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Preliminary Computational Analysis of Pyrazinamide-Based Derivatives Reveals Possible Inhibition of SARS-CoV-2 RNA-Dependent RNA Polymerase, and Their Possible Use as Antiviral Agents

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Abstract

Pyrazinamide is a pyrazine analog currently used to treat tuberculosis. It shares a core structure similar to that of favipiravir, which is a promising drug candidate that may inhibit the SARS-CoV-2 RNA-dependent RNA polymerase. This feature could be an opportunity for further drug development of anti-COVID-19 medication starting from a pyrazinamide core structure. This study aimed to determine and predict the most effective pyrazinamide-based analogs against the SARS-CoV-2 RNA-dependent RNA polymerase by using combined ligand- and structure-based computational analysis. This study performed a rational *in silico* study to screen pyrazinamide-like molecules from a commercially available ZINC database, with a similarity score higher than 0.40, and then these screened for acceptable pharmacokinetic properties, and then to further conduct molecular docking analysis with SARS-CoV-2 RNA-dependent RNA polymerase. The results showed that compound **12**, having a dichloropyrimidine core structure had a similarity score of 0.446. It exerted the most binding affinity with RNA-dependent RNA polymerase, with estimated docking scores of −5.72, −5.25, −7.06, −7.00 and −4.63 kcal/mol in intact, ribosylated, mono-phosphoribosylated, di-phosphoribosylated and tri-phosphoribosylated forms, respectively. Watson-Crick base-pairing of compound **12** indicated that it favored binding with the uracil nucleoside of the RNA template. Compound **12** was confirmed as the lead compound, being a pyrazinamide-like molecule, and so might be a most promising candidate molecule, as an adenine analog RNA-dependent RNA polymerase inhibitor. It is suggested that the antiviral effect of this lead compound should be studied further as part of a drug discovery and development process.

Keywords: Pyrazinamide, Favipiravir, *In silico* analysis, COVID-19, SARS-CoV-2, RNA-dependent RNA polymerase

Introduction

In December 2019, an epidemic of coronavirus disease of 2019 (COVID-19) infection originated from Wuhan, China, and then spread rapidly throughout the world [1]. Over the next 2 years, it affected most of the countries in the world, infected more than 500 million people and caused more than 6.2 million deaths [1]. This infection is caused by a novel beta-coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has been classified by the World Health Organization (WHO) into 5 main variants including the Alpha, Beta, Gamma, Delta and Omicron variants [2]. This virus can spread from an infected person's respiratory tract in small respiratory droplets and decreases the capability of the lower respiratory tracts [3]. To reduce the harmful consequences of COVID-19 infection, such as respiratory failure, or multi-organ dysfunction, treatment with antiviral agents linked to prevention with the optimal vaccine is the main therapeutic approach for this emerging disease [1].

SARS-CoV-2 is a coronavirus with a positive-sense single-linear RNA (+ssRNA) genetic segment that has close genetic similarities to coronaviruses most likely of bat origin (e.g. SARS, MERS and SADS) [3]. The analysis of phylogenetic and evolutionary relationships between SARS-CoV-2 and SARS-CoV reveals that the SARS-CoV-2 genome shares approximately 80 % nucleotide sequence similarity with that of SARS-CoV. Notably, the RNA-dependent RNA polymerase (RdRp) and nucleotidyl-transferase (NiRAN) of SARS-CoV-2 show a shared sequence of about 98.1 and 93.2 %, respectively, which suggests this is a promising target for new antiviral agents [4]. RdRp is a polymerase that catalyzes the replication of RNA from an RNA template at the key active sites of RdRp as ASP760 and ASP761 [5].

