatcalc.blogspot.com/2013/10/shapiro-wilk-test-calculator.html, offering researchers a practical tool to assess the normality of their data.

2.4.2. Mann-Whitney U Test

The Mann-Whitney U Test [30] is a non-parametric statistical method that does not require assumptions about the data distribution. This test is especially useful for comparing two independent samples and is applicable to ordinal or continuous data that may not follow a normal distribution, making it advantageous when working with small sample sizes.

Sample Preparation: The Mann-Whitney U test (https://www.socscistatistics.com/tests/mannwhitney/default2.aspx) requires two independent samples, denoted as Sample 1 and Sample 2. These samples can consist of any form of continuous or ordinal data.

Ranking: The data from both samples are combined and sorted in ascending order. Each value is assigned a rank. In cases of ties, the average rank is assigned to the tied values.

The U statistic is derived for two samples using the following formulas:

$$U_1 = n_1 \cdot n_2 + \frac{n_1 \cdot (n_1 + 1)}{2} - R_1$$

$$U_2 = n_1 \cdot n_2 + \frac{n_2 \cdot (n_2 + 1)}{2} - R_2$$

In these equations, R_1 and R_2 represent the rank sums of Sample 1 and Sample 2, respectively, while n_1 and n_2 denote the sizes of the two samples. The final U statistic is defined as the smaller value between U_1 and U_2.

The Z statistic is calculated using the formula:

$$\mathrm{Z} = \frac{U - \mu_U}{\sigma_U}$$

where μ_U represents the expected value of U, and σ_U signifies the standard deviation of U. The Z statistic is utilized for hypothesis testing.

$$\mu_U = \frac{n_1 \cdot n_2}{2}$$

$$\sigma_U = \sqrt{\frac{n_1 \cdot n_2 \cdot (n_1 + n_2 + 1)}{12}}$$

The null hypothesis (H_0) posits that the two samples are derived from the same distribution, suggesting that their medians are equal. In contrast, the alternative hypothesis (H_1) asserts that the medians of the two samples differ. The U statistic is then used to compute the p-value, which can be determined through statistical tables or software. After estimating μ_U and σ_U for the U statistic, the Z statistic is calculated.

This study aims to classify different small molecules within the same molecular template based on their binding energies, which inherently involves the issue of multiple testing. When conducting multiple tests, each individual test carries a certain probability of producing a Type I error. As the number of tests increases, the cumulative probability of committing at least one Type I error also rises, potentially leading to misleading conclusions. To reduce the risk of false positives in

the context of multiple comparisons, researchers often apply correction methods that adjust the significance threshold to account for the number of tests conducted. In this study, multiple testing correction is performed using the False Discovery Rate (FDR) method to account for the potential occurrence of Type I errors across multiple comparisons.

2.4.3. False Discovery Rate

The statistical principle underlying FDR is to manage the trade-off between type I errors (false positives) and the ability to detect true signals. By focusing on the proportion of false discoveries rather than absolute counts, FDR provides a practical approach to hypothesis testing in high-dimensional settings.

The goal of FDR is to control the expected proportion of incorrect rejections (false discoveries) among all rejections, rather than strictly controlling the probability of any false discovery (as in family-wise error rate, FWER). This makes FDR a more flexible and powerful approach in situations involving large numbers of hypotheses.

FDR is mathematically defined as:

$$FDR = E\left[\frac{V}{R}\right]$$

where:

V: The number of false positives (incorrectly rejected null hypotheses). R: The total number of rejected hypotheses (both true and false rejections). If $R = 0$, then $\frac{V}{R}$ is defined to be 0. This definition emphasizes the expectation of the proportion of false positives among the rejections, providing a measure of error that scales with the number of rejections.

The Benjamini-Hochberg procedure assumes that p-values are uniformly distributed under the null hypothesis and adjusts the significance thresholds dynamically to control the proportion of false positives. The expected number of false discoveries is calculated based on the number of true null hypotheses (m_0) and the significance level, while the ratio $\frac{V}{R}$ reflects the error proportion. The most common method to control FDR is the Benjamini-Hochberg procedure, which works as follows [31]:

Sort P-Values: Arrange the p-values of all tests in ascending order, $p_{(1)}$, $p_{(2)}$, …, $p_{(m)}$, where m is the total number of hypotheses. Compute thresholds: For each test, compute a threshold:

$$Threshold_k = \frac{k}{m} \cdot Q$$

where k is the rank of the p-value, m is the total number of tests, and Q is the desired FDR level (set to 0.05 in this study). Identify significant results: Find the largest k such that:

$$P_{(k)} \leq Threshold_k$$

All hypotheses with p-values less than or equal to this threshold are considered significant.

