



## Targeting SARS-CoV-2 non-structural protein 16: a virtual drug repurposing study

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### ABSTRACT

Non-Structural Protein 16 (nsp-16), a viral RNA methyltransferase (MTase), is one of the highly viable targets for drug discovery of coronaviruses including SARS-CoV-2. In this study, drug discovery of SARS-CoV-2 nsp-16 has been performed by a virtual drug repurposing approach. First, drug shape-based screening (among FDA approved drugs) with a known template of MTase inhibitor, simefungin was done and best compounds with high similarity scores were selected. In addition to the selected compounds, 4 nucleoside analogs of anti-viral (Raltegravir, Maraviroc and Favipiravir) and anti-inflammatory (Prednisolone) drugs were selected for further investigations. Then, binding energies and interaction modes were found by molecular docking approaches and compounds with lower energy were selected for further investigation. After that, Molecular dynamics (MD) simulation was carried to test the potential selected compounds in a realistic environment. The results showed that Raltegravir and Maraviroc among other compounds can bind strongly to the active site of the protein compared to simefungin, and can be potential candidates to inhibit NSP-16. Also, the MD simulation results suggested that the Maraviroc and Raltegravir are more effective drug candidates than Simefungin for inhibiting the enzyme. It is concluded that Raltegravir and Maraviroc which may be used in the treatment of COVID-19 after invitro and invivo studies and clinical trial for final confirmation of drug effectiveness.

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## 1. Introduction

Coronaviruses are members of the family *Coronaviridae* which can cause several lethal zoonotic infections in human including severe acute respiratory syndrome SARS-CoV, Middle East respiratory syndrome (MERS-CoV), and more recently SARS-related coronavirus-2 (SARS-CoV-2; COVID-19) (Memish et al., 2020). Fehr and Perlman have provided a review on coronaviruses and discussed their replication and pathogenicity, and current therapeutics strategies (Fehr & Perlman, 2015).

Because of pandemic potential and the absence of any effective treatments for new lethal coronavirus, SARS-CoV-2, the researchers have focused on the treatment and drug discovery for the prevention of the outbreak and stop viral infections. Recently, Boopathy et al, have reviewed the structure of novel coronavirus, Mechanim of action and trial test of antiviral drugs in the lab and patients with COVID-19 (Boopathi et al., 2020). Based on the reports, several potential drug candidates have been proposed for the treatment of COVID-19 including: oseltamivir (Muralidharan et al., 2020; Rosa & Santos, 2020), lopinavir/ritonavir (Arabi et al., 2020; Cao et al., 2020), Cobicistat,

Darunavir (Pant et al., 2020), Tocilizumab (Bennardo et al., 2020; Luo et al., 2020; Michot et al., 2020; Zhang et al., 2020), nucleoside analogues and nucleotide inhibitors (Elfiky, 2020a), remdesivir (Cao et al., 2020; Hendaus, 2020; Elfiky, 2020b), tenofovir, ribavirin, sofosbuvir, galidesivir (Elfiky, 2020b), antibiotics (Sodhi & Etminan, 2020) and Chloroquine and hydroxychloroquine (Ferner & Aronson, 2020; Sahraei et al., 2020; Scuccimarri et al., 2020). Recently, various phytochemical including Belachinal, Macaflavanone E and Vibsanol B (Gupta et al., 2020), Flavone and Coumarine derivatives (Khan et al., 2020), Saikosaponins (Sinha et al., 2020), Crocin, digitoxigenin and  $\beta$ -Eudesmol (Aanouz et al., 2020),  $\delta$ -Viniferin, Myricitrin, Taiwanhomoflavone A, Lactucopicrin 15-oxalate, Nymfolide A, Afzelin, Biorobin, Hesperidin and Phyllaemblicin B (Joshi et al., 2020) and Theophylline and prymidone derivatives (Sarma et al., 2020) have been reported as proposed drug candidates for COVID-19. Furthermore, other reports have shown the antiviral potential of intravenous immunoglobulin and systemic steroids, an angiotensin-converting enzyme 2 (ACE2)-based peptide, 3CLpro inhibitor (3CLpro-1) and a novel vinylsulfone protease inhibitor against SARS-CoV-2 (Lai et al., 2020). Rosa and Santos have summarized 24 clinical trials for more than 20 medicines (Rosa and

Santos, 2020). Despite all these extensive efforts by researchers to discover an effective drug, vaccine (Enayatkhani et al., 2020) or definitive treatment of COVID-19, so far, no effective treatment has been found for it.

It is a fact that from discovery to bring a new approved drug to the market takes several years and 2 billion dollars on average. Therefore, due to the time consuming process of new drug discovery by wet lab experiments, it seems that the repositioning of existing drugs may be the best solution for this sudden pandemic infectious disease, at this time (Prasad & Mailankody, 2017). All pharmacokinetics and toxicological properties of approved drugs were examined and evaluated in preclinical and clinical trials by Food and Drug Administration of USA (FDA) and they don't need to pass any safety tests and take less time to reach the market (Cha et al., 2018). Although drug repurposing has some limitations, but it can avoid expensive costs associated with early-stage testing of the hit compounds and facilitate the discovery of new classes of medicines (Ma et al., 2013). Recently, a review article, published by Sohraby et al, have reported the basic principles and recent advances in drug repositioning by structure-based virtual screening and highlighted the powerful synergy of in-silico techniques (Sohraby et al., 2019). So far, several studies have been done on drug discovery of SARS-CoV-2 by using drug repurposing approach (Elmezayen et al., 2020; Liu et al., 2020; Sang et al., 2020; Shah et al., 2020).

Finding the main target in drug repurposing studies is a key challenge. Coronaviruses by a non-segmented, positive-sense RNA genome (~30 kb) encode a highly conserved and novel genes mixture, as well as genetic elements necessary for infection and pathogenesis, raising the possibility of common targets for attenuation and treatment design (Menachery et al., 2017).

Li et al., have reviewed general features, molecular immune pathogenesis, diagnosis and treatment of SARS-CoV-2. They have mentioned that the genome of coronaviruses contains a 5' cap structure along with a 3' poly (A) tail act as a mRNA for translation of the replicase polyproteins ( Li et al., 2020). The replicase gene encodes the nonstructural proteins; nsps 1–16. Nsp16 with its cofactor nsp-10, forms a heterodimer and stimulates 2'-O-methyltransferase (2'-O-MTase) activity. In addition to 2'-O-MTase activity, nsp-16 modifies the genetic material of the virus and make it look more like the human RNA and shields viral RNA from MDA5 recognition (Bouvet et al., 2010; Decroly et al., 2008; 2011; Fehr & Perlman, 2015; Ke et al., 2012; Menachery et al., 2017; Züst et al., 2011) and the innate immune responses, which play an important role in controlling the replication and infection of coronavirus (Canrong Wu et al., 2020), are blocked. Therefore, if a drug can be developed to inhibit nsp16, the immune system would be able to detect the virus and eradicate it faster. Importantly, the broad conservation of 2-O-MTase in a number of other viral families including CoVs provides a broadly applicable approach ideal for targeting viral infections. According to reports published by Menachery et al, both vaccine and drug treatment approaches have been conceived to target 2-O-MTase activity of nsp-16 for

COVs treatment and other emergent viral infections (Menachery et al., 2014). In this study, the efforts have been made to discover potential nsp-16 inhibitors among the FDA-approved drugs by repurposing approach and computational drug design methods including virtual screening, molecular docking and molecular dynamics simulation. We hope that the knowledge offered in this investigation resulted to progress in clinical studies and treatment of SARS-CoV-2 infections.

## 2. Experimental

### 2.1. Retrieval of protein structure

The three-dimensional (3D) structure of SARS-CoV-2 nsp16 (PDBID: 6W4H) was accessed from RCSB.

