AFRICA ENVIRONMENTAL REVIEW Journal
ISSN: 1727-8341

AER Journal Volume 5, Issue 1, pp. 261-277, June, 2022

RESEARCH ARTICLE
Available Online at *http://ojs.uoeld.ac.ke/*

Discovery of Withaphysalin A and Limonin as Potential SARS-COV2 Papain-Like Protease Inhibitors

S. S. Barasa*, W. O. Okenyo and T. A. O. Akenga
Department of Chemistry and Biochemistry, University of Eldoret, Kenya
**Corresponding Author's Email: sbarasa@uoeld.ac.ke*

Abstract

Protease inhibitors are strategically and functionally important components of drug-hunters' arsenal against pathogenic viruses including SARS-COV2, SARS-COV, MERS, Ebola and HIV. Drug discovery efforts reported in this work target the papain-like protease (PLpro) alias non-structural protein 3 (nsp3) of SARS-COV2. An in-house database comprising of 6264 phytochemicals derived from 1560 Kenyan medicinal plants, as well as the COCONUT-Mitishamba database provided phytochemical scaffolds used in this in silico structure-based virtual screening study. Overall, 170 phytochemicals from our in-house database and 58 phytochemicals from the COCONUT-Mitishamba database had desirable binding affinities of less than -9.0 kcal/mol. Withaphysalin A (11.5 kcal/mol) and Limonin (-11.1 kcal/mol) are the best compounds targeting the nsp3 of SARS-COV2 from our in-house database. We studied protein-ligand interactions of top-binding molecules to gain insight on their potential modulatory effects on the SARS-COV2 virus. Although experimental validation of the results obtained and other further tests need to be done, these findings will accelerate the drug design and development process.

Keywords: SARS-CoV2, Virtual Screening, nsp3, Protein-Ligand Interactions, Papain-Like Protease, PLpro

INTRODUCTION

Drug discovery efforts against SARS-COV2, although unrelenting, are yet to bear tangible and variant-proof outcomes. Research and development (R&D) quest for a cheap, orally available pill that is effective against existing and emerging SARS-COV2 variants is ongoing but yet to hit this constantly evasive target. This is despite the elucidation of a repertoire of viral as well as humanoid druggable targets for COVID-19 (Gil et al., 2020; Khataniar et al., 2022; Raj et al., 2021). Due to their inherent pharmacodynamic and pharmacokinetic liabilities, many COVID-19 drug candidates have thus far failed to progress to beyond phase-3 clinical trials or approvals (Stader et al., 2020). This has led to wastage of time and money in the race against finding a lasting cure for COVID-19. Efforts to repurpose existing drugs such as remdsivir, chloroquine, hydroxychloroquine, ivermectin, lopinavir and ritonavir have met limited success as effective COVID-19 drugs. (Xu et al., 2021; Khataniar et al., 2022).

SARS-CoV-2 encodes two proteases that are tasked with the cleavage of viral polyproteins (pp1a and pp1b) into 16 non-structural protein (nsps).(Klemm et al 2020) One of the proteases, 3-chymotrypsin-like "main" protease (3CLpro or Mpro, encoded by nsp5) cleaves the polyprotein into mature nonstructural proteins (nsp4, nsp5, nsp6, nsp7, nsp8, nsp9, nsp10, nsp11, nsp12, nsp13, nsp14, nsp15 and nsp16). The other viral protease, the papain-like protease (PLpro, encoded within nsp3), cleaves the polyprotein into three mature nonstructural

proteins (nsp1, nsp2 and nsp3). (Klemm et al., 2020; Jiang et al., 2022) Apart from the proteolytic functions, viral proteases serve to dampen the hosts immune response through the removal of ubiquitin and ubiquitin-like modifications.(Klemm et al., 2020; Pitsillou et al., 2020) Normally, in the event of viral infection, the inflammatory and interferon systems of the host are upregulated in order to respond to the attack. Dampened immune responses occasioned by these viral proteases result in the calamitous 'cytokine storms' of COVID-19 (Mattoo et al., 2022; Moustaqil et al., 2021).

Both 3CLpro and PLpro have been the subject of previous drug discovery efforts against COVID-19 (Jade et al., 2021; Anirudhan et al., 2021). In a case of failed efficacy, a repurposed drug combination of viral protease inhibitors lopinavir and ritonavir was ineffective in two large randomized controlled trials in hospitalized COVID-19 patients (Recovery, 2020). In a move granting credence to continued R&D efforts in seeking potential treatments against COVID-19, the drug company Pfizer unveiled two new 3C-like protease (3CLpro) inhibitors that differ in their bioavalibility profiles. Nirmatrelvir (PF-07321332) is administered orally to patients while Lufotrelvir (PF-07304814) can only be administered intravenously.(Boras et al., 2021) In December 2021, Nirmatrelvir (co-packaged with ritonavir), was granted emergency FDA authorization for the treatment of patients with COVID-19 (Lamb et al., 2022).

PLpro is particularly attractive as a polypharmacological COVID-19 drug target. This is because, a part from promoting the human host's immunity against the virus, inhibition of PLpro suppresses viral infection making SARS-COV2 less transmissible (Klemm et al., 2020) This makes PLpro a dual-pronged target worthy of pursuit. Recent and current drug discovery efforts targeting PLpro of SARS-COV2 exist.(Jamalan et al., 2021; Ma et al., 2021; Shin et al., 2020; Virdi et al., 2020; Lim et al., 2021; Yan & Gao, 2021). These efforts have witnessed the discovery of potential small molecule inhibitors of the PLpro of SARS-COV2. Notable among these is a naphthalene based drug previously developed as SARS-COV PLpro inhibitor (GRL0617), (Fu et al., 2021) a peptidomimetic inhibitor (VIR251), (Rut et al., 2020) a phenylthiophene inhibitor (XR8-23),(Shen et al., 2021) a phase 1 clinical trial antineoplastic drug (YM155),(Zhao et al., 2021) a selenoorganic drug (ebselen) (Weglarz-Tomczak et al., 2021) and a component of component of a Chinese herbal preparation (tanshinone).(Jiang et al 2022) (Figure 1)

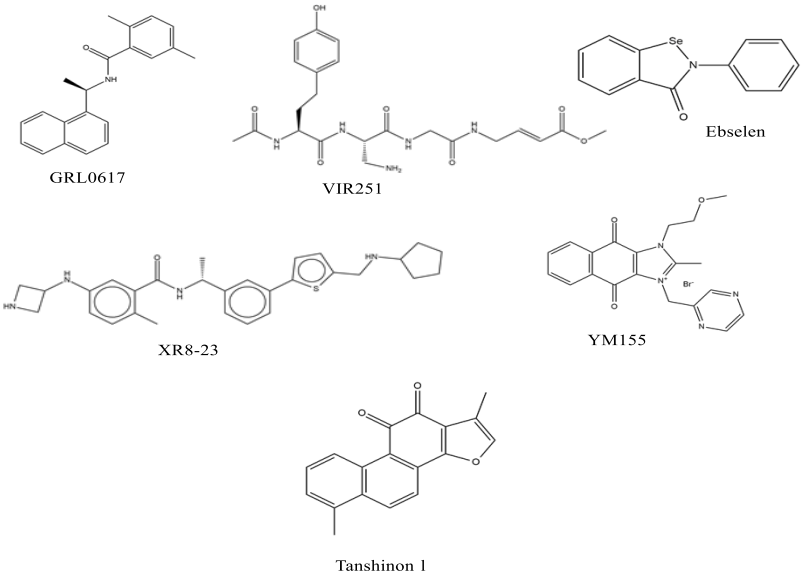


Figure 1: Structures of potential PLpro SARS-CoV-2 inhibitors from current literature.

