



## ADMET & MOLECULAR DOCKING STUDIES OF NOVEL ZANAMIVIR ANALOGS AS NEURAMINIDASE INHIBITORS

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Accepted on: 29-12-2011; Finalized on: 25-02-2012.

### ABSTRACT

Now days, Influenza is one of the highly potent contagious pathogen spreading and threatening all over the world by causing dreadful respiratory diseases like Swine flu for which enzyme Neuraminidase (NA) is the major drug target. Neuraminidase, otherwise called as sialidases, exists as a mushroom shape projection on the surface; catalyze the hydrolysis of terminal sialic acid residues from the newly formed virions and from the host cell receptors. Reports of the recent studies reveal that enzyme neuraminidase is resistant to drugs like Oseltamivir. In the present study, we have used commercial computational tools like Accelry's Discovery Studio 2.5 to identify the novel analogs that established better binding than the Zanamivir. From the docking studies, we found that substitution of hydroxyl group with methyl is having better dock score and higher interaction energy than Zanamivir.

**Keywords:** Influenza, H1N1, Neuraminidase, Zanamivir, ADMET, Molecular docking.

### INTRODUCTION

Influenza, commonly referred to as the flu, is an infectious disease caused by RNA viruses of the family Orthomyxoviridae (the influenza viruses), that affects birds and mammals. Influenza spreads around the world in seasonal epidemics, resulting in the deaths of between 250,000 and 500,000 people every year, and millions in pandemic years<sup>1</sup>. The impact of Influenza is felt globally each year when the disease develops in approximately 20 percent of the world's population. Although vaccination is the primary strategy for the prevention of influenza, there are number of likely scenario for which vaccination is inadequate and effective antiviral agents would be of utmost importance. In the course of pandemic, vaccine supplies would be inadequate. Antiviral agents thus form an important part of a rational approach to epidemic influenza and are critical to planning for a pandemic<sup>2</sup>.

The two classes of antiviral drugs used against influenza are neuraminidase inhibitors and M2 protein inhibitors (adamantane derivatives). Neuraminidase inhibitors are currently preferred for flu virus infections since they are less toxic and more effective<sup>3</sup>.

The antiviral drugs amantadine and rimantadine block a viral ion channel (M2 protein) and prevent the virus from infecting cells<sup>4</sup>. These drugs are sometimes effective against influenza A if given early in the infection but are always ineffective against influenza B because B viruses do not possess M2 molecules.

Neuraminidase (NA or N) is a glycoprotein, which is found as projections on the surface of the virus. It forms a tetrameric structure with an average molecular weight of

220,000. The NA molecule presents its main part at the outer surface of the cell, spans the lipid layer, and has a small cytoplasmic tail<sup>5</sup>. NA acts as an enzyme, cleaving sialic acid from the Haemagglutinin (HA) molecule, from other NA molecules and from glycoproteins and glycolipids at the cell surface. Hence it is also known as sialidase. It also serves as an important antigenic site, and in addition, seems to be necessary for the penetration of the virus through the mucin layer of the respiratory epithelium<sup>6</sup>.

In the present study, we have designed 9 novel Zanamivir analogs, performed docking studies using CDocker of Accelry's Discovery Studio 2.5 and identified 7 ligands that established better binding affinity towards the enzyme neuraminidase than Zanamivir. Also, we evaluated these analogues for predicted ADME and Toxicity studies.