3. Results

This study utilizes the RNA-elongating NS5-NS3 complex of the dengue virus, a known structural element involved in the elongation replication mechanism, as the template for molecular docking simulations. Two types of templates were employed: one derived from the Protein Data Bank and the other generated through artificial intelligence via AlphaFold 3. The Cryo-EM structure from the Protein Data Bank does not specify a particular dengue serotype; therefore, AlphaFold 3 was utilized to simulate the complex structures of the four dengue serotypes based on genomic data obtained from the National Center for Biotechnology Information. Each template was paired with seven different ligands for molecular docking calculations. The binding stability and affinity of each ligand were assessed using binding energy as a metric, where lower binding energy values indicate higher stability and affinity. The same template was employed to evaluate the binding stability and affinity of the seven ligands under identical grid box conditions, specifically targeting the RdRp domain within the NS5 subunit of the complex.

To assess the homogeneity of the results, the non-parametric Mann-Whitney U test was used. The binding energy values of small molecules AT-9010 and RK-0404678 were compared as reference points for interaction with the template, with Oseltamivir (a neuraminidase inhibitor) designated as the control group. The remaining four small molecules were categorized based on their respective binding profiles. The Shapiro-Wilk test was employed to assess whether the sample distributions, derived from the pairings of each template with its corresponding ligand, followed a normal distribution. For a sample size of 50, the critical value for the Shapiro-Wilk test at a significance level of $\alpha = 0.05$ is 0.954.

Subsequently, the Mann-Whitney U test was applied with a two-tailed approach, and FDR method was employed as a multiple comparison correction to interpret the statistical significance of the results. Ligands within the same category were grouped and indicated within parentheses, with relationships between them denoted by an "=" sign.

The RNA-elongating NS5-NS3 template of Dengue virus (DENV) was derived from the Cryo-EM structure of the NS5-NS3 RNA-elongation complex (PDB ID: 8GZR), as illustrated in Figure 1. The binding energies (mean ± 95% CI) for the seven ligands were recorded as follows: AT-9010 (−8.23 ± 0.24 kcal/mol), RK-0404678 (−7.03 ± 0.18 kcal/mol), Oseltamivir (−5.37 ± 0.25 kcal/mol), Remdesivir (−6.64 ± 0.13 kcal/mol), Favipiravir-RTP (−7.68 ± 0.27 kcal/mol), Abacavir (−6.2 ± 0.2 kcal/mol), and Ribavirin (−6.24 ± 0.21 kcal/mol). The W values obtained from the Shapiro-Wilk test for the respective ligands were as follows: AT-9010 (0.986), RK-0404678 (0.936), Oseltamivir (0.91), Remdesivir (0.948), Favipiravir-RTP (0.966), Abacavir (0.883), and Ribavirin (0.96). The results of the Shapiro-Wilk test indicated that the samples for AT-9010 and Favipiravir-RTP followed a

Model [template : ligand]	Binding Energy (mean ± 95%CI) kcal/mol	calculated Shapiro-Wilk statistic W
A: [8GZR : AT-9010]	-8.23 ± 0.24	0.986◎
B: [8GZR : RK-0404678]	-7.03 ± 0.18	0.936
C: [8GZR : Oseltamivir]	-5.37 ± 0.25	0.91
D: [8GZR : Remdesivir]	-6.64 ± 0.13	0.948
E: [8GZR : Favipiravir-RTP]	-7.68 ± 0.27	0.966◎
F: [8GZR : Abacavir]	-6.2 ± 0.2	0.883
G: [8GZR : Ribavirin]	-6.24 ± 0.21	0.96◎

the NS5-NS3 RNA-elongation complex derived from (PDB ID: 8GZR)
CI: confidence interval; critical value of W (5% significance level): 0.954; ◎: indicates that the sample exhibits a normal distribution

P-value from the Mann-Whitney U test	A: [8GZR : AT-9010]	B: [8GZR : RK-0404678]	C: [8GZR : Oseltamivir]
A: [8GZR : AT-9010]			
B: [8GZR : RK-0404678]	< 0.00001		
C: [8GZR : Oseltamivir]	< 0.00001	< 0.00001	
D: [8GZR : Remdesivir]	< 0.00001	0.00072	< 0.00001
E: [8GZR : Favipiravir-RTP]	0.00236	0.00214	< 0.00001
F: [8GZR : Abacavir]	< 0.00001	< 0.00001	< 0.00001
G: [8GZR : Ribavirin]	< 0.00001	< 0.00001	< 0.00001