RdRP is another interesting target that has led to the development of several inhibitors. Molnupiravir (2-oxopyrimidine analog, clinical trial phase III) [6], galidesivir (4-amino-pyrrolopyrimidine analog, clinical trial phase I) [7], remdesivir (4-amino-pyrrolotriazine analog, launched) [1,8], favipiravir (2-oxopyrazine-3-carboxamide analog, clinical trial phase III) [9] and ribavirin (1,2,4-triazole-3-carboxamide analog, clinical trial phase I) [10]. Each of these promising drugs require their conversion from prodrugs into triphosphate active forms and then subsequent incorporation into the viral RNA template, resulting in blocking of viral RNA replication which may then induce lethal mutagenesis of RNA viruses [11]. The action of these inhibitors can suppress the SARS-CoV-2 viral load which helps patients to recover from the infection. Among these inhibitors, favipiravir (FPV) is a distinctive compound that requires enhancement of its activity, due to the lack of statistical significance on the clinical trial in moderately ill COVID-19 patients [12]. FPV is a substituted pyrazinamide (PZA) derivative that was shown preclinically to inhibit the RdRp of SARS-CoV-2 [13]. It has similar features to the previously studied PZA antimycobacterial agent, these studies have reported that PZA and its derivatives show antifungal, antibacterial, and as well as antiviral activities [14]. Hence, the exploration of FPV analogs, by searching for chemical similarity of the PZA core structure, is a strategy that is expected to generate new compounds for the treatment of COVID-19 infection.

Molecular docking has been used in the screening of new substances that interact with target proteins to develop inhibitors of certain diseases [15]. We aimed to design the inhibitors of RdRp by using a molecular docking approach to investigate the binding affinity of FPV and PZA-like compounds at the molecular level. We hypothesized that our selected PZA-derivatives required intracellular conversion into their active forms, including ribosylated, mono-phosphoribosylated, di-phosphoribosylated and tri-phosphoribosylated forms, before viral RdRp inhibition can occur (**Figure 1**). We decided to perform molecular docking for these derivatives and their phosphoribosylated forms against the active site of the RdRp enzyme. In an attempt to understand the possible action of the best binding derivative against RdRp through lethal mutagenesis induction [16], we also illustrated the possible interaction of this derivative upon the RNA template at the Watson-Crick base-pairing level.

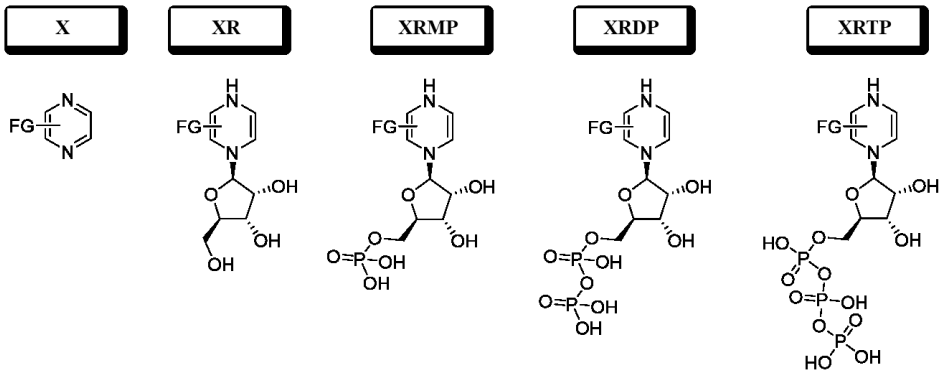


Figure 1 Design of chemical transformations into phosphoribosylated forms to create PZA-like molecules.

Materials and methods

Selection and preparation of PZA analogs

The 2D structure of PZA was drawn and then converted into the simplified molecular-input line-entry system (SMILES) format using ChemDraw Professional 16.0. The PZA molecule was used to search for new similar molecules using Swiss Similarity, a web tool that can compute the similarity of all compounds that are available in the ZINC database of commercially-available compounds for virtual screening [17].

In this work, the compounds that had a similarity score of more than 0.40 were selected and these PZA analogs were submitted into SwissADME (http://www.swissadme.ch/index.php), to compute their physicochemical and pharmacokinetic properties [18]. All compounds that were judged to be orally active drugs, with favorable pharmacokinetic properties, were included in this analysis (**Figure 2**) [19]. The criteria for the selection of PZA analogs included: 1) the compounds that fell within Lipinski's rule of five criteria (molecular weight < 500, number of H-bond donors < 10, number of H-bond acceptors < 5 and logP < 5) [18]; 2) the compounds that showed high gastrointestinal (GI) absorption; 3) the compounds that did not possess a P-glycoprotein (Pgp) substrate.

