Supporting information

**Molecular dynamics simulations suggest SARS-CoV-2 3CLpro mutations in Beta and Omicron variants do not alter binding affinities for cleavage sites of non-structural proteins**

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BA.1 SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDVVYCPRHVICTSEDMLNPNYEDLLIR 60

BETA SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDVVYCPRHVICTSEDMLNPNYEDLLIR 60

H41A SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDVVYCPRAVICTSEDMLNPNYEDLLIR 60

WT SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDVVYCPRHVICTSEDMLNPNYEDLLIR 60

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BA.1 KSNHNFLVQAGNVQLRVIGHSMQNCVLKLKVDTANPKTPKYKFVRIQPGQTFSVLACYNG 120

BETA KSNHNFLVQAGNVQLRVIGHSMQNCVLKLRVDTANPKTPKYKFVRIQPGQTFSVLACYNG 120

H41A KSNHNFLVQAGNVQLRVIGHSMQNCVLKLKVDTANPKTPKYKFVRIQPGQTFSVLACYNG 120

WT KSNHNFLVQAGNVQLRVIGHSMQNCVLKLKVDTANPKTPKYKFVRIQPGQTFSVLACYNG 120

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BA.1 SPSGVYQCAMRHNFTIKGSFLNGS**C**GSVGFNIDYDCVSFCYMHHMELPTGVHAGTDLEGN 180

BETA SPSGVYQCAMRPNFTIKGSFLNGS**C**GSVGFNIDYDCVSFCYMHHMELPTGVHAGTDLEGN 180

H41A SPSGVYQCAMRPNFTIKGSFLNGS**C**GSVGFNIDYDCVSFCYMHHMELPTGVHAGTDLEGN 180

WT SPSGVYQCAMRPNFTIKGSFLNGS**C**GSVGFNIDYDCVSFCYMHHMELPTGVHAGTDLEGN 180

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BA.1 FYGPFVDRQTAQAAGTDTTITVNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKYNYE 240

BETA FYGPFVDRQTAQAAGTDTTITVNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKYNYE 240

H41A FYGPFVDRQTAQAAGTDTTITVNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKYNYE 240

WT FYGPFVDRQTAQAAGTDTTITVNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKYNYE 240

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BA.1 PLTQDHVDILGPLSAQTGIAVLDMCASLKELLQNGMNGRTILGSALLEDEFTPFDVVRQC 300

BETA PLTQDHVDILGPLSAQTGIAVLDMCASLKELLQNGMNGRTILGSALLEDEFTPFDVVRQC 300

H41A PLTQDHVDILGPLSAQTGIAVLDMCASLKELLQNGMNGRTILGSALLEDEFTPFDVVRQC 300

WT PLTQDHVDILGPLSAQTGIAVLDMCASLKELLQNGMNGRTILGSALLEDEFTPFDVVRQC 300

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BA.1 SGVT 304

BETA SGVT 304

H41A SGVT 304

WT SGVT 304

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**Figure S1. Multiple alignment of the amino acid sequence of 3CLproH41A, 3CLproWT, 3CLproBeta and 3CLproBA.1.** Global multiple alignment (using Clustal W, [[1]](https://paperpile.com/c/rXGgCp/bUyT)) of 3CLpro amino acid sequences from different SARS-CoV-2 strains. This alignment shows that the Beta and Omicron variants have substitutions at K90R (K3353R) and P132H (P3395H) respectively.

