Blocking catalytic metal ion binding sites to develop antiviral therapies: exemplified using SARS-CoV-2 and HIV

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Article

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Abstract

Despite widely disseminated COVID19 vaccinations, infections continue. Treating COVID19 by inhibition of the RNA dependent-RNA polymerase of the causative virus, SARS-CoV2, is a helpful strategy. In this manuscript we describe a method of inhibiting SARS-CoV2 and other viral polymerases by blocking the binding of catalytic metal ions to the catalytic site in these polymerases. We performed an ~900,000 small molecule, in silico virtual screening for small molecule compounds that would bind the metal ion site on nsp12; the SARS-CoV-2 replicase. We also tested seven of the best scoring "hit" compounds in an in vitro activity assay for HIV reverse transcriptase. We found that even though the in silico screen for compounds had be targeted at nsp12, our compounds, at 10 µM, still had up to 24.4% inhibitory activity on HIV-RT in an enzymatic assay. Docking to a model of HIV-RT found that these seven molecules dock in overlapping pockets an near the catalytic metal ion binding site, occluding it. Presumably these molecules inhibit HIV-RT in the same fashion they were intended to inhibit SARS-CoV-2's nsp12. Further development of compounds that target catalytic metal ion binding sites can generate antivirals for a variety of viruses or even broad-spectrum antiviral therapeutics.

Introduction

COVID19 is a viral respiratory illness; a pandemic of which began in early December of 2019 [1, 2]. The now widely recognized illness is caused by the SARS-CoV2 virus. Vaccines are now available in the United States. Other countries have also developed vaccines. However, these may not be the best option for all individuals due to the potential autoimmune responses and other complications from vaccines [5]. Additionally, the available COVID19 vaccines may have limited effectiveness against new strains of the SARS-COV2 virus, such as Omicron [6]. Consequently, a threat from COVID19 and a need for effective therapeutics exists. Additionally, as suggested by the findings in this manuscript, COVID19 therapeutics may be able to be repurposed for the treatment of other viral infections.

SARS-CoV2, the virus that causes COVID-19, is a new member of the Orthocoronavirinae subfamily; genus Sarbecovirus. It is a novel coronavirus, and though homologous, it is not MERS-CoV or SARS-CoV [4]. This novel coronavirus has a similar replication mechanism as other coronaviruses, West Nile virus, Marburg virus, Ebola virus, dengue virus, and hepatitis C virus (HepC) which utilizes a positive-sense, single strand of RNA to encode its genome. The genome of SARS-CoV2 is replicated by non-structural protein 12 (nsp12), an RNA-dependent RNA polymerase (RdRp). This RNA dependency is like reverse transcriptase in HIV and HCV replicase (NS5B) in HepC.[7, 8]. The RdRp mechanism is also seen in other positive strand RNA viruses, such as rhinoviruses, that cause common colds, and, not surprisingly, the homologous MERS-CoV and SARS-COV. [7, 8]. An existing drug, remdesivir, targets the SARS-CoV-2 virus's nsp12 [9, 10].

Inhibition of viral polymerases has proven effective in treating viral infections, such as in the cases of HIV and HepC, and the effectiveness of this strategy has led to compassionate use of the drug, remdesivir, in the case of SARS-CoV2 [11–13]. While using existing drugs speeds the treatment to the field more quickly, the threat of second, third, and even more subsequent waves of COVID19, or other pandemic coronaviruses, necessitate treatments specifically targeted to coronaviruses and that have increased potency. This study sought novel, non-nucleoside inhibitors of SARS-CoV2's nsp12. This strategy will allow for incorporating molecules from a greater chemical space to find more potent coronavirus nsp12-specific lead molecules. Additionally, we discovered that by targeting the viral polymerase's catalytic metal ion binding areas, we found compounds were also partially effective against HIV1 reverse transcriptase displaying up to 24.4% inhibition of HIV-RT at 10 µM compound. These compounds could be helpful against COVID19 and may be developable into a wider spectrum of antivirals.

