Supporting Information

Diagrama, Histograma

Descrição gerada automaticamente

**Figure S1.** Distribution of physicochemical properties and synthetic accessibility score (SAScore) of 37 fragments selected from virtual screening.MW: molecular weight; cLogP: logarithm of calculated octanol:water partition coefficient (P); HBD: number of hydrogen bond donor atoms; HBA: number hydrogen bond acceptor atoms; Csp3: proportion of sp3 hybridized carbon atoms.



**Figure S2.** Chemical structures of the 34 fragments purchased for experimental validation against the main SARS-CoV-2 (Mpro), clustered according to the Butina algorithm. A – Clusters containing at least two fragments, B – Clusters containing only one fragment (singletons).

Gráfico

Descrição gerada automaticamente

**Figure S3.** Concentration-response curves of 4 hit compounds (737, 281, 183 and 048, see main text for nomenclature) against Mpro activity.

Diagrama

Descrição gerada automaticamente

**Figure S4.** Compound 818 interactions with protein 6W79 (left) and 7K40 (right) based on the posed obtained from use of the unrestricted protocol.

Diagrama

Descrição gerada automaticamente

**Figure S5.** Compound 818 interactions with protein 6W79 (left) and 7K40 (right) based on the posed obtained from use of the restricted protocol.

Mapa de jogo de vídeo game com personagem de desenho animado

Descrição gerada automaticamente com confiança baixa

**Figure S6.** Compound 818 top score binding mode with 7K40.Mpro molecular surface is colored by subsite: S1 (red), S2 (green), S3 (blue) and S1’ (yellow).

**Table S1**: Physicochemical properties of 37AI-generated fragments selected for purchase.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| MolPort ID | MW | cLogP | HBD | HBA | SAScore | Csp3 |
| MolPort-001-978-204 | 284.34 | 3.56 | 1 | 4 | 1.64 | 0.07 |
| MolPort-001-488-948 | 206.27 | 2.64 | 1 | 3 | 1.72 | 0.20 |
| MolPort-003-250-760 | 298.37 | 3.95 | 1 | 4 | 1.70 | 0.13 |
| MolPort-001-490-649 | 220.30 | 2.89 | 1 | 3 | 1.76 | 0.27 |
| MolPort-001-488-988 | 192.24 | 2.25 | 1 | 3 | 1.69 | 0.11 |
| MolPort-008-299-715 | 279.41 | 1.27 | 1 | 4 | 2.16 | 0.62 |
| MolPort-001-644-741 | 295.41 | 0.51 | 1 | 5 | 2.23 | 0.62 |
| MolPort-035-835-271 | 279.34 | -0.64 | 1 | 6 | 2.00 | 0.62 |
| MolPort-002-141-795 | 218.23 | 2.33 | 0 | 3 | 1.92 | 0.17 |
| MolPort-009-188-048 | 220.30 | 2.56 | 0 | 4 | 2.39 | 0.27 |
| MolPort-000-476-145 | 201.23 | 1.58 | 0 | 4 | 2.15 | 0.18 |
| MolPort-002-087-958 | 272.30 | 3.28 | 0 | 4 | 2.10 | 0.38 |
| MolPort-000-881-866 | 204.18 | 1.72 | 0 | 4 | 2.00 | 0.09 |
| MolPort-000-869-497 | 261.75 | 3.57 | 0 | 2 | 1.90 | 0.27 |
| MolPort-000-145-183 | 282.17 | 4.17 | 0 | 2 | 1.99 | 0.21 |
| MolPort-002-050-562 | 255.25 | 2.56 | 0 | 2 | 1.70 | 0.07 |
| MolPort-002-665-737 | 238.25 | 1.81 | 0 | 3 | 1.87 | 0.07 |
| MolPort-001-613-317 | 225.30 | 2.16 | 1 | 5 | 2.36 | 0.13 |
| MolPort-001-987-455 | 219.27 | 2.10 | 1 | 4 | 1.93 | 0.10 |
| MolPort-000-477-378 | 251.36 | 1.53 | 2 | 3 | 2.11 | 0.42 |
| MolPort-000-477-559 | 251.36 | 1.53 | 2 | 3 | 2.18 | 0.42 |
| MolPort-000-900-636 | 218.30 | 2.29 | 1 | 2 | 1.65 | 0.46 |
| MolPort-000-876-515 | 204.27 | 1.89 | 1 | 2 | 1.63 | 0.42 |
| MolPort-006-067-818 | 262.74 | 2.60 | 1 | 1 | 2.35 | 0.36 |
| MolPort-000-151-211 | 247.32 | 3.17 | 1 | 4 | 1.97 | 0.15 |
| MolPort-002-616-689 | 291.38 | 1.90 | 1 | 6 | 2.95 | 0.57 |
| MolPort-002-001-389 | 214.65 | 2.13 | 0 | 3 | 1.82 | 0.30 |
| MolPort-000-002-336 | 190.20 | 1.74 | 1 | 3 | 1.96 | 0.20 |
| MolPort-046-893-813 | 276.37 | 2.13 | 1 | 5 | 2.31 | 0.46 |
| MolPort-002-312-078 | 249.31 | 1.62 | 0 | 3 | 2.21 | 0.50 |
| MolPort-001-757-547 | 234.08 | 1.72 | 2 | 2 | 1.64 | 0.22 |
| MolPort-000-998-473 | 232.24 | 1.28 | 1 | 3 | 2.19 | 0.25 |
| MolPort-001-799-281 | 234.08 | 2.11 | 2 | 3 | 2.71 | 0.22 |
| MolPort-001-990-142 | 254.72 | 1.70 | 1 | 3 | 1.87 | 0.42 |
| MolPort-000-153-326 | 214.61 | 1.77 | 1 | 3 | 1.83 | 0.13 |
| MolPort-001-757-947 | 272.30 | 1.30 | 0 | 3 | 1.79 | 0.40 |
| MolPort-008-304-480 | 248.33 | 1.17 | 0 | 4 | 2.02 | 0.62 |

