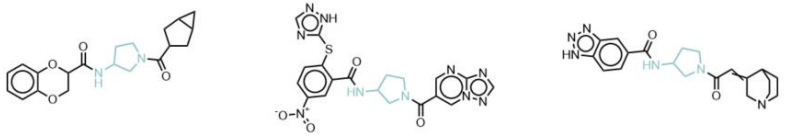
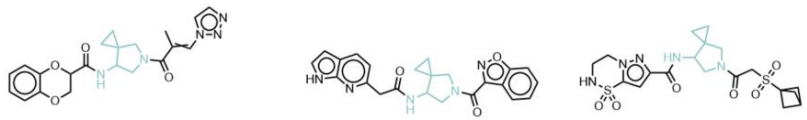
De-Novo Generated Top 100 Scoring Compounds:
Moving away from Pyridine N-oxides

- 316 diverse acids available from Enamine Building Blocks and created 10 libraries of about 93,000 compounds each.
- 10 diamine core libraries, 93’026 molecules per library = ~ 1 million compounds.

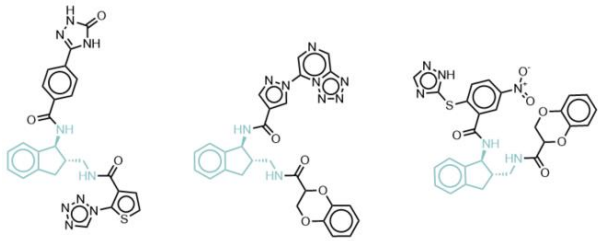
Core 1



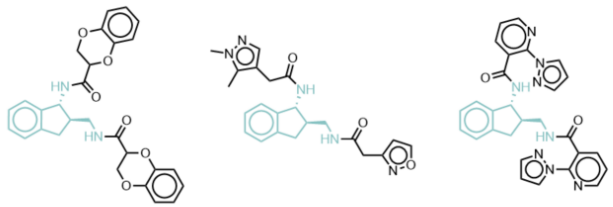
Core 2



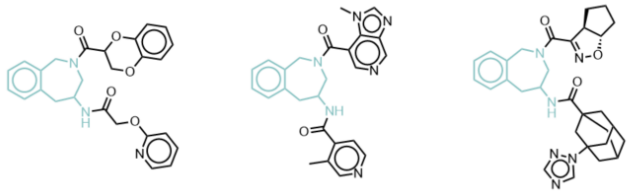
Core 3a



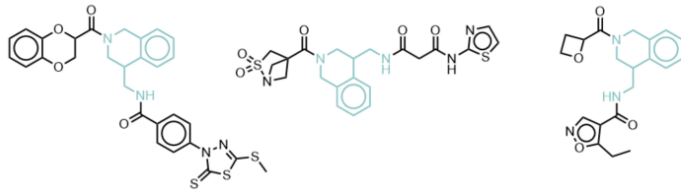
Core 3b



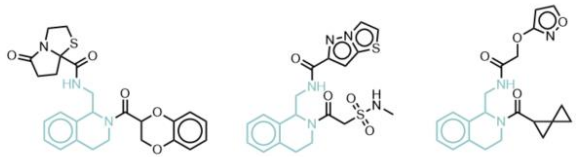
Core 4



Core 5



Core 6



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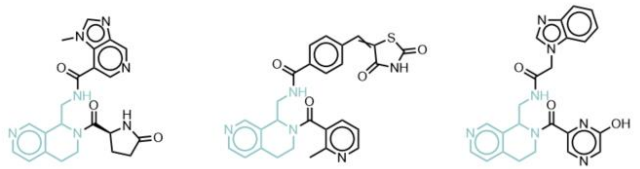
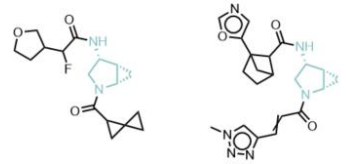
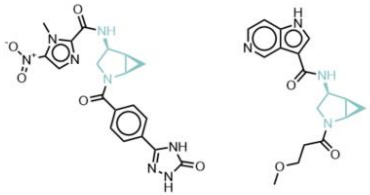


