

# Identification of Potential Inhibitors against the Human Influenza A Virus Targeting the CPSF30 and RNA Binding Domains of the NS1 Protein: An E-Pharmacophore approach.

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## ABSTRACT

**Background:** Common occurrences of influenza virus strains resistant to known neuraminidase and matrix protein inhibitors like oseltamivir and amantadine respectively necessitate the development of newer antivirals. The virus' non-structural protein (NS1) through its effector domain (ED) and RNA binding domain (RBD) plays significant roles in overcoming the host antiviral response and regulating the virus replication cycle respectively. **Aim:** This work attempts to identify potential NS1 based inhibitors against influenza, by interrupting the above stated mechanisms through using insilico drug design methods. **Methods:** E-pharmacophore models, were generated by docking a fragment library at the active sites of both the domains using GlideXP. Based on the energy-optimized pharmacophore obtained, the Phase program was used to screen Asinex's MedChem building blocks to find suitable hits with the essential pharmacophore features. **Results:** The docking complexes of the top ranking compounds at the ED formed hydrogen bond contacts with Gly184, Asn188, and hydrophobic contacts with Ile119, Trp187 which are critical for binding the F3 zinc finger of the cleavage and polyadenylation specificity factor (CPSF30). The top ranking compounds at the RBD made hydrogen bond contacts with critical residues Arg38 of both its chains and showed better docking score and space occupancy when compared to the top ranking compounds at the ED. **Conclusion:** The compounds identified and their backbone structural scaffolds could be further used to design drug like compounds targeting the NS1 protein of influenza. Further invitro studies would be required for testing their antiviral activity as well as for ligand optimization.

**Key words:** Influenza, Non structural (NS1) protein, Molecular docking, E-pharmacophore mapping, Virtual screening.

## INTRODUCTION

Influenza A viruses are common pathogens that cause acute respiratory diseases in a wide variety of avian and mammalian species including human. The virus has a complex replicative cycle and undergoes rapid evolutionary divergence because of antigenic drift and antigenic shift, which causes local epidemics and worldwide pandemics with significant infection and high mortality rates.<sup>1</sup> Though vaccination is the primary means of preventing and controlling the

disease, still antiviral drugs play an important role in containing the disease during epidemics and pandemics by checking the virus spread and alleviating the symptoms. Oseltamivir and Zanamivir, the known neuraminidase inhibitors, are two approved antiviral drugs recommended for use against the recently circulating influenza viruses.<sup>2</sup> However, drug resistant influenza virus strains to these antivirals have recently emerged,<sup>3</sup> while Adamantanes (amantadine

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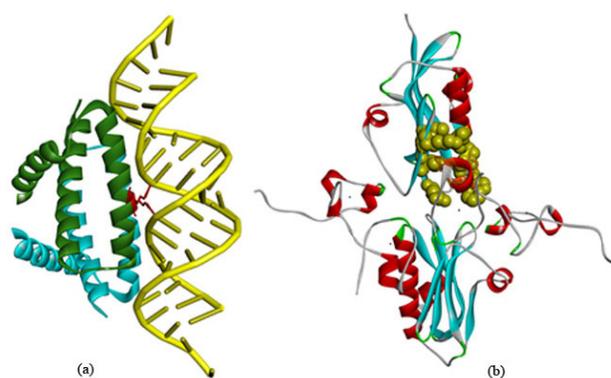


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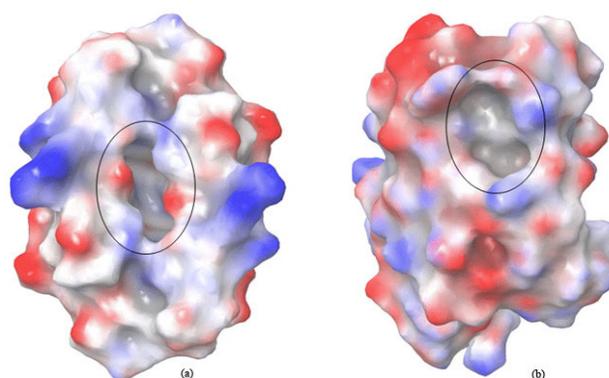
and rimantadine; M2 ion channel inhibitors) are not recommended as many influenza strains are now resistant to these drugs.<sup>4</sup> In view of the resistance to the existing drugs it becomes vital to explore novel drug targets for the development of influenza antivirals.

In addition to hemagglutinin and neuraminidase, the non structural protein 1 (NS1) of Influenza A viruses (NS1A) is a key factor for virulence,<sup>5</sup> and participates in both protein-RNA as well as protein-protein interactions.<sup>6</sup> Considering the role of NS1A in the pathogenicity of the virus, a few reports have considered NS1 as novel antiviral drug target.<sup>7,8</sup> The NS1A has a strain-specific length of 230–237 amino acids, and an approximate molecular mass of 26 kDa.<sup>1</sup> It is synthesized in infected cells, but not incorporated into the virions.<sup>9</sup> The viral NS1 protein has been observed to antagonize host immune responses.<sup>10,11</sup> Furthermore it regulates the virus replication cycle, together with viral RNA replication, viral protein synthesis and host cell physiology. Broadly NS1A is divided into two different functional domains: (i) an N-terminal (NS1A<sub>N</sub>) RNA-binding domain (RBD; residues 1-73) which binds double stranded RNA in a sequence independent manner, and (ii) a C-terminal (NS1A<sub>C</sub>) effector domain (ED; residues 74-230) which mainly interacts with host-cell proteins and also stabilizes the RBD.<sup>12</sup>