Materials And Methods

The virtual screening we carried out was performed before a SARS-CoV2 nsp12 macromolecule experimentally solved structure was available and used a homology model made from SARS-CoV nsp12 6nur.pdb, chain A [14]. A methodology like that posted online at http://home.fatsilicodatapharm.com was used for the virtual screening. Streamlined and better resourced versions of these methods may be useful in future viral outbreaks.

Homology modeling

The macromolecule model of SARS-CoV2 nsp12 was created using Bioinformatics Toolkit [15] as part of a personal interest project while the author was in an academic role. The template used was the cryo-EM structure of SARSs-CoV nsp12, 6nur.pdb, chain A [14]. At the time of beginning of our preliminary screening (circa March 15, 2020), there were no available models of SARS-CoV2 nsp12. A BLAST alignment of the R1ab's sequence from https://viralzone.expasy.org/8996 [16] was done using HHpred. This showed the region of the polypeptide homologous to the SARS-CoV nsp12. A homology model was then produced using MODELLER. An unstructured region of the N-terminus was deleted in the finished model used. The homology model had a backbone rmsd of 0.66 Å when fit to the first SARS-CoV2 cryo-EM structure, 6m71.pdb in DEEPVIEW [17, 18].
The models were prepared for docking in AutoDock MGL Tools 1.5.6 [19]. The SARs-CoV2 nsp12 and HIV-RT docking model's hydrogen were added and then merged into nonpolar hydrogen. Gasteiger charges were calculated and AutoDock4 atom types were added. The nsp12 docking model was then saved as a .pdbqt file. For the HIV RT model 1rtd.pdb1, the structure's biological assembly had RNA and small molecules removed, leaving only chains A and B. The HIV RT model was then processed similarly as described.

Virtual screening ligand preparation

Clean Drug Like sets 50 through 56 were downloaded from the ZINC12 Database (zinc12.docking.org) [20]. The compressed .mol2 files were decompressed and then split using Raccoon [21], available from the Olson laboratory, which also produces AutoDock. A Linux script that employs nested "for each" loops and files in AutoDock MGL Tools 1.5.6, based on scripts in the tutorial, UsingAutoDock4forVirtualScreening_v4.pdf from Scripps Institute, was used to set up ligand files in the proper .pdbqt file for screening. This script also split the ZINC ligand files into 100 folders, numbered 0 to 99, based on the first two numbers in the ZINC Database entry number.

Running the virtual screening

AutoDock Vina [22] was used for the virtual screening of ~900,000 compound ligand files. Each set was run by a separate instance of a Linux shell script that employed nested "for each" loop that traversed through the folders set up in the "Virtual screening ligand preparation" section to run AutoDock Vina on each individual, processed ZINC .pdbqt ligand file. A 16 by 16 by 18 angstrom search area, centered at approximately CYS 623 in our model (CYS622 in 6m71.pdb), was used as our pocket of interest (POI) for the screening. Residues have been renumbered in this manuscript to reflect the now accepted numbering. The first sets to finish in the virtual screening, Clean Drug Like 50, Clean Drug Like 52 and Clean Drug Like 53 were immediately analyzed in order to purchase lead compounds. Using scripts, log files from each docking were combined, and the best score for each compound was extracted into a new file. These were then sorted. ZINC compound files scoring ~10.1 kcal/mol or better, with more negative scores being better, were included in our lists of "hit" compounds. Analysis and sorting were performed using custom shell scripts and Calc in Libre Office as available on Centos 7.

HIV-RT dockings

Structure files of the small molecules of interest in this manuscript were docked to a 20x20x20 angstrom search box centered on the alpha carbon of 1rtd.pdb1 chain A MET184 [23]. MET184 corresponds structurally to the same position on HIV-RT as CYS 623 on SARS-CoV-2 nsp12 in relation to the catalytic metal ions binding site.

Molecular Representation

Molecules in this manuscript were rendered in AutoDock MGL Tools 1.5.6 [19] or Biovia Discovery Studio Viewer [24].