To date, no SARS-COV2 PLpro inhibitor has been approved. African medicinal plants constitute a novel yet untapped source of potential drug leads against the SARS-COV2 and other pathogenic viruses. Against this backdrop and in line with our research programme on drug discovery using Kenyan medicinal plants, we decided to commence a structure-based virtual screening project targeting the papain-like (PLpro) protease of SARS-COV2. Herein, we report the discovery of Withaphysalin A and Limonin as potential SARS-COV2 papain-like protease inhibitors. Protein-ligand interactions of the top ranked compounds (based on their binding affinity) are then further analyzed to rationalize the how these phytochemicals may potentially modulate the PLpro drug target. This study provides a basis for further computational and experimental investigations, including *in vitro* and *in vivo* studies.

MATERIALS AND METHODS

Preparation of the SARS-CoV-2 Nsp3 Papain Like Protease

The crystal structure of SARS Cov-2 nsp13 helicase enzyme was retrieved from the Protein Databank (PDB) with a PDB ID of 6W9C, at a good resolution of 2.70Å. The structure was then treated in Pymol software (Schrodinger & DeLano, 2020) by addition of hydrogen atoms, removal of co-crystalized ligands, and fixing of the sidechains.

Structure-Based Drug-Likeness Test

A set of Kenyan medicinal plants reported to have antiviral activity were compiled. From these plants, literature searches were done to obtain the small molecules present in them. These small molecules were then uploaded to the SwissADME online software to select only the drug-like compounds. The Lipinski rule of five was applied in selecting the candidates for virtual screening (Daina et al., 2017)

Structure-Based Virtual Screening

Spatial data files (sdf) were downloaded from our in-house database as well from the COCONUT-Mitishamba database.(Sorokina et al., 2021) Only the small molecules that passed the drug-likeness test were used in the docking process. The structure of Nsp3 papain like protease macromolecule was obtained from the Protein databank. An automated in-built docking engine i.e., Pyrx software was utilized to screen the

compounds that had been prepared for docking (Dallakyan & Olson, 2015).

The Protein databank for the Nsp3 papain like protease macromolecule was 6w9c (Osipiuk et al., 2021). The compounds were ranked based on the approximations of their respective binding affinities (in kcal/mol). The top hits were selected and further post docking analysis was done using a different docking program i.e., Autodock Vina (Trott Olson, 2010).

Molecular Dynamics Simulations

To understand the molecular dynamics of the nsp3 papain like protease, the protein was subjected to molecular dynamics simulations using the GROMOS 43 force field within Gromacs (Abraham et al., 2015) In this regard, calculations were done to determine the number of hydrogen bonds, Solvent Accessible Surface Area (SASA), root mean square fluctuation (rmsf), root mean square deviation (rmsd), and the radius of gyration (Rg).(Abraham et al., 2015).

RESULTS AND DISCUSSION

Structure of Nsp3 Papain-Like Protease

Figure 2 shows our Pymol (Schrodinger and DeLano, 2020) rendering of the crystal structure of SARS-COV2 PLpro. The crystal structure was solved by Osipiuk et al (2021) and deposited within the protein data bank (PDB id 6W9C). As shown, the nsp3 structure comprises of at least six domains.

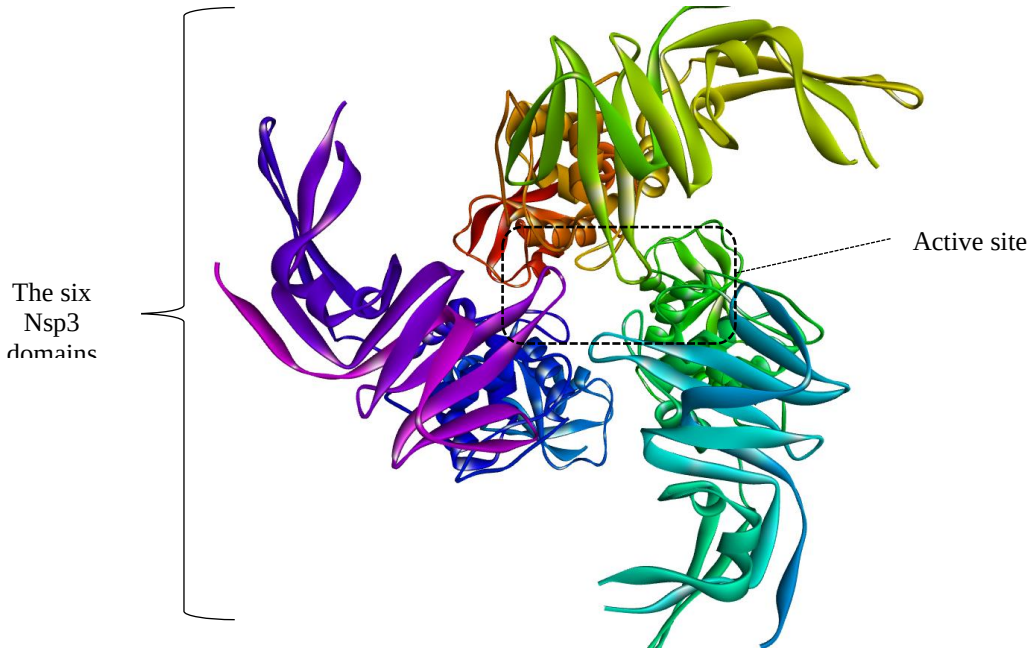


Figure 2: Structural representation of SARS-CoV-2 Nsp3 Papain-Like Protease.

Databases of Compounds Derived from Kenyan Medicinal Plants

Our in-house database comprised of 1560 medicinal plants were compiled from the various Kenyan communities. Literature searches resulted in a total of 6264 compounds reported from these plants. The COCONUT-Mitishamba database (Sorokina et al., 2021) enabled us to download 1010 additional compounds.

SwissADME Analysis

SwissADME analysis involves prediction of adsorption, distribution, metabolism, and excretion parameters including medicinal chemistry and drug-like nature of one or a multiple of small molecules to support drug

design and development. This process reduces the number of unnecessary small molecules which have no drug-like properties (Daina et al., 2017).

All the compounds that passed this process had no violations on the Lipinski's rule of five, molecular weight or the Veber, Mugge, Egan, and Ghose filters. The SMILES of the small molecules were used for SwissADME analysis by feeding them into the online server (SwissADME) (Daina et al., 2017)

Thus, SwissADME analysis of 6264 compounds from our in-house database and 1010 compounds from the COCONUT-Mitishamba database (Sorokina et al., 2021) reduced the number of phytochemicals to 1758 and 610, respectively. These compounds had a good gastrointestinal absorption and the recorded average bioavailability score was 0.55.