False Disvovery Rate (FDR)	A: [8GZR : AT-9010]	B: [8GZR : RK-0404678]	C: [8GZR : Oseltamivir]	Interpretation of Results
A: [8GZR : AT-9010]				
B: [8GZR : RK-0404678]	*			C > B > A
C: [8GZR : Oseltamivir]	*	*		
D: [8GZR : Remdesivir]	*	*	*	C > D > B > A
E: [8GZR : Favipiravir-RTP]	*	*	*	C > B > E > A
F: [8GZR : Abacavir]	*	*	*	C > F > B > A
G: [8GZR : Ribavirin]	*	*	*	C > G > B > A

*: statistically significant as determined by FDR correction; NS: not statistically significant

Figure 1. Molecular docking interactions and variability analysis of the RNA-elongating NS5-NS3 complex (PDB ID: 8GZR) with ligands.

normal distribution, whereas the samples for RK-0404678, Oseltamivir, Remdesivir, Abacavir, and Ribavirin deviated from normality. The statistical analysis results and the corresponding inferences are summarized below (**Figure 1**):

Mann-Whitney U test and FDR results:

Classification by ligand binding energy: Oseltamivir > RK-0404678 > AT-9010

For Remdesivir classification: Oseltamivir > Remdesivir > RK-0404678 > AT-9010

For Favipiravir-RTP classification: Oseltamivir > RK-0404678 > Favipiravir-RTP > AT-9010

For Abacavir classification: Oseltamivir > Abacavir > RK-0404678 > AT-9010

For Ribavirin classification: Oseltamivir > Ribavirin > RK-0404678 > AT-9010

The RNA-elongating NS5-NS3 template of Dengue virus serotype 1 (DENV-1) was derived from an AlphaFold 3 model, with the following model parameters and metrics: Seed: 1482632268, ipTM = 0.43, and pTM = 0.53, as depicted in **Figure 2**. The binding energies (mean ± 95% CI) for the seven ligands were as follows: AT-9010 (−7.92 ± 0.31 kcal/mol), RK-0404678 (−6.69 ± 0.3 kcal/mol), Oseltamivir (−5.15 ± 0.15 kcal/mol), Remdesivir (−6.11 ± 0.06 kcal/mol), Favipiravir-RTP (−7.47 ± 0.27 kcal/mol), Abacavir (−6.01 ± 0.09 kcal/mol), and Ribavirin (−5.73 ± 0.15 kcal/mol). The W values obtained from the Shapiro-Wilk test for the respective ligands were as follows: AT-9010 (0.946), RK-0404678 (0.876), Oseltamivir (0.934), Remdesivir (0.929), Favipiravir-RTP (0.952), Abacavir (0.882), and Ribavirin (0.852). The results of the Shapiro-Wilk test indicated that none of the samples conformed to a normal distribution. The outcomes of the statistical tests and the corresponding inferences are summarized as follows (**Figure 2**):

Mann-Whitney U test and FDR results:

Classification by ligand binding energy: Oseltamivir > RK-0404678 > AT-9010

Model [template : ligand]	Binding Energy (mean ± 95%CI) kcal/mol	calculated Shapiro-Wilk statistic W
A: [DENV1 : AT-9010]	-7.92 ± 0.31	0.946
B: [DENV1 : RK-0404678]	-6.69 ± 0.3	0.876
C: [DENV1 : Oseltamivir]	-5.15 ± 0.15	0.934
D: [DENV1 : Remdesivir]	-6.11 ± 0.06	0.929
E: [DENV1 : Favipiravir-RTP]	-7.47 ± 0.27	0.952
F: [DENV1 : Abacavir]	-6.01 ± 0.09	0.882
G: [DENV1 : Ribavirin]	-5.73 ± 0.15	0.852

The template predicted by AlphaFold 3 is based on the NS5-NS3 RNA-elongation complex of DENV1
CI: confidence interval; critical value of W (5% significance level): 0.954; ◎: indicates that the sample exhibits a normal distribution