The RBD is a symmetrical homodimer each consisting of three  $\alpha$ -helices,<sup>13</sup> that bind to double stranded RNA (dsRNA) (Figure 1a). It protects the virus against the antiviral state induced by interferon (INF)  $\alpha/\beta$  by blocking the activation of 2'-5'-oligo(A) synthetase/RNase L pathway.<sup>14,15</sup> Several key residues on the concave dsRNA-binding surface, such as Arg35, Arg38, Ser42 and Thr49, are directly involved in dsRNA binding. A conserved residue, Arg38, from each of the NS1A<sub>N</sub> monomers is paired to interact with the dsRNA at its major groove. The Arg38-Arg38 pair and Arg35-Arg46 pairs are crucial for dsRNA binding, with the former penetrating into the bound dsRNA and the latter probably critical for the stabilization of NS1A RBD dimer.<sup>16</sup> Earlier studies by Wang *et al.*<sup>17</sup> had suggested that the residues critical for dimer formation, such as Asp12, Arg19, Asp29, Arg35 and Arg46, are also important for dsRNA binding. Further, although Arg37, Lys41 and Arg44 on the dsRNA-binding surface were proposed to have important roles in dsRNA binding, the only residue absolutely required for dsRNA binding was shown to be Arg38. The conserved deep pocket observed beneath the concave dsRNA-binding surface was suggested as an inviting target for structure-function studies and antiviral drug design (Figure 2a).<sup>18</sup> A glycerol molecule binding site identified at this pocket under the invariable



**Figure 1: (a) Influenza NS1A Receptor Binding Domain (RBD)-dsRNA complex gripped by Arg38 pair from both monomers and (b) tetrameric complex of effector domain (ED). Interacting residues of NS1A shown in CPK and CPSF30 residues shown in sticks (generated with Discovery Studio Visualizer 3.5, Accelrys Software Inc.).**



**Figure 2: (a) NS1A-RBD domain and (b) NS1A-ED with the potential drug binding pockets encircled (generated with Schrodinger Software Suite 2013-1).**

Arg38 pair from the crystal structure firmly proposed that some chemical compound might bind to this pocket and induce the disruption of the Arg38 pair.<sup>16</sup> Beside this the RBD also overlaps with the retinoic acid-inducible gene I (RIG-I) binding domain.<sup>19</sup> The RIG-I protein functions as an intracellular viral sensor which can be activated by influenza virus ssRNA. The binding of RIG-I to RBD of NS1 inhibits the viral detection ability of the cell.<sup>14</sup>

The NS1A effector domain (ED) forms dimers in solution and each monomer contains seven  $\beta$ -strands and three  $\alpha$ -helices.<sup>20</sup> The ED has been found to interact relatively with four different proteins: elongation initiation factor 4GI (eIF4GI), protein kinase R (PKR), poly(A)-binding protein II (PAB II) and the cleavage and polyadenylation specificity factor 30 kDa subunit (CPSF30).<sup>14</sup> The CPSF30 binding domain is at the base of the largest

$\alpha$ -helix.<sup>21</sup> The binding of the ED to CPSF30 inhibits the maturation and export of host cellular antiviral m-RNAs.<sup>22</sup> The second and third zinc fingers (F2F3) of CPSF30 have been shown to mediate the binding of NS1A.<sup>23</sup> NS1 mutations involving the CPSF30 binding site (residues 184-188) weaken CPSF30 binding which validates NS1 effector domain as potential drug target.<sup>22,23</sup> The crystal structure of the F2F3:NS1A complex is a tetramer, in which two F2F3 molecules wrap around two NS1A effector domains that interacts with each other in a head-to-head orientation (Figure 1b). The F2F3 binding pocket on the surface of NS1A is largely a hydrophobic pocket (Figure 2b), primarily defined by amino acid residues Lys110, Ile117, Ile119, Gln121, Val180, Gly183, Gly184, and Trp187, which interacts with aromatic side chains of residues Tyr97, Phe98, and Phe102 of the F3 Zn finger of the corresponding F2F3 molecule.<sup>24</sup> Mutational studies showed that, amino acids Gly184, Trp187 and Gln121 are required for the formation of the F2F3:NS1A complex.<sup>24</sup> The eight residues of NS1 reported above are not only conserved among influenza A viruses isolated from humans but are also conserved in H5N1 viruses isolated from humans and in the pandemic 1918 virus (A/Brevig Mission/1/18).<sup>24</sup> A mutation at position 186 and 187 of this site induces host cell INF  $\beta$  and attenuates viral replication.<sup>23,25</sup> Thus developing inhibitors against CPSF30 binding site of the NS1 ED would be an ideal influenza antiviral.

In this study we attempted the identification of potent inhibitors against the major functional binding sites on the two domains of the influenza NS1 protein. An energy optimized pharmacophore (e-pharmacophore) model was developed for potential NS1 inhibitors by utilizing a combined ligand and structure based approach based on the e-pharmacophore module of Schrodinger software suite. The model was used to screen the MedChem Building Blocks database from Asinex of nearly seven thousand compounds. Hits matching the model were further docked to identify the compounds binding effectively to the major active sites of both the NS1 domains. Thus the current study will broaden the identification of potential NS1 based antivirals against influenza and their chemical features.