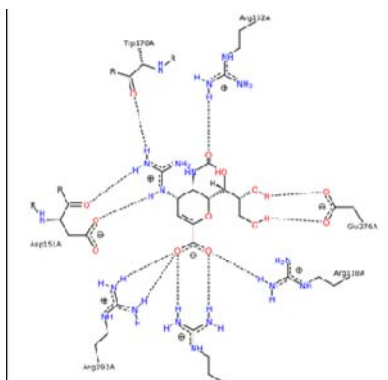
### MATERIALS AND METHODS

**Ligand & Protein Selection:** The enzyme Neuraminidase was retrieved from the RSCB PDB website (<http://www.pdb.org>) with PDB ID: 3B7E<sup>7</sup>. Protein retrieved from the database is in complex with Zanamivir which is useful in identification of its active binding sites. Active binding sites were identified by using an online tool, PoseView ([www.poseview.de/poseview/wizard](http://www.poseview.de/poseview/wizard)) given in Fig:1, and also verified by using graphic interface of Accelry's Discovery Studio 2.5

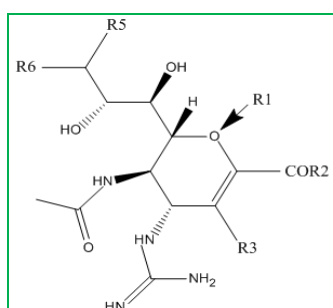
Analog of Zanamivir, a Neuraminidase inhibitor were drawn by selecting Zanamivir as Lead moiety and altering its carboxylic acid groups using Accelry's Symyx Draw 4.0 according to the Lipinski's rule. Zanamivir analogs were



listed in Table: 1 and modifications of Zanamivir structure were shown in Fig:2.



**Figure 1:** Identification of the active binding site of the protein enzyme Neuraminidase (PDB ID: 3B7E) using Poseview tool



**Figure 2:** Modifications of the Zanamivir structure.

**Table 1:** Substituted functional groups of newly designed Zanamivir analogs

Ligand No	Ligand Name	R1	R2	R3	R5	R6
	ZANAMIVIR	O	OH	H	H	OH
1	ZANAMIVIR ETHYL	O	ETHYL	H	H	OH
2	Z. HYDROXY REPLACES METHYL	O	OH	H	H	METHYL
3	Z.ISOPROPYL	O	ISOPROPYL	H	H	OH
4	Z.METHYL	O	METHYL	H	H	OH
5	Z.PYRAN ALPHA METHYL	O	OH	ALPHA METHYL	H	OH
6	Z.PYRAN BETA METHYL	O	OH	BETA METHYL	H	OH
7	Z.METHYL 2	O	OH	H	METHYL	OH
8	Z.PYRIDINE	N	OH	H	H	OH
9	Z.THIOL	S	OH	H	H	OH

**Processing of Target Protein:** Internal ligands and the crystallographic water molecules were removed from the protein and missing Hydrogen's was added<sup>8</sup>. Crystallographic disorders and unfilled valances were corrected. Geometrical optimization/ energy minimization of the protein was performed by employing CHARMM27 force field and RMSD of 0.01 using Discovery Studio's proprietary algorithm, Smart Minimizer<sup>9</sup>.

**Ligand Preparation:** Ligands were designed and geometrically optimized by using the "Smart Minimizer" minimization algorithm of Discovery Studio 2.5 by employing CHARMM27 Force field with RMSD of 0.0001.

**ADME & Toxicity Profiles:** For all the newly designed ligands, ADME properties were studied by using ADME

Descriptors protocol of the Discovery studio 2.5 in which we predicted the levels for Blood-Brain-Barrier (BBB) penetration<sup>10</sup>, Intestinal Absorption<sup>11</sup>, Aq. Solubility<sup>12</sup>, Hepatotoxicity<sup>13</sup>, Cytochrome P450 inhibition<sup>14</sup>, and Plasma Protein Binding (PPB) levels<sup>15</sup>.

Toxicity profiles of the analogs were studied by using "toxicity prediction-Extensible" protocol of Discovery Studio 2.5 which follows Bayesian Model<sup>16</sup>. Toxicity profiles like Aerobic Biodegradability, AMES mutagenicity, Developmental Toxicity Potential, ocular and Skin irritancy, and carcinogenicity using various animal models (*In silico*) were predicted.