Structure-Based Virtual Screening of Drug-Like Phytochemicals

From the preceding steps, 1758 compounds (derived from our in-house database) and the 610 compounds from the COCONUT-Mitishamba online database (Sorokina et al., 2021) were made available for virtual screening against nsp3 papain like protease receptor 6W9C. The receptor was prepared by evaluation of charge, building of the missing proteins, receptor grid generation, and tautomer evaluation. The virtual screening was then performed using Pyrx software.(Dallakyan & Olson, 2015).

Overall, 170 compounds (from our in-house database) and 58 small molecules from the COCONUT-Mitishamba database (Sorokina et al., 2021) recorded a binding affinity of less than -9 kcal/mol. As shown in Table 1 Withaphysalin A (with a binding affinity of -11.5 kcal/mol) is the top ranking compound from our in-house database. Table 1 also shows the docking scores and smile notations of the top 20 hits in this work. The docking scores range from -11.5 kcal/mol to -10.2 kcal/mole. This indicates that our in-house database is rich in scaffolds that are amenable to hit-to-lead progression.

In the same vein, and as indicated in Table 2, the COCONUT-Mitishamba database (Sorokina et al., 2021) has unexpectedly proven to hold a good number of hits that have favourable binding affinities against the PLpro of SARS-COV2. Indeed, the top 20 hits have binding affinities that range from -11.6 kcal/mol to -10.1 kcal/mol.

The chemical structures of the top 20 hits (Table 3) indicate compounds from a range of chemical classes including steroids, flavones, phenols, furanolactones, a benzopheanthridine alkaloid as well as a 3-hydroxy carboxylic acid. It is worth noting that steroids are overly represented in this top 20 compounds hit list. A keen observer will note that a medicinal plant *Datura metel* is a source of the three withanolide steroids in this top 20 hit list.

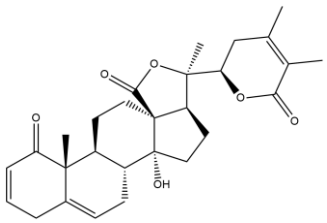
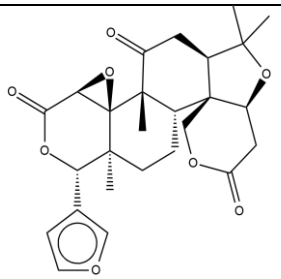
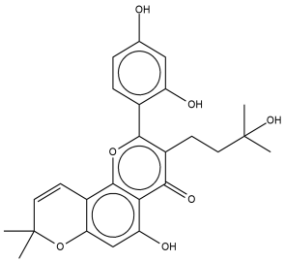
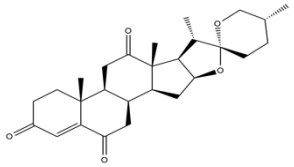
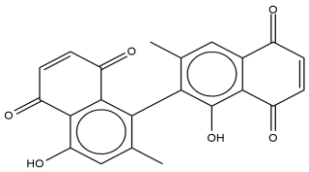
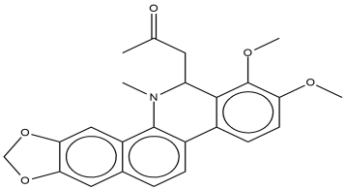
Table 1: Docking scores and smile notations of the top 20 hits

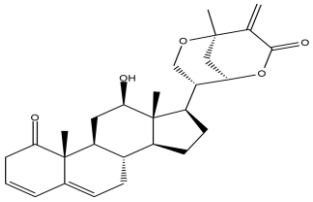
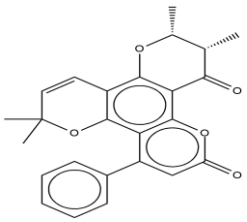
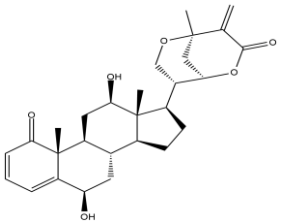
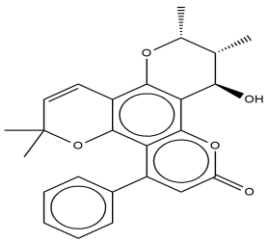
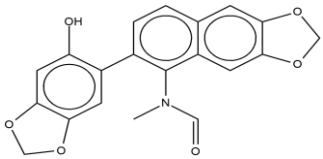
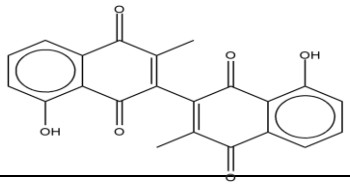
COMPOUND	Docking score	SMILES
Withaphysalin_A	-11.5	C1=CCC2=CC[C@@H]3[C@@H]([C@]2(C1=O)C)CC[C@@]12[C@]3(CC[C@@H]1[C@@](OC2=O)([C@@H]1OC(=O)C(=C(C1)C)C)C)O
Limonin	-11.1	C1[C@@H]2[C@@]([C@]34[C@@](C1)([C@@H](OC(=O)[C@H]3O4)c1cocc1)C)(C)C(=O)C[C@@H]1[C@]32COC(=O)C[C@@H]3OC1(C)C
Morusinol	-10.9	c12cc(c3c(c1C=CC(O2)(C)C)oc(c(c3=O)CCC(C)(C)O)c1ccc(cc1O)O)O
25R-Spirostan-4-ene-3612-trione	-10.8	C1C(=O)C=C2[C@](C1)([C@@H]1[C@H](CC2=O)[C@H]2[C@](C(=O)C1)([C@@H]1[C@H](C2)O[C@]2([C@H]1C)OC[C@@H](CC2)C)C)C
Isodiospyrin	-10.7	c1(c(c(c2c(c1)C(=O)C=CC2=O)O)c1c2c(c(cc1C)O)C(=O)C=CC2=O)C
8-acetonyldihydrocherythrine	-10.7	COc1c(OC)ccc2c1C(CC(=O)C)N(C)c1c2ccc2c1cc1OCOc1c2
Withametelin_M	-10.6	C1C=CC2=CC[C@@H]3[C@@H]([C@]2(C1=O)C)C[C@H]([C@]1([C@H]3CC[C@@H]1[C@@H]1CO[C@]2(C(=C)C(=O)O[C@@H]1C2)C)C)O
Inophyllum_C	-10.5	c12c(c3c(c4c1oc(=O)cc4c1ccccc1)OC(C=C3)(C)C)O[C@@H]([C@@H](C2=O)C)C
Withametelin_K.	-10.5	C1=CC=C2[C@](C1=O)([C@@H]1[C@@H](C[C@H]2O)[C@H]2[C@]([C@@H](C1)O)([C@H](CC2)[C@@H]1CO[C@]2(C(=C)C(=O)O[C@@H]1C2)C)C)C
Inophyllum_D	-10.3	c12C=CC(Oc1c1c(c3c2O[C@@H]([C@@H]([C@H]3O)C)C)oc(=O)cc1c1ccccc1)(C)C
Integriamide	-10.3	c12c(cc3c(c1)ccc(c3N(C=O)C)c1cc3c(cc1O)OCO3)OCO2
3'3'-Biplumbagin	-10.3	c12c(C(=O)C(=C(C1=O)C1=C(C(=O)c3c(C1=O)c(ccc3)O)C)C)cccc2O
Ekeberin_C1	-10.3	C1C(=O)C([C@H]2[C@](C1)([C@@H]1[C@@](C(=O)C2)([C@]23[C@@](CC1)([C@@H](OC(=O)[C@H]2O3)c1cocc1)C)C)C)(C)C
Erysenegalensein_J	-10.3	C1(C=Cc2c(O1)c(c1c(c2O)C(=O)[C@@]2([C@@H](O1)Oc1c2ccc(c1)O)O)CC=C(C)C)(C)C
Glabrocoumarin	-10.3	c1cc(c2c(c1c1c(=O)oc3c(c1)ccc(c3)O)OC(C=C2)(C)C)O
Neocaesalpin_R	-10.3	C1[C@]2([C@](C(CC1)(C)C)([C@@H]([C@@H]([C@@H]1[C@H]2C=C2C(=CC(=O)O2)[C@@H]1C)O)OC(=O)c1ccccc1)O)C
Withametelin_L.	-10.3	C1=CCC2=CC[C@@H]3[C@@H]([C@]2(C1=O)C)C[C@H]([C@]1([C@H]3CC[C@@H]1[C@@H]1CO[C@]2(C(=C)C(=O)O[C@@H]1C2)C)C)O
Hydroxyvernolide	-10.2	C1(=C)[C@H]2[C@@H](OC1=O)/C=C/1\CO[C@H]([C@]3([C@@H](CC1)O3)C[C@@H]2OC(=O)C(=C)CO)O
Praecanson_A	-10.2	c1(c(c(c2c(c1)OC(C=C2)(C)C)OC)/C(=C/C(=O)c1ccccc1)/OC)OC
3-O-methylcalopocarpin	-10.2	COc1cc2OC[C@@H]3[C@H](c2cc1CC=C(C)C)Oc1c3ccc(c1)O