Core 8a

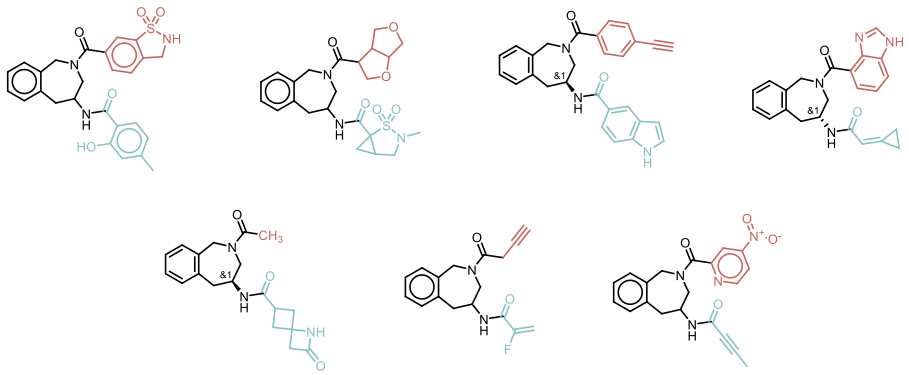


Core 8b



De-Novo Generated Top 100 Scoring Compounds:
Moving away from Pyridine N-oxides whilst retaining cores

1) Diversification of the amine cores with commercially available and diverse Enamine carboxylic acids afforded 93'026 compounds, which were sent for virtual screening (Maestro Glide).

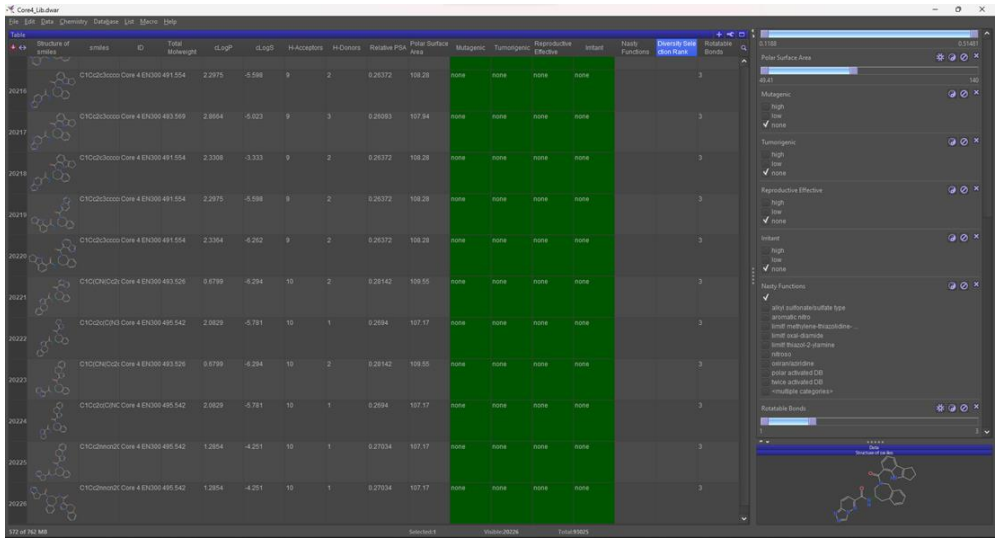


2) DataWarrior triage

Total molweight < 500 Da
cLogP < 5
cLogS < 1
H Acceptors < 10
H Donors < 5
Rotatable bonds < 3
Polar Surface Area < 140 Angstroms
Mutagenic ***X***
Tumorigenic ***X***
Reproductive effective ***X***
Irritant ***X***
Nasty functions ***X***

3) Virtual Screening with PyRx 0.8 / AutoDock Vina 1.2

- Reduced to 20'226 molecules.
- DataWarrior's 'diversity selection rank' in order of structural diversity.
- Selected the top 1000 most diverse compounds for virtual screening using PyRx 0.8/AutoDock Vina 1.2. PyRx - Virtual Screening Tool download | SourceForge.net