**Molecular Docking:** Protein – ligand interactions between Zanamivir, its analogs and Neuraminidase enzyme were identified by using CDOCKER docking protocol of Discovery Studio 2.5. CDOCKER is a molecular dynamics (MD) simulated-annealing-based algorithm. The ligand side chains are conformationally sampled and are subjected to core-constrained protein docking, using a modified CHARMM-based CDOCKER method to generate top poses along with CDOCKER energies<sup>17-19</sup>.

## RESULTS AND DISCUSSION

Enzyme Neuraminidase with PDB ID: 3B7E retrieved from PDB website is geometrically optimized and screened for its active binding sites. Zanamivir analogs were designed according to Lipinski's Rule of Five and geometrically optimized to their least energy – high stability state and their molecular properties like, Molecular wt.(MW), surface area, volume, AlogP are listed in the Table: 2. Optimized ligands are used for the ADMET studies and also for Molecular Docking studies along with the optimized protein.

**Table 2:** Molecular properties of novel Zanamivir analogs

Ligand No	Mol MW	Mol Surface Area	Volume	No of HB donors	No of HB acceptor	AlogP
Zanamivir	332.31	333.81	200.99	8	8	-3.395
1	360.363	368.32	227.75	7	8	-2.821
2	330.337	337.78	209.91	7	7	-1.983
3	374.39	385.34	242.84	7	8	-2.443
4	346.336	353.05	211.97	7	8	-3.17
5	343.336	353.23	216.08	8	8	-2.949
6	346.336	353.23	216.08	8	8	-2.949
7	346.336	350.78	218.49	8	8	-3.018
8	331.325	333.81	203.05	9	8	-3.848
9	348.375	344.92	212.07	8	8	-2.837

**ADME & Toxicity Studies:** ADME (Absorption, Distribution, Metabolism and Excretion) profile, also called as pharmacokinetic profile of Zanamivir and its analogs are tabulated in the Table: 3. All the ligands have optimum Aq. Solubility and better drug like properties without BBB penetration, thus chances of causing CNS effects are marginal. All the analogs are Cytochrome P450 enzyme non-inhibitors. All the ligands show low intestinal absorption and the plasma-protein binding are less than 90%. Dose dependent hepatotoxicity is found for the ligands 2, 3, 5, and 6.

**Table 3:** ADME profiles of the Zanamivir analogs

Ligand NO	BBB level	Absorption level	Solubility level	Hepatotoxicity level	CYP2D6 level	PPB level
Zanamivir	4	3	4	0	0	0
1	4	3	4	1	0	0
2	4	3	4	0	0	0
3	4	3	4	1	0	0
4	4	3	4	0	0	0
5	4	3	4	1	0	0
6	4	3	4	1	0	0
7	4	3	4	0	0	0
8	4	3	5	0	0	0
9	4	3	4	0	0	0

**Table 4:** Toxicity profile of the novel Zanamivir analogs obtained by using Toxicity prediction tool of Discovery studio 2.5

Ligand	Aerobic Bio-Degradability	AMES Mutagenicity	Developmental Toxicity Potential	Ocular Irritancy	Skin Irritancy	Carcinogenicity				
						Rodent	Female Mouse	Male Mouse	Female Rat	Male Rat
Zanamivir	YES	NO	YES	YES	NO	NO	NO	NO	NO	NO
1	YES	NO	YES	YES	YES	NO	NO	NO	NO	NO
2	YES	NO	YES	YES	YES	NO	NO	NO	NO	NO
3	YES	NO	YES	NO	YES	NO	NO	NO	NO	NO
4	YES	NO	YES	YES	YES	NO	NO	NO	NO	NO
5	YES	NO	YES	YES	NO	NO	NO	NO	NO	NO
6	YES	NO	YES	YES	NO	NO	NO	NO	NO	NO
7	YES	NO	YES	YES	YES	NO	NO	NO	NO	NO
8	YES	NO	YES	YES	NO	NO	NO	NO	NO	NO
9	YES	NO	YES	YES	NO	NO	NO	NO	NO	NO

