International Journal for Research in Engineering Application & Management (IJREAM)
ISSN : 2454-9150 Vol-05, Issue-06, Sep 2019

In Silico analysis of Physicochemical, Pharmacokinetic and Toxicological properties of NS3 Protease inhibitors of West Nile Virus

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Abstract: West Nile Virus (WNV) can cause neurological disease and death in people. NS3 protease of WNV has potential to work as drug target protein since it was involved in fundamental of viral replication. Inhibition of protease could be considered as a strategy for treatment of WNV infection. In this study, we performed molecular docking analysis of antiviral drug Favipiravir derivatives against NS3 Protease (PDB Id: 3E90) of WNV using Autodock 4.2 Autodock 4.2 and ArgusLab 4.0.1 program and found two potent inhibitors CID22674959 and CID135001386 of them with optimal binding energy-5.48, -5.61 kcal/mol with AutoDock and -5.43, -5.67 kcal/mol with ArgusLab, respectively. Further, ADME/Tox properties were calculated for inhibitors using preADMET and SwissADME web tools. Both inhibitors showed satisfactory results for oral administration, because they have adapted to the Lipinski, Veber and Egan parameters. In relation to the penetration of the blood-brain barrier the inhibitors analyzed showed penetration values < 1 and high gastrointestinal absorption (HIA). Compound CID135001386 showed a lower strength in plasma protein binding in relation to the compound CID22674959. Other ADME/Tox properties of proposed inhibitors showed positive and satisfactory results.

Keywords: Autodock, Favipiravir derivatives, NS3 protease, preADMET, SwissADME, West Nile Virus.

I. INTRODUCTION

West Nile fever is a zoonotic disease caused by West Nile virus (WNV). West Nile virus is mainly transmitted to human through the bites of infected Culex mosquitoes. WNV is maintained in nature by transmission between birds and mosquitoes; as birds are the natural hosts of the virus. Humans, horses and other mammals can be infected. West Nile fever can cause severe neurological illness and death in people, however about 80% of people who are infected show no symptoms. Right now there is no antiviral treatment avaiabe for human WNV infection [27]. WNV has positive sense 11-kb RNA genome, which encodes a polyprotein. This polyprotein translates to give three basic (C, prM, and E) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins. NS3 protease has potential target since it is involve in viral replication [6] [26].

To be effective as a drug, a potent molecule must reach its target in the body in sufficient concentration, and stay there in a bioactive form long enough for the expected biologic events to occur. Drug development involves assessment of absorption, distribution, metabolism and excretion (ADME) increasingly earlier in the discovery process. Absorption is the exchange of a medication from its site of organization to the circulation system. Once a medication is consumed into the circulation system it can be conveyed all through the body. This procedure is called distribution. Medications are dispensed with from the body either unaltered through the kidneys and bile, or they may experience compound changes that enable them to be all the more effortlessly discharged. This procedure is called biotransformation or digestion. The total expulsion of the medication from the body is alluded to as end. Preclinical in vivo toxicology considers should be led in Rats and mice for intense and ceaseless presentation.

In this study, Drug development for West Nile infection has been in advance for quite a few years. In this study, we docked Favipiravir derivatives with West Nile virus NS3 Protease using AutoDock4.2 and ArgusLab 4.0.1 tool. The docked complexes stability was evaluated by molecular dynamics simulation. In Silico physicochemical, pharmacokinetic and toxicological properties of inhibitors were perfomed by preADMET and SwissADME web tools [7] [15] [24].

II. METHODOLOGY

A. Molecular docking

Docking of Favipiravir derivatives against NS3 Protease (PDB Id: 3E90) structure was done using molecular docking program AutoDock 4.2 and ArgusLab 4.0.1. [12].

 DOI : 10.35291/2454-9150.2019.0443

Docking program Autodock 4.2 is designed to predict how small molecules bind to a receptor of known 3D structure. It actually consists of two main programs: AutoDock and Auto Grid, where AutoDock performs the docking of the ligand to a set of grids depicting the target protein; Auto Grid pre-calculates these grids. In additions to using them for docking, the atomic affinity grids can be visualized. Docking of favipiravir derivatives with IMPDH structure was carried out [20]. Gasteiger charges were added to the ligand and maximum 6 number of active torsion are given to the lead compound using AutoDock tool. Kollaman charges and solvation terms were added to the protein structure using AutoDock tool. The Grid for docking calculation was centered to cover the protein binding site residues and accommodate ligand to move freely. During the docking procedure a Lamarckian Genetic Algorithm (LGA) were used for flexible ligand rigid protein docking calculation [12]. Docking parameters were as follows: 30 docking trials, population size of 150, maximum number of energy evaluation rangesof 25,0000, maximum number of generations is 27,000, mutation rate of 0.02, cross-over rate of 0.8, Other docking parameters were set to the software's default values.

B. Physicochemical, Pharmacokinetic and Toxicological Properties of the Inhibitors

A drug should have good oral absorption and must follow the Lipinski rules by satisfying the following parameters: molecular weight of less than 500 Da, logP (lipophilicity) less than five (5); maximum of five (5) hydrogen donor groups and maximum of ten (10) groups acceptors binding intestinal permeability.