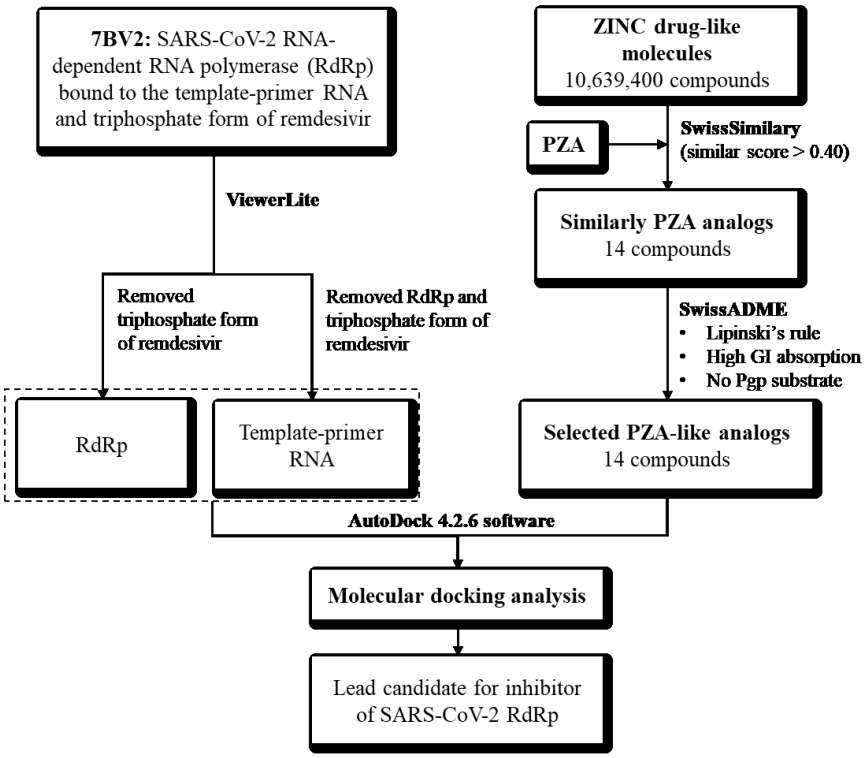


Figure 2 Design strategy for PZA-like analogs targeting the SARS-CoV-2 RdRp.

Preparation of the structure of PZA and its analogs

The activated forms of PZA and selected PZA-like analogs including ribosylated (XR), mono-phosphoribosylated (XRMP), di-phosphoribosylated (XRDP) and tri-phosphoribosylated (XRTP) forms, were drawn on ChemDraw Profession 16.0 (PerkinElmer Informatics, Inc.), followed by 3-dimensional (3D) structure transformation, using Chem3D Professional 10.0 (PerkinElmer Informatics, Inc.). The energy minimizations of PZA and its analogs were performed using Chem3D Professional 10.0, by using the MM2 force field to avoid any steric clashes of the freely rotatable bonds.

Preparation of structure of SARS-CoV-2 RdRp

A Crystal structure of a SARS-CoV-2 RdRp, a complex of the 3 proteins NSP7, NSP8 and RNA polymerase/NSP12, bound with the template-primer RNA and triphosphate form of remdesivir (RTP) (PDB ID: 7BV2) [20], was prepared by removing all water molecules, any solvent, RTP and template-primer RNA, using the ViewerLite program.

Molecular docking of PZA and its analogs onto the SARS-CoV-2 RdRp

The docking score (kcal/mol) and inhibitory constant (Ki) of PZA, PZA-like compounds, and their phosphoribosylated forms were docked and then analyzed, using AutoDock 4.2.6 software [21]. Each energy-minimized compound was submitted into the well-prepared targets with default parameters of docking procedures. The binding site sphere radius of ligand-target interaction was fixed at 60 Å, covering the catalytic site of RdRp, according to previous studies (Asp760 and Asp761). The molecular docking protocol was obtained from the active site of the SARS-CoV-2 RdRp with a molecular grid at 0.375 Å grid spacing. The location of grid was located at x = 97.657, y = 85.355 and z = 94.429 Å. Docking results of all analogs with SARS-CoV-2 RdRp were evaluated using the best docking score and inhibitory constant from all clusters of each conformational structure. Virtual analysis of the best results was then viewed and analyzed using UCSF Chimera (University of California, San Francisco) [22] and LigPrep 3.4 (Schrödinger Inc.).

