In-silico drug designing of novel Morpholino based Physcion drug candidate and investigation of inhibition effects on Covid-19 RNA Dependent - RNA polymerase non structural protein 12 (nsp 12) with ADMET study

Abstract

Recent explosion of highly fatal pandemic corona virus *Covid-19* in human population. The *Covid-19* is a positive sense single stranded enveloped virus which belongs to the *Coronaviridae family* required non-structural proteins 12 (nsp12), a RNA dependent-RNA polymerase as an important machinery for the viral genome replication and transcription processes. There are various RNA polymerase inhibitors are currently using in clinical activities to treat *Covid-19* infections but their treating efficacy is not up to much impressive particularly in aged people. In this study, we docked Morpholino based physcion drug candidate against RNA polymerase target (*PDB ID : 6NUR*). We designed drug candidate using Chemsketch software and further it was proceeded to molecular docking using AutoDock Vina 4.0 software. UCSF Chimera software was used for visualization of 3-Dimensional structure of ligand - protein docked pose. Moreover the docked drug candidate was checked for ADMET properties. Hence, this study supports the emergence of developing an efficient new drugs to combat Covid-19 infections. From this computational study we identified the designed drug candidate have high potential of inhibition of virus RNA Dependent - RNA polymerase minimum binding energy of - *8.76*. To identify the inhibition potential of designed ligand, we used Remdesivir resulted minimum binding energy of - *7.25* as a positive control. These findings supports emergency discovery of anti-viral drug candidate to combat *Covid-19* infections all over the world.

Keywords

Covid-19, RNA dependent-RNA polymerase, Morpholino based physcion drug, Chemsketch software, AutoDock Vina 4.0 software, UCSF Chimera software, ADMET, minimum binding energy, Remdesivir.

1. Introduction

Based on the statistics from World Health Organization (WHO), Covid-19 novel viral strain has been emerged first in Wuhan, China and represents a pandemic infections to public health. Initially, the infection was unable to identify causing agent and then the Chinese Center for Disease Control and Prevention (CDC) and local CDCs found illness is because of novel virus from coronavirus (CoV) family. They are large family of positive sense single stranded and many animals are acting as reservoirs for those viruses. From current studies, the therapeutic methods to cure this infection are supportive only and there is no available vaccine to prevent this infection in the human community [1]. The transmission of COVID-19 occurred by human to human with an aggressive 1 to 4^{th} degree infections in human community. Currently there approximately 22,000 deaths has been reported in all over the world.

From the data received from December 2019, an emergence of COVID-19 infections in human health, the anti-viral drug Remdesivir was reported by World Health Organization (WHO). Previous case of Ebola virus, Remdesivir was used to inhibit RNA-Dependent RNA polymerase which replaces Adenosine Tri Phosphate (ATP) by polymerization process before chain termination and it was considered as the nucleotide analog [2]. In-vitro assay of Remdesivir exhibited 1.76 μM (Effective Concentration) against corono virus[3]. Currently, the Remdesivir was administrated in COVID-19 infected patients to control the disease states. But there efficacy is not up to the mark in aged peoples. Hence there is a requirement of new drugs to combat and control the COVID-19 aggressive nature in community of human population in whole world.

The important enzymatic machinery of COVID-19 includes Spike protein, Nucleocapsid protein, Matrix protein, envelop protein and RNA dependent RNA polymerase non-structural proteins 12 (nsp 12) [4]. COVID-19 RNA-dependent RNA polymerases belongs to a class of nucleic acid polymerases. It involved in replication and transcription of viral genomes possess single stranded RNA and single stranded RNA-dependent polymerase activity. It has complex non-structural proteins (nsp). The aspartate amino acid residue present on beta turn of protein structure acting as the highly conserved domain and enables them to access nucleotide channel [5,6].

Morpholino backbone block binding of other complementary molecules in RNA molecule using complementary nucleotide analog. Additionally the structure of morpholino resembles the structure of naturally occurred nucleotide and exhibited resistant to nuclease activity.