P-value from the Mann-Whitney U test	A: [DENV1 : AT-9010]	B: [DENV1 : RK-0404678]	C: [DENV1 : Oseltamivir]
A: [DENV1 : AT-9010]			
B: [DENV1 : RK-0404678]	< 0.00001		
C: [DENV1 : Oseltamivir]	< 0.00001	< 0.00001	
D: [DENV1 : Remdesivir]	< 0.00001	0.03846	< 0.00001
E: [DENV1 : Favipiravir-RTP]	0.02574	0.00012	< 0.00001
F: [DENV1 : Abacavir]	< 0.00001	0.00634	< 0.00001
G: [DENV1 : Ribavirin]	< 0.00001	< 0.00001	< 0.00001

False Disvovery Rate (FDR)	A: [DENV1 : AT-9010]	B: [DENV1 : RK-0404678]	C: [DENV1 : Oseltamivir]	Interpretation of Results
A: [DENV1 : AT-9010]				
B: [DENV1 : RK-0404678]	*			C > B > A
C: [DENV1 : Oseltamivir]	*	*		
D: [DENV1 : Remdesivir]	*	*	*	C > D > B > A
E: [DENV1 : Favipiravir-RTP]	NS	*	*	C > B > (A=E)
F: [DENV1 : Abacavir]	*	*	*	C > F > B > A
G: [DENV1 : Ribavirin]	*	*	*	C > G > B > A

*: statistically significant as determined by FDR correction; NS: not statistically significant

Figure 2. Molecular docking interactions and variability analysis of the RNA-elongating NS5-NS3 complex (DENV1), modeled using AlphaFold 3, with ligands.

For Remdesivir classification: Oseltamivir > Remdesivir > RK-0404678 > AT-9010

For Favipiravir-RTP classification: Oseltamivir > RK-0404678 > (AT-9010 = Favipiravir-RTP)

For Abacavir classification: Oseltamivir > Abacavir > RK-0404678 > AT-9010

For Ribavirin classification: Oseltamivir > Ribavirin > RK-0404678 > AT-9010

The RNA-elongating NS5-NS3 template of Dengue virus serotype 2 (DENV-2) was derived from an AlphaFold 3 model, with the following model parameters and metrics: Seed: 10631558, ipTM = 0.47, and pTM = 0.56, as shown in **Figure 3**. The binding energies (mean ± 95% CI) for the seven ligands were as follows: AT-9010 (−7.21 ± 0.2 kcal/mol), RK-0404678 (−6.43 ± 0.27 kcal/mol), Oseltamivir (−4.96 ± 0.21 kcal/mol), Remdesivir (−6.14 ± 0.12 kcal/mol), Favipiravir-RTP (-6.94 ± 0.2 kcal/mol), Abacavir (−6.08 ± 0.21 kcal/mol), and Ribavirin (−6.27 ± 0.24 kcal/mol). The W values obtained from the Shapiro-Wilk test for the respective ligands were as follows: AT-9010 (0.95), RK-0404678 (0.909), Oseltamivir (0.909), Remdesivir (0.72), Favipiravir-RTP (0.97), Abacavir (0.859), and Ribavirin (0.921). The results of the Shapiro-Wilk test indicated that the sample for Favipiravir-RTP followed a normal distribution, whereas the samples for AT-9010, RK-0404678, Oseltamivir, Remdesivir, Abacavir, and Ribavirin deviated from normality. The outcomes of the statistical tests and the corresponding inferences are summarized below (**Figure 3**):

Mann-Whitney U test and FDR results:

Classification by ligand binding energy: Oseltamivir > RK-0404678 > AT-9010

For Remdesivir classification: Oseltamivir > (RK-0404678 = Remdesivir) > AT-9010

Model [template : ligand]	Binding Energy (mean ± 95%CI) kcal/mol	calculated Shapiro-Wilk statistic W
A: [DENV2 : AT-9010]	-7.21 ± 0.2	0.95
B: [DENV2 : RK-0404678]	-6.43 ± 0.27	0.909
C: [DENV2 : Oseltamivir]	-4.96 ± 0.21	0.909
D: [DENV2 : Remdesivir]	-6.14 ± 0.12	0.72
E: [DENV2 : Favipiravir-RTP]	-6.94 ± 0.2	0.97©
F: [DENV2 : Abacavir]	-6.08 ± 0.21	0.859
G: [DENV2 : Ribavirin]	-6.27 ± 0.24	0.921

The template predicted by AlphaFold 3 is based on the NS5-NS3 RNA-elongation complex of DENV2
CI: confidence interval; critical value of W (5% significance level): 0.954; ©: indicates that the sample exhibits a normal distribution