## MATERIALS AND METHODS

### Protein and ligand preparation

The protein structures for influenza NS1A ED and RBD were downloaded from Protein Data Bank (PDB) (PDB IDs 2RHK and 2ZKO, respectively). A single chain for

the ED comprising of the F2F3 CPSF30-binding site of NS1A<sub>C</sub> and both the chains of NS1A<sub>N</sub> RBD were considered. These proteins were prepared by using the Protein Preparation Wizard,<sup>26</sup> which is part of the Maestro software package (Maestro, v9.4, Schrödinger, LLC, New York, NY). The proteins were pre-processed and the H-bond network was optimized by adding hydrogens to all atoms in the structures. The OPLS\_2005 force field was used to run a restrained minimization on the structures.<sup>27</sup>

Drug like fragments (Glide Fragment library) from Schrodinger's website (<http://www.schrodinger.com/productpage/14/5/78/>) were downloaded while MedChem Building Blocks (<http://www.asinex.com/building-blocks.html>) a drug like compounds database was obtained from Asinex (<http://www.asinex.com>). The latter is a collection of seven thousand drug like compounds which mainly consists of primary amines, secondary amines, carboxylic acids, aldehydes and poly-functional building blocks with low molecular weight and is ideal for lead optimization and fragment based screening.

The 3D molecular structure files of the compounds were prepared using the 'LigPrep' version 2.6 of Schrödinger suite (Schrödinger, LLC, New York, NY, 2013).

Hydrogen atoms were added, unsuitable molecules were filtered out based on their properties, unwanted molecules such as water and small ions were removed. This was followed by neutralizing charged groups, generation of ionization and tautomeric states using 'Epik', also from Schrodinger, for generating low energy ring conformations and optimizing the geometries.

### Docking of compounds from Drug-like fragment library

For docking, the ligands and the receptor grid to be used was specified and Glide XP module of Schrodinger was applied. The receptor grid for the NS1A ED, was generated around amino acid residues Lys110, Ile117, Ile119, Gln121, Val180, Gly183, Gly184, and Trp187 whereas for the NS1 RBD, the grid was generated around residues Arg35, Arg38, Ser42, Arg46 and Thr49. The grid size was kept at the default value of 20 Å.

The Glide XP mode (Glide version 5.9, Schrödinger, LLC, New York, NY, 2013) was chosen from among HTVS (High Throughput Virtual Screening), SP (Standard Precision), and XP (Extra Precision) modes for the docking of drug like fragments and compounds.<sup>28</sup> The glide fragment library which was downloaded was docked using the XP mode to the proteins (NS1A – ED/ RBD). The 'Write XP' descriptor information was selected to visualize the interaction terms. The docked

poses were viewed in the pose viewer and in XP Visualizer of Schrodinger Suite, Epik version 2.4 (Schrödinger LLC, New York, NY, 2013).

### E-pharmacophore generation

E-Pharmacophore suite was used for the pharmacophore hypothesis generation. GLIDE XP and the docking post processing script were used to generate the pharmacophore hypothesis. The glide scoring terms were computed and energies were mapped onto the atoms.<sup>29</sup> Hydrogen-bond acceptor sites were represented as vectors along the hydrogen bond axis in accordance with the hybridization of the acceptor atom. Hydrogen-bond donors were represented as projected points, located at the corresponding hydrogen-bond acceptor positions in the binding site. Each pharmacophore feature site was first assigned an energetic value equal to the sum of the Glide XP contributions of the atoms comprising the site. This allows sites to be quantified and ranked on the basis of these energetic terms. The best four or less features were selected based on the score. The hypothesis was generated with the selected features and was further used for database screening.

### Phase database preparation and screening

Database generation and screening with the pharmacophore model was carried out using Phase version 3.5 of Schrodinger Suite (Schrödinger, LLC, New York, NY, 2013).<sup>30</sup> The MedChem Building Blocks database was converted to a 3D molecular structure compound database using Phase. The structures were ensured to be all-atom structures with reasonable 3D geometries. The input structures were represented in 2D form, without explicit hydrogen atoms, or with counter ions and solvent molecules. The structures were generated at physiological pH and high energy ionization/tautomer states were removed; other parameters were kept default. During the screening of the database, molecules were required to match a minimum of 3 sites for a hypothesis with 3 or 4 sites. Database hits were ranked in the order of fitness score, a measure of how well the aligned ligand conformer matches the hypothesis based on RMSD site matching, vector alignments, and volume terms. The fitness scoring function is an equally weighted composite of these three terms and ranges from 0 to 3, as implemented in the default database screening of Phase. The ligands that yielded fitness score of more than 1.0 were further docked into the binding sites of the specific receptor by using Glide XP and compounds with best glide scores were reported.

## RESULTS AND DISCUSSION

In this study we have considered the NS1 protein domains defining the ED (PDB ID: 2RHK) and RBD (PDB ID: 2ZKO) as the target for docking as well as for developing the e-pharmacophore model. As the e-pharmacophore approach combines aspects of structure based and ligand based techniques, it has been shown to produce enhanced enrichments over using ligand information alone. The method incorporates structural and energetic information using the scoring function in Glide XP.<sup>32</sup> As both the targets lack a co-crystallized ligand, in order to generate protein-ligand interaction information for designing the pharmacophore model, the molecules from the glide fragment library which are available for download (<http://www.schrodinger.com/Glide/Fragment-Library>) were docked using the XP mode to the respective NS1A binding sites.