Over 50% of the drug candidates did not succeed due to ADME/Tox deficiencies during development. To avoid this failure at the development a set of in vitro ADME/Tox screens has been implemented with the aim of discarding compounds in the discovery phase that are likely to fail further down the line. The preADMET server calculates parameters such as human intestinal absorption, cellular permeability Caco-2 in vitro, cell permeability Maden Darby Canine Kidney (MDCK), skin permeability, plasma protein binding, and penetration of the blood brain barrier, carcinogenicity and mutagenicity [15]. SwissADME compute physicochemical descriptors as well as to predict ADME parameters, pharmacokinetic properties and druglike nature of small molecules to support drug discovery [24].

III. RESULTS AND DISCUSSION

A. Molecular Docking

Optimal interactions and the best score were used as criteria to interpret the best conformation among the conformations generated by AutoDock and ArgusLab tools. Both docking programs showed almost similar results as shown in table 1.

Table 1: Binding energy of Favipiravir derivatives resulted by AutoDock 4.2 and ArgusLab 4.0.1.

Sl. No.	Pubchem CID/drug	Binding Energy (Kcal/mol) from AutoDock	Binding Energy (Kcal/mol) from ArgusLab
1	22674959	-5.48	-5.43
2	135001386	-5.61	-5.67

B. Physicochemical, Pharmacokinetic and Toxicological Properties of compounds

Inhibitors in SMILES format were entered in the online SwissADME web tool for generating Physicochemical parameters such as molecular weight, the number of heavy atoms, the number of aromatic heavy atoms, the fraction of carbon bond saturation (Csp3), number of rotatable bonds, the number of H-bond acceptors, the number of H-bond donors, Molar Refractivity and TPSA [7]. Drugs entering the brain typically have a TPSA value of less than 60–70 $Å^2$ [14]. Both inhibitors had TPSA >70 $Å^2$. Druglikeness of inhibitors were accessed by five different rule-based filters such as Lipinski [16] filter implemented rule-of-five, Ghose [10], Veber [28], Egan [9] and Muegge [21] methods, respectively. In analyzing the parameters of inhibitos, we observed that all had values within Lipinski, Veber and Egan parameters, as shown in table 2, 3 & 4.

Table 2: Physicochemical properties of inhibitors.

Sl.No.	PubChem CID/ Drug Name	Molecular Weight (g/mol)	Donor	Acceptor	logP
1	22674959	157.104	2	4	-0.6
2	135001386	154.129	3	4	-1.3

 DOI : 10.35291/2454-9150.2019.0443

Table 3: Physicochemical Properties of compounds

Physicochemical Parameter	Compound CID22674959	Compound CID135001386
Molecular weight	157.10 g/mol	154.13 g/mol
Num. heavy atoms	11	11
Num. arom. heavy atoms	6	6
Fraction Csp3	0.00	0.00
Num. rotatable bonds	1	1
Num. H-bond acceptors	4	4
Num. H-bond donors	2	3
Molar Refractivity	32.91	37.36
TPSA	88.84 Å²	114.86 Å²

Table 4: Druglikeness of inhibitors.

Parameters	Compound CID22674959	Compound CID135001386
Lipinski	Yes; 0 violation	Yes; 0 violation
Ghose	No; 4 violations: MW<160, WLOGP<-0.4, MR<40, #atoms<20	No; 4 violations: MW<160, WLOGP<-0.4, MR<40, #atoms<20
Veber	Yes	Yes
Egan	Yes	Yes
Muegge	No; 1 violation: MW<200	No; 1 violation: MW<200
Bioavailability Score	0.55	0.55

Pharmacokinetics evaluates individual ADME behaviors of the molecule under investigation. The SwissADME predictions for passive human gastrointestinal absorption (HIA) and blood-brain barrier (BBB) permeation both consist in the readout of the BOILED-Egg model [4] as shown in table 5. Human gastrointestinal absorption (HIA) properties are determinant for the drug development that purports to be administered orally. Analyzing the ADME tests of inhibitors using preADMET, it was observed that the compounds presented high human gastrointestinal absorption (HIA) 71.913015 and 55.088598 as shown in table 6. Caco-2 cell model and MDCK cell model has been recommended as a reliable *in vitro* model for the prediction of oral drug absorption [31]. Inhibitor calculated, it was found that the P_{CaCO2} (nm/s) was low permeability, having 17.0714 nm/s and 10.4662 nm/s, standard range is (P_{CaCO2} >70 nm/sec) [32]. The cell permeability in vitro in MDCK system is used as a tool for the rapid analysis of permeability [13]. Analyzing the data in this MDCK system in the inhibitors shown in Table 6, it is verified that the permeability can be classified into low 0.633704 nm/s and 0.578405 nm/s.

Table 5: Pharmacokinetics of compounds

Parameters	Compound CID22674959	Compound CID135001386
GI absorption	High	High
BBB permeant	No	No
Log K_p (skin permeation)	-7.66 cm/s	-8.19 cm/s

Table 6: Absorption properties of compounds.