P-value from the Mann-Whitney U test	A: [DENV2 : AT-9010]	B: [DENV2 : RK-0404678]	C: [DENV2 : Oseltamivir]
A: [DENV2 : AT-9010]			
B: [DENV2 : RK-0404678]	< 0.00001		
C: [DENV2 : Oseltamivir]	< 0.00001	< 0.00001	
D: [DENV2 : Remdesivir]	< 0.00001	0.42952	< 0.00001
E: [DENV2 : Favipiravir-RTP]	0.13104	0.0056	< 0.00001
F: [DENV2 : Abacavir]	< 0.00001	0.07672	< 0.00001
G: [DENV2 : Ribavirin]	< 0.00001	0.35238	< 0.00001

False Disvovery Rate (FDR)	A: [DENV2 : AT-9010]	B: [DENV2 : RK-0404678]	C: [DENV2 : Oseltamivir]	Interpretation of Results
A: [DENV2 : AT-9010]				
B: [DENV2 : RK-0404678]	*			C > B > A
C: [DENV2 : Oseltamivir]	*	*		
D: [DENV2 : Remdesivir]	*	NS	*	C > (B=D) > A
E: [DENV2 : Favipiravir-RTP]	NS	*	*	C > B > (A=E)
F: [DENV2 : Abacavir]	*	NS	*	C > (B=F) > A
G: [DENV2 : Ribavirin]	*	NS	*	C > (B=G) > A

*: statistically significant as determined by FDR correction; NS: not statistically significant

Figure 3. Molecular docking interactions and variability analysis of the RNA-elongating NS5-NS3 complex (DENV2), modeled using AlphaFold 3, with ligands.

For Favipiravir-RTP classification: Oseltamivir > RK-0404678 > (AT-9010 = Favipiravir-RTP)

For Abacavir classification: Oseltamivir > (RK-0404678 = Abacavir) > AT-9010

For Ribavirin classification: Oseltamivir > (RK-0404678 = Ribavirin) > AT-9010

The RNA-elongating NS5-NS3 template of Dengue virus serotype 3 (DENV-3) was derived from an AlphaFold 3 model, with the following model parameters and metrics: Seed: 812015825, ipTM = 0.46, and pTM = 0.54, as shown in **Figure 4**. The binding energies (mean ± 95% CI) for the seven ligands were as follows: AT-9010 (−8.86 ± 0.28 kcal/mol), RK-0404678 (−6.58 ± 0.25 kcal/mol), Oseltamivir (−5.67 ± 0.08 kcal/mol), Remdesivir (−6.98 ± 0.15 kcal/mol), Favipiravir-RTP (−8.61 ± 0.34 kcal/mol), Abacavir (−6.62 ± 0.12 kcal/mol), and Ribavirin (−6.14 ± 0.15 kcal/mol). The W values obtained from the Shapiro-Wilk test for the respective ligands were as follows: AT-9010 (0.939), RK-0404678 (0.852), Oseltamivir (0.987), Remdesivir (0.895), Favipiravir-RTP (0.957), Abacavir (0.941), and Ribavirin (0.935). The results of the Shapiro-Wilk test indicated that the samples for Oseltamivir and Favipiravir-RTP followed a normal distribution, whereas the samples for AT-9010, RK-0404678, Remdesivir, Abacavir, and Ribavirin deviated from normality. The outcomes of the statistical tests and corresponding inferences are summarized below (**Figure 4**):

Mann-Whitney U test and FDR results:

Classification by ligand binding energy: Oseltamivir > RK-0404678 > AT-9010

For Remdesivir classification: Oseltamivir > RK-0404678 > Remdesivir > AT-9010

Model [template : ligand]	Binding Energy (mean ± 95%CI) kcal/mol	calculated Shapiro-Wilk statistic W
A: [DENV3 : AT-9010]	-8.86 ± 0.28	0.939
B: [DENV3 : RK-0404678]	-6.58 ± 0.25	0.852
C: [DENV3 : Oseltamivir]	-5.67 ± 0.08	0.987◎
D: [DENV3 : Remdesivir]	-6.98 ± 0.15	0.895
E: [DENV3 : Favipiravir-RTP]	-8.61 ± 0.34	0.957◎
F: [DENV3 : Abacavir]	-6.62 ± 0.12	0.941
G: [DENV3 : Ribavirin]	-6.14 ± 0.15	0.935