### E-pharmacophore development and database screening

An E-pharmacophore model encompassing the major interactions between the protein-ligand complexes was developed based on the docking poses of 441 fragments from the glide fragment library. Of the seven pharmacophore sites that were predicted, only four sites were chosen based on the score. The final hypothesis consists of an aromatic ring (R), two H-bond donors (D), and one H-bond acceptor (A) for NS1 ED whereas for NS1 RBD the hypothesis consisted of two hydrogen bond acceptors (A), one hydrogen bond donor (D) and one negative ionizable (N). Based on these pharmacophore hypotheses, screening of compounds were performed against Asinex's MedChem Building Blocks Database containing 7,000 compounds. 130 compounds with a fitness score of more than 1 were shortlisted for the ED while 38 compounds with fitness score of more than 1 were shortlisted for the RBD. Further docking of these compounds was carried out and four best compounds from each of the binding sites were proposed as potential inhibitors based on their docking score and space occupied within the binding pocket. The distances and the geometry of the hypotheses are shown in Figure 3.

The Glide scores of the four best compounds at the ED site were in the range -6.0 to -6.7 and Glide energies were between -23 to -31 kcal mol<sup>-1</sup> (Table 1). Similarly the Glide scores and Glide energies of the four best compounds at the RBD site were found to be between -7.3 to -8.2 and -29 to -39 kcal mol<sup>-1</sup> (Table 2).

### Docking at NS1 ED

The best hit obtained from screening the MedChem Building Block Database based on the highest score and space occupation at the ED binding pocket was

Table 1: Pharmacophore results for the best hit compounds, and Glide XP docking results at ED						
Compound ID	Interaction (D...H-A)#	H-bond Length (Å)	Residues in hydrophobic contacts	Fitness score	Glide Score	Glide energy (kcal/mol)
BAS 00337509	NH(Gln121)...O OH...O(Asn188) OH...O(Gly184)	2.41 2.30 2.35	None	1.477	-6.7	-31.237
BAS 09627795	NH...O(Val180) NH...O(Gly184) NH...O(Asn188)	2.07 1.83 1.93	Ile 119, Trp187	1.276	-6.6	-29.746
BAS 09627796	NH...O(Val180) NH...O(Gly184) NH...O(Asn188)	2.08 1.83 1.93	Ile 119, Trp187	1.520	-6.5	-28.6
BAS 09627799	NH...O(Val180) NH...O(Gly184) NH...O(Asn188)	2.17 2.36 2.45	Ile 119, Trp187	1.443	-6.0	-23.923

# D- donor, H- Hydrogen, A- Acceptor

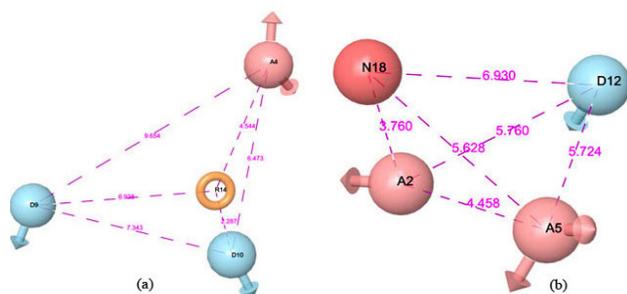


Figure 3: (a) Pharmacophore model for NS1A ED (DDAR) (b) Pharmacophore model for NS1A RBD (AADN).

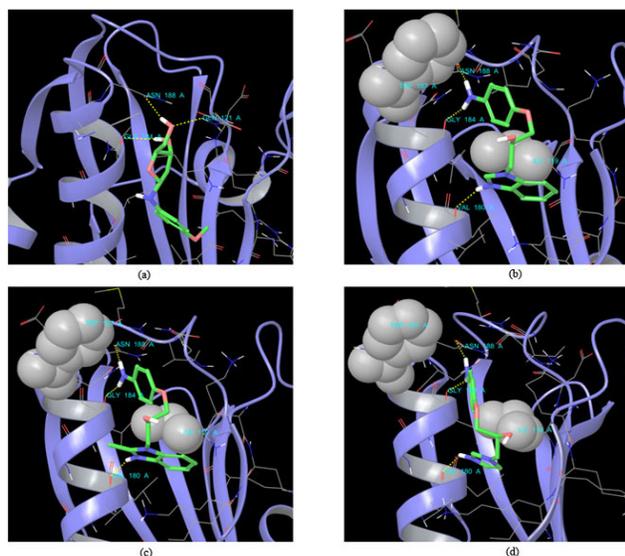


Figure 4: Binding mode of the ligands at NS1A ED (a) BAS 00337509 (b) BAS 09627795 (c) BAS 09627796 (d) BAS 09627799. Residues with Hbond contact in yellow line. Residues in CPK represents hydrophobic interaction.