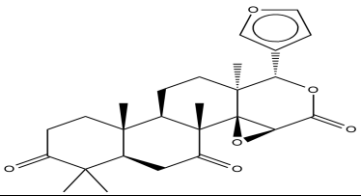
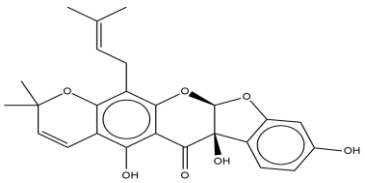
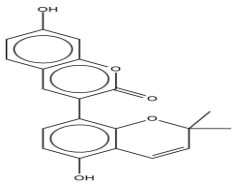
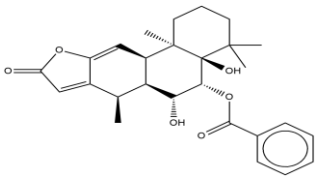
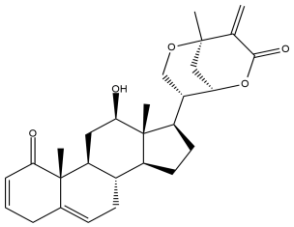
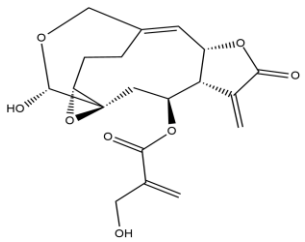
Table 2: Docking scores and smile notations of the top 20 hits (COCONUT-Mitishamba Database)

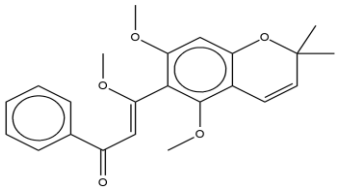
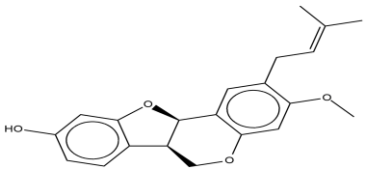
COMPOUND	Docking Score	SMILES
CNP0350592	-11.6	O=C1C=2C(O)=CC=CC2C3OC=4C=C(OC)C(=C(O)C4C5=C(C=C(O)C1=C53)C)C(=O)C
CNP0350368	-11.3	O=C(OC1(C)C2C=3C=C4OC(=CC(=O)C4=CC3OC2OC1(C)C)C=5C=CC=CC5)C
CNP0289849	-11.2	O=C1C=C(C(=O)C2=C1C(O)=C3C(O)=CC=CC3=C2C=4C=CC=5C(=O)C6=CC(=CC(O)=C6C(=O)C5C4)C)C
CNP0350459	-11.2	O=C1C=2C(O)=CC=CC2C(C3=CC=C4C=C5C(=CC4=C3O)C(=O)CC(O)(C5)CC)C=6C=CC=C(O)C16
CNP0298793	-11.1	O=C1C2=C(O)C=3C=C(C=CC3C=C2CC(O)(C)C1)C4=C5C=CC=C(O)C5=C(O)C=6C(=O)CC(O)(C)CC64
CNP0350315	-11	O=CC12CCC3OC3C2(OC(=O)C)CCC4C5=C(OC)C(=O)C(C6COC(=O)CC6)C5(C)CCC41
CNP0337385	-10.6	O=C1C=2C(O)=CC=CC2CC3=C1C(O)=CC(=C3C=4C(O)=CC(OC)=C(C4O)C(=O)C)C
CNP0106794	-10.5	O=C1OC(C)(C)C2(C=C1)CC(=O)C3(C)C(CCC(C(=O)C4=COC=C4)C53OC5C(=O)OC)C2(C)C
CNP0350354	-10.5	O=C(OC1CC2C(C(=O)CCC2(C)C3(C)CCC4(OC(=O)C)C(=CCC4C5=COC=C5)C13C)(C)C)C
CNP0260728	-10.4	O=C1C2=CC=C3OC(C=CC3=C2OC=C1C=4C=C(OC)C=5OCOC5C4OC)(C)C
CNP0300492	-10.4	O=C1C=2C(O)=CC=CC2C(C3=CC(=CC(O)=C13)C)C4C=5C=CC=C(O)C5C(=O)C6=C(O)C=C(C=C64)C
CNP0313475	-10.4	O=C1C=2C=CC=C(O)C2C(=O)C3=C(O)C=C(C(=C13)C=4C(O)=CC(OC)=C(C(=O)C)C4O)C
CNP0140079	-10.3	O=C1OC(C2=COC=C2)CC3C1CC4OC45C6(C(=O)CC7C(C(=O)CCC7(C)C6CCC35C)(C)C)C
CNP0309850	-10.3	O=C1C=2C=CC=C(O)C2C(=O)C3=C(O)C=C(C(=C13)C4=C(O)C=C(O)C(C(=O)C)=C4O)C
CNP0227861	-10.2	O=C1C=2C(O)=CC(O)=CC2OC(C3=CC=4C=CC(OC4C(=C3)CC=C(C)C)(C)C)C1
CNP0324724	-10.2	O=C1C2=CC=C(OC)C(=C2OC3COC4=CC=5OCOC5C=C4C13O)CC=C(C)C
CNP0221435	-10.1	O=C1C2=C(O)C=C3OC(C(=C)C)CC3=C2OC4COC5=CC(OC)=C(OC)C=C5C14
CNP0258562	-10.1	O=C1C=2C=CC=C(O)C2C(=O)C3=C(O)C=C(C(=C13)C4=C(O)C=C(O)C(C(=O)C)=C4OC)C
CNP0270955	-10.1	O=C1OC(C2=COC=C2)C3(C)CCC4C5(C=CC(=O)C(C)(C)C5CC(=O)C4(C)C63OC16)C
CNP0310156	-10.1	O=C1OC23C(=C1)C(=CC(N4CCCC42)C3)C5CC6=CC(=O)OC67CC5N8CCCC87