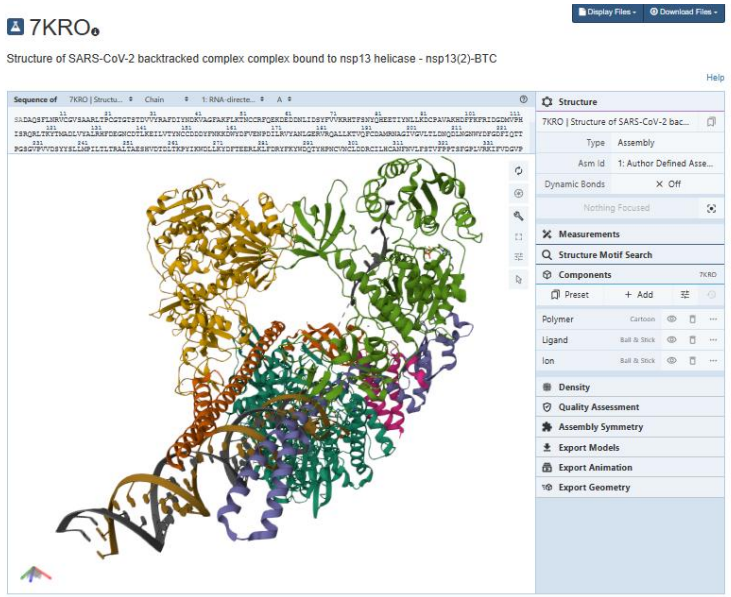
De-Novo Generated Top 100 Scoring Compounds:
Virtual Screen with PyRx 0.8 / AutoDock VINA

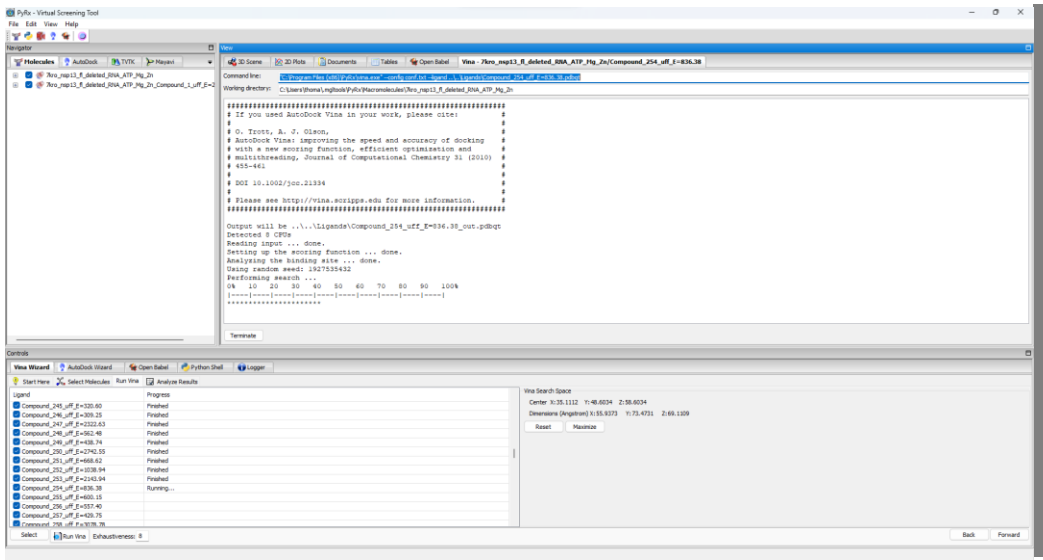
- First docked to pdb 7kro (ATP/(+)ssRNA bound state as part of replicase-transcriptase complex (RTC) cryo-EM structure.

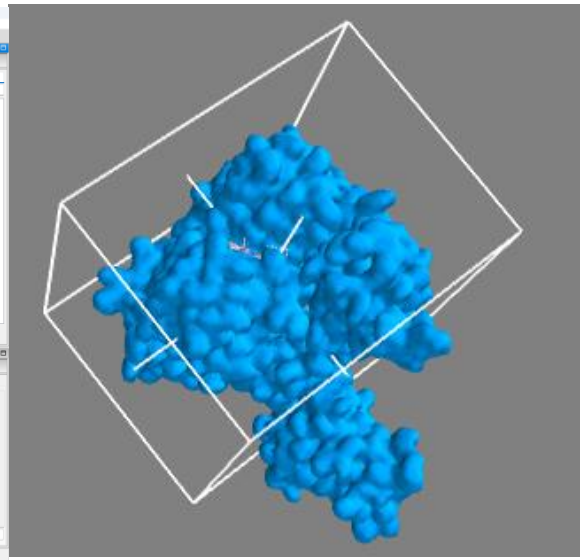
- Backtracking ssRNA ; counteracting the RdRp/nsp12.