MW: molecular weight; cLogP: logarithm of calculated octanol:water partition coefficient (P); HBD: number of hydrogen bond donor atoms; HBA: number hydrogen bond acceptor atoms; SAScore: synthetic accessibility score; Csp3: proportion of sp3 hybridized carbon atoms.

**Table S2**: Screening of 34 fragment-like compounds against SARS-CoV-2Mpro.

|  |  |  |
| --- | --- | --- |
| Compound | % Residual activity | SD (%) |
| Control w/o DMSO | 100.60 | 9.65 |
| Control DMS0 1% | 100.00 | 9.71 |
| MolPort-008-304-480 | 109.1 | 12.61 |
| MolPort-000-869-497 | 99.89 | 6.60 |
| MolPort-001-990-142 | 97.49 | 6.05 |
| MolPort-003-250-760 | 96.68 | 17.06 |
| MolPort-002-050-562 | 95.88 | 10.92 |
| MolPort-001-757-547 | 95.59 | 2.61 |
| MolPort-002-087-958 | 94.83 | 0.41 |
| MolPort-001-488-988 | 93.71 | 3.32 |
| MolPort-001-490-649 | 92.61 | 5.07 |
| MolPort-035-835-271 | 92.46 | 10.53 |
| MolPort-002-312-078 | 91.48 | 3.01 |
| MolPort-001-987-455 | 89.9 | 1.84 |
| MolPort-008-299-715 | 88.86 | 2.24 |
| MolPort-001-757-947 | 87.97 | 3.64 |
| MolPort-000-477-378 | 86.92 | 8.58 |
| MolPort-001-644-741 | 84.3 | 1.34 |
| MolPort-001-488-948 | 84.21 | 5.96 |
| MolPort-000-998-473 | 82.67 | 4.36 |
| MolPort-000-151-211 | 82.35 | 9.22 |
| MolPort-000-002-336 | 81.98 | 7.11 |
| MolPort-000-900-636 | 81.24 | 5.68 |
| MolPort-001-978-204 | 81.05 | 8.93 |
| MolPort-002-001-389 | 78.61 | 4.81 |
| MolPort-000-153-326 | 76.97 | 1.03 |
| MolPort-001-613-317 | 76.48 | 8.38 |
| MolPort-046-893-813 | 75.48 | 6.51 |
| MolPort-002-616-689 | 72.04 | 3.28 |
| MolPort-001-799-281 | 66.85 | 4.69 |
| MolPort-000-145-183 | 59.28 | 6.95 |
| MolPort-009-188-048 | 48.77 | 6.67 |
| MolPort-002-665-737 | 11.85 | 8.10 |
| MolPort-006-067-818 | 6.60 | 9.72 |
| MolPort-000-881-866 | N.D. | N.D. |
| MolPort-000-876-515 | N.D. | N.D. |

\* % Residual activity values were calculated from mean Initial velocities (Vo; n = 3) for hydrolysis of the fluorogenic substrate MCA-AVLQSGFR-Lys(Dnp)-Lys-NH2by SARS-CoV-2 Mpro using control with added 1% DMSO as 100%. \*\* Not determined (N.D.). Compound MolPort-000-881-866 could not be tested due to interference with assay read out. Compound MolPort-000-876-515 was insoluble at assay conditions.