### 2.2. Screening of FDA approved drugs

The nsp16 protein, from SARS-COV-2, is a S-adenosylmethionine (SAM)-dependent (nucleoside-2'-O)-methyltransferase. Based on the previous reports (Chrebet et al., 2005), MTase inhibitors such as sinefungin bind to the S-adenosylmethionine binding pocket and suppress coronavirus MTase activity of nsp16 (Chen & Guo, 2016; Decroly et al., 2011).

The structure of Sinefungin is similar to SAM structure with a similarity score of 0.8 (obtained from drug bank). Therefore, we used SAM and this small molecule as two templates for shape-based screening and the best compounds from 1516 FDA-approved drugs were selected through score similarity. The SwissSimilarity (<http://www.swissimilarity.ch/>) (Zoete et al., 2016) and drug bank (<https://www.drugbank.ca/>) (Wishart et al., 2017), two online platforms, were used to identify and screen some chemical hits from FDA approved drugs library with respect to SAM and sinefungin as reference structures for shape based screening. The smile format and chemical structures of SAM and sinefungine were retrieved from pubchem (<https://pubchem.ncbi.nlm.nih.gov/>) (Wang et al., 2009). All screened drugs were ranked according to their predicted score values. Top common structures in both shape-based screening were selected. In addition to these compounds, due to inhibitory activity of nucleoside analogs against methyl transferase, 4 nucleoside analogs including 3 FDA-approved anti-viral drugs (Maraviroc, Raltegravir and Fivapiravir) and one anti-inflammatory drug (Prednisolone) were also selected based on accurate literature review and drug accessibility for further investigations in the future. Raltegravir has been reported as a 2-O'-methyl transferase inhibitor, previously using a predicted model (Khan et al., 2020). Also, based on the reports, Favipiravir, has shown to be a useful drug against SARS-COV-2 in initial clinical trials conducted in Wuhan and Shenzhen (Li et al., 2020). In this study, this compound was selected with this aim to investigate its methyl transferase inhibitory potential as a nucleosid analog. Furthermore, Prednisolone as a DNA methyltransferase inhibitor, was selected for its inhibitory activity against RNA methyl transferase activity of nsp16 (Harshitha and Nair, 2020).

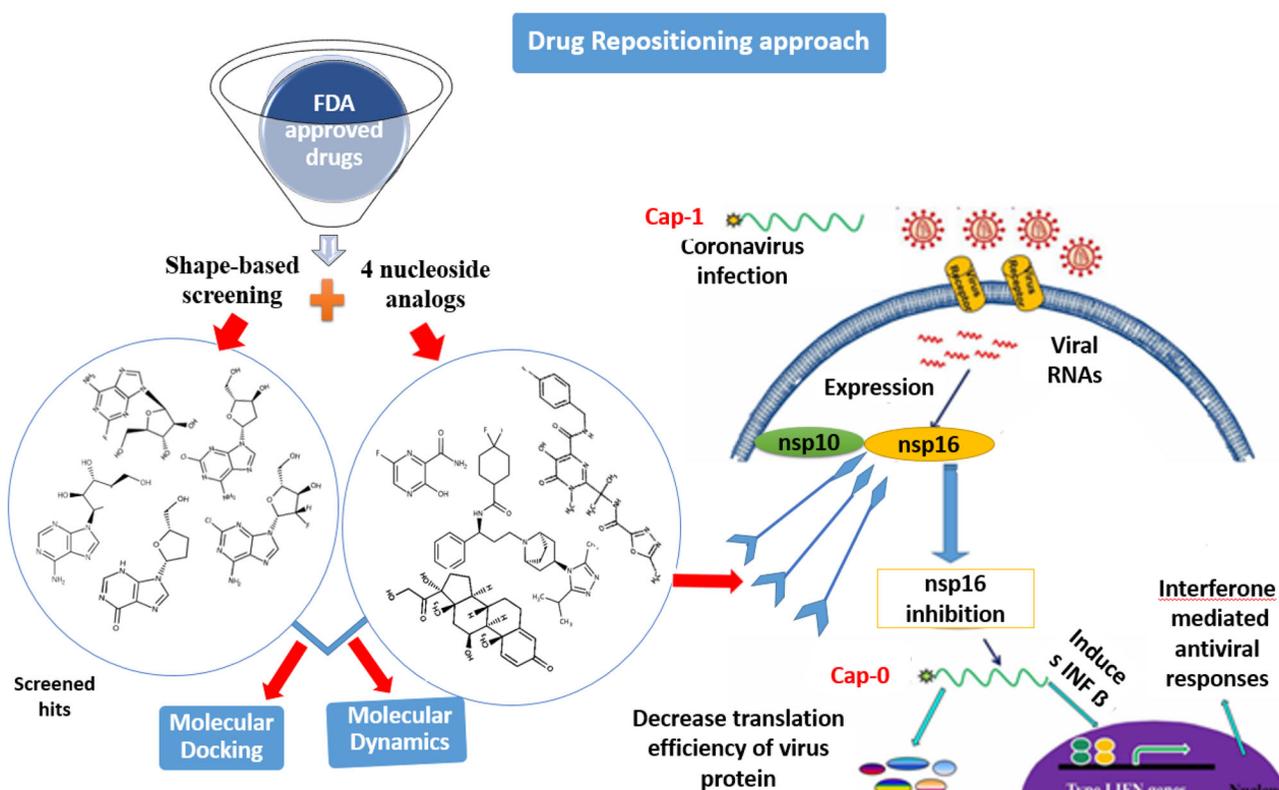


Figure 1. The flowsheet diagram of our designed work.

Table 1. 5 top Similarity scores of screened drugs to SAM or Sinefungin.

Ligand	SwissSimilarity web tool		DrugBank database	
	Score similarity to sinefungin	Score similarity to SAM	Score similarity to sinefungin	Score similarity to SAM
Cladribine	0.343	0.129	0.80	0.73
Vidarabine	0.631	0.287	0.88	0.794
Fludarabine	0.202	0.093	0.83	0.77
Clofarabin	0.188	0.082	0.74	0.72
Didanosine	0.12	–	0.70	–
SAM	0.283	1	0.81	1
Sinefungin	1	–	1	0.81

### 2.3. Molecular docking

Local docking experiments were performed using two different algorithms: AutoDock 4.2 (Goodsell, 2009), and AutoDock Vina (Trott & Olson, 2010) and blind docking experiments were done by SwissDock (Bitencourt-Ferreira & de Azevedo, 2019; Grosdidier et al., 2011b). For local docking, the search space was restricted to SAM binding groove. While for blind docking, whole cavities of protein were selected to examine the possibility and potential sites in nsp-16.