DNA Polymerase Assay

We carried out polymerase activity assay of the purified wild-type HIV-1 RT using homopolymeric poly (rA/dT) 18 or heteropolymeric DNA TP, which consisted of 21-mer DNA PBS primer annealed with 49-mer U5-PBS DNA templates (3'CAG GGA CAA GCC CGC GGT GAC GAT CTC TAA AAG GTG TGA CTG ATT TTC C-5'). Assays were done in a 100-µl volume containing 50 mM Tris-HCl (pH 7.8), 100 µg ml⁻¹ bovine serum albumin, 2 mM MgCl₂, 1 mM dithiothreitol, 100 nM TP, 50 µM dNTPs, and 21 nM enzyme. The homopolymeric rA/dT₁₈ TP, the reaction mixture contained 20 µM of TTP and 2 µCi of ³H TTP and heteropolymeric TP; all four dNTPs (50 µM each) were included, with one of them being α³²P-labeled (0.4 µCi/nmol of dNTP). Reactions were performed at 37°C for varying times, in the presence and absence of selected small molecules then terminated by the addition of ice-cold 5% trichloroacetic acid (TCA) containing 5 mM inorganic pyrophosphate. We collected insoluble acid materials on Whatman GF/B filters and counted for radioactivity in a liquid scintillation counter.

Results

Virtual screening and compound of interest selection

Our homology modeling resulted in a model that shifted one residue compared to the now accepted numbering for SARS-CoV-2 nsp12. Residue references in this manuscript have been adjusted to reflect the currently accepted numbering. As stated in the "Materials and Methods" section, our model has a RMSD deviation of 0.66 Å when fit to the first SARS-CoV2 cryo-EM structure, 6m71.pdb in DEEPVIEW [17, 18]. We targeted the search area depicted in Fig. 1 as our POI. This search area included the surmised metal ion binding sites for SARS-CoV-2. In our screening of about 900,000 compounds from ZINC12's "Clean Drug Like" we counted small molecule compounds from these sets that scored the same as or better than ~10.1 kcal/mol in AutoDock Vina, with more negative scores being better, as hit molecules. This amounted to the best scoring < 0.05% of the screened small molecule compounds or about 400 to 500 molecules.
From this top 0.05% we were able to test a selected, seven of these small molecule compounds (Enamine: Kiev, Ukraine) in an HIV1 RT activity assay. We have analyzed these seven small molecules in this manuscript. They are ZINC8938064 (2-[[2-(3,4-dihydro-2H-1,5-benzodioxepin-7-yl)pyrrolidin-1-yl)methyl]-5-(5-methyl-3-phenyl-1,2-oxazol-4-yl)-1,3,4-oxadiazole), ZINC12545143 (2-(4-oxo-3H-phthalazin-1-yl)-N-(2-phenylquinolin-4-yl)acetamide), ZINC12939070 ((2S)-1-(2,2-dimethyl-3-oxo-4H-quinoxalin-1-yl)-1-oxopropan-2-yl)-9-oxo-2,3-dihydro-1H-pyrrolo[2,1-b]quinazoline-6-carboxylate), ZINC27385605 ((3S)-3-(1,3-benzoxazol-2-yl)piperidin-1-yl)-[3-(2,3-dihydroindol-1-ylsulfonyl)phenyl]methanone), ZINC12939070 ((2S)-1-(2,2-dimethyl-3-oxo-4H-quinoxalin-1-yl)-1-oxopropan-2-yl)-9-oxo-2,3-dihydro-1H-pyrrolo[2,1-b]quinazoline-6-carboxylate), ZINC27385605 ((3S)-3-(1,3-benzoxazol-2-yl)piperidin-1-yl)-[3-(2,3-dihydroindol-1-ylsulfonyl)phenyl]methanone), ZINC30280395 ((3S)-3-(1,3-benzoxazol-2-yl)piperidin-1-yl)-[3-(2,3-dihydroindol-1-ylsulfonyl)phenyl]methanone), ZINC12733075 (N-dibenzofuran-2-yl-4-(4-oxo-1H-quinazolin-2-yl)butanamide), and ZINC29112491 ([3-(3,4-dihydro-1H-isouquinolin-2-ylsulfonyl)phenyl]-[2-(4-fluorophenyl)morpholin-4-yl]methanone). Figure 2 shows chemical drawings of the compounds in this manuscript. All are non-nucleotides. Interestingly, while each contains at least one phenol ring, each also represents a novel scaffold in different chemical space.