Table 3: Chemical structures and plant sources of the top 20 hits (In house database)

COMPOUND	**STRUCTURE**	**PLANT SOURCE**
Withaphysalin A		*Physalis minima L.*
Limonin		*Citrus spp*
Morusinol		*Morus alba*
25R-Spirostan-4-ene-3,6,12-trione		*Tribulus terrestris*
Isodiospyrin		*Diospyros abyssinica*
8-acetonyldihydrochelerythrine		*Zanthoxyllum gilleti*

Withametelin M		*Datura metel*
Inophyllum C		*Calophyllum braziliense*
Withametelin K.		*Datura metel*
Inophyllum D		*Calophyllum braziliense*
Integriamide		*Toddalia asiatica*
3'3'-Biplumbagin		*Plumbago zeylanica*

Ekeberin C1		*Ekebergia capensis Sparrm.*
Erysenegalensein J		*Erythrina senegalensis*
Glabrocoumarin		*Glycyrrhiza glabra*
Neocaesalpin R		*Caesalpinia pulcherrima*
Withametelin L.		*Datura metel*
Hydroxyvernolide		*Vernonia amygdalina*

Praecanson A		*Tephrosia pumila*
3-O-methylcalopocarpin		*Erythrina burttii*

Binding Analysis Experiments

In order to visually inspect how the top binding compounds interact with the PLpro macromolecule, we utilized Pymol (Schrodinger and DeLano, 2020) and the respective docking output files. As exemplified in Figure 3, Withaphysalin A is bound within the active site of the protease. Similarly, Figure 4 depicts how CNP0350592 is bound within the active site of the SARS-COV2 nsp3.

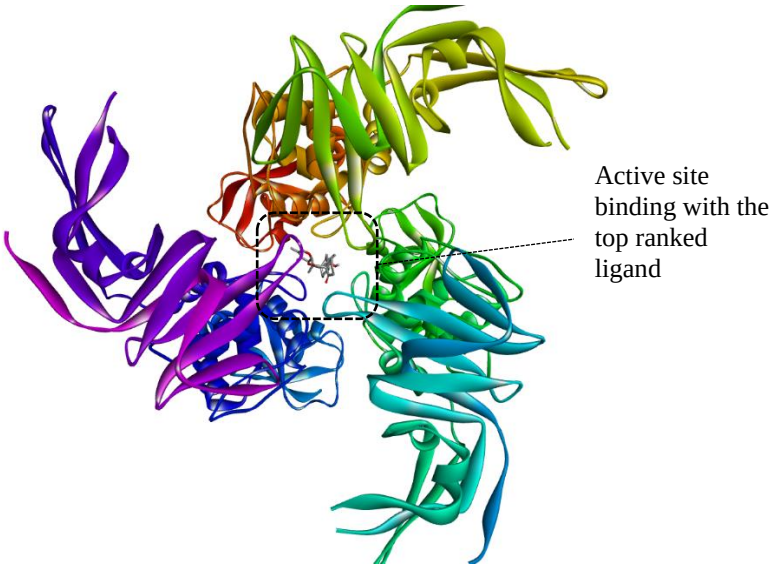


Figure 3: Binding of Withaphysalin A (Binding Affinity of -11.5 kcal/mol) to the Active Site via Different Domains of the Protein

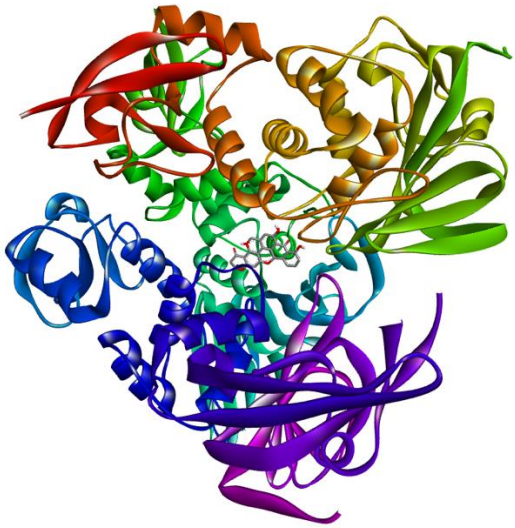


Figure 4: Binding of CNP0350592 from the COCONUT-Mitishamba DATABASE (Binding Affinity of -11.6kcal/mol) to the Active Site via Different Domains of the Protein

Ligand-Protein Interactions Analysis

Ligand binding capacity is quite vital for the regulation of biological functions. Protein-ligand interactions occur through molecular mechanics which involves the changes in conformation among the low binding affinity and high binding affinity states of a specified ligand. As shown in Figure 5, in this study, the main types interactions observed between the ligand and the protein at the active sites were van der Waals, conventional hydrogen bond, Carbon Hydrogen bond, Pi-Donor Hydrogen bond, Pi-sigma, and Pi-Alkyl interactions. The protein-ligand interactions were performed on the top ranked compounds of the two databases i.e., Withaphysalin A, CNP0350592, CNP0350368, and Limonin. These compounds recorded a binding affinities of -11.5 kcal/mol, -11.6 kcal/mol, -11.3 kcal/mol, and -11.1 kcal/mol respectively.

