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Targeting the NS5 Protein of Zika Virus

Ilham Mohammed A. Alshiraihi[1], Jeffrey Bennett[2], Megan Hamrick[2], Michaela Good[2], Krista Henderson[2], Christine Sobolewski[2], Melissa A. Edwards[1], and Mark A. Brown[1, 3, 4]

[1]Cell and Molecular Biology Program, Colorado State University, Fort Collins, CO, USA
[2]Department of Chemical and Biological Engineering, Colorado State University, Fort Collins, CO, USA
[3]Department of Clinical Sciences, Colorado State University, Fort Collins, CO USA
[4]Epidemiology Section, Colorado School of Public Health, Colorado State University, Fort Collins, CO, USA
Address Correspondence to: Mark.Brown@colostate.edu

***Abstract*—Non-structural protein 5 (NS5) is highly conserved and plays an essential role among Flaviviruses. The recent structural elucidation of the Zika virus (ZIKV) NS5 variant paves the way for the development of structural-based inhibitors for the clinical management of ZIKV infections. Herein, we present our ongoing study design and findings for targeting ZIKV NS5 for drug discovery.**

Keywords—Zika virus; Inhibitor; NS5

I. INTRODUCTION

Zika virus (ZIKV) is a member of the genus of Flaviviruses within the family, *Flaviviridae* [1]. First identified in 1947, it is an arbovirus transmitted by *Aedes* mosquitos [2, 3]. Although it was previously associated with serious symptoms in only rare circumstances, its emergence in the Americas has coincided with a significant increase in ZIKV-associated microcephaly [4] and Guillain-Barre syndrome [5]. It has now become a global health concern resulting in advisories from the World Health Organization.

The virus itself is an enveloped, single-stranded, positive-sense RNA molecule of 10,794 kb with flanking 5' and 3' noncoding regions and a polyprotein-encoding open reading frame [6]. The NS5 protein is approximately 103 kDa and is comprised of an N-terminal RNA capping domain that has methyl transferase enzymatic activity and a C-terminal RNA-dependent RNA polymerase domain [7]. The ZIKV genome maintains a methylated 5'cap which facilitates translation, stability, and circumvention of host immune mechanisms [8].

The NS5 RNA-dependent polymerase is essential for replication of viral RNA in Flaviviruses. It initiates RNA synthesis by generating negative-sense RNA from the positive-strand template [9]. The product, in turn, provides a template for the production of positive-stranded RNA during viral replication. The 5' end of the viral RNA molecule includes a cap comprised of a guanine nucleotide tethered to the first nucleotide of the RNA. The cap is methylated at the guanine N-7 position and the 2' OH on the ribose associated with the first adenosine [10]. Viral mutations that eliminate N-7 methylation prevent further replication of the virus [8].

The Flaviviral methytransferase domain of NS5 includes multiple ligand-binding sites, including a site for the methyl donor S-adenosyl methione (SAM), and a GTP cap binding pocket. There is also a positively charged RNA binding site [11]. Although this domain catalyzes two separate methylation reactions, there is only a single SAM-binding site. N7 methylation precedes 2'-O methylation and involves a stem loop. For this, the capped RNA associates with the RNA-binding site whereby the RNA stem loop facilitates presentation of the cap to the methyl donor. The methylated product subsequently binds in the GTP binding pocket and the RNA is resituated such that the ribose of the first nucleotide is methylated at its 2'-OH [12]. Thus, the GTP and SAM binding sites represent potential targets for the inhibition of NS5. Our current approach targets the SAM cofactor binding site.

II. MATERIALS AND METHODS

The PyMOL software "build" tool is used to generate two small molecule template backbones (8-carbon and 10-carbon) off the SAM amine group (**Figure 1**) to position at the NS5 cofactor binding site. To determine which functional groups to place off the backbones, the surrounding atoms in the cofactor pocket that will interact with the small molecule are considered with respect to their ability to form hydrogen bonds of relevance. Upon selection of potential functional groups, a library of small molecules comprised of the adenosine from SAM and the built backbone-functional group arrangement is generated. This library of several thousand distinct small molecules is tested using the computational language Python. Each small molecule is docked into the NS5 cofactor pocket by mapping the adenosine to the SAM adenosine coordinates. This ensures that the small molecules will displace SAM.