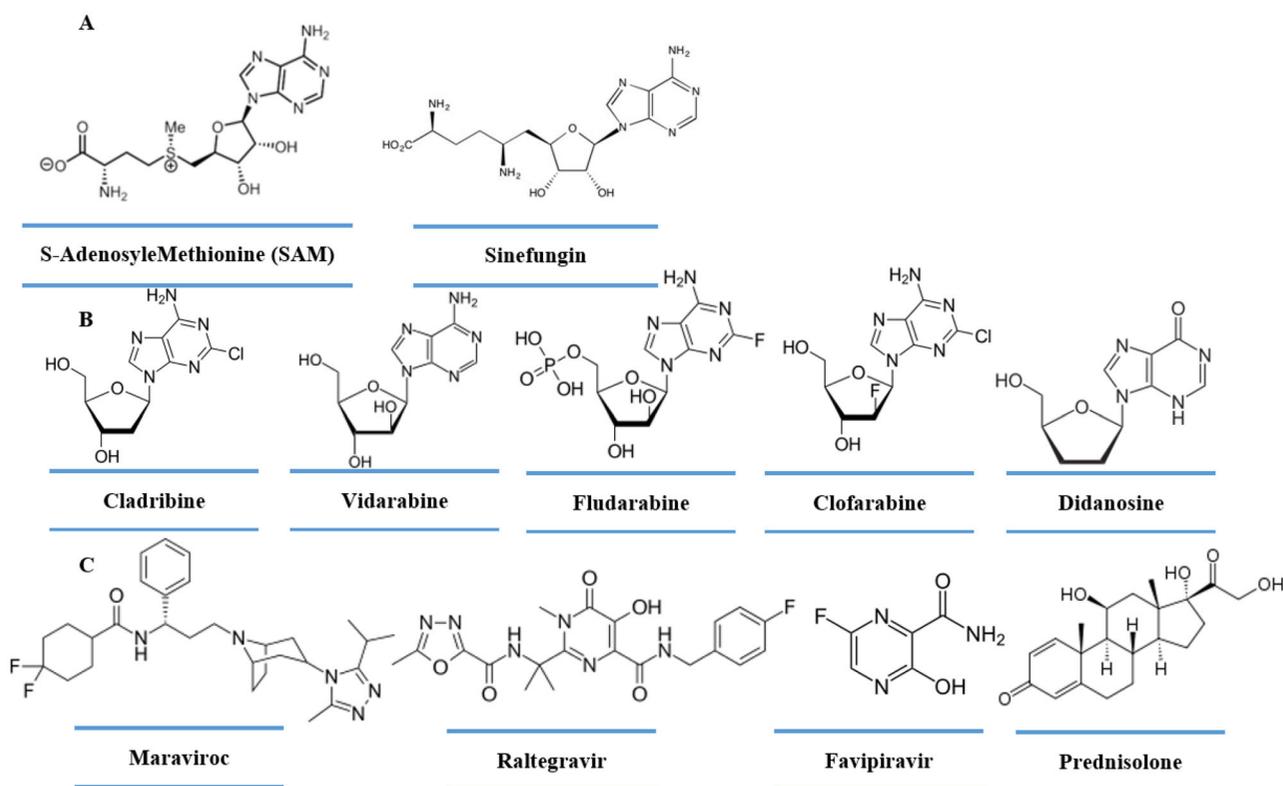
#### 2.3.1. Autodock vina

In this study, a valuable tool for computer-aided drug discovery and an open-source program, Autodock vina in PyRx0.8 (Dallakyan and Olson, 2015), was used to perform molecular docking. Briefly, UCSF Chimera software (Huang et al., 2014) was employed for energy minimization of nsp-16 by using Gasteiger algorithm and amber force field. Then it was saved in pdb format and uploaded in PyRx 0.8. Ligands were imported and energy minimization was performed via software

OpenBabel. The SAM-binding groove was placed in the center of a simulation box. The box dimension was  $46 \times 50 \times 46$  cubic angstroms. All the other parameters were kept as default.

#### 2.3.2. Autodock 4.2

Molecular docking was performed on the optimized SARS-COV-2 nsp16 (PDB ID:6W4H, chain A) by AutoDock 4.2 software. The pdb structure (6W4H) of SARS-COV-2 nsp-16 was observed for sequence break by using pymol molecular visualization system software. Then the protein structure was refined for hetero-atoms and water molecules to demarcate active sites of proteins. Further, the gasteiger charges and hydrogen atoms were added to the drug target to maintain coordination between various interactions by using UCSF Chimera software. Finally the drug target was saved in pdb format with their respective pdb IDs for docking studies. To find the suitable binding position of a ligand on the protein, combination of energy evaluation through pre-calculated grids of the potential affinity employing different search



**Figure 2.** (A) 2D structure of SAM, Sinefungine (reference ligand), (B) 5 top hit compounds obtained shape-based similarity screening and (C) Four FDA approved antiviral and anti-inflammatory nucleoside analogs.

algorithms is performed by Autodock. At first, grid box was created. Three-dimensional structure of receptor was constructed and optimized using Polak-Ribiere conjugate gradient algorithm and AMBER95 force field implemented in Hyper Chem (Hyper Cube Inc., Gainesville, FL) (Froimowitz, 1993). The ligands were stored by ChempSpider server (<http://www.chemspider.com>) (Pence and Williams, 2010). Using a plain text editor all the water molecules were removed, then missing hydrogens and Kollman united atom charges and polar hydrogens were added to the protein. Finally, non-polar hydrogens were merged to their corresponding carbons, and desolvation parameters were assigned to each atom. Then, rotatable bonds were assigned. For flexible docking, rotatable bonds in the ligands were kept free. Each prepared protein structure was uploaded and saved in pdb format, and the ligands under examination were also uploaded and saved in pdbqt format. The grid was set around the active site of the drug target for site-specific docking whereas the grid was maximized to surround the entire protein surface for docking. It was set to 48 x, 48 Y, and 50Z grid points (x, y and z), with spacing between grid points kept at 0.375 Å and the coordinate of central grid point of maps was adjusted as -8.278 x -14.333 y, and 8.250 z points (x, y and z). The Lamarckian genetic algorithm was selected to find the best conformers. For each box, one hundred independent docking runs were carried out. After completion of docking, the dock results were saved for the observation of binding affinities and bonding interactions between ligand-target were analyzed by Ligplot software (Wallace et al., 1995). Labeling of ligand and the protein binding sites were performed by chimera 1.7 s.

### 2.3.3. Swissdock

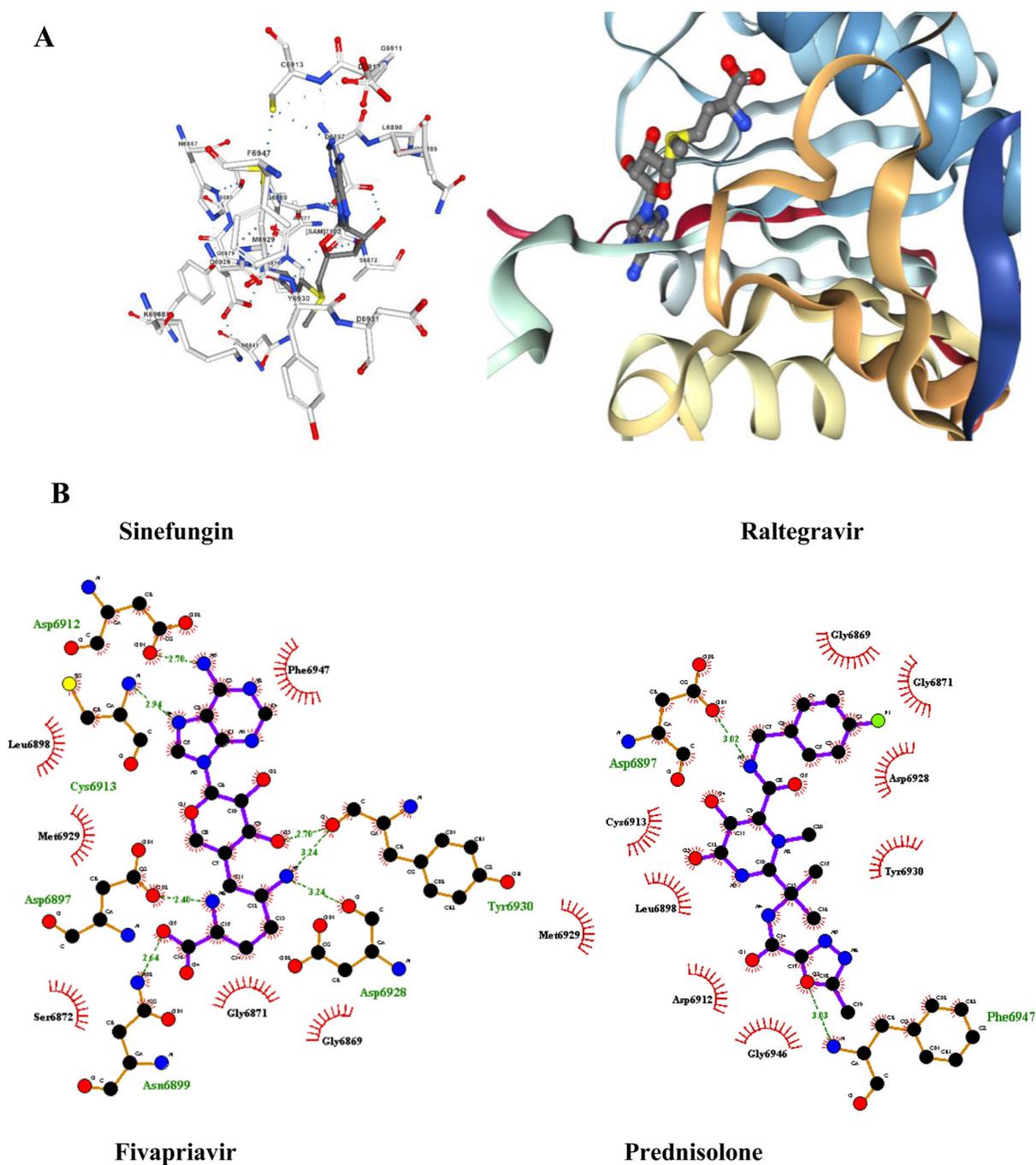
Docking experiment by SwissDock (Grosdidier et al., 2011b) web server is also carried out based on the EADock DSS engine using a multiobjective scoring function designed around the CHARMM22 force field and FACTS solvation model (Grosdidier et al., 2011a). Here, the protein structure was selected via PDB ID (6W4H; chain A) Also, the ligand structures were selected through the ligand name and verified by using zinc database on SwissDock. To perform blind docking, the binding modes are generated in the vicinity of all target cavities. Also, docking type was set on accurate. The results were rendered in UCSF Chimera (Pettersen et al., 2004).