Watson-Crick base-pair docking of PZA and its analogs on RNA nucleotide

The compounds that had the best binding affinity against the SARS-CoV-2 RdRp were docked against the nucleotide base in the template-primer RNA by using AutoDock 4.2.6 software [21]. Virtual analysis

of top 10 ranked interactions was then viewed and analyzed using UCSF Chimera (University of California, San Francisco) [22]. The atomic distance between the core structure of the selected PZA-like compounds and purine/pyrimidine bases of the RNA template was generally considered to be from 2.7 to 3.3 Å which indicated the hydrogen bond lengths in Watson-Crick base pairs [23].

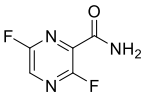
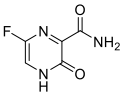
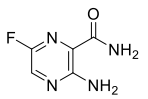
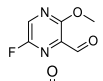
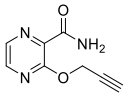
Results and discussion

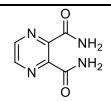
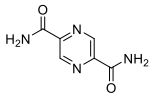
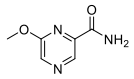
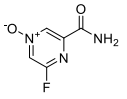
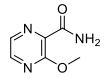
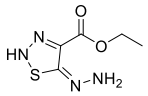
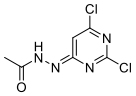
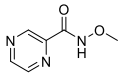
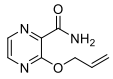
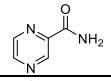
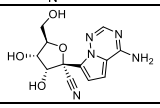
Combining vaccination with the development of new antiviral chemotherapeutics is one of the most effective ways to control catastrophic viral pandemics, particularly COVID-19. Inhibition of the SARS-CoV-2 viral replication *via* blocking RdRp has been investigated in antiviral drug development such as molnupiravir, galidesivir, remdesivir, bemnifosbuvir, favipiravir and ribavirin [1,24]. Pyrazinamide which is currently used to treat tuberculosis also shares a core structure similar to that of favipiravir. Therefore, pyrazinamide could be a promising drug candidate that may inhibit the SARS-CoV-2 RNA-dependent RNA polymerase. This study determined and predicted the most effective pyrazinamide-based analogs against the SARS-CoV-2 RNA-dependent RNA polymerase by using combined ligand- and structure-based computational analysis.

Molecular docking is the use of computational study of ligand interaction with targets to find effective drug candidates. Many molecular docking studies have been used to examine the interaction of compound libraries to the target proteins of interest before the confirmation of preclinical studies with successful results [15]. The combined ligand and structure-based approach were used in this study to rank PZA-like molecules from the data set. We hypothesized that the top-ranked PZA-like molecules, with high to moderate values of similar scores, might possess the structural features to inhibit SARS-CoV-2 RdRp. After method validation by redocking (median RMSD was 0.00 Å), the results showed that all PZA-like molecules were located at the RdRp catalytic site with characteristic orientation, illustrating that these molecules might effectively affect the function of RdRp. Docking scores in terms of estimated inhibition constants of all molecules were likely to be strongly governed by the hydrogen bonding interaction between each ligand and the amino acids surrounding key active sites of RdRp as Asp760 and Asp761.

The 10,639,400 commercially available ZINC drug-like molecules were scored for similarity to PZA by using the SwissSimilarity Web Tool. The results revealed 14 molecules having similarity scores of more than 0.4 (the range of 0.431 - 0.817) (**Table 1**). Among these PZA-like molecules, favipiravir (FPV) was ranked as the second (compound **2**) with a similarity score of 0.778. After SwissADME submission, 14 molecules that fell within Lipinski's rule of 5 criteria, including molecular weight, number of the rotatable bonds, number of H-bond donors, number of H-bond acceptors, and log*P* were selected [19]. All of the 14 molecules showed high GI absorption and did not possess P-glycoprotein (Pgp) substrate. Compound **12**, dichloropyrimidin-ylidene core structure, was the only pre-selected PZA-like molecule that possessed the likely structure suggestive of being a CYP1A2 inhibitor *via* SwissADME pharmacokinetics prediction. The physicochemical and pharmacokinetic properties of these 14 PZA-like molecules together with PZA and remdesivir (RDV) are summarized in **Table 2**.