Derivative	PubChem CID	Absorption			
		HIA (%)	P_{Caco-2} (nm/sec)	MDCK(nm/sec)	Skin Permeability
1	22674959	71.913015	17.0714	0.633704	-4.90937
2	135001386	55.088598	10.4662	0.578405	-5.19886

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The skin permeability is used in the pharmaceutical industry to assess the risk chemical products in case there is accidental contact with skin [25]. Proposed inhibitors showed negative permeability values as shown in table 5 & 6. The binding to plasma proteins can alter the half-life of the drug in the body of the individual [11] [23]. According to table 5 & 7 calculated inhibitors binded loosly to plasma proteins. Both inhibitors analyzed showed had BBB values <1. The Ames test [2] assesses mutagenicity of the Inhibitors as shown in table 8. Both inhibitors showed positive prediction as a mutagen. Carcinogenicity is a toxicity that causes cancer in body. On analyzing carcinogenicity in mice and rat the inhibitors were predicted as positive, i.e., have no carcinogenic evidence as shown in table 8.

Table 7: Distribution properties in percentages of PPB and penetration of the blood brain barrier for four compounds.

Derivative	CID	Distribution	
		PPB (%)	BBB
1	22674959	3.983510	0.100174
2	135001386	0.000000	0.0647327

Table 8: Toxicological properties of mutagenicity (Ames test) and carcinogenicity (mouse and rat).

Derivative	CID	Ames Test	Carcinogenicity	
			Mouse	Rat
1	22674959	mutagen	positive	positive
2	135001386	mutagen	positive	positive

SwissADME predict Lipophilicity of compounds based on five predictive models, such as XLOGP3, an atomistic method including corrective factors and knowledge-based library [5], WLOGP [30], MLOGP [18] [19], SILICOS-IT, an hybrid method relying on 27 fragments and 7 topological descriptors and finally iLOGP, our in-house physics-based method relying on free energies of solvation in n-octanol and water calculated by the Generalized-Born and solvent accessible surface area (GB/SA) model. The consensus log Po/w is the arithmetic mean of the values predicted by the five proposed methods. Lipophilicity and Water Solubility of both compounds were shown in table 9 &10.

Table 9: Lipophilicity of compounds

Parameter	Compound CID22674959	Compound CID135001386
Log $P_{o/w}$ (iLOGP)	0.55	-0.08
Log $P_{o/w}$ (XLOGP3)	-0.56	-1.34
Log $P_{o/w}$ (WLOGP)	-0.57	-1.54
Log $P_{o/w}$ (MLOGP)	-1.30	-1.90
Log $P_{o/w}$ (SILICOS-IT)	0.69	-0.45
Consensus Log $P_{o/w}$	-0.24	-1.06

SwissADME predict water solubility of compounds based on two topological methods as ESOL model [8] and second one was adapted from Ali [1]. SwissADME third predictor for solubility was developed by SILICOS-IT.

Table 10: Water Solubility of compounds

Parameters	Compound CID22674959	Compound CID135001386
Log *S* (ESOL)	-0.80	-0.29
Solubility	2.50e+01 mg/ml ; 1.59e-01 mol/l	7.92e+01 mg/ml ; 5.14e-01 mol/l
Class	Very soluble	Very soluble
Log S (SILICOS-IT)	-1.42	-0.57
Solubility	6.04e+00 mg/ml ; 3.85e-02 mol/l	4.12e+01 mg/ml ; 2.67e-01 mol/l
Class	Soluble	Very soluble
Log *S* (SILICOS-IT)	-4.08	-0.78
Solubility	4.09e-02 mg/ml ; 8.36e-05 mol/l	2.59e+01 mg/ml ; 1.68e-01 mol/l
Class	Moderately soluble	Soluble

 DOI : 10.35291/2454-9150.2019.0443

IV. CONCLUSION

Two Favipiravir derivatives CID22674959 and CID135001386 had binding energy -5.48, -5.61 kcal/mol with AutoDock and -5.43, -5.67 kcal/mol with ArgusLab, respectivley. Both the docking tool predicts almost same binding energy. Optimization of docked protein - inhibitor complexes shows its stability. Physicochemical, Pharmacokinetic and Toxicological properties of predicted Favipiravir derivatives of NS3 protease showed satisfactory results for oral administration, since they quaify the Lipinski, Veber and Egan parameters. It was observed that the compounds presented had high human gastrointestinal absorption and low permeability as P_{CaCO2} and MDCK system. Predicted inhibitors had negative skin permeability and low BBB values. The Ames test assessed that both inhibitors had positive prediction as a mutagen. On analyzing carcinogenicity in mice and rat, both inhibitors have no carcinogenic evidence. Predicted lipophilicity and water solubility of both inhibitors showed satisfactory results.

Therefore, it is predicted that Favipiravir derivatives CID22674959 and CID135001386 could be promising drug like compounds for NS3 Protease as drug target yet experimental studies have to confirm it. The present study may provide the information about potential derivatives for synthetic medicinal chemist as chemotherapeutic agents to fight against the increasing burden of West Nile virus.

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 DOI : 10.35291/2454-9150.2019.0443