Physcion is a naturally available bioactive molecules by plants, endophytic fungi which is a derivative of anthraquinone. It exhibits various biological activities such as laxative, anti-hepatic, anti-inflammatory, anti-microbial and anti-cancer activity (nasopharyngeal carcinoma) in in-vivo assays [7]. In order to find new antiviral compound, physcion was evaluated to inhibit viral replication, anti-absorption and virucidal activities. Further it was identified as inhibitor against CVB4 and RSV infections [8].

Hence, from our study, the molecular docking results are suggest to use as anti-polymerase therapeutic drug candidate against the novel strain of Coronavirus 2019 infections.

2. Methodology

2.1 Protein Target Preparation

The crystalline structure of protein target (RNA Dependent - RNA Polymerase) was not available in the Protein Data Bank Databases. To modelled targeted protein, Sequences in FASTA format was retrieved from NCBI public database. The retrieved sequence was used to build a protein model by using SWISS-MODEL serve. Template search with BLAST and HHBlits has been performed against the SWISS-MODEL template library. The target sequence was searched with BLAST against the primary amino acid sequence contained in the SMTL. An initial HHblits profile has been built using the procedure outlined in [9], followed by 1 iteration of HHblits. For each identified template, the template's quality has been predicted from features of the target-template alignment. The templates with the highest quality have then been selected for model building [10-12]. The existed protein model IN PDB (*Id: 6NUR*) was selected as the template for further docking study based on sequence similarity.

2.2 Ligand Designing

The Morpholino based - physcion drug candidate was designed by using Chemsketch free software and further selected for molecular docking study [Figure No.1]. Moreover, the designed ligand structures closely resembles the structure of Remdesivir with slight modification in replacement of nucleoside analog with morpholino analog. The modelled targeted protein was proceeded to virtual screening of potential drug candidate against COV2 2019 virus infection inhibition.

2.3 Protein optimization & Energy Minimization

The targeted protein was opened with word pad to remove the coordinates of other ligands which is not a part of protein and hence consider as a hetero atom [HETATM]. For Protein optimization, all the hetero atoms were removed till before the END of our protein. Now the optimized protein is save and run for the further studies. The energy minimization was done by using Swiss PDB Viewer software.

After ligand drawing with the help of chemsketch software and the structures were saved as MDL Molfiles [V2000]. Open Babel software is used for converting the .mol file pdb format of designed ligand for proceeding the docking study. AutoDock Vina 4.0 is used in this project for docking study. Target protein were open as read molecule and converted to PDBQT format using MGL Tools (version - 1.5.6rc3). The polar hydrogen atoms, kollman charge was added. A systematic analysis of ligand binding poses generated by AutoDock Vina shows that the highest accuracy is achieved when the dimensions of the search space are 2.9 times larger than the radius of gyration of a docking compound [13]. The grid size was generated automatically with the help of installed MGL tools to increase the accuracy of docking studies. Docking with AutoDock Vina 4.0 employ the Lamarckian genetic algorithm to generate a better conformations of a lead compound. Totally Ten docking runs were performed. The scoring of the generated docking poses and ranking of the ligands is based on the AutoDock Vina RMSD (Root Mean Square Deviation) empirical scoring function approximating the binding affinity in kcal/mol. The docked ligands were arranged in an order of minimum binding energy and the pdb files were visualized by using UCSF Chimera (3D visualizing software).

2.5 Hydrogen Bond Analysis

The hydrogen bond formation contributes the strong stability of protein-ligand complex and its important in enzymatic catalysis reaction to stabilize a ligand in a binding pocket of target protein. The analysis of hydrogen bond to determine the hydrogen bond donor - acceptor pair. More the aminoacids residues in a protein, the more number of hydrogen bond formation. The hydrogen bond analysis was done by using Protein-Ligand Interaction Profiler (PLIP) web tool [14].

3. Results and Discussions

3.1 Ligand Designing

Computer aided drug designing have became an efficient method to identify new potential drug candidates using various Bioinformatics tools. This methodology create an impact in drug discovery process with a significant low cost development in less time duration. Then the designed compound was synthetically made using synthetic chemistry processes and further investigating their in-vivo bioactivity screening, pharmacokinetic properties.