Table 1 Chemical structure of PZA-like molecules obtained from SwissSimilarity website.

Compound	Zinc Code	Similarity	Structure
1	ZINC39065329	0.817	
2[a]	ZINC13915654	0.778	
3	ZINC39065039	0.773	
4	ZINC39062882	0.749	
5	ZINC39089963	0.616	

Compound	Zinc Code	Similarity	Structure
6	ZINC00967329	0.591	
7	ZINC34352310	0.533	
8	ZINC02507592	0.521	
9	ZINC39065149	0.494	
10	ZINC39248535	0.485	
11	ZINC01650651	0.482	
12	ZINC41078044	0.446	
13	ZINC39064861	0.433	
14	ZINC44549071	0.431	
PZA	ZINC00002005	1.000	
RDV[b]	ZINC84586789	-	

[a]Favipiravir, [b]Non-phosphorylated form of remdesivir

Table 2 Physiochemical and pharmacokinetic properties of pre-selected PZA-like molecules obtained from SwissADME website.

Com-pound	MW	HBA	HBD	*c*LogP	*P*gp sub.	CYP 1A2 inh.	CYP 2C19 inh.	CYP 2C9 inh.	CYP 2D6 inh.	CYP 3A4 inh.
1	159.09	5	1	0.32	No	No	No	No	No	No
2[a]	157.10	4	2	−0.27	No	No	No	No	No	No
3	156.12	4	2	−0.44	No	No	No	No	No	No
4	156.11	5	0	0.58	No	No	No	No	No	No
5	177.16	4	1	0.00	No	No	No	No	No	No
6	166.14	4	2	−1.24	No	No	No	No	No	No
7	166.14	4	2	−1.12	No	No	No	No	No	No
8	153.14	4	1	−0.09	No	No	No	No	No	No
9	158.11	4	2	−1.71	No	No	No	No	No	No
10	153.14	4	1	−0.31	No	No	No	No	No	No
11	188.21	4	2	0.33	No	No	No	No	No	No
12	221.04	4	1	1.16	No	Yes	No	No	No	No

Com-pound	MW	HBA	HBD	*c*LogP	*P*gp sub.	CYP 1A2 inh.	CYP 2C19 inh.	CYP 2C9 inh.	CYP 2D6 inh.	CYP 3A4 inh.
13	153.14	4	1	−0.14	No	No	No	No	No	No
14	179.18	4	1	0.26	No	No	No	No	No	No
PZA	123.11	3	1	−0.37	No	No	No	No	No	No
RDV[b]	291.26	7	4	−1.32	Yes	No	No	No	No	No

[a]Favipiravir, [b]Non-phosphorylated form of remdesivir

For further evaluation, all the PZA-like molecules, together with PZA and RDV, were individually docked against the catalytic site of SARS-CoV-2 RdRp. The phosphoribosylated forms of each screened molecule were docked and analyzed, based either on their docking score as shown in **Figure 3**. The results of the intact (X), ribosylated (XR), mono-phosphoribosylated (XRMP), di-phosphoribosylated (XRDP) and tri-phosphoribosylated (XRTP) forms. Of all of the intact forms, compound **12** showed the lowest estimated BE (−5.72 kcal/mol) compared to all of the other screened compounds. Its estimated BE was better than that of the PZA, RDV and FPV (−4.06, −4.04 and −4.31 kcal/mol, respectively). The ribosylated compound **11** showed the lowest estimated BE (−5.53 kcal/mol) which is better than that of the estimated BE of ribosylated PZA, RDV, and FPV (−4.57, −5.17 and −4.88 kcal/mol, respectively). The mono-phosphoribosylated compound **12** showed the lowest estimated BE (−7.06 kcal/mol) which is better than that of the estimated BE of mono-phosphoribosylated PZA, RDV and FPV (−4.66, −5.95 and −4.81 kcal/mol, respectively). The di-phosphoribosylated compound **12** showed the lowest estimated BE (−7.00 kcal/mol) which is better than that of the estimated BE of di-phosphoribosylated PZA, RDV and FPV (−5.40, −3.62 and −4.23 kcal/mol, respectively). The tri-phosphoribosylated compound **2** or FPV showed the lowest estimated BE (−6.16 kcal/mol) which is better than that of the estimated BE of tri-phosphoribosylated PZA and RDV (−4.86 and −4.28, respectively).