- Used **PyRx 0.8** to run virtual screen using **AutoDock Vina v1.2 scoring function** > protein prepared via DockPrep in **Chimera 1.16** with hydrogens added. All ligands energy minimised before running.

- Unbiased virtual screen (RecA domains, ATP site + RNA channel protein grid) using energy-minimised ligands and exhaustiveness = 8 conformers.

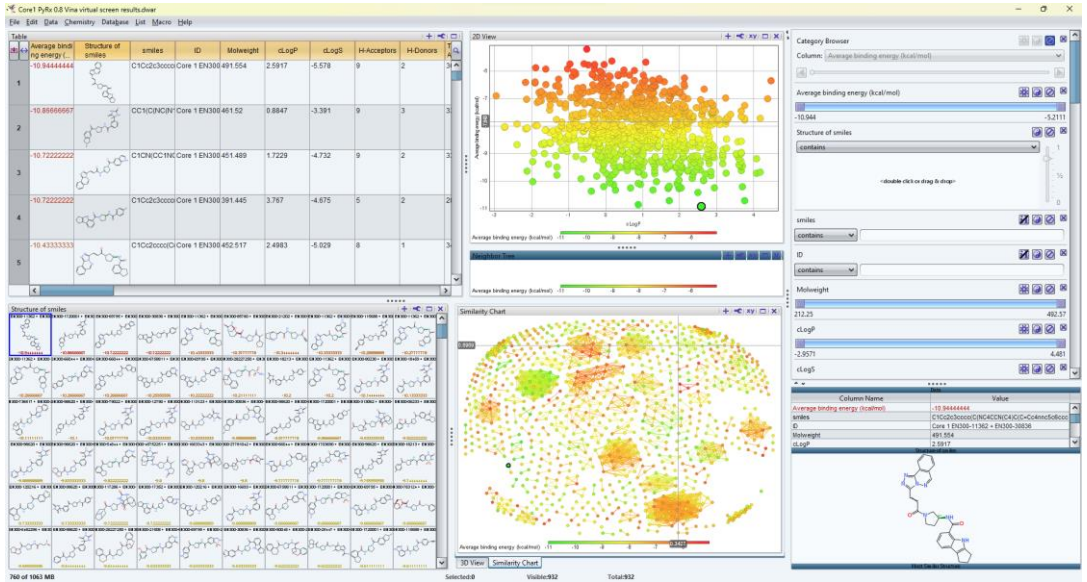




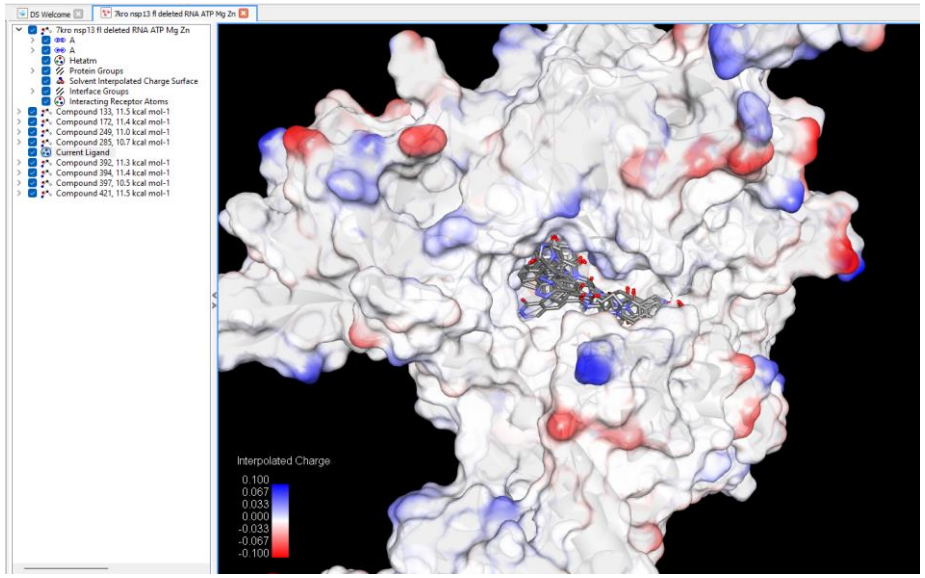


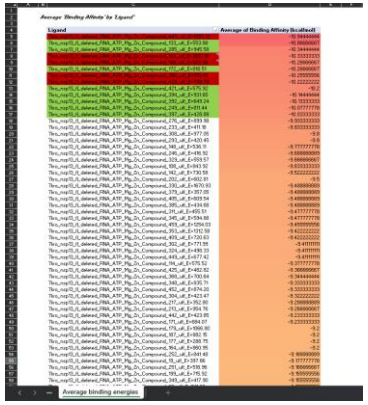
De-Novo Generated Top 100 Scoring Compounds:
Virtual Screen with PyRx 0.8 / AutoDock VINA (Core 1, pdb 7kro)

- Ranked average binding free energy scores, then applied cutoff at **-10.0 kcal/mol** (lead-like binding energy)

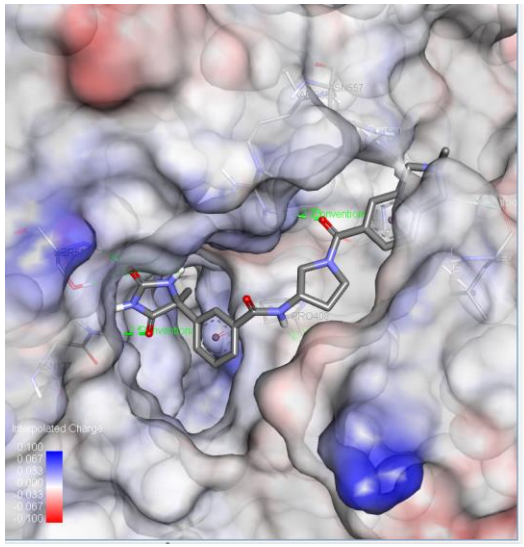


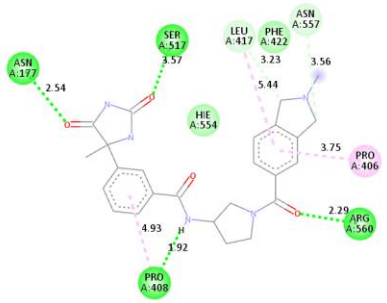
- **Visually inspected** minimum energy poses to validate, eliminating incorrect geometries (cis-amides).