The template predicted by AlphaFold 3 is based on the NS5-NS3 RNA-elongation complex of DENV3
CI: confidence interval; critical value of W (5% significance level): 0.954; ◎: indicates that the sample exhibits a normal distribution

P-value from the Mann-Whitney U test	A: [DENV3 : AT-9010]	B: [DENV3 : RK-0404678]	C: [DENV3 : Oseltamivir]
A: [DENV3 : AT-9010]			
B: [DENV3 : RK-0404678]	< 0.00001		
C: [DENV3 : Oseltamivir]	< 0.00001	< 0.00001	
D: [DENV3 : Remdesivir]	< 0.00001	0.00028	< 0.00001
E: [DENV3 : Favipiravir-RTP]	0.27134	< 0.00001	< 0.00001
F: [DENV3 : Abacavir]	< 0.00001	0.05876	< 0.00001
G: [DENV3 : Ribavirin]	< 0.00001	0.0096	< 0.00001

False Disvovery Rate (FDR)	A: [DENV3 : AT-9010]	B: [DENV3 : RK-0404678]	C: [DENV3 : Oseltamivir]	Interpretation of Results
A: [DENV3 : AT-9010]				
B: [DENV3 : RK-0404678]	*			C > B > A
C: [DENV3 : Oseltamivir]	*	*		
D: [DENV3 : Remdesivir]	*	*	*	C > B > D > A
E: [DENV3 : Favipiravir-RTP]	NS	*	*	C > B > (A=E)
F: [DENV3 : Abacavir]	*	NS	*	C > (B=F) > A
G: [DENV3 : Ribavirin]	*	*	*	C > G > B > A

*: statistically significant as determined by FDR correction; NS: not statistically significant

Figure 4. Molecular docking interactions and variability analysis of the RNA-elongating NS5-NS3 complex (DENV3), modeled using AlphaFold 3, with ligands.

For Favipiravir-RTP classification: Oseltamivir > RK-0404678 > (AT-9010 = Favipiravir-RTP)

For Abacavir classification: Oseltamivir > (RK-0404678 = Abacavir) > AT-9010

For Ribavirin classification: Oseltamivir > Ribavirin > RK-0404678 > AT-9010

The RNA-elongating NS5-NS3 template of Dengue virus serotype 4 (DENV-4) was derived from an AlphaFold 3 model, with the following model parameters and metrics: Seed: 1527850668, ipTM = 0.45, and pTM = 0.53, as depicted in **Figure 5**. The binding energies (mean ± 95% CI) for the seven ligands were recorded as follows: AT-9010 (−9.47 ± 0.29 kcal/mol), RK-0404678 (-6.59 ± 0.19 kcal/mol), Oseltamivir (−5.37 ± 0.13 kcal/mol), Remdesivir (−6.47 ± 0.12 kcal/mol), Favipiravir-RTP (−8.91 ± 0.24 kcal/mol), Abacavir (−6.18 ± 0.17 kcal/mol), and Ribavirin (−5.83 ± 0.15 kcal/mol). The W values calculated from the Shapiro-Wilk test for the respective ligands were as follows: AT-9010 (0.976), RK-0404678 (0.945), Oseltamivir (0.967), Remdesivir (0.976), Favipiravir-RTP (0.985), Abacavir (0.874), and Ribavirin (0.968). The results of the Shapiro-Wilk test indicated that the samples for AT-9010, Oseltamivir, Remdesivir, Favipiravir-RTP, and Ribavirin followed a normal distribution, while the samples for RK-0404678 and Abacavir deviated from normality. The results of the statistical tests and corresponding inferences are summarized as follows (**Figure 5**):

Mann-Whitney U test and FDR results:

Classification by ligand binding energy: Oseltamivir > RK-0404678 > AT-9010

For Remdesivir classification: Oseltamivir > (RK-0404678 = Remdesivir) > AT-9010

For Favipiravir-RTP classification: Oseltamivir > RK-0404678 > Favipiravir-RTP > AT-9010

Model [template : ligand]	Binding Energy (mean ± 95%CI) kcal/mol	calculated Shapiro-Wilk statistic W
A: [DENV4 : AT-9010]	-9.47 ± 0.29	0.976©
B: [DENV4 : RK-0404678]	-6.59 ± 0.19	0.945
C: [DENV4 : Oseltamivir]	-5.37 ± 0.13	0.967©
D: [DENV4 : Remdesivir]	-6.47 ± 0.12	0.976©
E: [DENV4 : Favipiravir-RTP]	-8.91 ± 0.24	0.985©
F: [DENV4 : Abacavir]	-6.18 ± 0.17	0.874
G: [DENV4 : Ribavirin]	-5.83 ± 0.15	0.968©