BAS 00337509 (2-Deoxy-N-(4-methoxyphenyl) pentofuranosylamine) with a Glide score of  $-6.7$  (Table 1). This compound formed hydrogen bond contacts with amino acid residues, Asn188, Gln121, Gly184 (Figure. 4a). The other top hits obtained were: BAS 09627795 (1-(4-Aminophenoxy)-3-(1H-benzimidazol-1-yl)-2-propanol), BAS 09627796 (1-(4-Aminophenoxy)-3-(2-methyl-1H-benzimidazol-1-yl)-2-propanol) and BAS 09627799 ((2S)-1-(4-Aminophenoxy)-3-(1H-imidazol-1-yl)-2-propanol). The structures of these compounds are shown in Figure 5. The docking complexes of these compounds showed that they made hydrogen bond contacts with Gly184, Asn188, and hydrophobic contacts with Ile119, Trp187 (Figure. 4). It can be noted that all these residues are known to be critical for the CPSF30 binding.<sup>23,24</sup>

### Docking at NS1 RBD

The best hit screened from the MedChem Building Block Database was BAS 02862810 (N-[(5-Hydroxy-3-pyridinyl) carbonyl] serine) with Glide score  $-8.2$  (Table 2). The compound formed hydrogen bond contacts with amino acid residues Arg38 from chain A and B and Asp39 from chain B (Figure 6). In addition, a pi-cation contact was formed with Arg19 (B) and Arg35 (A) and, salt bridge contacts were formed by Asp12 (B) and Asp39 (B). Of these, residues Asp12, Arg19, and Arg35 are known to be critical for dimer formation and dsRNA binding<sup>17</sup> while Arg38 was shown to be one of the most critical residues for RNA binding.<sup>16,17</sup>

Other compounds that were identified based on the docking scores were: BAS 02912613 (N-[(4-Methylphenyl)sulfonyl]histidine) (ii) BAS 08978615: (3-(6-Amino-

Table 2: Pharmacophore results for the best hit compounds, and Glide XP docking results at RBD					
Compound ID	Interaction (D...H-A) <sup>§</sup>	H-bond Length (Å)	Fitness score	Glide Score	Glide energy (kcal/mol)
BAS 02862810	NH(Arg38A)...O NH(Arg38B)...O NH(Arg38B)...O OH...O(Asp39B) NH...O(Asp39B)	2.02 2.28 2.14 2.03 2.20	1.326	-8.2	-29.661
BAS 02912613	NH...O(Asp12B) NH(Arg19B)...O NH...O(Asp39B) NH...O(Asp39A)	2.39 1.95 2.03 1.78	1.196	-7.9	-39.238
BAS 08978615	NH...O(Asp12B) NH(Arg38A)...O NH(Arg38B)...O OH...O(Asp39A) NH...O(Asp39A) NH...O(Asp39B)	2.43 2.07 2.09 1.84 1.63 2.38	1.263	-7.6	-36.411
BAS 01947678	NH(Arg35A)...O NH(Arg38A)...O NH(Arg38B)...O NH...O(Asp39A)	2.05 1.91 2.13 2.35	1.090	-7.3	-34.071

<sup>§</sup>D- donor, H- Hydrogen, A- Acceptor

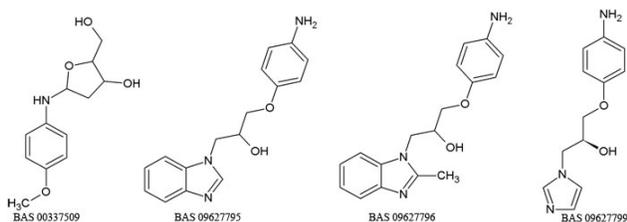


Figure 5: Structures of the best hits found at the CPSF30 binding site of NS1A ED.

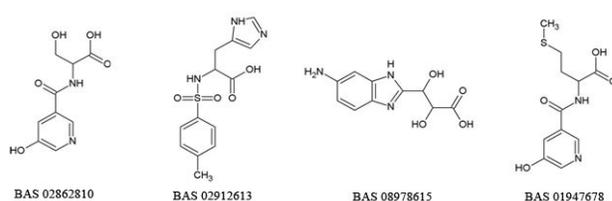


Figure 7: Structures of the best hits found at the ds-RNA binding site of NS1 RBD.

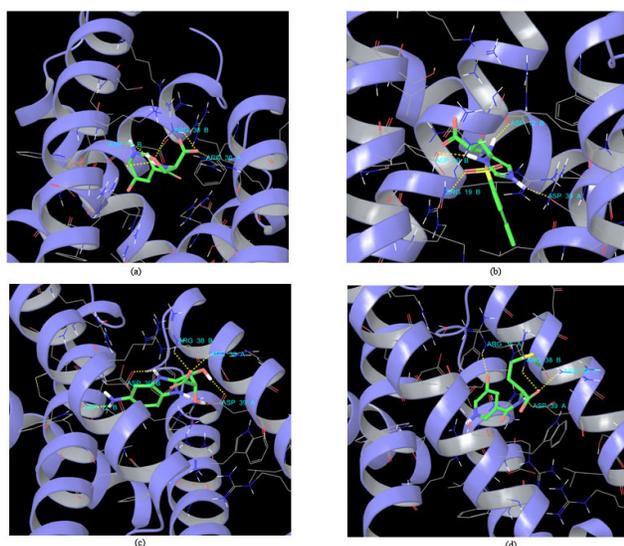


Figure 6: Binding mode of ligands at NS1A RBD (a) BAS 02862810 (b) BAS 02912613 (c) BAS 08978615 (d) BAS 01947678.