### 2.4. Molecular dynamics simulations (MD)

The dynamics of the interactions between mentioned protein and drugs were then investigated using molecular dynamics (MD) simulations. Optimized Drug-protein complexes obtained from the docking step were used as initial structures for further MD analysis. The topology information for all drugs was prepared through Automated Topology Builder (ATB) server (Malde et al., 2011). Simulations were performed in GROMACS package (version 2018) by using gromos 53a6 force field (Abraham et al., 2015). In this study, a SPC/E (Extended Simple Point Charge) model of water was selected and the neutralization of the systems was done by adding appropriate amount of Na or Cl ions (Binder, 1997). Also the steepest descend algorithm was applied for energy minimization of system in order to eliminate the undesirable atomic contacts. In the next, the temperature and pressure

**Table 2.** Theoretical binding free energies ( $\Delta G_{\text{binding}}$  (kcal mol<sup>-1</sup>)) as obtained by three different molecular docking experiments; Autodock vina, Autodock 4.2 and SwissDock.

Ligand	Drug category	Local docking		Blind docking Swissdock
		Autodock vina	Autodock 4.2	
Cladribine	Anticancer	-6.4	-6.59	-9.14
Vidarabine	Antiviral	-6.2	-5.04	-8.88
Fludarabine	Anticancer	-6.5	-5.77	-7.65
Clofarabine	Anticancer	-6.3	-6.05	-9.14
Maraviroc	Antiviral	-8.3	-9.73	-9.15
Raltegravir	Antiviral	-10.4	-8.3	-8.21
Fivapiravir	Antiviral	-5.3	-5.27	-6.79
Didanosine	Antiviral	-6.2	-5.95	-7.41
Prednisolone	Immunosuppressive and Anti-Inflammatory Agents	-7.7	-6.66	-7.38
Sinefungin	Anti-infective/Nucleoside Analog	-7.2	-7.24	-8.14



**Figure 3.** (A) SAM interactions in nsp-16. (B) Ligplot of binding interactions of drugs in the SAM binding groove of SARS-COV-2 nsp-16. Salt bridges are in yellow spheres connected by solid lines. (C) a cartoon representation of the docked ligands in the protein.

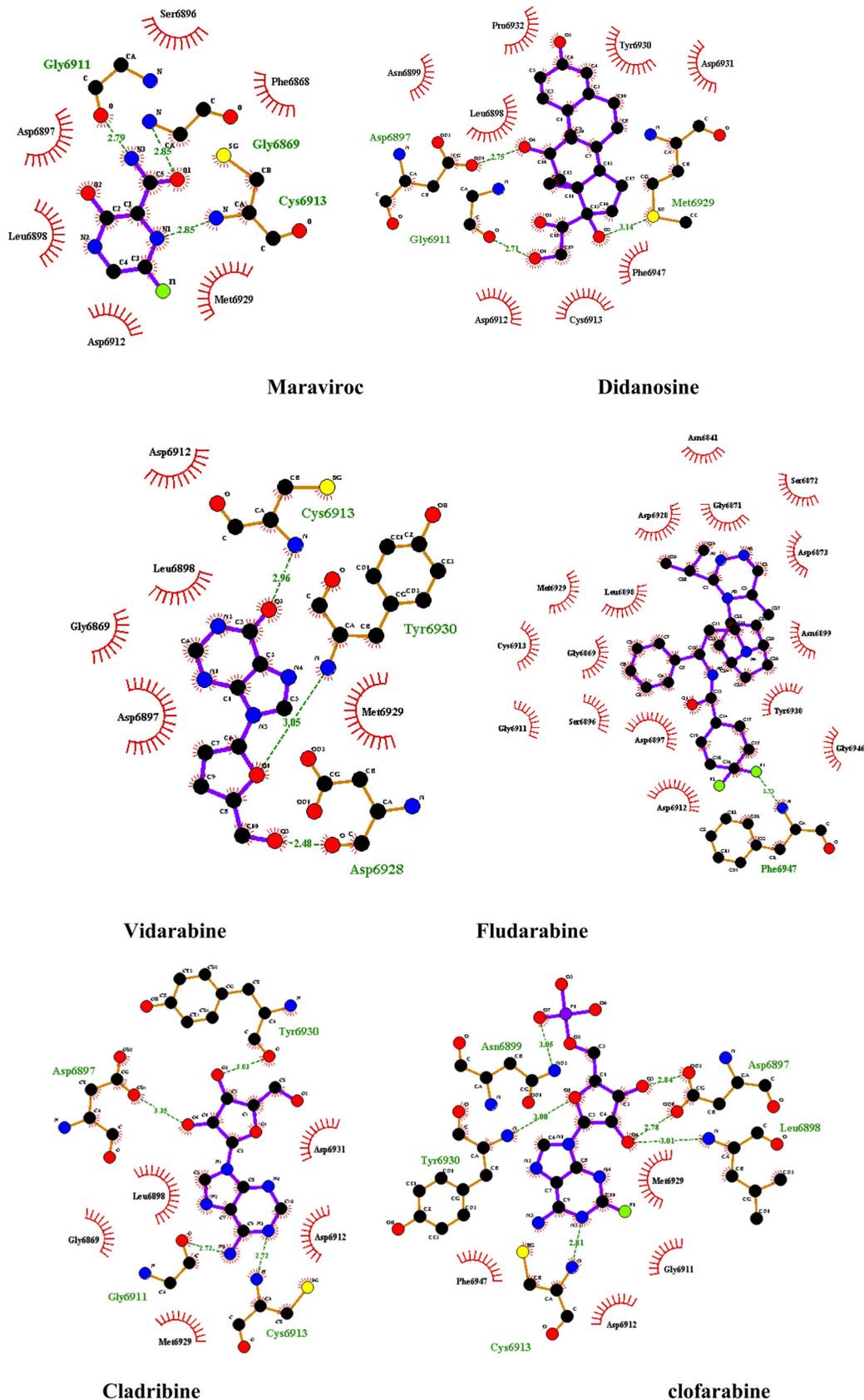


Figure 3. Continued

were coupled by applying NVT and NPT ensembles, in 310K and 1 bar respectively using v-rescale thermostat and parinello-rahman barostat (Hess et al., 2008). All bonds were constrained in their equilibrium values using LINCS algorithm.