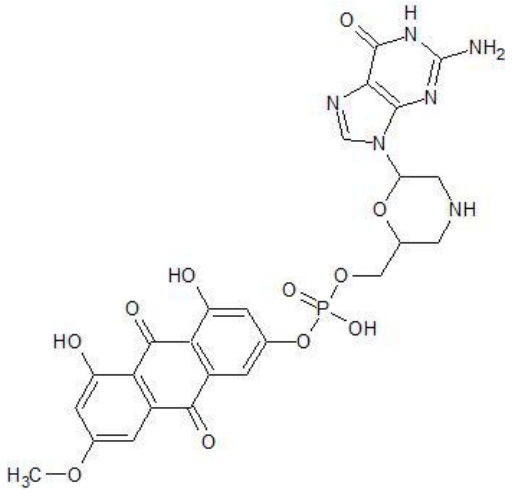

Figure No.1 : The above ligand was designed by using Chemsketch free software.

3.2 Energy Minimization

The energy minimization was run with steepest descent method instead of using MD simulation in order to change the protein coordinates in such a way as to reduce the

potential energy. The energy minimized model was opened with word pad in order to remove the spdbv coordinates it put during energy minimization run of steepest method with 10.000Å cutoff. The spdbv coordinates removed energy minimized is the final refined model for further studies.

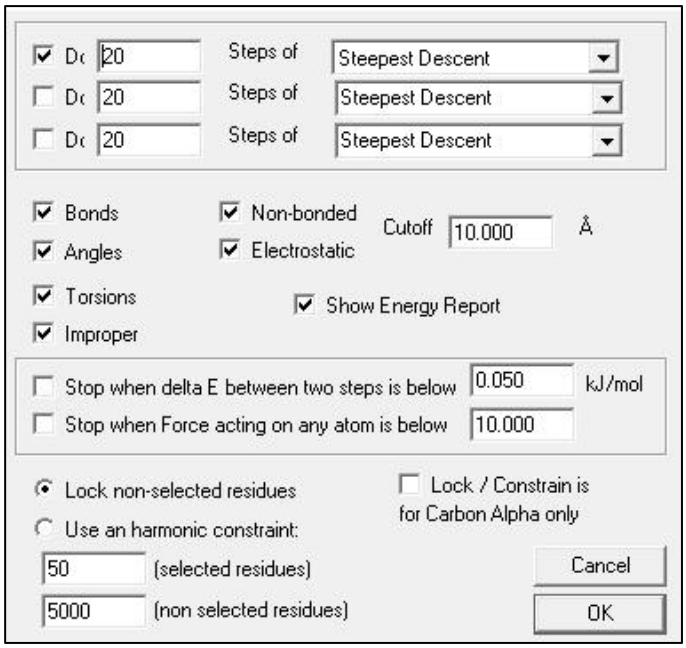

Figure No.2 : The above figure depicted the Energy Minimization default parameters in order to remove the close overlaps of the LJ cores.

3.4 Molecular Docking algorithm

By using the cygwin command prompt software we create GLG file and DLG file to generate the RMSD value for the identification lead compound which showing minimum binding energy with the docked ligands [Table No.1]. For the better resolution of RMSD we have separate the cygwin command into I and II of docking algorithm.