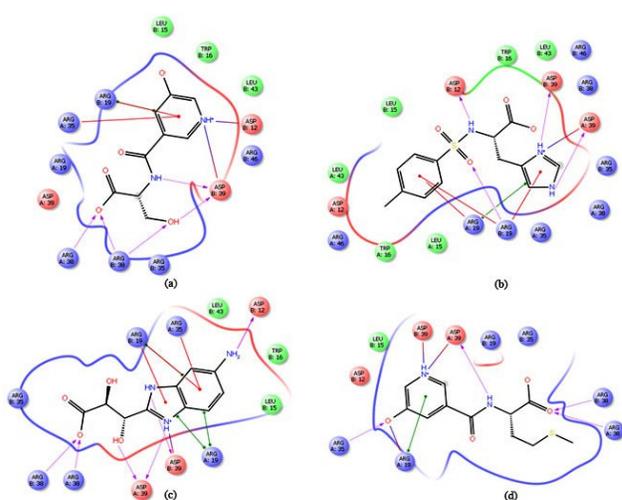


Figure 8: Contacts made by ligands at RBD other than H-bonds (a) BAS 02862810 (b) BAS 02912613 (c) BAS 08978615 (d) BAS 01947678. Green lines denote pi-pi stacking interaction, orange line denotes pi-cation interaction, and red blue line denotes salt bridge contact.

1H-benzimidazol-2-yl)-2,3-dihydroxypropanoic acid) (iii) BAS 01947678: (N-[(5-Hydroxy-3-pyridinyl)carbonyl]methionine). The structures of the compounds are shown in Figure. 7. Most of these compounds also formed hydrogen bond contacts with Arg38 residue of both the chains. Few other prominent contacts comprising of pi-cation, pi-pi stacking and salt bridge contacts with important residues were also found (Figure 8).

## CONCLUSIONS

The main objective of this work was to identify potential NS1 protein based inhibitors for influenza based on the CPSF30 binding effector and RNA binding domain of the protein. This is the first report wherein we identified the essential pharmacophore features of the ligands that are vital for binding at the two domains of NS1.

Screening of the MedChem Building Blocks database from Asinex showed that the compound BAS 00337509 (2-Deoxy-N-(4-methoxyphenyl) pentofuranosylamine) was, the best hit at the NS1 ED. The compound formed hydrogen bond contacts with Gln121, Gly184 and Asn188 all of which are residues which are known to interact with the aromatic side chain residues of the F3 zinc finger of CPSF30. In addition to this, three compounds belonging to the amino-phenoxy- groups were found to have good space occupying effect and hydrogen bond contacts with Gly184, Asn188. These compounds also formed hydrophobic contacts with the other critical residues Ile119 and Trp187. Based on these observations, the identified compounds could possess the ability to prohibit the interaction of NS1 ED with the CPSF30 of the host protein and thus reduce the virulence of the virus. Similarly, a recent molecular docking study screening the H1N1 Influenza NS1 CPSF30 binding pocket, revealed four novel compounds with high binding energy that can interact at the pocket by strong hydrophobic interactions, which was similar to the three aromatic residues (Tyr97, Phe98 and Phe102) of F3.<sup>31</sup>

Screening of the same database for potential NS1 RBD inhibitors showed compound BAS 02862810 (N-[(5-Hydroxy-3-pyridinyl) carbonyl] serine) to have the best docking score. Most importantly it made hydrogen bond contacts with critical residues Arg38 of both chains which in turn could result in the disruption of Arg38 pair. As has been reported in the literature, this could further lead to the decrease of dsRNA-binding affinity by NS1A and eventually lead to the attenuation of virulence. A recent review describes how biochemical, cell-based and nucleic-acid based approaches are being used to identify NS1 antagonists. Compounds including

epigallocatechin gallate (EGCG), have been reported to show good *in vitro* antiviral activity, and showed significant inhibition of RNA-NS1 binding.<sup>32</sup>

Finally, it was also noted in this study that the top hit compounds showed better docking score and space occupancy at the NS1A RBD when compared to the ED. The results from the present studies are thus indicative that the RBD may be a better pocket for drug intervention compared to the ED. Though there is no experimental data to support this point, our results provide vital information for developing new drugs that target the RNA binding site of NS1.

This study has limitations, in that the database screened for obtaining both the E-pharmacophore models as well as for identifying likely drug candidates was not exhaustive. Thus, a larger database of compounds needs to be screened to find more potent compounds with better binding affinities. Moreover the stability of the top hit compounds thus found can be further evaluated by implementing molecular dynamics studies. This would help understand the in-depth molecular mechanism of drug binding. Subsequently the potential lead compounds could be tested *in-vitro* for their antiviral activity and clinical research.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS USED

**NS1:** Non-structural protein; **NS1A:** Non structural protein 1 of Influenza A viruses; **NS1AN:** N- terminal of NS1A protein; **NS1AC:** C- terminal of NS1A protein; **ED:** effector domain; **RBD:** RNA binding domain; **dsRNA:** Double stranded RNA; **INF:** Interferon; **RIG-I:** Retinoic acid-inducible gene I; **eIF4GI:** Elongation initiation factor 4GI; **PKR:** Protein kinase R; **PAB II:** Poly(A)-binding protein II; **CPSF30:** Cleavage and polyadenylation specificity factor 30; **F2F3:** Second and third zinc fingers of CPSF30; **e-pharmacophore:** Energy optimized pharmacophore; **PDB:** Protein data bank; **GLIDE:** Grid based ligand docking with energetic; **HTVS:** High throughput virtual screening; **SP:** standard precision; **XP:** Extra precision; **EGCG:** Epigallocatechin gallate.