Electrostatics and Van der Waals interaction were calculated by the cutoff of 1 nm. Finally the production phase of MD simulations were done on all systems using the leap frog algorithm (van Aalten et al., 1996). The trajectories were

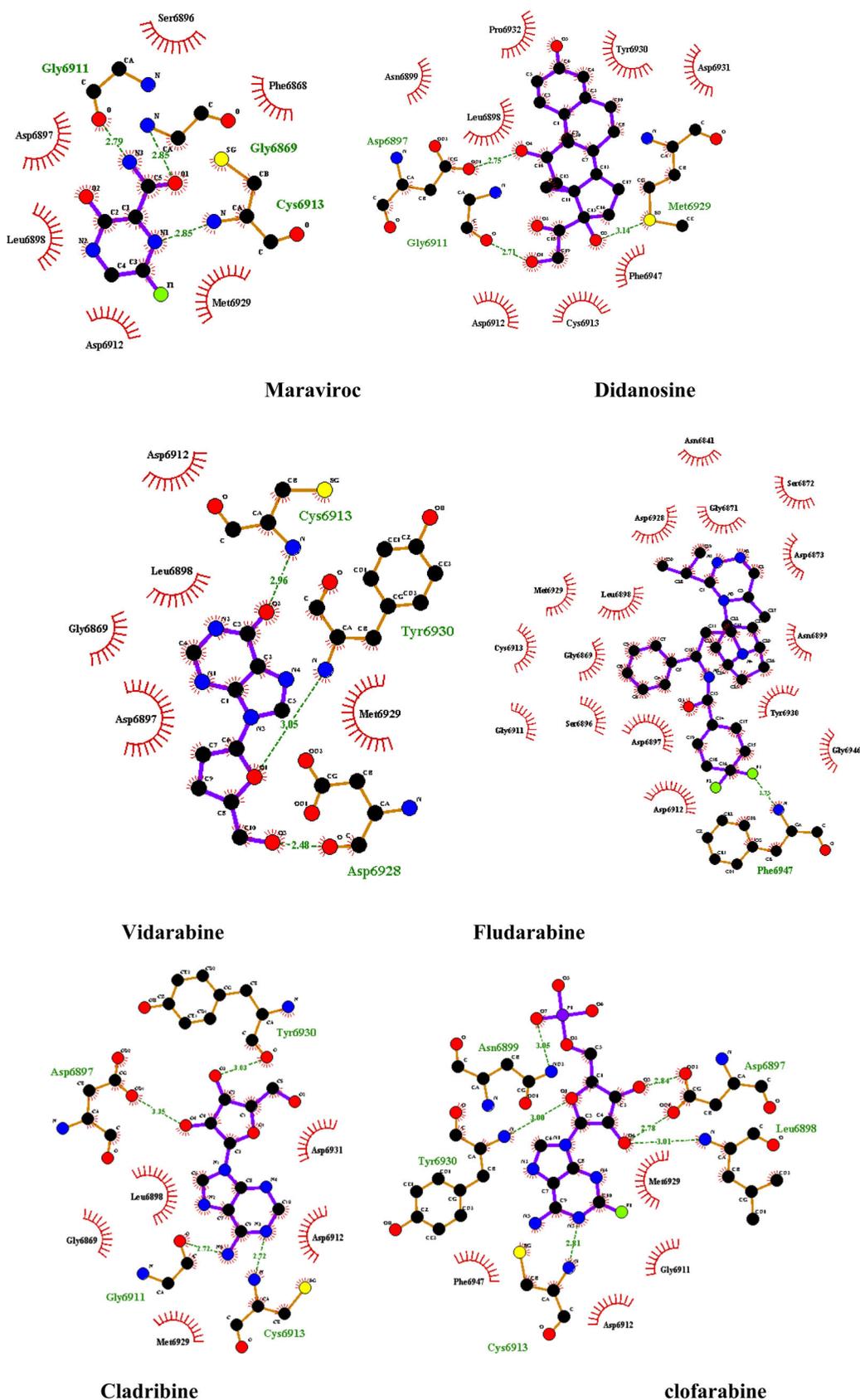


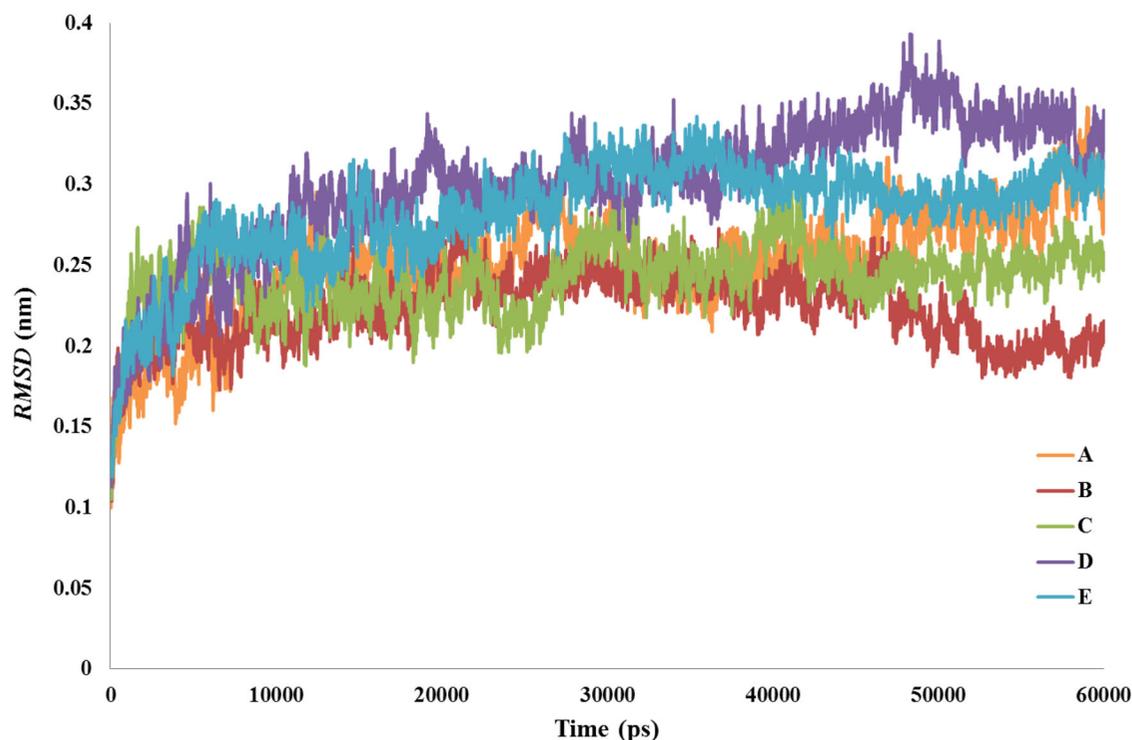
Figure 3. Continued

analyzed using the built-in function of gromacs. In this regard the equilibration in system and mean fluctuations of each residue were calculated by Root Mean Square Deviation (RMSD) and Root Mean Squared Fluctuation (RMSF) tools,

respectively. Solvent Accessible Surface Area (SASA) and Radius of gyration (Rg) also were measured to investigate the structural variation in protein. The changes in flexibility and main component of protein movement in different