./autogrid4.exe -p X.gpf -l X.glg &

./autodock4.exe -p X.dpf -l X.dlg &

Tail -f X.dlg

Grep '^DOCKED' X.dlg | cut -c9- > X_run.pdbqt

Cut -c-66 X_run.pdbqt > X_run.pdb

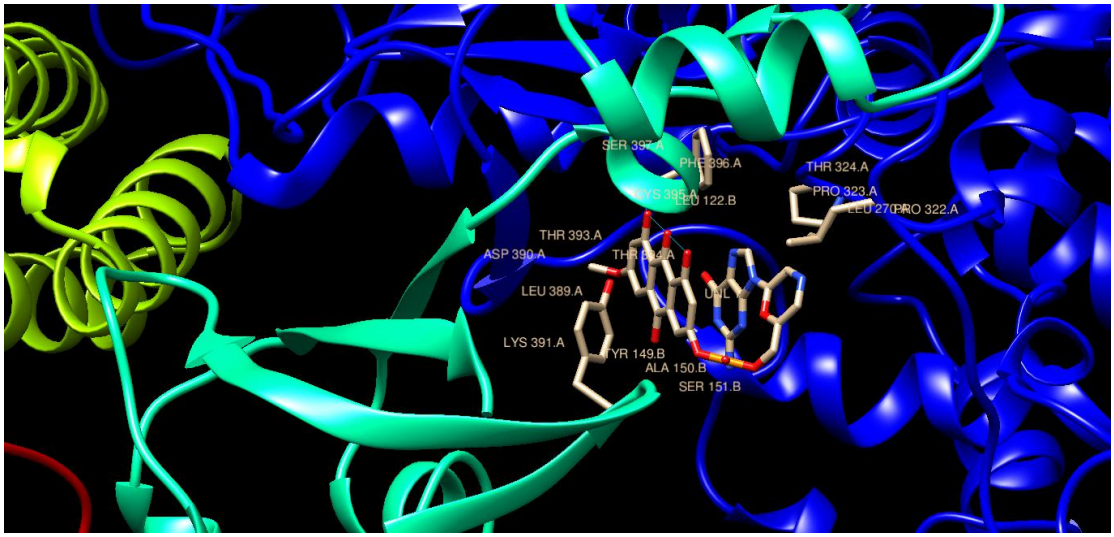

Figure No.3 : The above docked pose of Morpholino based designed ligand candidate interact with amino acids of protein target (Ser 397.A, Leu 122.B, Asp 390.A, Leu 389.A, Tyr 149.B, Thr 393.A, Ala 150.B, Lys 391.A, Ser 151.B, Thr 394.A, Pro 322.A, Leu 270.A, Pro 323.A, Thr 324.A, Phe 396.A, Leu 122.B, Cys 395.A).

Table No.1 : AutoDock Vina4.0 Interaction Profile of Docked Ligands

S.No	**Ligands**	**Binding energy (Kcal /mol)**	**Inhibition constant Ki**
1.	Morpholino based physcion Drug Candidate	- 8.76	376.49 nM
2.	Remdesivir	- 7.25	4.86 µM

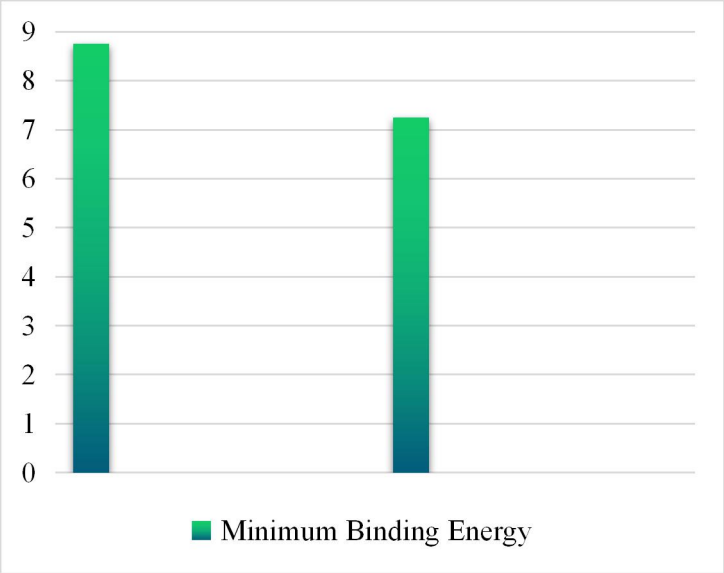

Figure No.4 : The above column bar chart represented the minimum binding energy of docked drug candidate (- 8.76) and Remdesivir (-7.25).

3.5 Hydrogen Bond Analysis

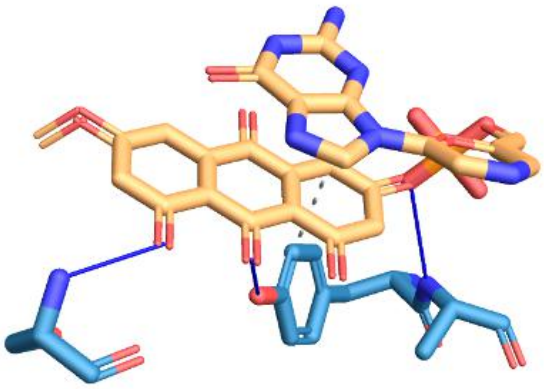

Figure No.5 : Hydrogen Bond analysis of Morpholino-based physcion form three hydrogen bonds with the targeted protein [Table No. 2].

