*pharmaceutics*

MDPI

*Article*

# Supplementary Materials

# for

# Identification of Triazolopyrimidinyl Scaffold SARS-CoV-2 Papain-Like Protease ($PL^{pro}$) Inhibitor

**Sebastjan Kralj [1], Marko Jukič [1,2,3,*], Miha Bahun [4], Luka Kranjc [4,5], Anja Kolarič [1], Milan Hodošček [6], Nataša Poklar Ulrih [4] and Urban Bren [1,2,3,*]**

[1] Faculty of Chemistry and Chemical Engineering, University of Maribor, Smetanova Ulica 17, SI-2000 Maribor, Slovenia; sebastjan.kralj1@um.si (S.K.); anja.kolaric2@um.si (A.K.)
[2] Faculty of Mathematics, Natural Sciences and Information Technologies, University of Primorska, Glagoljaška Ulica 8, SI-6000 Koper, Slovenia
[3] Institute of Enviormental Protection and Sensors, Beloruska Ulica 7, SI-2000 Maribor, Slovenia
[4] Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia; miha.bahun@bf.uni-lj.si (M.B.); luka.kranjc@nib.si (L.K.); natasa.poklar@bf.uni-lj.si (N.P.U.)
[5] National Institute of Biology, Večna Pot 111, SI-1000 Ljubljana, Slovenia
[6] National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia; milan@cmm.ki.si
* Correspondence: marko.jukic@um.si (M.J.); urban.bren@um.si (U.B.)

Table of Interactions

**Table S1.** Key interactions responsible for GRL0617 binding with SARS-CoV-2 PLpro.

| *Bond type* | *Amino-Acid residue (PLpro)* | *Atom number (GRL0617)* | *Distance (Å)* | *Angle (°)* |
|---|---|---|---|---|
| *Hydrophobic interaction* | P247 | C12 | 3.73 | / |
| | P248 | C14 | 3.76 | / |
| | Y264 | C14 | 3.67 | / |
| | Y268 | C13 | 3.50 | / |
| | Y268 | C20 | 3.86 | / |
| *Hydrogen bond* | D164(O) | N2 (amid) | 2.82 | 118.6 |
| | Y268(OH) | N01 | 3.43 | 129.54 |
| | Q269(N, backbone) | O7 | 3.14 | 146.54 |
| *T-shaped π-π interaction* | Y268 | Naphthalene group | 4.73 | 72.75 |

## Minimization of the Open $PL^{pro}$ Conformation

This was achieved using the CHARMM-GUI Solution Builder for the molecular dynamics program CHARMM[34,57]. Minimization included 50 steps of Steepest Descent (SD) and 500 steps of Adopted basis Newton-Raphson (ABNR), with a further 1 ns equilibration equilibration with the NVT ensemble applied, the temperature was set at 310.15 K using the HOOVER thermostat and the integration time-step set at 1fs. VDW interactions were cutoff between 10-12 Å using the force switch method (VFSWitch). For the calculation of electrostatic interactions, the particle-mesh Ewald summation was used, bonds to hydrogens were constrained using the SHAKE algorithm and the CHARMM 36m force-field was used.

**ROC Study**

To ensure the best docking protocol was used on the full library, the docking program CmDock (https://gitlab.com/Jukic/cmdock/) was tested on a subset of compounds. This included a random selection of a 1000 decoy drug-like molecules with the average molecular weight of 400 Da, and fifteen known non-covalent inhibitors with structural information deposited on the PDB (Supplementary-Table S2). The structure of the open conformation (PDB ID: 7CJM) and the closed conformation (PDB ID: 6WX4) were selected with all docking calculations, with the binding site prepared separately in each of the chosen programs. Using the CmCavity (https://gitlab.com/Jukic/cmdock/) program, the binding site was defined automatically around the coordinates of the co-crystallized ligand (GRL0617). The generated .grd file was inspected in PyMol to ensure that the binding site has been defined correctly.