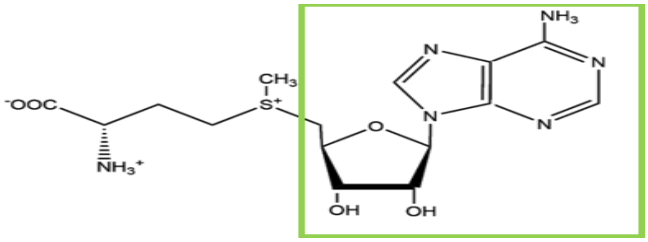

Figure1: S-adenosyl methione. The specificities of the inhibitor candidates are assessed with the sugar and adenosine (highlighted in green) and designed side chains built off the amine.

Following docking of the inhibitor candidate into the pocket, a trie tree data structure is developed to model the bond angle rotations between the carbons in the backbone. As a result of Brownian motion [13], the conformations of cellular molecules vary over time. The trie tree models this motion to predict the conformation most likely to occur *in vivo*. Thus, the trie tree eliminates rotations likely to cause the small molecule to clash in the binding pocket. Rotation angles are set in 30 degree increments (**Figure 2**). Clashing or drifting of carbons out of the pocket are deemed unviable and the potential inhibitors resulting from that angle are eliminated. The trie tree method provides several inhibitor conformations corresponding with each original molecule. **Figure 3** provides a scaled-down representation of a trie tree corresponding to an eight-carbon small molecule.

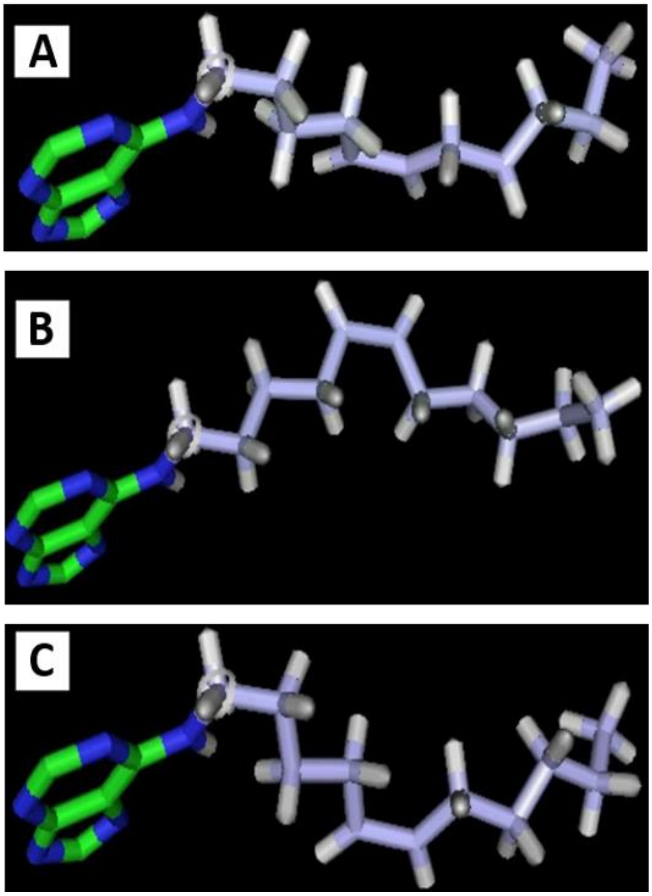

Figure 2: Bond angle rotations of the eight-carbon backbone molecule.