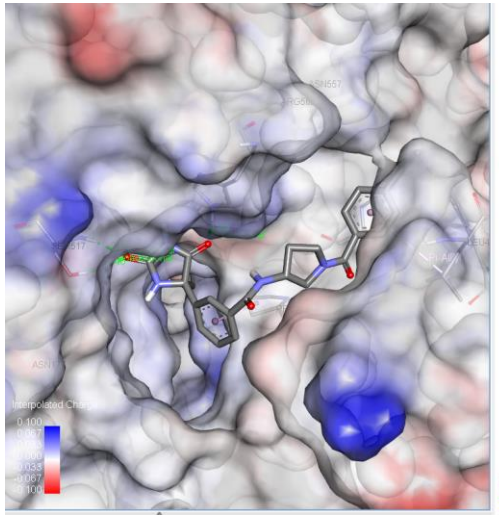


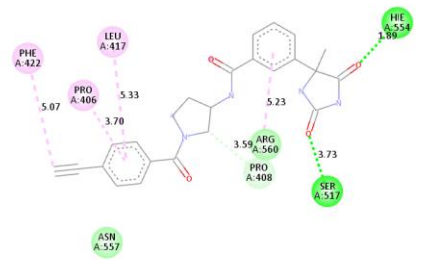


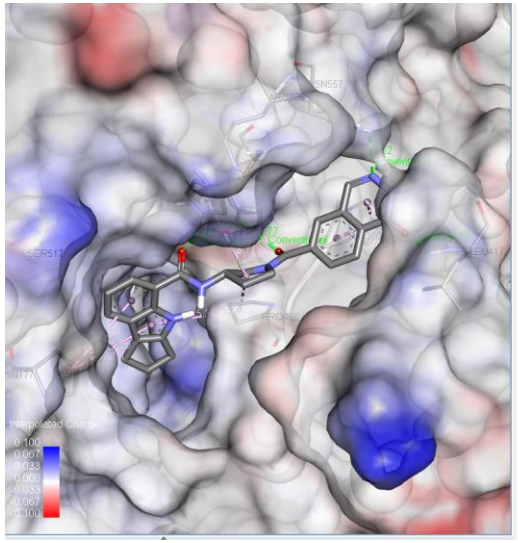
De-Novo Generated Top 100 Scoring Compounds:
Virtual Screen with PyRx 0.8 / AutoDock VINA (Core 1 , pdb 7kro)