The template predicted by AlphaFold 3 is based on the NS5-NS3 RNA-elongation complex of DENV4
CI: confidence interval; critical value of W (5% significance level): 0.954; ©: indicates that the sample exhibits a normal distribution

P-value from the Mann-Whitney U test	A: [DENV4 : AT-9010]	B: [DENV4 : RK-0404678]	C: [DENV4 : Oseltamivir]
A: [DENV4 : AT-9010]			
B: [DENV4 : RK-0404678]	< 0.00001		
C: [DENV4 : Oseltamivir]	< 0.00001	< 0.00001	
D: [DENV4 : Remdesivir]	< 0.00001	0.85716	< 0.00001
E: [DENV4 : Favipiravir-RTP]	0.00614	< 0.00001	< 0.00001
F: [DENV4 : Abacavir]	< 0.00001	0.00108	< 0.00001
G: [DENV4 : Ribavirin]	< 0.00001	< 0.00001	< 0.00001

False Disvovery Rate (FDR)	A: [DENV4 : AT-9010]	B: [DENV4 : RK-0404678]	C: [DENV4 : Oseltamivir]	Interpretation of Results
A: [DENV4 : AT-9010]				
B: [DENV4 : RK-0404678]	*			C > B > A
C: [DENV4 : Oseltamivir]	*	*		
D: [DENV4 : Remdesivir]	*	NS	*	C > (B=D) > A
E: [DENV4 : Favipiravir-RTP]	*	*	*	C > B > E > A
F: [DENV4 : Abacavir]	*	*	*	C > F > B > A
G: [DENV4 : Ribavirin]	*	*	*	C > G > B > A

*: statistically significant as determined by FDR correction; NS: not statistically significant

Figure 5. Molecular docking interactions and variability analysis of the RNA-elongating NS5-NS3 complex (DENV4), modeled using AlphaFold 3, with ligands.

For Abacavir classification: Oseltamivir > Abacavir > RK-0404678 > AT-9010

For Ribavirin classification: Oseltamivir > Ribavirin > RK-0404678 > AT-9010

Based on the AlphaFold 3 predicted template modeling (pTM) scores, the pTM scores for all four DENV serotypes exceeded 0.5, suggesting that the predicted fold of the complex is likely to closely resemble the actual structure. However, when evaluating the interface predicted template modeling (ipTM) scores, it was observed that all values were below 0.5, indicating that the AI-generated structure does not reliably confirm the predicted relative positions of the subunits within the complex. Nonetheless, utilizing five templates—including the Cryo-EM structure and AlphaFold 3-generated structures of the RNA-elongating NS5-NS3 complex—along with molecular docking simulations involving seven small molecule ligands, the affinity of these small molecules for the RdRp domain of the complex was assessed. AT-9010 demonstrated the highest affinity, followed by Favipiravir-RTP. In molecular docking simulations for DENV serotypes 1, 2, and 3, both AT-9010 and Favipiravir-RTP exhibited no statistically significant differences in their binding affinities.

4. Discussion

AT-752, a guanosine analogue prodrug, demonstrates antiviral activity against dengue virus (DENV). Upon entry into infected cells, AT-752 is metabolized into 2'-methyl-2'-fluoro guanosine 5'-triphosphate (AT-9010), which functions as an RNA chain terminator, thereby inhibiting RNA synthesis [32]. AT-9010 exerts its effects on the full-length NS5 protein of DENV through multiple mechanisms. Importantly, AT-9010 does not significantly interfere with the synthesis of the primer pppApG [27]. Instead, it targets two enzymatic activities associated with

NS5: RNA 2'-O-methyltransferase and RdRp during the RNA elongation step. The crystal structure of the DENV2 MTase domain in complex with AT-9010, resolved at a resolution of 1.97 Å, reveals that AT-9010 binds to the GTP/RNA cap binding site. This binding mechanism inhibits 2'-O methylation without affecting N7-methylation activity [27]. Additionally, AT-9010 exhibits a 10- to 14-fold selectivity against GTP at the active site of the NS5 RdRp across all four DENV serotypes (DENV1-4), contributing to the termination of viral RNA synthesis [27]. Experimental evidence suggests that the primary mechanism by which AT-9010 inhibits viral replication involves its interaction with the RNA 2'-O-methyltransferase rather than direct effects on RdRp.