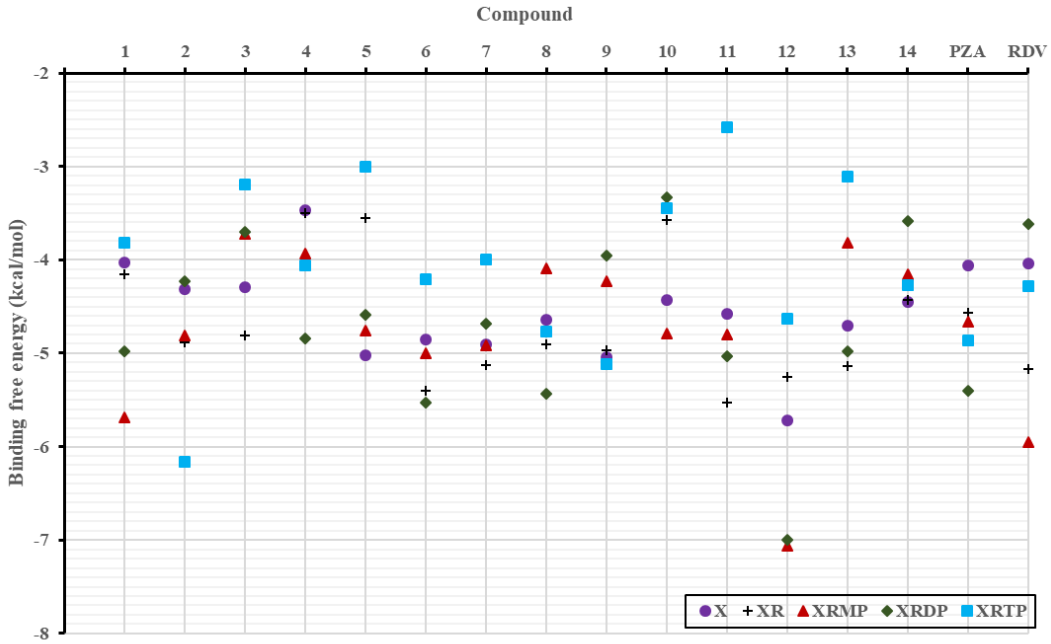


Figure 3 Estimated docking score of PZA-like molecules, targeting the SARS-CoV-2 RdRp in intact forms (X), ribosylated forms (XR), mono-phosphoribosylated forms (XRMP), di-phosphoribosylated forms and tri-phosphoribosylated forms (XRTP) (from AutoDock 4.2.6 software).

The best form of PZA, interacting with SARS-CoV-2 RdRp, was the di-phosphoribosylated form, with an estimated BE of −5.40 kcal/mol. Moreover, the docking analysis of current RdRp inhibitors for the treatment of COVID-19, including compound **2** (FPV) and RDV, was also determined. The best forms of

FPV and RDV interacting with SARS-CoV-2 RdRp were the tri-phosphoribosylated and mono-phosphoribosylated forms, with estimated BE of −6.16 and −5.95 kcal/mol, respectively. Compound **2**, 6-fluoro-3-hydroxy-pyrazine analog (FPV) showed the relevant binding affinity against SARS-CoV-2 RdRp according to the previous studies which supported our hypothesis [25-27]. However, other pyrazine analogs (compound **1-10** and compound **13-14** except compound **2**) showed fluctuating estimated inhibition constant values. This finding indicated that the pyrazine core structure might not show any relevant interaction with SARS-CoV-2 RdRp.