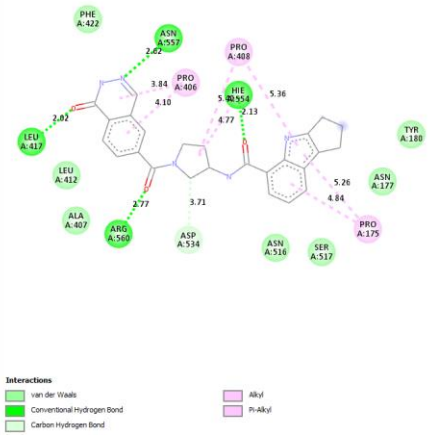


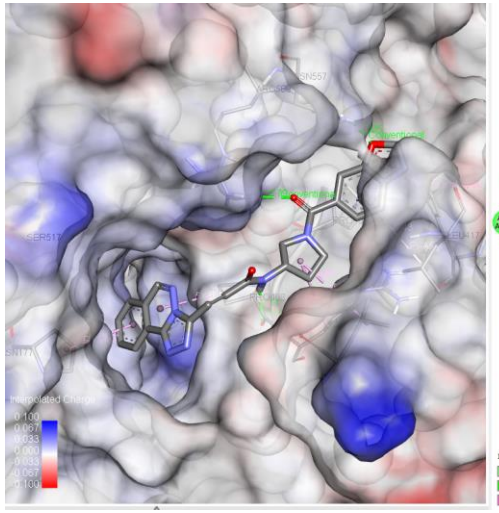


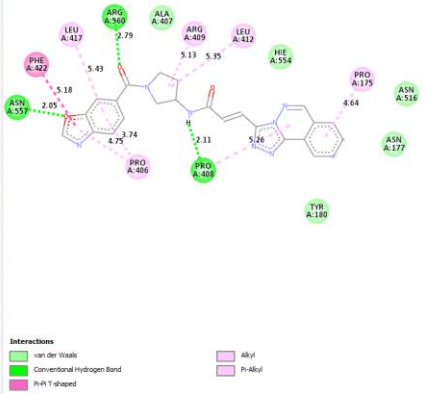