Table No.2 : Hydrogen Bond Interaction profile of Morpholino-based physcion drug candidate

Residue	AA	Distance H-A	Distance D-A	Donor Angle
149B	TYR	3.29	3.84	119.41
150B	ALA	3.18	3.92	132.62
397A	SER	2.03	2.99	166.38

3.6 ADMET Prediction

To compute physiochemical properties and ADME parameter we use SwissADME web tool [15]. From this computation, we predict physiochemical properties, lipophilicity, water solubility, pharmacokinetics, druglikeness and medicinal chemistry [Table No. 3-8]. The toxicity models were predicted by using Prediction of Toxicity of Chemicals (ProTox-II), a virtual web tool lab for predicting toxicity nature of designed ligand molecule. The compound toxicity prediction enable to reduce an experiment on animals and also reduce animal testing with suggesting animal Ethics 3R [16-18].

Table No.3 : Physiochemical Properties

Formula	$C_{25}H_{23}N_6O_{11}P$
Molecular weight	614.46 g/mol
Number of heavy atoms	43
Number of aromatic heavy atoms	21
Fraction Csp3	0.24
Number of rotatable bonds	7
Number of H-bond acceptors	14
Number of H-bond donors	6
Molar Refractivity	149.50
TPSA	260.25 Å

Table No.4 : Lipophilicity

iLOGP	1.49
XLOGP3	-2.74
WLOGP	-0.12
MLOGP	-1.90
SILICOS-IT	-0.24
Consensus Log P $_{o/w}$	-0.70

Table No.5 : Water Solubility

Log S (ESOL)	-1.82
Solubility	9.24e +00 mg/ml; 1.50e-02 mol/l

Class	Very soluble
Log S (Ali)	-2.17
Solubility	4.12e+00 mg/ml; 6.71e-03 mol/l
Class	Soluble
Log S (SILICOS-IT)	-4.68
Solubility	1.29e-02 mg/ml; 2.10e-05 mol/l
Class	Moderately soluble

Table No.6 : Pharmacokinetics

GI absorption	Low
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log Kp (Skin permeation)	-11.99 cm/s

Table No.7 : Druglikeness

Lipinski	No; 3 violations: MW>500, NorO>10, NHorOH>5
Ghose	No; 2 violations: MW>480, MR>130
Veber	No; 1 violation: TPSA>140
Egan	No; 1 violation: TPSA>131.6
Muegge	No; 5 violations: MW>600, XLOGP3<-2, TPSA>150, H-acc>10, H-don>5
Bio-availability Score	0.17

Table No.8 : Medicinal Chemistry

Pains	1 alert: quinone_A
Brenk	1 alert: phosphor
Leadlikeness	No; 1 violation: MW>350

Synthetic accessibility	5.17

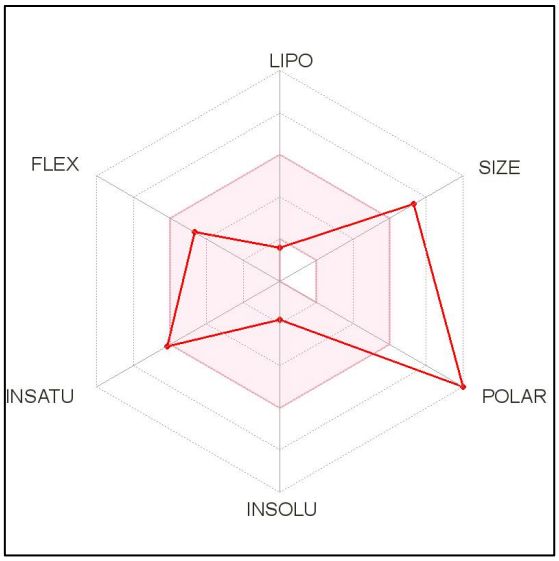

Figure No.6 : The ADME radar chart is intended to quickly illustrate the confidence of positive results compared to the average of its class with an available datasets. The colored Zone is the suitable physiochemical space for oral bioavailability. Lipophilicity : -0.7 < XLOGP3 < +5.0, Size : 150 g/mol < MV <500 g/mol, Polarity : 20 $Å^2$ < TPSA < 130 $Å^2$, Insolubility : 0 < Log S (ESOL) < 6, Insaturation : 0.25 < Fraction Csp3 < 1, flexibility : 0 < Number of rotatable bonds < 9.