According to molecular docking analysis, the most likely candidate compound from which to develop a new antiviral agent, *via* SARS-CoV-2 RdRp inhibition, was compound **12**, possessing the best binding affinity toward RdRp in most forms (intact, mono-phosphoribosylated, and di- phosphoribosylated forms). The binding analysis of the most suitable candidate in this study, compound **12**, was examined and is shown in **Figure 4**. Binding mode analysis of the intact form of compound **12** against SARS-CoV-2 RdRp, revealed that the heteroatoms of compound **12** interact *via* H-bonding interaction with the H-bond donor or acceptor amino acids, including Lys621 and Ser795, with a bond distance of 2.90 and 3.15 Å, respectively. The ribosylated compound **12** binds with SARS-CoV-2 RdRp inside the active site, by H-bond interactions with His439, Ser814 and Asp833 residues with bond distances of 3.25, 3.21 and 3.03 Å, respectively, and the estimated BE was −5.25 kcal/mol. The mono-phosphoribosylated compound **12** binds with SARS-CoV-2 RdRp in a good conformational position inside the catalytic site, by H-bond interactions with Tyr619, Lys621, Cys622 and Asp623 residues. The nitrogen atom of the pyrimidine ring in the mono-phosphoribosylated compound **12** interacts with the nucleoside of template-primer RNA. The di-phosphoribosylated compound **12** binds with SARS-CoV-2 RdRp in a good conformational position inside the catalytic site, by H-bond interactions with Lys551, Arg555, Lys621 and Asp760 residues with bond distances of 2.69, 3.02, 2.98 and 2.70 Å, respectively. The tri-phosphoribosylated compound **12** binds with SARS-CoV-2 RdRp inside the active site again by H-bond interactions with Lys551, Tyr619, Lys621, Cys622 and Asp760, and the estimated BE was −4.63 kcal/mol.

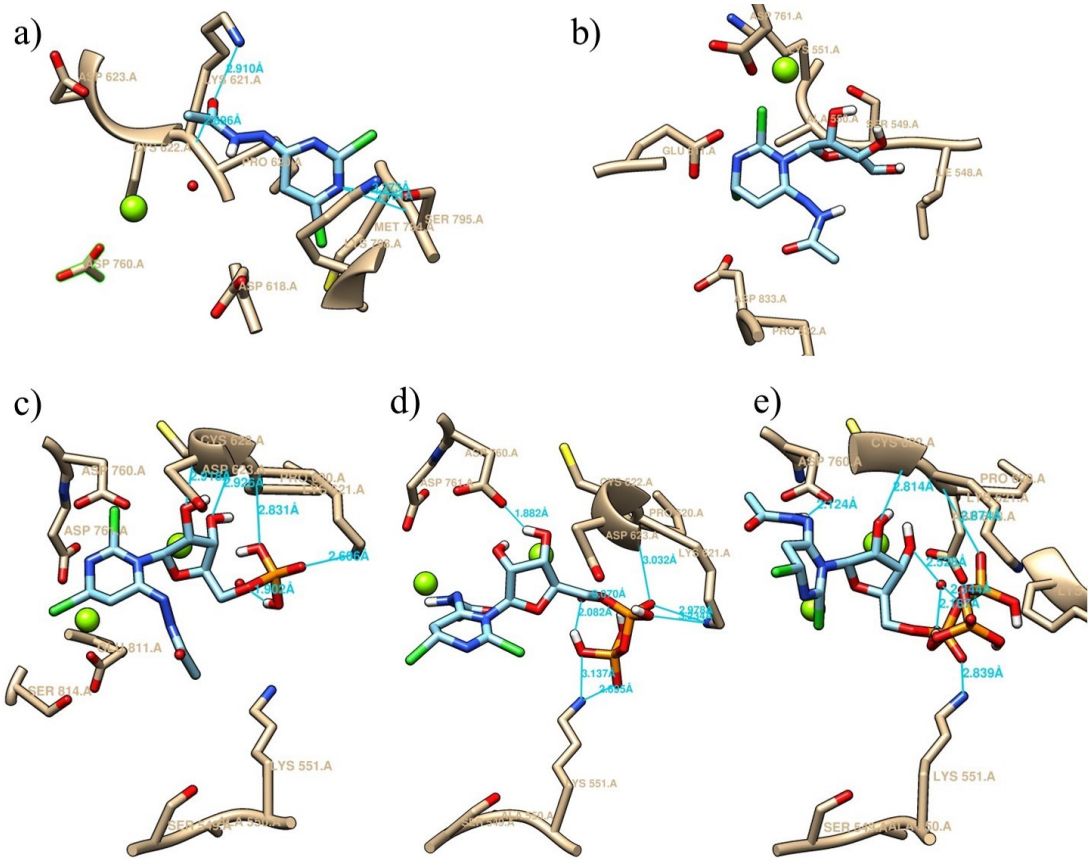


Figure 4 Binding mode analysis of compound **12**, a) ribosylated form, b) mono-phosphoribosylated form, c) di-phosphoribosylated form, d) and tri-phosphoribosylated form, e) toward SARS-CoV-2 RdRp (7BV2) (from Chimera software).