**Table S1.** Hydrogen bonding occupancies of potential 3CLpro inhibitors.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Inhibitor** | **Chemical structure** | **Residues (HBA)** | **Residues (HBD)** | **ΔG (kJ/mol)** | **Ref.** |
| Amentoflavone | Shape  Description automatically generated with low confidence | Arg188 (15.5%), **His164 (78.8%)** | His41(33.8%), Tyr54 (23.8%), Gln192(45.1%) | -73.8 | [[2]](https://paperpile.com/c/rXGgCp/dSkgp) |
| Dalpanitin | Diagram, schematic  Description automatically generated | Asn142 (33%), **Arg188 (82.3%)** | **Gln192 (74.4%)**, Thr190 (18%), Asn142 (24.7%), Glu166 (67%) | -44.3 | [[2]](https://paperpile.com/c/rXGgCp/dSkgp) |
| Hinokiflavone | Diagram, engineering drawing  Description automatically generated | His41 (19.7%), Thr (10.7%) | Thr26 (39.9%), His41 (40.1%), Arg188 (12.3%), Gly143 (10.4%) | -80.9 | [[2]](https://paperpile.com/c/rXGgCp/dSkgp) |
| Naringin | Shape  Description automatically generated with medium confidence | Thr45 (25.2%), His41 (23.2%), His164 (39.8%), Met49 (24.7%) | Ser46 (42.7%), **Ser144 (72.2%),****Cys145 (82%)** | -69.0 | [[2]](https://paperpile.com/c/rXGgCp/dSkgp) |
| Dihydrostreptomycin | Shape  Description automatically generated with low confidence | **Glu166 (88.9%), Asn142 (66.5%)**, Gln189 (36%), His41 (0.8%) | **Glu166 (55.2%),** Cys145 (13.8%), Asn142 (10%) | -365.0 | [[3]](https://paperpile.com/c/rXGgCp/tCaqM) |
| Viomycin | Shape  Description automatically generated with medium confidence | **Glu166 (57.9%)**, Asn142 (21.7%), Gln189 (16.2%), His41 (9.37%) | **Glu166 (45.8%),** Cys145 (13.1%), Asn142 (49.0%) | -377.2 | [[3]](https://paperpile.com/c/rXGgCp/tCaqM) |
| Fenoterol | Shape  Description automatically generated with medium confidence | **His41 (56.7%)** | Asn142 (9.8%) | -221.8 | [[3]](https://paperpile.com/c/rXGgCp/tCaqM) |
| Carvedilol |  | **Glu166 (63.8%),**  | **His41 (42%)** | -203.7 | [[4]](https://paperpile.com/c/rXGgCp/azenD) |
| Nebivolol | Shape  Description automatically generated with medium confidence | His41 (59.5%) | Gln192 (75.3%) | -202.7 | [[4]](https://paperpile.com/c/rXGgCp/azenD) |

References

1. [Thompson, J.D.; Higgins, D.G.; Gibson, T.J. CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice. *Nucleic Acids Res.* **1994**, *22*, 4673–4680.](http://paperpile.com/b/rXGgCp/bUyT)

2. [Sawant, S.; Patil, R.; Khawate, M.; Zambre, V.; Shilimkar, V.; Jagtap, S. Computational Assessment of Select Antiviral Phytochemicals as Potential SARS-Cov-2 Main Protease Inhibitors: Molecular Dynamics Guided Ensemble Docking and Extended Molecular Dynamics. *In Silico Pharmacol* **2021**, *9*, 44.](http://paperpile.com/b/rXGgCp/dSkgp)

3. [de Souza, A.S.; de Souza, R.F.; Guzzo, C.R. Quantitative Structure-Activity Relationships, Molecular Docking and Molecular Dynamics Simulations Reveal Drug Repurposing Candidates as Potent SARS-CoV-2 Main Protease Inhibitors. *J. Biomol. Struct. Dyn.* **2021**, 1–18.](http://paperpile.com/b/rXGgCp/tCaqM)

4. [Hamed, M.I.A.; Darwish, K.M.; Soltane, R.; Chrouda, A.; Mostafa, A.; Abo Shama, N.M.; Elhady, S.S.; Abulkhair, H.S.; Khodir, A.E.; Elmaaty, A.A.; et al. β-Blockers Bearing Hydroxyethylamine and Hydroxyethylene as Potential SARS-CoV-2 Mpro Inhibitors: Rational Based Design, , , and SAR Studies for Lead Optimization. *RSC Adv.* **2021**, *11*, 35536–35558.](http://paperpile.com/b/rXGgCp/azenD)