**Table 3.** Binding energies, inhibition constants and H-bond interaction of compounds against NSP-16.

Ligand	$\Delta G_{\text{binding}}$ (kcal mol <sup>-1</sup> )	Inhibition Constant (K <sub>i</sub> )	H-Bond Interaction
Cladribine	-6.59	10.49 $\mu\text{M}$	Asp 6897 Cys 6913, Tyr 6930
Vidarabine	-5.04	194.14 $\mu\text{M}$	Gly 6911 Asp 6897 Cys 6913, Tyr 6930
Fludarabine	-5.77	58.92 $\mu\text{M}$	Asp6897 Tyr 6930, Asn6899, Leu6898 Cts 6913
Clofarabin	-6.05	36.77 $\mu\text{M}$	Tyr 6930, Cys 6913, Gly 6911, Leu 6898
Maraviroc	-9.73	73.54 nM	phe 6947
Prednisolone	-6.66	13.07 $\mu\text{M}$	Asp6897, Gly6911, Met6929
Didanosine	-5.95	43.7 $\mu\text{M}$	Cys6913, Tyr 6930, Asp 6928
Raltegravir	-8.3	818.66 nM	Phe 6947, Asp 6897
Favipiravir	-5.27	136.87 $\mu\text{M}$	Gly 6911, Gly 6869, Cys 6913
Sinefungin	-7.24	4.93 $\mu\text{M}$	Asp6897, Asp 6912, Asp 6928, Tyr 6930, Asn6899, Leu6898

**Figure 4.** Time evolutions of RMSD of C-alpha atoms for different systems of (a) Free protein, (b) Protein-Maraviroc, (c) Protein-Prednisolone, (d) Protein-Raltegravir and (e) Protein-Sinefungin.

conditions were analyzed by Principal Component Analysis (PCA) tool.

### 3. Results and discussion

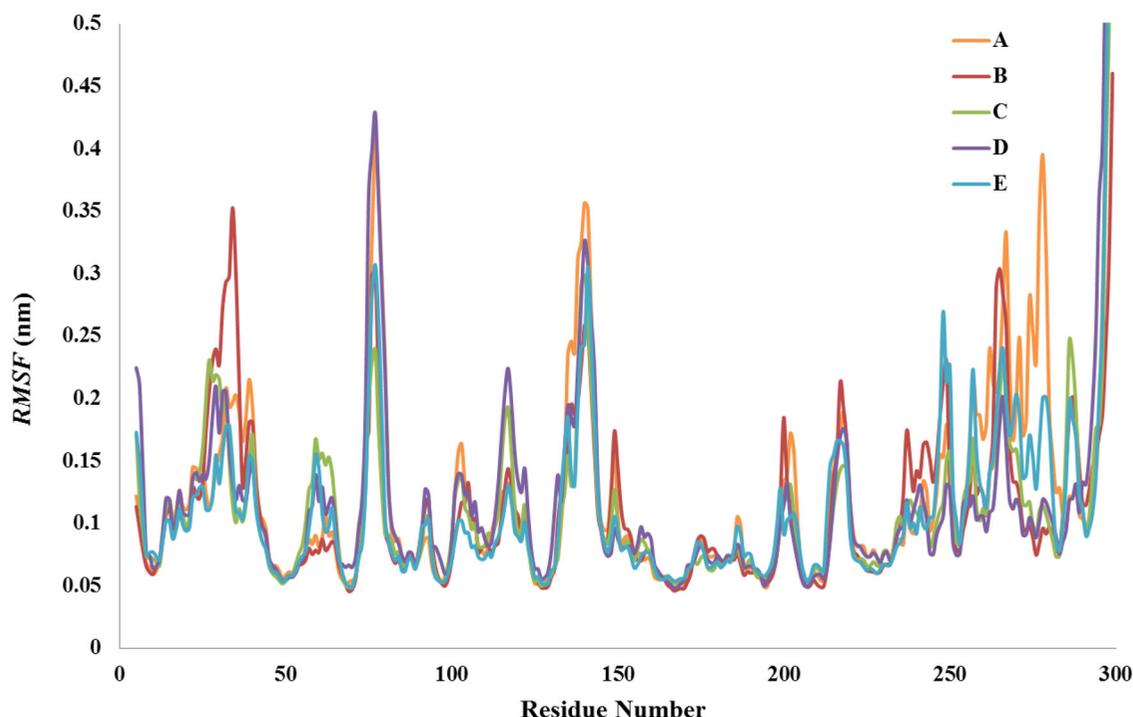
In drug repositioning approach, in order to predict the possible therapeutic potency of known drugs against a target the shape-based screening, molecular docking methods, molecular dynamics simulation and an accurate literature review need to be performed (Hassan et al., 2019). Thus, in this study we used these methods to find the potential hits for inhibiting nsp-16 as a target in COVID-19, The overall research diagram which depicted the basic hierarchy of our newly designed work has been illustrated in Figure.1.

#### 3.1. Drug screening

As mentioned in the introduction and method sections, the 2'-O-MTase activity of nsp-16 prevents virus detection by cell innate immunity mechanisms. Nsp16 is an S-adenosyl-l-

methionine (SAM)-dependent 2'-O-MTase that its activity is regulated by nsp10 binding. The methyl donor SAM plays an important role in the complex formation of nsp10/nsp16 and enhancing RNA binding. Actually, small conformational changes of the enzyme are induced by SAM binding and RNA affinity and methylation increase by nsp10/nsp16. Thus, it is expected that the SAM analogues such as sinefungin through entering in the SAM binding site and inhibiting of 2'-O-MTase activity of nsp-16, elicit strong antiviral responses (Aouadi et al., 2017).

Based on these facts, here, it was decided to screen similar compounds to SAM or sinefungin (as a known SAM analog) from among 1516 FDA approved drugs by two online platforms SwissSimilarity (Zoete et al., 2016) and DrugBank database. Then, 5 top drugs with good structural resemblance to reference compounds (SAM and Sinefungin) were identified (Table 1). In addition to these compounds, four other antiviral and anti-inflammatory nucleoside analogs including maraviroc, raltegravir, favipiravir and prednisolone were selected based on the literature review for further investigations (Figure 2).



**Figure 5.** The values of RMSF in (a) Free protein, (b) Protein-Maraviroc, (c) Protein-Prednisolone, (d) Protein-Raltegravir and (d) Protein-Sinefungin systems.

**Table 4.** Total energy of Protein-drug interaction. All values were calculated as kJ/mol.

Hits	$E_{vdw}$	$E_{ele}$	$E_{total}$
Maraviroc	-138.24	-1125.73	-1263.97
Raltegravir	-131.31	-503.69	-634.10
Prednisolone	-65.57	-80.47	-146.04
Sinefungin	-88.64	-469.66	-558.31

### 3.2. Molecular docking results

Molecular docking is a powerful approach to study the binding affinity and investigating the binding interactions of ligands within the active region of target proteins (Meng et al., 2011). Because docking programs are computationally not experimentally, it is hard to pretend which program can be more accurate for docking and it is not expected to have a full correlation between their results. For blind docking, the SwissDock server is an excellent tool and for local docking AutoDock is a standard method. In this study two defined docking modes (AutoDock Vina and AutoDock 4.2) based on the Lamarckian algorithm were performed as direct docking and blind docking was done by SwissDock server. The predicted active site docking (site-specific docking) was performed at known active site of protein (binding site of SAM) to examine binding affinities of mentioned ligands against nsp-16. Then those compounds which consistently passed binding energy thresholds of  $\leq 7$  kcal/mol (at least in two different algorithms) were selected as best docked compounds for MD simulation. To evaluate the potential candidates, all the screened drugs were docked against nsp-16 separately with three different docking algorithms: AutoDock Vina and AutoDock 4.2 for local docking and SwissDock for blind docking. The results analyzed on the basis of the lowest binding energy values ( $\text{kcal mol}^{-1}$ ).