Closed PLpro conformation docking cavity:
Total volume 5627.75 A^3
Cavity #1 Size=45022 points; Vol=5627.75 A^3; Min=(-7.5,11.5,-13); Max=(19.5,39.5,10); Center=(4.66316,25.1299,-2.88701); Extent=(27,28,23)
Cav = 45022; total = 45022
Open PLpro conformation docking cavity
Total volume 5557 A^3
Cavity #1 Size=44456 points; Vol=5557 A^3; Min=(-7.5,11.5,-13); Max=(19.5,39.5,10.5); Center=(4.49022,25.1067,-2.85059); Extent=(27,28,23.5)
Cav = 44456; total = 44456
The docking protocol used was defined in the docking parameter file, and was as follows:

```
RBT_PARAMETER_FILE_V1.00
TITLE GRL0617 docking to Plpro

#cavitiy
SECTION MAPPER
    SITE_MAPPER
RbtLigandSiteMapper
    REF_MOL GRL0617_7cjm.sdf
    RADIUS 10.0
    SMALL_SPHERE 1.0
    MIN_VOLUME 100
    MAX_CAVITIES 1
    VOL_INCR 0.0
    GRIDSTEP 0.5
     END_SECTION

 #restraint
SECTION CAVITY
     SCORING_FUNCTION
RbtCavityGridSF
     WEIGHT 1.0
END_SECTION
#end restraint
```

**ROC Curves for docking protocol**

Custom KNIME software workflows for ROC curve generation were used for both the open and closed conformation and on all three docking programs, resulting in a total of 6 ROC curves. As stated earlier the results among the docking programs were comparable, and CmDock was chosen based on its speed and ease of use.

**Table S2**: Table of known inhibitors for SARS-CoV-PLpro

| Molecule name IUPAC | Chemical formula/ name | PDB ID | Reference |
|---|---|---|---|
| 5-amino-2-methyl-N-[(1R)-1-naphthalen-1-ylethyl]benzamide | C20 H20 N2 O GRL0617 | 7CJM | Fu et. al |
| 1-(2-methoxyethyl)-2-methyl-3-(pyrazin-2-ylmethyl)benzo[f]benzimidazol-3-ium-4,9-dione | C20 H19 N4 O3 YM155 | 7D7L | Zhao et al.[58] |
| N-[(3-acetamidophenyl)methyl]-1-[(1R)-1-naphthalen-1-ylethyl]piperidine-4-carboxamide | C27 H31 N3 O2 Compound S43 | 7E35 | Shan et al.[59] |
| 5-[(carbamoylcarbamoyl)amino]-2-methyl-N-[(1R)-1-(naphthalen-1-yl)ethyl]benzamide | C22 H22 N4 O3 Snyder 495 | 7JIT | Osipiuk et al.[12] |
| 5-(acryloylamino)-2-methyl-N-[(1R)-1-(naphthalen-1-yl)ethyl]benzamide | C23 H22 N2 O2 Snyder530 | 7JIW | Osipiuk et al.[12] |
| 3-amino-2-methyl-N-[(1R)-1-(naphthalen-1-yl)ethyl]benzamide | C20 H20 N2 O Snyder441 | 7JN2 | Osipiuk et al.[12] |
| 5-[(E)-(hydroxyimino)methyl]-2-methyl-N-[(1R)-1-(naphthalen-1-yl)ethyl]benzamide | C21 H20 N2 O2, Snyder496 | 7KOL | Osipiuk et al.[12] |
| 5-[(azetidin-3-yl)amino]-N-[(1R)-1-{3-[5-({[(1S,3R)-3-hydroxycyclopentyl]amino}methyl)thiophen-2-yl]phenyl}ethyl]-2-methylbenzamide | C29 H36 N4 O2 S XR8-89 | 7LBR | Ratia et al. |
| 5-[(azetidin-3-yl)amino]-2-methyl-N-[(1R)-1-(3-{5-[(pyrrolidin-1-yl)methyl]thiophen-2-yl}phenyl)ethyl]benzamide | C28 H34 N4 O S XR8-24 | 7LBS | Ratia et al. |
| 5-[(azetidin-3-yl)amino]-N-[(1R)-1-{3-[5-({[(1R,3S)-3-hydroxycyclopentyl]amino}methyl)thiophen-2-yl]phenyl}ethyl]-2-methylbenzamide | C29 H36 N4 O2 S XR8-83 | 7LLF | Ratia et al. |
| N-[(1R)-1-(3-{5-[(acetylamino)methyl]thiophen-2-yl}phenyl)ethyl]-5-[(azetidin-3-yl)amino]-2-methylbenzamide | C26 H30 N4 O2 S XR8-69 | 7LLZ | Ratia et al. |
| 5-(azetidin-3-ylamino)-2-methyl-~{N}-[(1~{R})-1-[3-[5-[[[(3~{R})-oxolan-3-yl]amino]methyl]thiophen-2-yl]phenyl]ethyl]benzamide | C28 H34 N4 O2 S XR8-65 | 7LOS | Ratia et al. |
| (1R)-N-[(1H-indol-3-yl)methyl]-N-methyl-1-(naphthalen-1-yl)ethan-1-amine | C22 H22 N2 Jun9-84-3 | 7RZC | Osipiuk et al.[12] |
| 4-({methyl[(1R)-1-(naphthalen-1-yl)ethyl]amino}methyl)phenol | C20 H21 N O Jun9-72-2 | 7SDR | Osipiuk et al.[12] |
| 5-amino-N-(naphthalen-1-yl)pyridine-3-carboxamide | C16 H13 N3 O Snyder608 | 7SGU | Osipiuk et al.[12] |
| N-(naphthalen-1-yl)pyridine-3-carboxamide | C16 H12 N2 O Snyder630 | 7SGW | Osipiuk et al.[12] |