## REFERENCES

- Palese P, Shaw ML. Orthomyxoviridae: the viruses and their replication. In: Knipe DM, Howley PM Editors. *Fields virology*, 5<sup>th</sup> ed. Philadelphia: Lippincott Williams & Wilkins; 2007;2:1647-90.
- McKimm-Breschkin JL. Neuraminidase inhibitors for the treatment and prevention of influenza. *Expert Opin Pharmacother*. 2002;3(2):103-12. <http://dx.doi.org/10.1517/14656566.3.2.103> PMID:11829724
- Kiso M, Ozawa M, Le MT, Imai H, Takahashi K, Kagawa S et al. Effect of an asparagine- to-serine mutation at position 294 in neuraminidase on the pathogenicity of highly pathogenic H5N1 influenza A virus. *J Virol*. 2011;85(10):4667-72. <http://dx.doi.org/10.1128/JVI.00047-11> PMID:21367898 PMID:PMC3126218
- CDC. Update: drug susceptibility of swine-origin influenza A (H1N1) viruses, April 2009. *MMWR Morb Mortal Wkly Rep*. 2009;58(16):433-5. PMID:19407738
- Solórzano A, Webby RJ, Lager KM, Janke BH, Garcia-Sastre A, Richt JA. Mutations in the NS1 protein of swine influenza virus impair anti-interferon activity and confer attenuation in pigs. *J Virol*. 2005;79(12):7535-43. <http://dx.doi.org/10.1128/JVI.79.12.7535-7543.2005> PMID:15919908 PMID:PMC1143661
- Krug RM, Yuan W, Noah DL, Latham AG. Intracellular warfare between human influenza viruses and human cells: the roles of the viral NS1 protein. *Virology*. 2003;309(2):181-9. [http://dx.doi.org/10.1016/S0042-6822\(03\)00119-3](http://dx.doi.org/10.1016/S0042-6822(03)00119-3).
- Basu D, Walkiewicz MP, Frieman M, Baric RS, Auble DT, Engel DA. Novel influenza virus NS1 antagonists block replication and restore innate immune function. *J Virol*. 2009;83(4):1881-91. <http://dx.doi.org/10.1128/JVI.01805-08> PMID:19052087 PMID:PMC2643796
- Walkiewicz MP, Basu D, Jablonski JJ, Geysen HM, Engel DA. Novel inhibitor of influenza non-structural protein 1 blocks multi-cycle replication in an RNase L-dependent manner. *J Gen Virol*. 2011;92(1):60-70. <http://dx.doi.org/10.1099/vir.0.025015-0> PMID:20881091 PMID:PMC3052532
- Lamb RA, Krug RM. Orthomyxoviridae: the viruses and their replication. In: Knipe DM et al. editors. *Fields virology*, 4th ed. Philadelphia: Lippincott Williams & Wilkins. 2001;1487-1503.
- Egorov A, Brandt S, Sereinig S, Romanova J, Ferko B, Katinger D, et al. Transfectant influenza A viruses with long deletions in the NS1 protein grow efficiently in Vero cells. *J Virol*. 1998;72(8):6437-41. PMID:9658085 PMID:PMC109801
- Kochs G, Koerner I, Thiel L, Kothlow S, Kaspers B, Ruggli N, et al. Properties of H7N7 influenza A virus strain SC35M lacking interferon antagonist NS1 in mice and chickens. *J Gen Virol*. 2007;88(5):1403-9. <http://dx.doi.org/10.1099/vir.0.82764-0> PMID:17412966
- Hale BG, Randall RE, Ortin J, Jackson D. The multifunctional NS1 protein of influenza A viruses. *J Gen Virol*. 2008;89(10):2359-76. <http://dx.doi.org/10.1099/vir.0.2008/004606-0> PMID:18796704
- Chien CY, Xu Y, Xiao R, Aramini JM, Sahasrabudhe PV, Krug RM, et al. Biophysical characterization of the complex between double-stranded RNA and the N-terminal domain of the NS1 protein from influenza A virus: Evidence for a novel RNA-binding mode. *Biochemistry*. 2004;43(7):1950-62. <http://dx.doi.org/10.1021/bi030176o> PMID:14967035
- Xia S, Monzingo AF, Robertus JD. Structure of NS1A effector domain from the influenza A/Udm/72 virus. *Acta Crystallogr D Biol Crystallogr*. 2009; 65(1):11-7. <http://dx.doi.org/10.1107/S0907444908032186> PMID:19153461 PMID:PMC2628972
- Min JY, Krug RM. The primary function of RNA binding by the influenza A virus NS1 protein in infected cells: Inhibiting the 2'-5' oligo (A) synthetase/RNase L pathway. *Proc Natl Acad Sci U S A*. 2006;103(18):7100-5. <http://dx.doi.org/10.1073/pnas.0602184103> PMID:16627618 PMID:PMC1459024
- Cheng Ao, Wong SM, Yuan YA. Structural basis for dsRNA recognition by NS1 protein of influenza A virus. *Cell Res*. 2009;19(2):187-95. <http://dx.doi.org/10.1038/cr.2008.288> PMID:18813227
- Wang W, Riedel K, Lynch P, Chien CY, Montelione GT, Krug RM. RNA binding by the novel helical domain of the influenza virus NS1 protein requires its dimer structure and a small number of specific basic amino acids. *RNA*. 1999;5(2):195-205. <http://dx.doi.org/10.1017/S135583829981621> PMID:10024172 PMID:PMC1369752