Compounds of interest and SARS-CoV2 nsp12 residue interactions

As part of the analysis of the hit molecules our virtual screening Table 1 shows the interacting residues predicted by our dockings in AutoDock Vina and detected through visualization in Discovery Studio Viewer [22, 24]. VAL166, ASP452, TYR455, LYS545, ARG553, ALA554, ARG555, THR556, VAL557, ALA558, PR0620, LYS621, CYS622, ASP623, ARG624, THR680, SER681, ASP760, and LYS798 were each contacted by at least one of the small molecule compounds. TYR455, ASP623, and ARG624 were predicted to be contacted by all of our small molecule compounds in each compound’s best docking pose. These residues are shown in red on the sequence alignment of nsp12 and HIV-RT in S1.

Figure 3 shows each compound in the POI as predicted by our dockings and depicted in Discovery Studio viewer. The center of our search area (CYS622) is shown in yellow stick. Our ubiquitous interacting residues TYR455, ASP623, and ARG624 are shown in black, red, and blue stick, respectively.

Inhibition of HIV-RT activity

Inhibition of HIV-1-RT by our small molecule compounds in an enzymatic activity assay of HIV1 reverse transcriptase is illustrated in Fig. 4. 10 µM of some of our small molecule compounds can inhibit HIV1-RT activity by as much as 24.4%, such as the case of ZINC27385605, that reduced activity to 75.6%. Experiments were carried out using either Mg$^{2+}$ or Mn$^{2+}$ as the catalytic ion. Data suggests that some compounds may be more effective against different catalytic ions being used for polymerase activity. For example, Mn$^{2+}$ZINC29112491 reduced activity by less than 7% versus DMSO control when Mn$^{2+}$ was the catalytic metal ion but reduced RT activity by nearly 20% when Mg$^{2+}$ was the catalytic ion present.

Small molecule docking to HIV-RT model

Upon running the docking of our 7 small molecules of interest to a model of HIV RT, we observed all seven molecules dock in overlapping pockets in close proximity to ASP185 and ASP186 that stabilize metal ion binding in reverse transcriptase (Fig. 5) [23]. This is evidence that our 7 compounds of interest all inhibit SAR CoV2 nsp12 and HIV-RT by the same mechanism. They also have lower, but still respectable AutoDock Vina scores ranging from ~ 7.8 to -9.1 kcal/mol (Table 2).

Discussion

Targeting a viral polymerase’s metal ion binding ability could be a practical strategy for therapeutically treating that virus’s corresponding infection. In this manuscript, we showed evidence that compounds designed to target the catalytic metal ion binding sites in SARS-CoV-2’s replicase, nsp12, in a virtual screening can partially inhibit HIV1 reserve transcriptase. This leads to the hypothesis that by targeting these catalytic metal ion binding sites in viral polymerases, we can find wide spectrum antivirals to increase our readiness for future viral pandemics.

The theoretical small molecule docking prediction that our compounds have some interacting residues in common, but not all, shows a strength of our not using different chemical space. Mutation of a residue may exclude some of our compounds from being effective but would presumably not affect all of the compounds’ binding to the same extent, leaving several other options for inhibiting viral polymerases.

This study has demonstrated that targeting the catalytic metal ion binding site on the viral replicase via properly executed virtual screening, coupled with in vitro verification testing is an effective strategy for finding a broad spectrum of potentially therapeutic inhibitors.

Declarations

Acknowledgements
Special thanks to Jason Kaelber and the Gaokerena Institute for Molecular Biology for funding that made this study possible, and to Rutgers University, where the corresponding author was employed at the beginning of this study. Thanks to Oleg Trott for assistance troubleshooting the HIV-RT file, and Abena Amankwaa, Joana Lopez, and T.W.C.'s other laboratory rotation students at Kean University for useful discussions.