A new library of conformations is created using the results of the trie tree, such that several hundred molecules evaluated, to date, have resulted in several million conformations. To determine the potential affinity of each small molecule inhibitor for the cofactor pocket, the overall binding energy is evaluated. The binding energy is the sum of the Lennard Jones attraction and repulsion potentials [14], hydrogen bond energies, and Lazaridis-Karplus solvation energies [15] between each side chain in the pocket and each atom in the molecule. Lower binding energies indicate a more favorable binding interaction between the small molecule and cofactor pocket of NS5. The conformation with the lowest energy is chosen to represent each small molecule. The energetic feasibility of the small molecules inhibiting SAM is provided by the altered SAM structure in the small molecule while the specificity is provided by the designed backbone, intended to prevent interaction with other proteins.

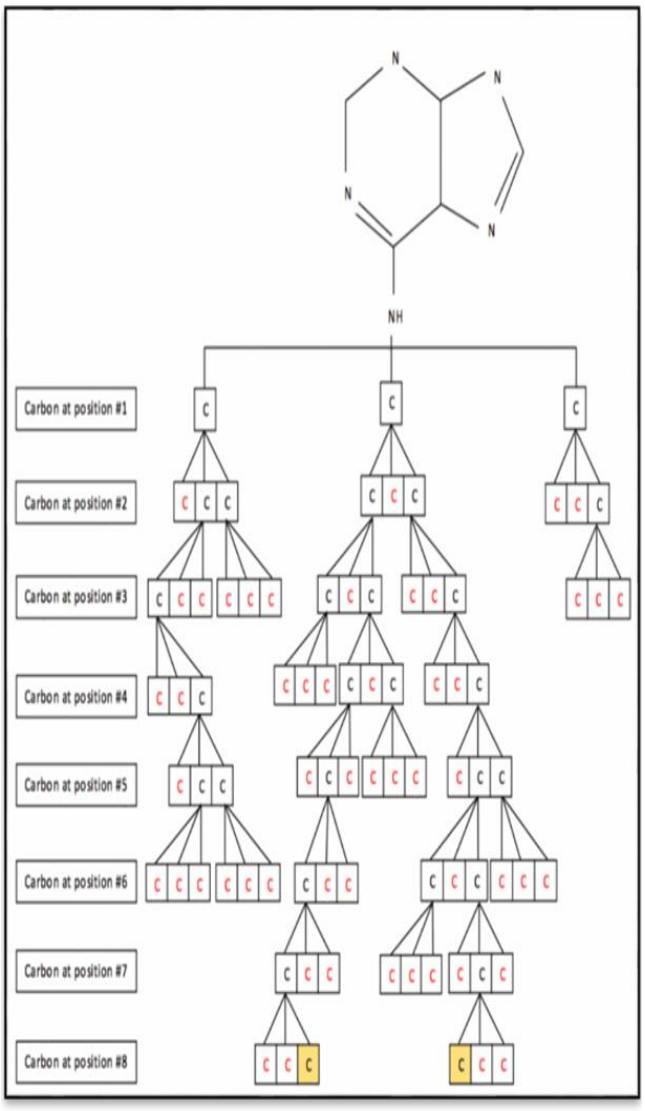

Figure 3: Representative trie tree showing eight levels of carbon rotations. The connection lines represent distinct bond angle rotations that the carbons can adopt (for simplicity, only three possible rotations for each carbon are shown). Carbons indicated in red are not viable rotations. The two highlighted carbons at position #8 represent two possible small molecule inhibitor conformations that could fit into the NS5 cofactor pocket without clashing. The path traced by this figure does not indicate the 3D structure of the inhibitor, but rather depicts a path showing how rotating bond angles between carbons may lead to a potential small molecule conformation.

III. RESULTS AND DISCUSSION

The methytransferase domain of ZIKV NS5 includes multiple ligand-binding sites. Included among

these is a cofactor binding pocket for the SAM methyl donor. **Figure 4** illustrates the structure of ZIKV NS5 as it is bound to SAM [7].