Compound **12** that exhibited the best binding affinity against the SARS-CoV-2 RdRp, was roughly docked against the nucleotide base in the template-primer RNA by using AutoDock 4.2.6 software. The pyrimidine core structure of compound **12** may bind to the uracil nucleoside of the RNA template, which is a superior option to that of other nucleosides **(Figure 5)**. Both chlorine atoms of compound **12** interact with oxygen atoms of the uracil pyrimidine ring with atomic distances of 2.978 and 3.208 Å which are within acceptable range. The nitrogen pyrimidine ring of compound **12** interacts with the nitrogen pyrimidine ring of the uracil ring with atomic distances of 3.007 Å. This finding indicates that compound **12** may act as an adenine analog of SARS-CoV-2 RdRp.

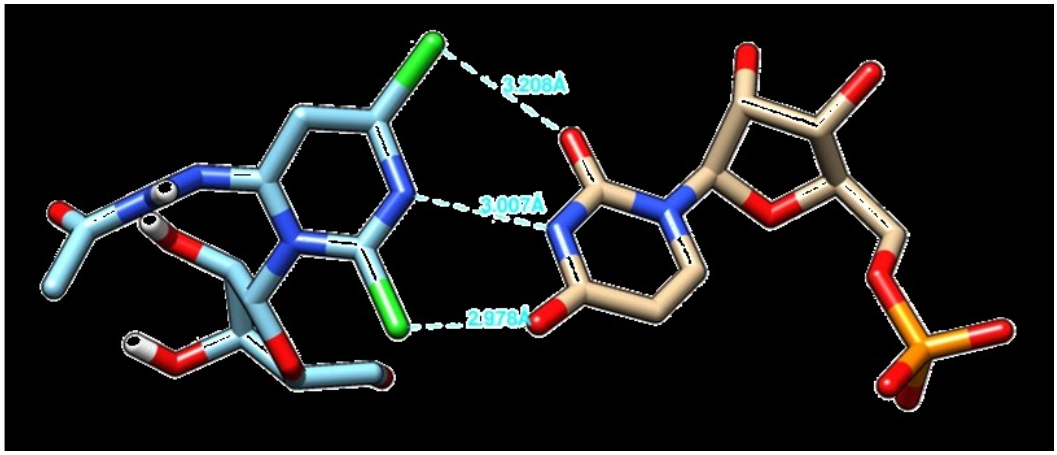


Figure 5 Binding mode analysis of compound 12 (Left) toward uracil nucleic acid (Right). The blue line denotes H-bonding interaction (from AutoDock 4.2.6 software).

The major findings of this study are as follows: 1) compound **12**, appears to be a promising compound that effectively binds to SARS-CoV-2 RdRp in most of its phosphoribosylated forms; 2) it might have a possible role in inhibiting viral replication, so may be considered as a possible antiviral agent to fight SARS-CoV-2 infection; 3) this rationale of *in silico* prediction of PZA-based molecules is an approach that can be used to screen and design drug candidates, which is less time consuming and provides essential information, to prioritize drug discovery and development processes in the ongoing COVID-19 situation [15]. However, this computational study assumed that all of the preselected molecules required their conversion intracellularly into their active forms. This study did not perform molecular dynamics to describe accuracy of bonding patterns. Therefore, preclinical studies would be required to confirm *in silico* hypothesis, their efficacy and toxicity.

Conclusions

This computational analysis was performed through chemical similarity analysis, ADME analysis, and structure-based virtual screening of PZA core structure. The results revealed that compound **12**, having a dichloro-pyrimidine core structure, showed the best binding affinity towards SARS-CoV-2 RNA-dependent RNA polymerase in most of its forms of phosphoribosylated active molecules, with higher values than FPV, whilst also exhibiting acceptable physicochemical characteristics and pharmacokinetic properties. This result suggests that *in silico* analysis has proved to be an advantageous tool for drug design, reducing the time required to ratify rational strategies for anti-COVID-19 drug development. However, preclinical studies are required to further evaluate its efficacy and toxicity.

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