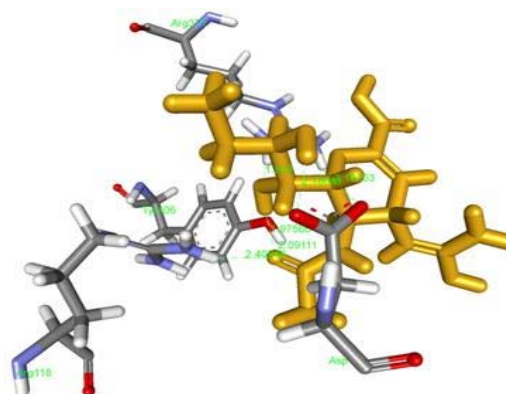
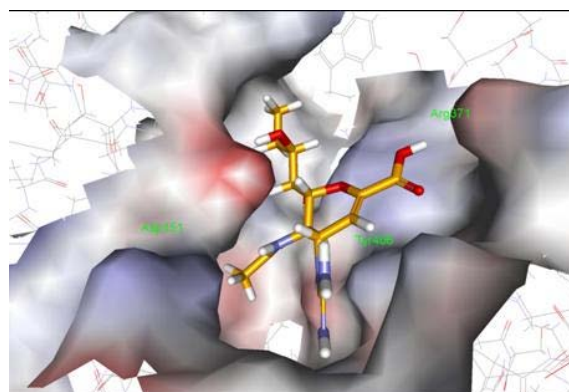
From the *In silico* toxicity studies, listed in Table: 4, we found that none of the analogs were exhibited carcinogenic and mutagenic potentials. All the ligands including Zanamivir have dose dependent toxicity potential and all are biodegradable in aerobic conditions. Except isopropyl derivative of the Zanamivir (ligand 3), remaining all other analogs are ocular irritants. Ligands 1, 2, 3, 4, and 7 show irritancy towards skin. As, these drugs are not useful as topical or ocular formulations, irritant nature of these ligands can be excluded.

**Table 5:** Docking score and interaction energies of the ligands with the protein 3B7E

Ligand No	Cdocking Score	Interaction Energy
Zanamivir	16.585	34.000
1	18.42	22.1808
2	29.311	43.0667
3	19.47	41.32
4	25.5624	38.5017
5	9.19	30.6308
6	10.5105	34.02
7	27.5626	48.203
8	21.5083	24.16
9	24.16	41.544

**Molecular Docking:** Protein – ligand interactions were identified by using CDOCKER docking protocol of Accelry's Discovery Studio 2.5. From the docking result listed in Table: 5 we found that Zanamivir has docking score of 16.585 with interaction energy of 34.00. Only pyran alpha and beta methyl substituted analogs (ligands 5, 6) are having lesser dock score when compared to Zanamivir. Methyl substituted analogs (ligand 2 and 7) have highest dock scores of 29.311 and 27.56 respectively. Out of 9

analog, 7 have better dock score than Zanamivir. Ligand 2 has 4 hydrogen bonds with the receptor residues like ARG 118, 371, TYR 406 and ASP 151 which can be visualized in the fig: 3. Binding site pocket of the docked protein can be visualized in the fig:4 along with ligand 2.

**Figure 3:** Binding interaction of Ligand 2 with enzyme Neuraminidase protein**Figure 4:** Binding site pocket of the protein 3B7E along with its docked ligand 2

## CONCLUSION

It can be concluded that methyl substitution at terminal hydroxyl group (ligand 2) and methyl at R<sub>6</sub> position (ligand 7) are have better binding interactions with enzyme Neuraminidase when compared to Zanamivir. The binding energies of the protein- ligand interactions also confirmed that the ligands will fit into the active pockets of receptor tightly. Even by considering the ADME & T profiles, respective analogs are have better profiles when compared to other analogs. These may hold better potential as drug candidates that inhibit the growth of influenza virus (H1N1). Further development of these analogs may lead to generation of novel high potent Neuraminidase inhibitors.

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