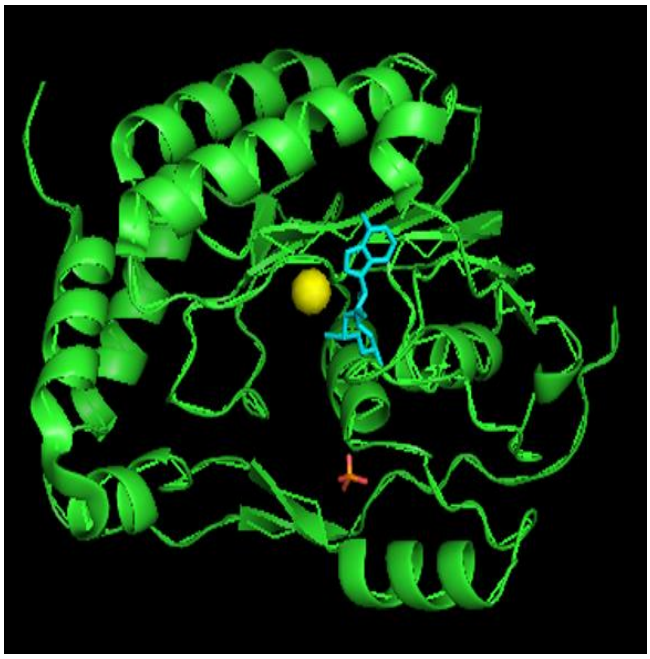

Figure 4: ZIKV NS5 bound to the SAM methyl donor. SAM is indicated in cyan; Chloride ion is indicated in yellow; Phosphate ion is indicated in red/orange.

The structure of ZIKV NS5 is highly similar to other reported flaviviral NS5 structures with the exception of moderately enhanced hydrophobicity at the GTP binding site [7]. ZIKV NS5 is comprised of a methyltransferase core which encompasses residues 54–223 including seven β strands and four α helices [7]. Flanking the core are α helices and β strands that establish the amino- and carboxyl-terminal wings. SAM binding occurs in a pocket comprised of two β strands (β1, β2), one α helix (αA), and a loop (residues 81–86) that includes a Rossman fold methylase consensus sequence [7]. The adenine of SAM is juxtaposed at the side chains of K105 and I147 while the ribose interacts with G106, E111, and T104. SAM methionine interacts with S56, D146, G86 and W87 [7]. D146 is integral to a motif that is essential for N7 and 2′O methylation [7, 9, 16, 17].

Using the approach outlined above, we have evaluated several hundred potential inhibitors. Of those molecules, the top candidates have been ranked with regard to their specificity and relative energies to the cofactor binding pocket of ZIKV NS5. Each candidate has a distinct chemical structure allowing it to fit uniquely in the cofactor pocket as illustrated by the comparison of potential inhibitors in **Figure 5**. Likewise, **Figure 6 (A)** illustrates a comparison between SAM and a candidate inhibitor highlighting the inhibitor's absence of a methyl group. **Figure 6 (B)** demonstrates how the inhibitor binds in the SAM binding pocket, thereby precluding SAM association with the NS5 methyltransferase core.

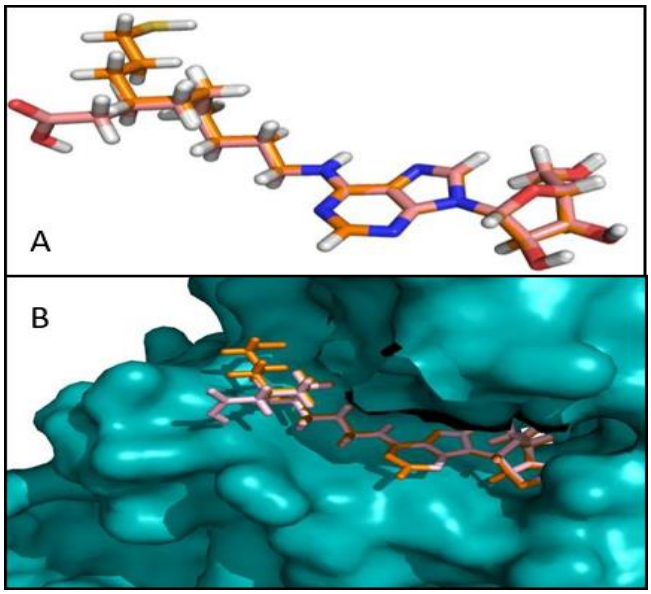

Figure 5: Physical and chemical variations of two candidates. **A)** Two potential inhibitors (pink) and (orange) superimposed to display varying chemical compositions. **B)** The inhibitors shown in the cofactor pocket illustrating spatial variations.