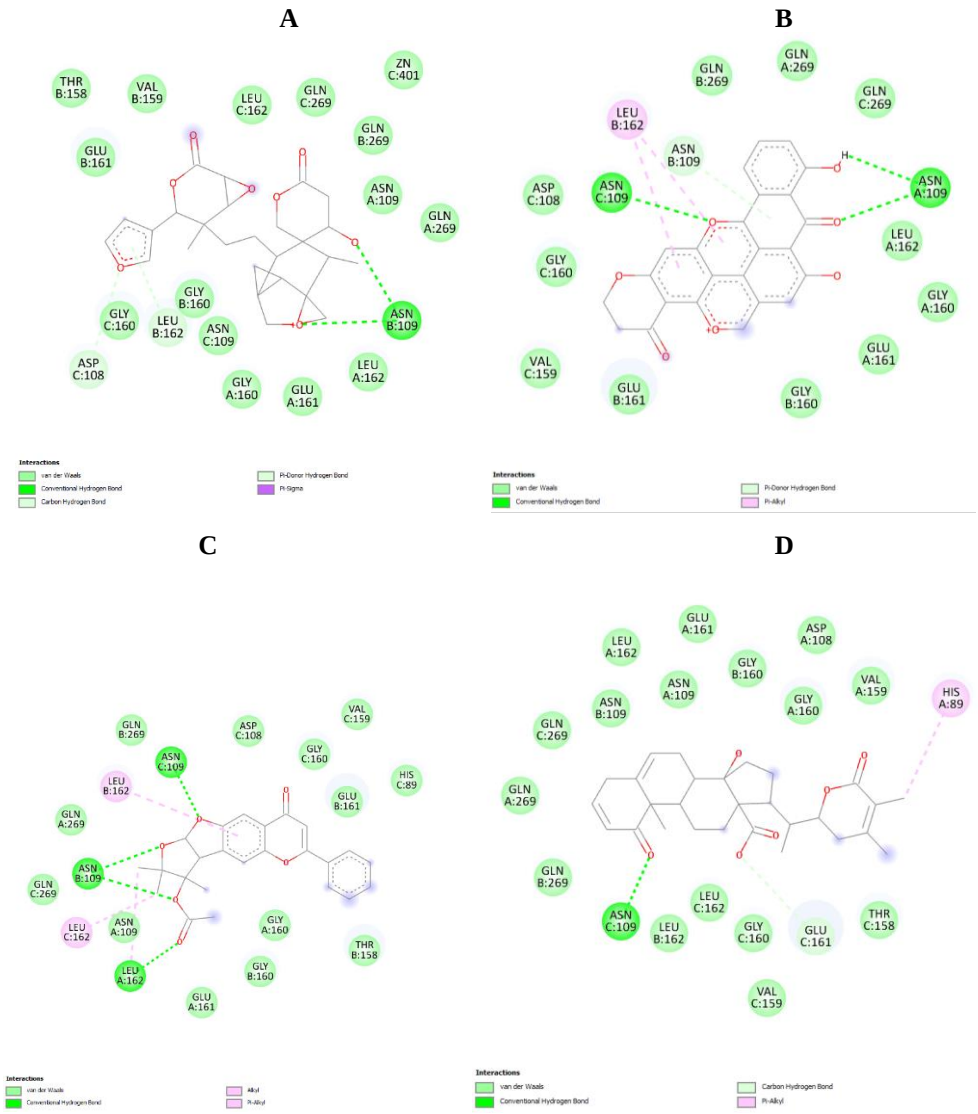


Figure 5: Protein-Ligand Interactions of Four Ligands from the Two Databases (See text). The dominant type of interaction was the van der Waals interactions and the conventional hydrogen bonds. (A) is Limonin, (B) is CNP0350592, (C) is CNP0350368, and (D) is Withaphysalin A.

Molecular Dynamics for Structural Stability Analysis

To provide the ultimate detail concerning the individual particle motions of the protein target receptor as a function of time, molecular dynamics simulations were performed. With the different parameters of simulations under the control of the user, the simulations are used to address specific questions about the properties of a model. In this study, the type of force field used for the study was the GROMOS96 43a1 force field and the simulation was conducted with a run-time of 1ns. The average root mean square deviation of the enzyme structure was found to be 2.61 Å ± 0.09. The fluctuations in the enzyme structure (rmsf) was also performed (see figure 6). Though there were fluctuations in the enzyme, the average residual movement implied that the enzyme structure was stable. The hydrogen bond interactions between the hydrogen bond donors and acceptors showed that fluctuations during the simulation are within an acceptable range. Overall, this PLpro system is a worthy and stable target. Simulations with the various top ligands bound to the complex remain to be investigated, as they are more computationally demanding.(Abraham et al., 2015).

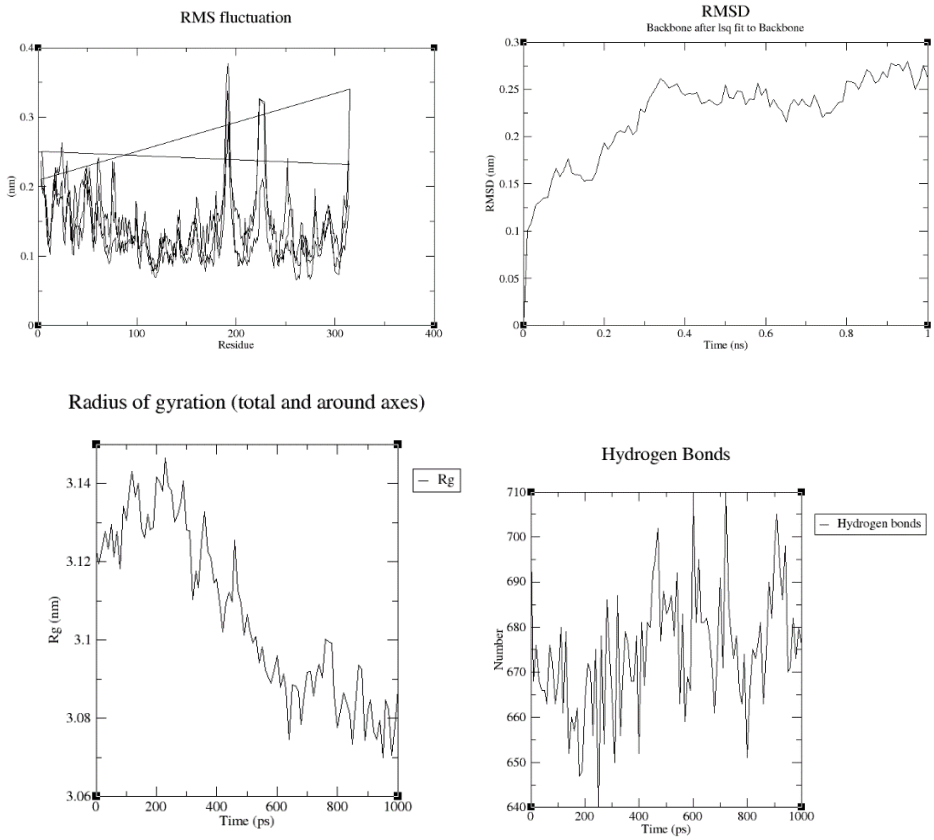


Figure 6: Molecular dynamics simulation analyses of the nsp3 Papain-Like Protease. The RMSF, RMSD, Radius of gyration, and hydrogen bond interactions were performed, respectively

CONCLUSION

Results of this structure-based virtual screening investigation of our in-house phytochemical database amply indicate that Withaphysalin A and Limonin are potential SARS-COV2 papain-like protease inhibitors. Based on their contribution to the top 20 phytochemicals list reported in this work, there is now a scientific basis for further investigations of 17 medicinal plants (*Caesalpinia pulcherrima*, *Calophyllum braziliense*, *Citrus* spp, *Datura metel*, *Diospyros abyssinica*, *Ekebergia capensis* Sparrm, *Erythrina burttii*, *Erythrina senegalensis*, *Glycyrrhiza glabra*, *Morus alba*, *Physalis minima L.*, *Plumbago zeylanica*, *Tephrosia pumila*, *Toddalia asiatica*, *Tribulus terrestris*, *Vernonia amygdalina*, *Zanthoxyllum gilleti*) for activity and subsequent antiviral drug development against SARS-COV2 PLpro and COVID-19. The top ranked compounds obtained from this study should be further analyzed *in vitro* and *in vivo* particularly for efficacy, specificity and ADMETox profiling.