As shown in Table 2, Maraviroc and Raltegravir drugs exhibited significant binding energy values compared to Sinefungin with all three algorithms. Also Autodock vina results, showed comparable docking energy value of prednisolone compared to Sinefungin. The other studied drugs possessed higher binding energies than the reference drug by two algorithms, Autodock 4.2 and Autodock vina as selected for local docking. Interestingly, the compounds which selected due to shape similarity to SAM and Sinefungin, had higher binding energy compared to Sinefungin in local docking. However, blind docking results by SwissDock exhibited that Cladribine, Vidarabine, Clofarabine along with Maraviroc and Raltegravir have lower binding energy compared to Sinefungin and other drugs.

The orientation and interactions of SAM in nsp-16 binding site has been illustrated in Figure 3A. Also, the detailed hydrophobic and hydrogen-bonding interactions and the interacting protein side chain residues in the SAM binding groove of nsp16 with 10 selected compounds has been shown in Figure 3B and 3C. Figure 3B shows that the interaction of all compounds with nsp-16 is derived by hydrophobic interaction and hydrogen bonds. However, hydrophobic interactions play a significant role in the interaction of Maraviroc and Raltegravir. Binding energy, inhibition constant and the residues participating in the hydrogen bond interactions for docked molecule with autodock 4.2 has been reported in Table 3.

However, molecular docking methods are the best approaches to study the binding conformation of ligands within the active region of target proteins but all these methods are probabilistic approaches. Therefore, further simulations (MD, etc) are needed on docking results in order to validate them.

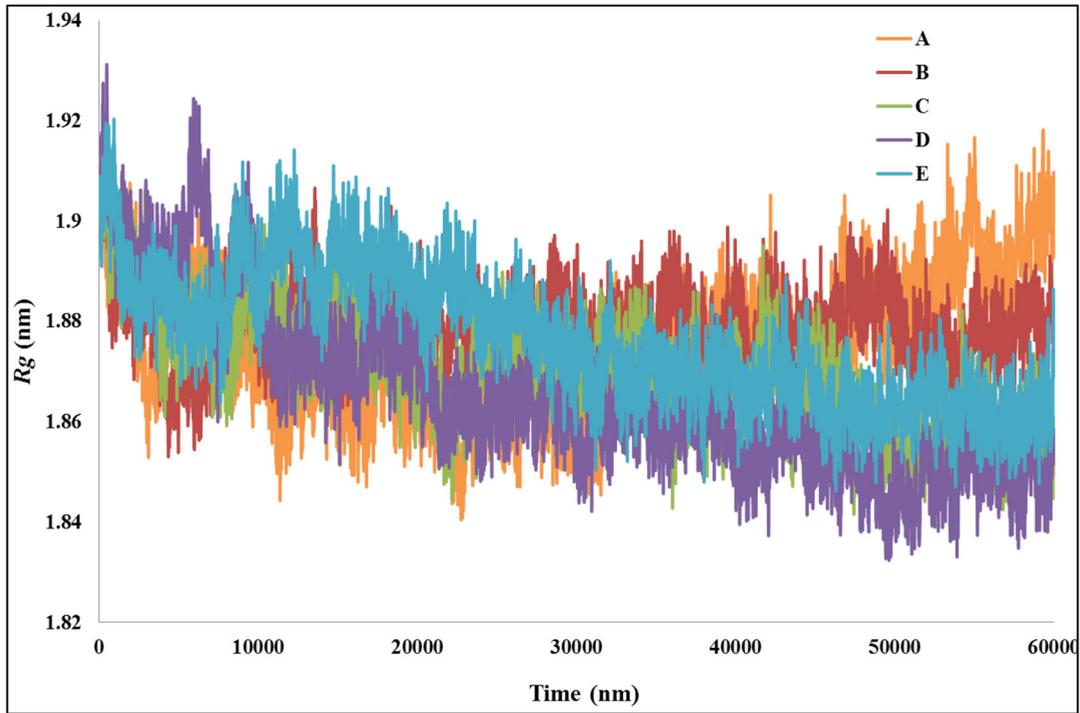


Figure 6. The value of  $R_g$  in (A) Free protein, (B) Protein-Maraviroc, (C) Protein-Prednisolone, (D) Protein-Raltegravir and (E) Protein-Sinefungin systems.

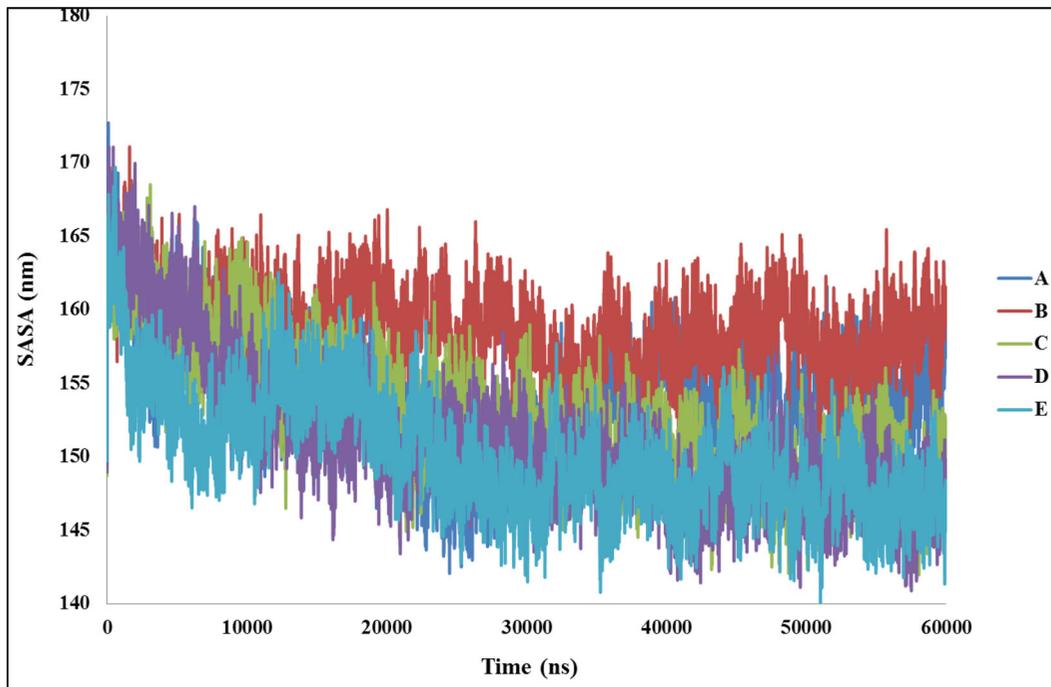
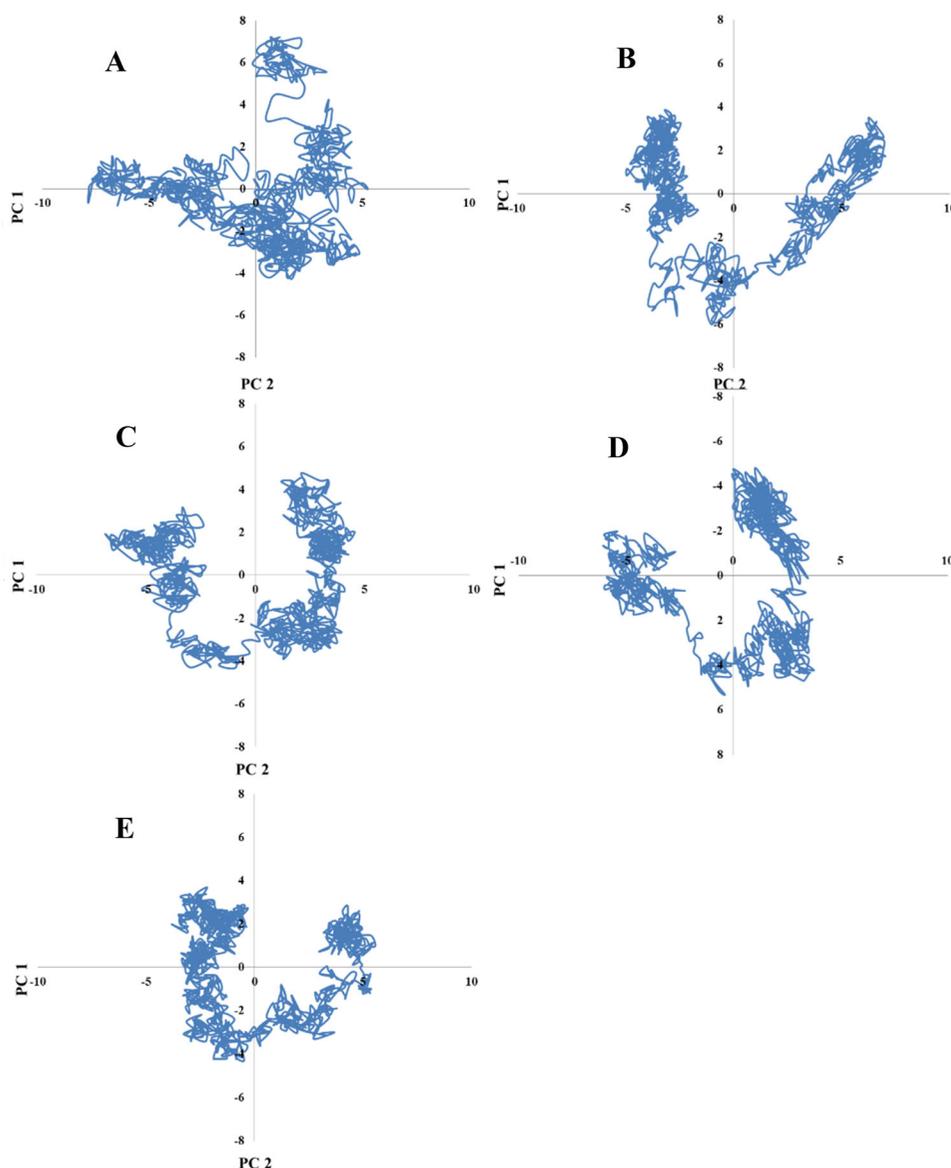