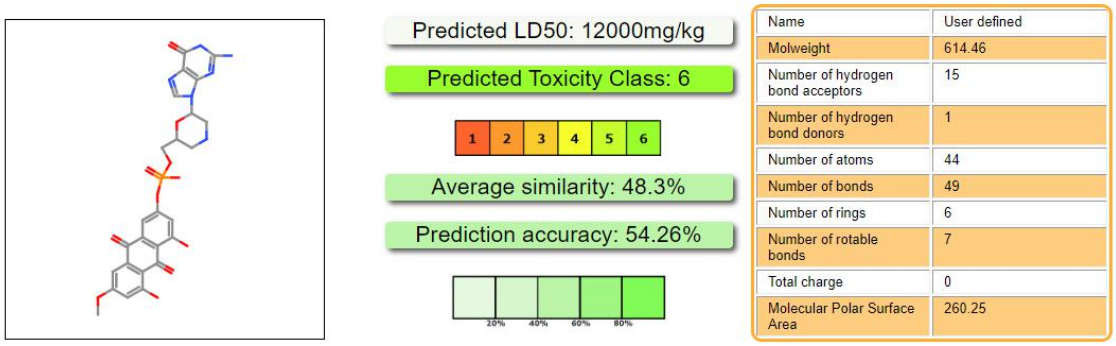

Figure No.7 : Oral toxicity prediction results for Morpholino based Physcion ligand candidate

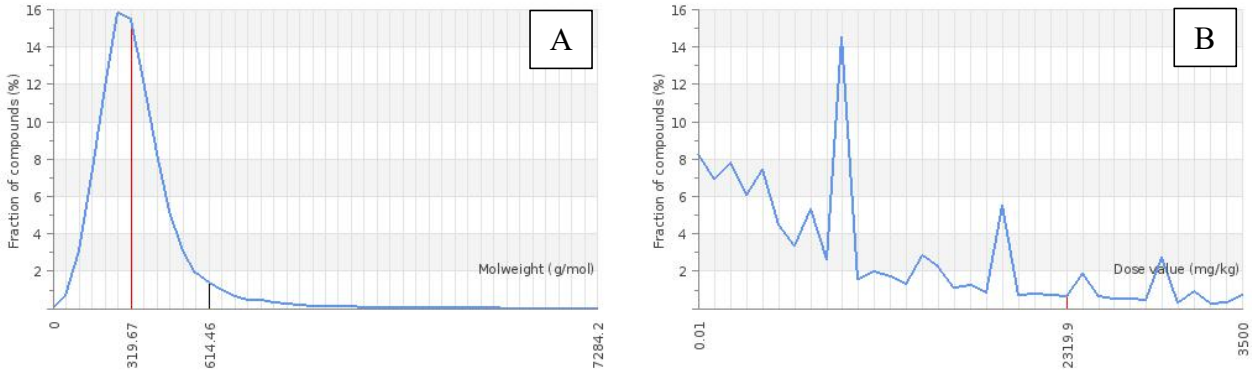

Figure No.8 : The distribution of molecular weight (g/mol) (A) with the mean value of existed database and distribution of dose value (B).

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.68
Toxicity end points	Carcinogenicity	carcino	Inactive	0.56
Toxicity end points	Immunotoxicity	immuno	Active	0.98
Toxicity end points	Mutagenicity	mutagen	Inactive	0.72
Toxicity end points	Cytotoxicity	cyto	Inactive	0.62
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.93
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.97
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.92
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.91
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.97
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.97
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.97
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.90
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.84
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.92

Figure No.9 : Toxicity Model profile of Morpholino based Physcion ligand candidate

Conclusion

The newly emerged fatal infection by Coronovirus in human community required an emergence of identifying a potent drug to combat the diseased states. Our study focused to design a morpholino based drug coupled with physcion which was resistant to nuclease activity inside the host cell. Moreover this study suggest to use our newly designed drug candidate to considered as anti-polymerase agent.

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