Acknowledgements

T.A wishes to acknowledge the University of Eldoret (through the directorate of research and innovation) for funding and infrastructural support. We wish to acknowledge the following former students for their contribution during the preliminary phase of this project: Anthony Warari, Muchina Kuria, Jackson Mwendwa, Shyline Jerotich, Daniel Owiny, Sara Adan and Shaun Jerotich.

References

Abraham M.J., T. Murtola, R. Schulz, S. Páll, J.C. Smith, B. Hess, and E. Lindahl, "GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers," SoftwareX, 1–2 19–25 (2015).

Anirudhan, V., Lee, H., Cheng, H., Cooper, L., & Rong, L. (2021). Targeting SARS-CoV-2 viral proteases as a therapeutic strategy to treat COVID-19. *Journal of medical virology*, *93*(5), 2722–2734. https://doi.org/10.1002/jmv.26814

Bafna, K., White, K., Harish, B., Rosales, R., Ramelot, T. A., Acton, T. B., Moreno, E., Kehrer, T., Miorin, L., Royer, C. A., García-Sastre, A., Krug, R. M., & Montelione, G. T. (2021). Hepatitis C virus drugs that inhibit SARS-CoV-2 papain-like protease synergize with remdesivir to suppress viral replication in cell culture. *Cell reports*, *35*(7), 109133. https://doi.org/10.1016/j.celrep.2021.109133

Boras, B., Jones, R. M., & Anson, B. J. (2021). Preclinical characterization of an intravenous coronavirus 3CL protease inhibitor for the potential treatment of COVID19. *Nat Commun*. 2021;12(1):6055. Published 2021 Oct 18. doi:10.1038/s41467-021-26239-2

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep* 7, 42717 (2017). https://doi.org/10.1038/srep42717

Dallakyan, S, Olson, A. J. (2015). Small-molecule library screening by docking with PyRx. Methods Mol Biol. 2015;1263:243-50. doi: 10.1007/978-1-4939-2269-7_19. PMID: 25618350.

Fu, Z., Huang, B., Tang, J., Liu, S., Liu, M., Ye, Y., Liu, Z., Xiong, Y., Zhu, W., Cao, D., Li, J., Niu, X., Zhou, H., Zhao, Y. J., Zhang, G., & Huang, H. (2021). The complex structure of GRL0617 and SARS-CoV-2 PLpro reveals a hot spot for antiviral drug discovery. *Nature communications*, *12*(1),488. https://doi.org/10.1038/s41467-020-20718-8

Gil, C., Ginex, T., Maestro, I., Nozal, V., Barrado-Gil, L., Cuesta-Geijo, M. Á., Urquiza, J., Ramírez, D., Alonso, C., Campillo, N. E., Martinez, A. (2020). COVID-19: Drug Targets and Potential Treatments. *J Med Chem*. 2020 Nov 12;63(21):12359-12386. doi: 10.1021/acs.jmedchem.0c00606. Epub 2020 Jun 26. PMID: 32511912; PMCID: PMC7323060.

Jade, D., Ayyamperumal, S., Tallapaneni, V., Joghee Nanjan, C. M., Barge, S., Mohan, S., & Nanjan, M. J. (2021). Virtual high throughput screening: Potential inhibitors for SARS-CoV-2 PL^{PRO} and $3CL^{PRO}$ proteases. *European journal of pharmacology*, *901*, 174082. https://doi.org/10.1016/j.ejphar.2021.174082

Jamalan, M., Barzegari, E., & Gholami-Borujeni, F. (2021). Structure-Based Screening to Discover New Inhibitors for Papain-like Proteinase of SARS-CoV-2: An *In Silico* Study. *Journal of proteome research*, *20*(1),1015–1026. https://doi.org/10.1021/acs.jproteome.0c00836

Jiang, H., Yang, P., Zhang, J. (2022). Potential Inhibitors Targeting Papain-Like Protease of SARS-CoV-2: Two Birds With One Stone. Front Chem. 2022 Feb 23;10:822785. doi: 10.3389/fchem.2022.822785. PMID: 35281561; PMCID: PMC8905519.

Khataniar, Ankita, Upasana Pathak, Sanchaita Rajkhowa, & Anupam N. Jha. (2022). "A Comprehensive Review of Drug Repurposing Strategies against Known Drug Targets of COVID-19" COVID 2, no. 2: 148-167. https://doi.org/10.3390/covid2020011

Klemm, T., Ebert, G., Calleja, D. J., Allison, C. C., Richardson, L. W., Bernardini, J. P., Lu, B. G., Kuchel, N. W., Grohmann, C., Shibata, Y., Gan, Z. Y., Cooney, J. P., Doerflinger, M., Au, A. E., Blackmore, T. R., van der Heden van Noort, G. J., Geurink, P. P., Ovaa, H., Newman, J., Riboldi-Tunnicliffe, A., & … Komander, D. (2020). Mechanism and inhibition of the papain-like protease, PLpro, of SARS-CoV-2. *The EMBO journal*, *39*(18), e106275. https://doi.org/10.15252/embj.2020106275

Lamb, Y. N. (2022). Nirmatrelvir Plus Ritonavir: First Approval. *Drugs*, 1–7. Advance online publication. https://doi.org/10.1007/s40265-022-01692-5

Lim, C. T., Tan, K. W., Wu, M., Ulferts, R., Armstrong, L. A., Ozono, E., Drury, L. S., Milligan, J. C., Zeisner, T. U., Zeng, J., Weissmann, F., Canal, B., Bineva-Todd, G., Howell, M., O'Reilly, N., Beale, R., Kulathu, Y., Labib, K., & Diffley, J. (2021). Identifying SARS-CoV-2 antiviral compounds by screening for small molecule inhibitors of Nsp3 papain-like protease. *The Biochemical journal*, *478*(13),2517–2531. https://doi.org/10.1042/BCJ20210244

Ma, C., Sacco, M. D., Xia, Z., Lambrinidis, G., Townsend, J. A., Hu, Y., Meng, X., Szeto, T., Ba, M., Zhang, X., Gongora, M., Zhang, F., Marty, M. T., Xiang, Y., Kolocouris, A., Chen, Y., & Wang, J. (2021). Discovery of SARS-CoV-2 Papain-like Protease Inhibitors through a Combination of High-Throughput Screening and a FlipGFP-Based Reporter Assay. *ACS central science*, *7*(7), 1245–1260. https://doi.org/10.1021/acscentsci.1c00519

Mattoo, S-u-S., Kim, S-J., Ahn, D-G., & Myoung. J. (2022). Escape and Over-Activation of Innate Immune Responses by SARS-CoV-2: Two Faces of a Coin. *Viruses*. 2022; 14(3):530. https://doi.org/10.3390/v14030530.