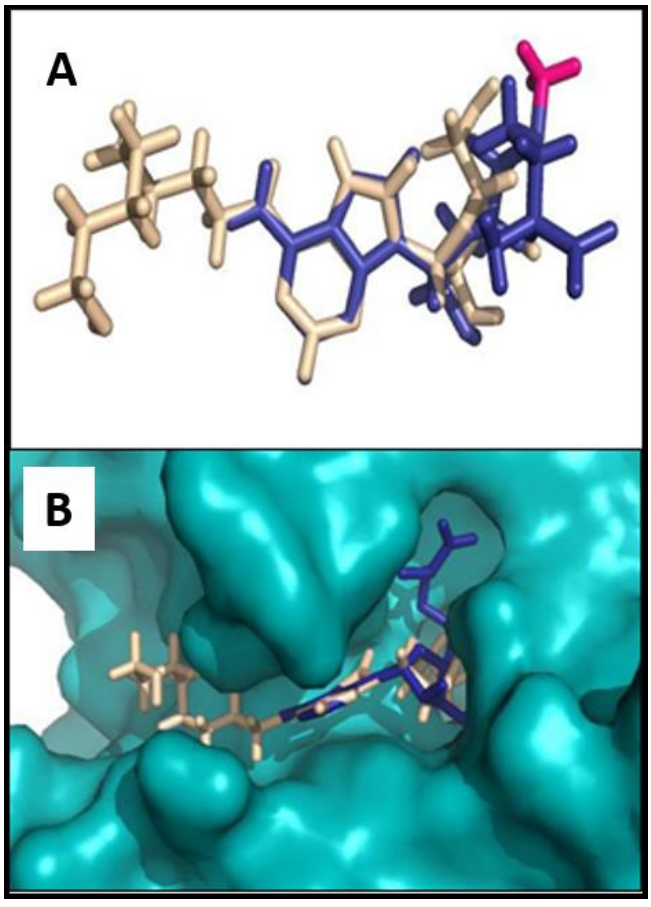

Figure 6: SAM and a Candidate Inhibitor **(A)** SAM (blue) superimposed on a potential inhibitor (tan). The donated methyl (magenta) is present in SAM but not in the inhibitor. **(B)** In the cofactor pocket, the inhibitor precludes association with SAM.

Inhibitors of greatest interest include those which inhibit the N7 methylation activity of ZIKV NS5, given the necessity of that step for replication of the virus. To simplify the screening process, RNA ligation fragments are generated according the protocol developed by Barral et al. for screening N7 methylation inhibitors of the Dengue virus NS5 [18]. This facilitates the side-by-side evaluation of 2'-O and N7 inhibition using a single substrate. Molecules that are proven most effective during the methylation screening are further analyzed in a plaque reduction assay where the 50% effective concentration (EC50) of each inhibitor is determined by the number of plaque forming units following introduction of the molecule in the plaque reduction assay.

IV. Conclusions

Given the growing number of individuals infected with ZIKV since its emergence in the Americas and the alarming increase in serious symptoms including ZIKV-associated microcephaly and Guillain-Barre syndrome, it is of paramount importance that a therapeutic be developed. Although a vaccine for a subset of the population was previously considered a sufficient solution based on the predominantly mild indications associated with past ZIKV infections, a safe, effective therapeutic is now in dire need. Monoclonal antibodies and other biologics that target the virus or ZIKV-infected cells are attractive but are both exceptionally challenging and, perhaps, prohibitively costly. A drug is likely to be a faster and significantly cheaper path. To that end, we have developed thousands of prospects for virtual screening and hundreds for *in vitro* screening. From these, a subset of the most promising candidates will be selected for more advanced screening with the goal of identifying several inhibitors for clinical stage testing.

Acknowledgments

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