**Author Contributions**

V.P. assisted in the experimental laboratory and in the writing of the manuscript. T.W.C secured funding, performed computational and experimental components of the study, and wrote the manuscript.

**Data Availability**

The datasets generated and/or analysed during the current study are not publicly available due to ongoing intellectual property filings but are available from the corresponding author on reasonable request.

**Fair Disclosure Statement (Patent Pending)**

Small molecule compound(s) discussed in this manuscript are covered under US patent application 17/472,647 invented and owned by Thomas W. Comollo, Ph.D, with a projected publication date of 3/16/2023.

**References**


Tables

Table 1. SARS-CoV2 non-structural protein 12 residues predicted to interact with our selected hit compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Vina score (kcal/mol)</th>
</tr>
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<tr>
<td>nsp12 residue</td>
<td>ZINC08938064 -10.4</td>
</tr>
<tr>
<td>VAL166</td>
<td>X</td>
</tr>
<tr>
<td>ASP452</td>
<td>X</td>
</tr>
<tr>
<td>TYR455</td>
<td>X</td>
</tr>
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<td>LYS545</td>
<td>X</td>
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</tr>
<tr>
<td>SER681</td>
<td>X</td>
</tr>
<tr>
<td>ASP760</td>
<td>X</td>
</tr>
<tr>
<td>LYS798</td>
<td>X</td>
</tr>
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</table>

Table 2. Scores of best scoring Vina docking to HIV-RT model conformations depicted in Figure 5.
### Figures

**Figure 1**

**Search area used in our virtual screening.** A. Homology model of SARS-CoV2 nsp12 and search area used in the virtual screening rendered in AutoDock MGL Tools 1.5.6.

<table>
<thead>
<tr>
<th>Small Molecule nsp12 Hit</th>
<th>Vina Score HIV-RT POI (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZINC12545143</td>
<td>-8.2</td>
</tr>
<tr>
<td>ZINC30280395</td>
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</tr>
<tr>
<td>ZINC29112491</td>
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<tr>
<td>ZINC27385605</td>
<td>-8.5</td>
</tr>
<tr>
<td>ZINC12939070</td>
<td>-8.3</td>
</tr>
<tr>
<td>ZINC12733075</td>
<td>-7.8</td>
</tr>
</tbody>
</table>
Figure 2

Small molecule "hits" featured in this manuscript. Selected "hits" from virtual screening, each using a different model of SARS-CoV2's nsp12, were purchased from Enamine Ltd (Kiev, Ukraine) and were characterized in a viral polymerase activity assay utilizing HIV reverse transcriptase. The small molecules from that pool, featured in this manuscript, have their structures and chemical names listed here. (patent pending)
Small molecule *hits* featured in this manuscript docked to snp12. Selected compounds were purchased from Enamine Ltd (Kyiv, Ukraine) the top docking conformations, returned by AutoDock Vina, of each are shown here in overlapping docking pockets when simulation was performed with our nsp12 model.
Figure 4

Inhibition of HIV1 - Reverse Transcriptase (HIV-RT) by our small molecule compounds. Modest inhibition was seen with all of our tested compounds compared to DMSO vehicle alone, some even exhibited greater than 20% inhibition of HIV-RT. Top shows results with Mn$^{2+}$ as catalytic metal ion. Bottom shows results with Mg$^{2+}$ as catalytic metal ion. Please note that the screening was performed for nsp12 from SARS-CoV2 while this assay is performed using HIV reverse transcriptase, a different viral polymerase.
Figure 5

Small molecule "hits" featured in this manuscript docked to HIV-RT structure, 1rtd.pdb. The small molecule compounds featured in this manuscript were docked to an HIV-RT macromolecule model made from 1rtd.pdb. In line representation are the top conformations from each molecule docking. In ball and stick are MET184, the center of our search area, and ASP185 and ASP186 that stabilize the binding of the catalytic magnesium ions.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- S1.pdf