Moustaqil, M., Ollivier, E., Chiu, H. P., Van Tol, S., Rudolffi-Soto, P., Stevens, C., Bhumkar, A., Hunter, D., Freiberg, A. N., Jacques, D., Lee, B., Sierecki, E., & Gambin, Y. (2021). SARS-CoV-2 proteases PLpro and 3CLpro cleave IRF3 and critical modulators of inflammatory pathways (NLRP12 and TAB1): implications for disease presentation across species. *Emerging microbes & infections*, *10*(1),178–195. https://doi.org/10.1080/22221751.2020.1870414

Osipiuk, J., Azizi, S. A., Dvorkin, S., Endres, M., Jedrzejczak, R., Jones, K. A., Kang, S., Kathayat, R. S., Kim, Y., Lisnyak, V. G., Maki, S. L., Nicolaescu, V., Taylor, C. A., Tesar, C., Zhang, Y. A., Zhou, Z., Randall, G., Michalska, K., Snyder, S. A., Dickinson, B. C., … Joachimiak, A. (2021). Structure of papain-like protease from SARS-CoV-2 and its complexes with non-covalent inhibitors. *Nature communications*, *12*(1), 743. https://doi.org/10.1038/s41467-021-21060-3

Pitsillou, E., Liang, J., Ververis, K., Lim, K. W., Hung, A., & Karagiannis, T. C. (2020). Identification of Small Molecule Inhibitors of the Deubiquitinating Activity of the SARS-CoV-2 Papain-Like Protease: *in silico* Molecular Docking Studies and *in vitro* Enzymatic Activity Assay. *Frontiers in chemistry*, *8*, 623971. https://doi.org/10.3389/fchem.2020.623971

Raj, K., Kaur, K., Gupta, G. D., & Singh, S. (2021). Current understanding on molecular drug targets and emerging treatment strategy for novel coronavirus-19. *Naunyn Schmiedebergs Arch Pharmacol*. 2021;394(7):1383-1402. doi:10.1007/s00210-021-02091-5

Recovery Collaborative Group (2020). Lopinavir-ritonavir in patients admitted to hospital with COVID-19 (RECOVERY): a randomised,

controlled, open-label, platform trial. Lancet (London, England), 396(10259), 1345–1352. https://doi.org/10.1016/S0140-6736(20)32013-4

Rut, W., Lv, Z., Zmudzinski, M., Patchett, S., Nayak, D., Snipas, S. J., El Oualid, F., Huang, T. T., Bekes, M., Drag, M., & Olsen, S. K. (2020). Activity profiling and crystal structures of inhibitor-bound SARS-CoV-2 papain-like protease: A framework for anti-COVID-19 drug design. *Science advances*, *6*(42),eabd4596. https://doi.org/10.1126/sciadv.abd4596

Schrödinger, L., & DeLano, W. (2020). *PyMOL*. Retrieved from http://www.pymol.org/pymol

Shen, Z., Ratia, K., Cooper, L., Kong, D., Lee, H., Kwon, Y., Li, Y., Alqarni, S., Huang, F., Dubrovskyi, O., Rong, L., Thatcher, G., & Xiong, R. (2021). Design of SARS-CoV-2 PLpro Inhibitors for COVID-19 Antiviral Therapy Leveraging Binding Cooperativity. *Journal of medicinal chemistry*, acs.jmedchem.1c01307. Advance online publication. https://doi.org/10.1021/acs.jmedchem.1c01307

Shin, D., Mukherjee, R., Grewe, D., Bojkova, D., Baek, K., Bhattacharya, A., Schulz, L., Widera, M., Mehdipour, A. R., Tascher, G., Geurink, P. P., Wilhelm, A., van der Heden van Noort, G. J., Ovaa, H., Müller, S., Knobeloch, K. P., Rajalingam, K., Schulman, B. A., Cinatl, J., Hummer, G., & Dikic, I. (2020). Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity. *Nature*, *587*(7835), 657–662. https://doi.org/10.1038/s41586-020-2601-5

Sorokina, M., Merseburger, P., & Rajan, K. (2021). COCONUT online: Collection of Open Natural Products database. J Cheminform 13, . https://doi.org/10.1186/s1,3321-020-00478-9

Stader, F., Khoo, S., Stoeckle, M., Back, D., Hirsch, H. H., Battegay, M., & Marzolini, C. (2020). Stopping lopinavir/ritonavir in COVID-19 patients: duration of the drug interacting effect. J Antimicrob Chemother. 2020 Oct 1;75(10):3084-3086. doi: 10.1093/jac/dkaa253. PMID: 32556272; PMCID: PMC7337877.

Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry, 31(2), 455-461.

Virdi, S. R., Bavisotto, V. R., Hopper, C. N., & Frick, N. D. (2020). Discovery of Drug-like Ligands for the Mac1 Domain of SARS-CoV-2 Nsp3. doi: https://doi.org/10.1101/2020.07.06.190413.

Weglarz-Tomczak, E., Tomczak, J. M., Talma, M., Burda-Grabowska, M., Giurg, M., & Brul, S. (2021). Identification of ebselen and its analogues as potent covalent inhibitors of papain-like protease from SARS-CoV-2. *Scientific reports*, *11*(1), 3640. https://doi.org/10.1038/s41598-021-83229-6

Xu, Y., Chen, K., Pan, J., Lei, Y., Zhang, D., Fang, L., Tang, J., Chen, X., Ma, Y., Zheng, Y., Zhang, B., Zhou, Y., Zhan, J., & Xu, W. (2021). Repurposing clinically approved drugs for COVID-19 treatment targeting SARS-CoV-2 papain-like protease. *International journal of biological macromolecules*, *188*,137–146. https://doi.org/10.1016/j.ijbiomac.2021.07.184

Yan, F., & Gao, F. (2021). An overview of potential inhibitors targeting non-structural proteins 3 (PL^{pro} and Mac1) and 5 ($3CL^{pro}/M^{pro}$) of SARS-CoV-2. *Computational and structural biotechnology journal*, *19*, 4868–4883. https://doi.org/10.1016/j.csbj.2021.08.036

Zhao, Y., Du, X., Duan, Y., Pan, X., Sun, Y., You, T., Han, L., Jin, Z., Shang, W., Yu, J., Guo, H., Liu, Q., Wu, Y., Peng, C., Wang, J., Zhu, C., Yang, X., Yang, K., Lei, Y., Guddat, L. W., & Yang, H. (2021). High-throughput screening identifies established drugs as SARS-CoV-2 PLpro inhibitors. *Protein & cell*, *12*(11), 877–888. https://doi.org/10.1007/s13238-021-00836-9