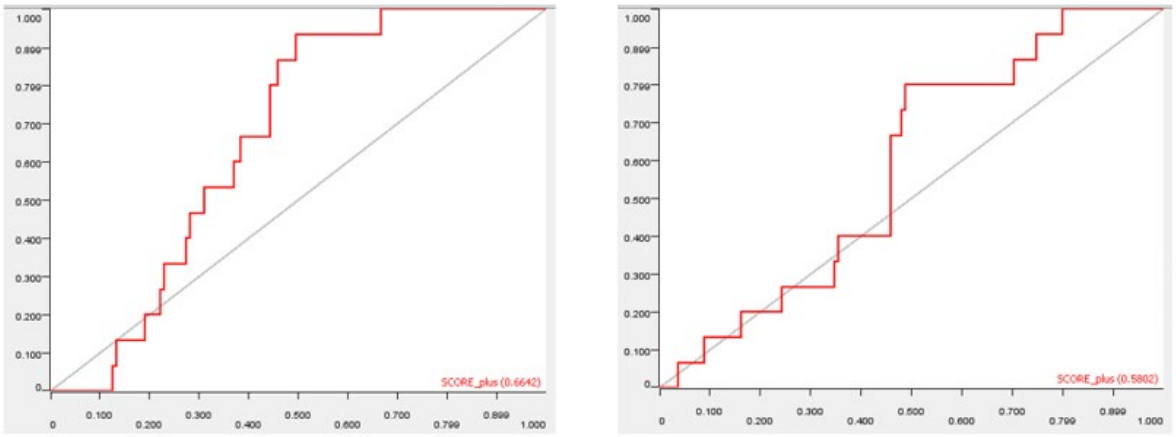



**Figure S1**: ROCS curves for conformations of the PLpro protein target

**Table S3**: MD results for selected docking hits

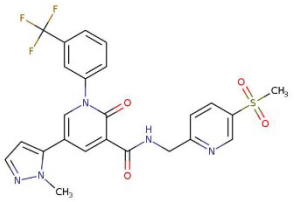
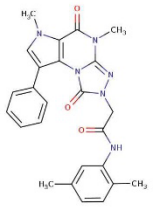
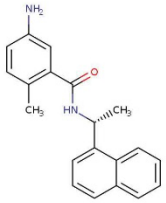
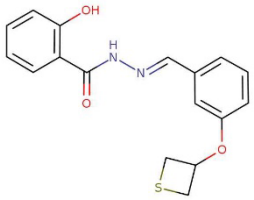
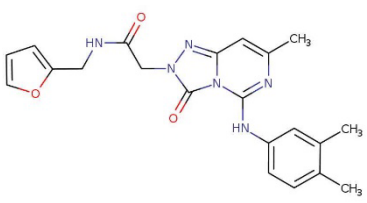
| Compound name | Structure |
|---|---|
| **Hit 903** |  |
| **Hit 922** |  |
| **GRL 0617** |  |
| **Hit 826** |  |
| **Hit 372** |  |