**Table S3.** SMILES for all 34 fragments obtained from the generative model.

|  |  |
| --- | --- |
| **Molecule Name** | **SMILES** |
| MolPort-001-978-204 | COc1ccccc1C(=O)Nc1nc2ccccc2s1 |
| MolPort-001-488-948 | CCC(=O)Nc1nc2ccccc2s1 |
| MolPort-003-250-760 | CCOc1ccccc1C(=O)Nc1nc2ccccc2s1 |
| MolPort-001-490-649 | CC(C)C(=O)Nc1nc2ccccc2s1 |
| MolPort-001-488-988 | CC(=O)Nc1nc2ccccc2s1 |
| MolPort-008-299-715 | CCCCNC(=S)N1CCN(c2ncccn2)CC1 |
| MolPort-001-644-741 | COCCCNC(=S)N1CCN(c2ncccn2)CC1 |
| MolPort-035-835-271 | COCCNC(=O)CN1CCN(CC1)c1ncccn1 |
| MolPort-009-188-048 | Cc1cc(C)n(C(=O)c2ccc(C)s2)n1 |
| MolPort-002-087-958 | O=C(Oc1ccc2ccc(=O)oc2c1)C1CCCCC1 |
| MolPort-000-881-866 | CC(=O)Oc1ccc2ccc(=O)oc2c1 |
| MolPort-000-869-497 | Cc1cc(C(=O)CCl)c(C)n1Cc1ccccc1 |
| MolPort-000-145-183 | Cc1cc(C(=O)CCl)c(C)n1-c1ccc(Cl)cc1 |
| MolPort-002-050-562 | Fc1ccc(CN2C(=O)C(=O)c3ccccc23)cc1 |
| MolPort-002-665-737 | O=C1N(Cc2ccncc2)c2ccccc2C1=O |
| MolPort-001-613-317 | Cc1ccc(C(=O)Nc2nncs2)s1 |
| MolPort-001-987-455 | Cc1cccc(C(=O)Nc2nncs2)c1 |
| MolPort-000-477-378 | Cc1ccc(NC(=S)NN2CCOCC2)cc1 |
| MolPort-000-900-636 | Nc1ccccc1C(=O)N1CCCCCC1 |
| MolPort-000-876-515 | Nc1ccccc1C(=O)N1CCCCC1 |
| MolPort-006-067-818 | Cc1ccc2[nH]c3CCN(Cc3c2c1)C(=O)CCl |
| MolPort-000-151-211 | CCOC(=O)c1cc(sc1N)-c1ccccc1 |
| MolPort-002-616-689 | CC1(C)Cc2c(sc3nc(C4CC4)n(N)c(=O)c23)CO1 |
| MolPort-002-001-389 | COc1ccc(OC)c(C(=O)CCl)c1 |
| MolPort-000-002-336 | CCOC(=O)c1ccc2nc[nH]c2c1 |
| MolPort-046-893-813 | Cc1nc(N)c2c(C)c(C(=O)N3CCCC3)sc2n1 |
| MolPort-002-312-078 | Cc1ccc(OC(C)C(=O)N2CCOCC2)cc1 |
| MolPort-001-757-547 | O=C(NCCO)c1ccc(Cl)c(Cl)c1 |
| MolPort-000-998-473 | Cc1ccc(N2C(=O)CC(=O)NC2=O)cc1C |
| MolPort-001-799-281 | OCCN=Cc1cc(Cl)cc(Cl)c1O |
| MolPort-001-990-142 | O=C(CCl)Nc1ccccc1N1CCOCC1 |
| MolPort-000-153-326 | O=C(CCl)Nc1ccccc1[N+](=O)[O-] |
| MolPort-001-757-947 | O=C(CN1C(=O)c2ccccc2C1=O)N1CCCCC1 |
| MolPort-008-304-480 | CC(C)CC(=O)N1CCN(c2ncccn2)CC1 |