- Yin C, Khan JA, Swapna GV, Ertekin A, Krug RM, Tong L, et al. Conserved surface features form the double-stranded RNA binding site of non-structural protein 1 (NS1) from influenza A and B viruses. *J Biol Chem*. 2007; 282(28):20584-92. <http://dx.doi.org/10.1074/jbc.M611619200> PMID:17475623
- Mibayashi M, Martinez-Sobrido L, Loo YM, Cárdenas WB, Gale M Jr, Garcia-Sastre A. Inhibition of retinoic acid-inducible gene I-mediated induction of beta interferon by the NS1 protein of influenza A virus. *J Virol*. 2007;81(2):514-24. <http://dx.doi.org/10.1128/JVI.01265-06> PMID:17079289 PMID:PMC1797471
- Bornholdt ZA, Prasad BV. X-ray structure of influenza virus NS1 effector domain. *Nat Struct Mol Biol*. 2006;13(6):559-60. <http://dx.doi.org/10.1038/nsmb1099> PMID:16715094
- Lin D, Lan J, Zhang Z. Structure and function of the NS1 protein of influenza A virus. *Acta Biochim Biophys Sin (Shanghai)*. 2007;39(3):155-62. <http://dx.doi.org/10.1111/j.1745-7270.2007.00263.x>
- Noah DL, Twu KY, Krug RM. Cellular antiviral responses against influenza A virus are countered at the posttranscriptional level by the viral NS1A protein via its binding to a cellular protein required for the 3' end processing of cellular pre-mRNAs. *Virology*. 2003;307(2):386-95. [http://dx.doi.org/10.1016/S0042-6822\(02\)00127-7](http://dx.doi.org/10.1016/S0042-6822(02)00127-7)
- Twu KY, Noah DL, Rao P, Kuo RL, Krug RM. The CPSF30 binding site on the NS1A protein of influenza A virus is a potential antiviral target. *J Virol*. 2006;80(8):3957-65. <http://dx.doi.org/10.1128/JVI.80.8.3957-3965.2006> PMID:16571812 PMID:PMC1440456
- Das K, Ma LC, Xiao R, Radvansky B, Aramini J, Zhao L, et al. Structural basis for suppression of a host antiviral response by influenza A virus. *Proc Natl Acad Sci USA*. 2008;105(35):13093-8. <http://dx.doi.org/10.1073/pnas.0805213105> PMID:18725644 PMID:PMC2522260
- Ayllon J, Russell RJ, Garcia-Sastre A, Hale BG. Contribution of NS1 effector domain dimerization to influenza A virus replication and virulence. *J Virol*. 2012;86(23):13095-8. <http://dx.doi.org/10.1128/JVI.02237-12> PMID:22993153 PMID:PMC3497675
- Sastry GM, Adzhigirey M, Day T, Annabhimoju R, Sherman W. Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. *J Comput Aided Mol Des*. 2013;27(3):221-34. <http://dx.doi.org/10.1007/s10822-013-9644-8> PMID:23579614
- Olsson MH, Søndergaard CR, Rostkowski M, Jensen JH. PROPKA3: Consistent Treatment of Internal and Surface Residues in Empirical pKa Predictions. *J Chem Theor Comput*. 2011;7(2):525-37. <http://dx.doi.org/10.1021/ct100578z> PMID:26596171
- Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, et al. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J Med Chem*. 2004;47(7):1739-49. <http://dx.doi.org/10.1021/jm0306430> PMID:15027865
- Salam NK, Nuti R, Sherman W. Novel method for generating structure-based pharmacophores using energetic analysis. *J Chem Inf Model*. 2009;49(10):2356-68. <http://dx.doi.org/10.1021/ci900212v> PMID:19761201
- Dixon SL, Smondyrev AM, Rao SN. PHASE: a novel approach to pharmacophore modeling and 3D database searching. *Chem Biol Drug Des*. 2006;67(5):370-2. <http://dx.doi.org/10.1111/j.1747-0285.2006.00384.x> PMID:16784462
- Zhang L, Zhao J, Ding G, Li X, Liu H. Screening of Potent Inhibitor of H1N1 Influenza NS1 CPSF30 Binding Pocket by Molecular Docking. *Advances in Infectious Diseases*. 2012;2:9296. <http://dx.doi.org/10.4236/aid.2012.24015>.
- Engel DA. The influenza virus NS1 protein as a therapeutic target. *Antiviral Res*. 2013;99(3):409-16. <http://dx.doi.org/10.1016/j.antiviral.2013.06.005> PMID:23796981 PMID:PMC4373342

## SUMMARY

- In view of the role of Influenza NS1 protein in the pathogenicity of the virus, this protein is being considered as a novel antiviral drug target
- We studied both the functional domains of NS1, the RNA-binding domain (RBD) and the effector domain (ED) as potential drug binding targets
- We generated the essential pharmacophore features of the ligands that are vital for binding at the two NS1 domains using Glide
- Based on E-pharmacophore models, Asinex's MedChem building blocks were screened for the identification of potential ligands using Phase
- Top ranking compounds at the RBD showed better docking scores and space occupancy when compared to the top ranking compounds at the ED
- The results provide vital information for developing new drugs that target the RNA binding site or the ED of NS1

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