Favipiravir, an antiviral agent initially developed by Fujifilm Toyama Chemical in Japan, is primarily used in the treatment of influenza, particularly for novel or severe strains that are resistant to existing antiviral drugs such as oseltamivir. Its mechanism of action involves inhibiting RdRp, thereby interfering with viral replication [33]. Favipiravir is metabolized intracellularly to its active form, favipiravir-RTP, which accumulates in direct proportion to increasing extracellular concentrations [34]. While favipiravir has proven effective against influenza and other RNA viruses, recent studies have raised concerns about its potential toxicity. Specifically, favipiravir has been shown to induce oxidative stress and DNA damage in cardiac and skin cells [35]. Furthermore, it has been associated with liver and kidney damage in animal models, leading to elevated serum enzyme levels, apoptotic cell death, and inflammation [36]. However, compared to ribavirin, favipiravir requires a 100-fold higher concentration to inhibit intracellular GTP levels, which may suggest lower toxicity [34].

During the COVID-19 pandemic, favipiravir was explored as a potential treatment for mild to moderate COVID-19 cases, with some studies indicating that it could accelerate viral clearance and alleviate symptoms [37]. However, its efficacy has varied across clinical trials, and further evidence is necessary to definitively establish its therapeutic role. In certain countries, such as India and Russia, favipiravir has been granted emergency use authorization for COVID-19 treatment [38]. Preclinical studies have also demonstrated favipiravir's antiviral activity against a broad range of RNA viruses, including the Ebola virus, Lassa fever virus, and paramyxoviruses [33]. Despite its broad antiviral spectrum, favipiravir has not been approved for these infections and remains under investigation.

The clinical efficacy of favipiravir for treating specific infections, such as COVID-19, remains inconclusive. While greater benefits have been observed in mild to moderate cases, its impact on severe cases appears limited [37]. Common adverse effects associated with favipiravir include diarrhea, elevated liver enzymes, and hyperuricemia [33] [39] [40]. Preclinical studies have also raised concerns regarding potential teratogenic effects, leading to contraindications during pregnancy [39], and patients are advised to use effective contraception during treatment. Additionally, given its mechanism of targeting viral RNA polymerase, prolonged use of favipiravir may increase the risk of antiviral resistance, necessitating

careful monitoring [33]. The production and availability of favipiravir are still limited due to high costs and restricted distribution in certain regions. It is currently approved for use only in a few countries, typically under emergency or specific indications [38]. When compared to other antiviral agents, such as remdesivir or molnupiravir, favipiravir's efficacy and safety profile are still under evaluation, and it has not yet become a global standard of care [39]. In conclusion, while favipiravir shows promise as an antiviral agent with potential efficacy against a variety of RNA viruses, its clinical utility is hindered by uncertain efficacy, safety concerns, and limited availability. Further well-designed clinical trials are required to determine its definitive role in antiviral therapy.

New drugs provide significant advantages in terms of innovation and targeted therapeutic approaches, effectively addressing clinical gaps that existing treatments may not be able to fill. However, their development is associated with high costs and considerable risks. In contrast, old drugs with new indications offer benefits such as reduced development costs, shorter time to market, and well-established safety profiles. Despite these advantages, they tend to offer less innovation and may not exhibit substantial efficacy in new therapeutic areas. In practice, pharmaceutical companies and research institutions typically select the most appropriate development pathway based on factors such as market demand, resource availability, and technological feasibility. The successful repurposing of old drugs can sometimes offer valuable insights or serve as a bridge toward the development of new drugs. Conversely, new drugs have the potential to provide breakthrough solutions that can significantly impact the therapeutic landscape.

5. Conclusion

This study suggests that Favipiravir-RTP warrants further human clinical trials to assess its therapeutic efficacy in treating DENV infection. Future research should focus on evaluating the drug's effectiveness across varying levels of dengue severity, determining the optimal dosage, and investigating potential adverse effects, thereby addressing both safety and efficacy concerns. Furthermore, this study has established a systematic approach for screening the repurposing of clinical drugs. This approach involves selecting small molecules with a solid research foundation as candidates, choosing or developing appropriate molecular templates, conducting molecular docking simulations to calculate binding energies, and utilizing these binding energy values to categorize potential candidates. The findings from these analyses will aid in determining the feasibility of repurposing clinical drugs for new therapeutic applications.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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