Figure 7. The values of SASA in (A) Free Protein, (B) Protein-Maraviroc, (C) Protein-Prednisolone, (D) Protein-Raltegravir and (E) Protein-Sinefungin systems

### 3. 3. Molecular dynamics simulation

To investigate the dynamics and changes in the structure of protein in complex with the drugs along with interaction energies related to binding of each one with MTase, MD simulations were performed. In this regards the mobility and changes in protein structures in Free State were compared to those for protein in complex with sinefungin, Raltegravir and

Maraviroc. The root mean square deviation (RMSD) as a measure of the global structural properties for the free protein and protein in complex with mentioned drugs for 60 nano seconds is seen in Figure 4. In this time the system reached to equilibration state and the analysis can be performed with acceptable accuracy. The mean of RMSD values fluctuated around 0.25 and nearly 0.25 nm in free protein and protein in complex with drugs, respectively. The value of *RMSD* for all



**Figure 8.** Principal Component Analysis of nsp-16 complexes. (A) Free Protein, (B) Protein-Maraviroc, (C) Protein-Prednisolone, (D) Protein-Raltegravir, and (E) Protein Sinefungin systems

simulations has reached to its equilibrium after 15 ns of simulation and fluctuates by the rest of time. In the protein-Sinefungin system the mean value of *RMSD* (0.28 nm) is higher than those for free protein (0.25 nm) which indicating some instability in protein. The least mean value of *RMSD* is related to the system containing Maraviroc as inhibitor (0.22 nm) which indicating that the more stability of the protein in presence of this drug. In the other hand the most sever fluctuation in *RMSD* (0.3 nm) which is also related to instability in protein is observed for the nsp-16-Raltegravir system.

Since distance deviations from the starting structure may not necessarily reflect the mobility of structural elements, another parameter, Root Mean Squared Fluctuation (RMSF), is used to obtain information on flexibility. To identify flexible regions in the molecule, RMSFs of the protein  $C\alpha$  atoms are illustrated in Figure 5. As can be seen from the illustrated results in Figure 5, the Maraviroc in the locations of around the residues 30, 150, 180, 200, 220 and 240 make the highest

fluctuation in the protein that can further put out the instability in its structure. Also in the location of residues 70-140 in the Raltegravir containing system the protein has higher fluctuations than other systems.

The changes in radius of gyration for protein in different systems were calculated and presented in Figure 6. From this figure it is concluded that the protein is undergo some compression in its 3D conformation and in the case of nsp-16 in complex with raltegravir the most compression than other systems is observed. These results are in agreement with those of SASA analysis in which because of compression in the protein structure, the amount of overall surface area is reduced for solvent accessibility (Figure 7).

As a result of unique structure of reach protein, their ordered local movements is also sole which changes in these motions affect the function of the protein and its interaction with other macro and micro molecules. The principal component analysis (PCA) discovers these punctual movements of

proteins and in this study was done in order to evaluate the effect of different drugs on the movement pattern of nsp-16. As can be seen from Figure 8, the most different patterns of 2D PCA analysis is related to that system containing Maraviroc and Sinefungin that indicate these drugs have a great potential to inhibit the enzymatic activity of nsp-16 by changing its structure and dynamics in addition to prevention of its native substrate from binding to protein.

Analyzing the protein-drug interaction energies in dynamic state were done for different systems using the MM/PBSA method. The mean values for equilibrium period of simulations are presented in Table 4. As can be seen from these results and in agreement with those of docking studies the Maraviroc and Raltegravir have the highest binding energy with protein and both interactions are stronger than that of sinefungin to MTase (Table 4).

#### 4. Conclusion

In order to discover effective drugs for inhibition of the nsp-16 and preventing SARS-COV-2 replication, a set exhaustive docking techniques and molecular dynamics simulation were performed. Compounds binding mode and energy were analyzed and ranked. Accordingly, based on docking results, three agents including Raltegravir, Maraviroc and prednisolone are proposed as potential inhibitors of nsp-16. The interatomic results showed the proposed compounds located in the SAM binding groove and revealed their ability in blocking the entrance of the nsp-16 active site and inhibiting nsp-16 enzyme activity. The MD simulation results exposed that Raltegravir and Maraviroc have better profiles with respect to their RMSD and RMSF and steadily stable behavior was observed in all docking complexes. Based on obtained results, it is concluded that Raltegravir and Maraviroc which may be used in the treatment of COVID-19 after clinical trial. Although subsequent *in vitro* and *in vivo* validation of antiviral effects will provide useful information for future researches, identification of drug candidates is an essential step in determining of timely and effective treatment approaches of COVID-19. We hope the sharing of our results with other scientists in anti-SARS-CoV-2 research lead to faster drug discovery for COVID-19 and clinical trials.

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#### Disclosure statement

No potential conflict of interest was reported by the author(s).

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