**Table S4**: RMSD for selected inhibitors during MD calculations

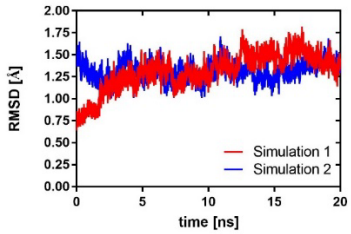
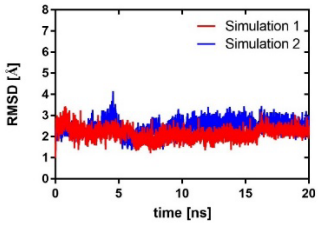
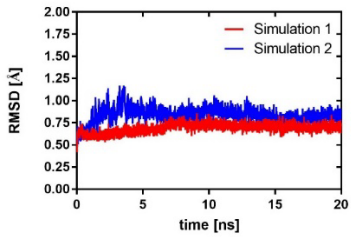
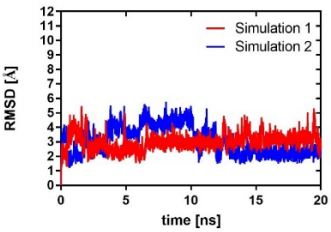
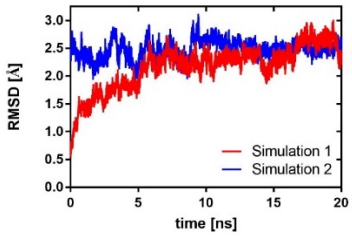
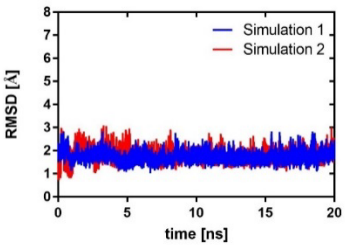
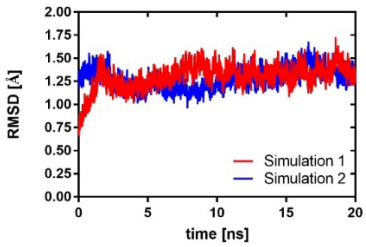
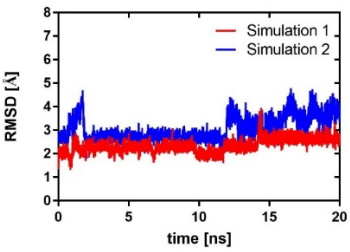
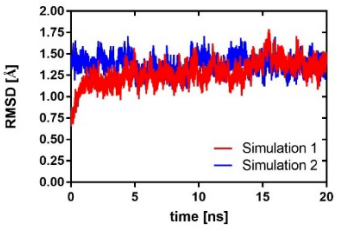
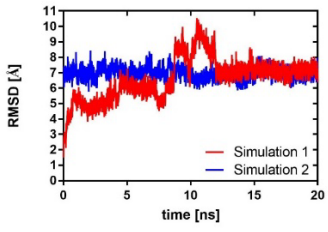
| Compounds ID | Average RMSD 1 ± SD[Å] | Average RMSD 2 ± SD[Å] | RMSD protein | RMSD ligand |
|---|---|---|---|---|
| **Hit 903** | 1.313 ± 0.208 | 1.316 ± 0.105 |  |  |
| **Hit 922** | 0.691 ± 0.051 | 0.837 ± 0.080 |  |  |
| **GRL 0617** | 2.166 ± 0.399 | 2.478 ± 0.165 |  |  |
| **Hit 826** | 1.302 ± 0.131 | 1.280 ± 0.110 |  |  |
| **Hit 372** | 1.276 ± 0.144 | 1.369 ± 0.099 |  |  |

**Table S5:** Oligonucleotide sequences used in this study.

| DNA primer | Sequence (from 5′ to 3′) |
|---|---|
| P1 | gtttaactttaagaaggagatatacatatggaagtgaggactattaaggtgtttacaacag |
| P2 | ctcagtggtggtggtggtggtgctcgagttttatggttgttgtgtaactgttttctttgtagaaaac |
| P3 | ctcgagcaccaccaccac |
| P4 | cagttattacccattgaaaaaggaagagtctgcaggcatcgtggtgtcac |
| P5 | caggaagattgtataagcaaatatttctgcagcaatggcaacaacgttgcgc |
| P6 | catatgtatatctccttcttaaagttaaacaaaattatttctagaggg |
| P7 | gacgagcgtgacaccacgatgcctgcagactcttccttttttcaatgggtaataactg |
| P8 | gttgttgccattgctgcagaaatatttgcttatacaatcttcctgtttttggg |
| P9 | gtttaactttaagaaggagatatacatatggaagtgaggactattaaggtgtttacaacag |
| P10 | aaaacatatgtatatctccttcttaaagttaaacaaaattatttctagaggg |