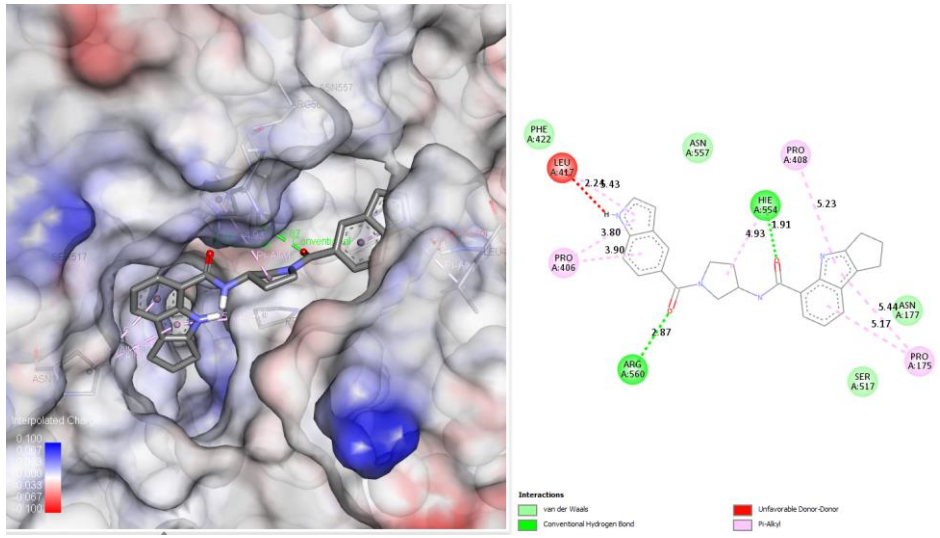


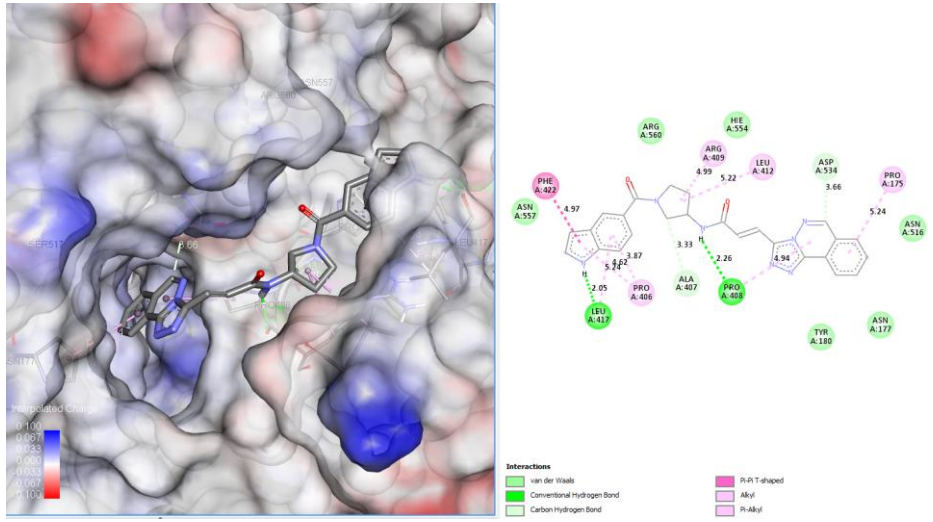


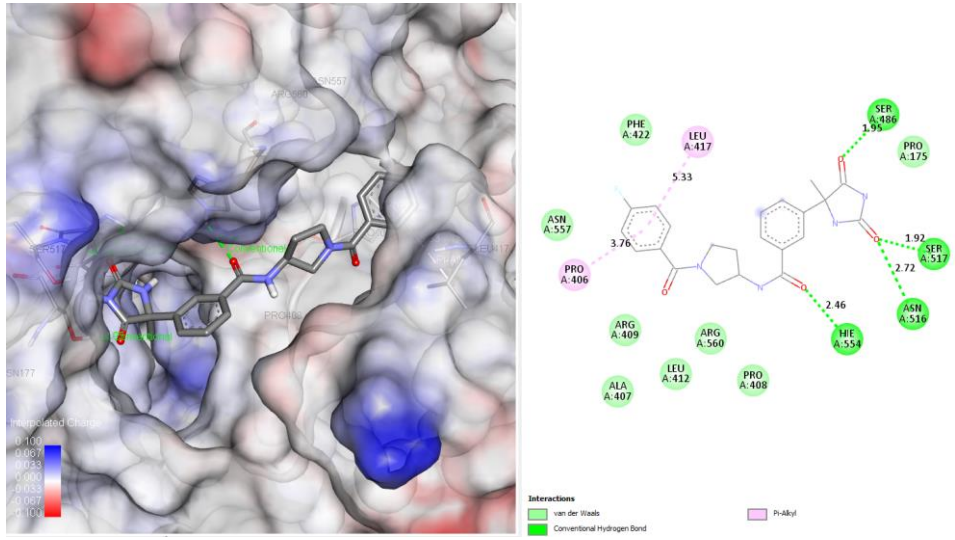


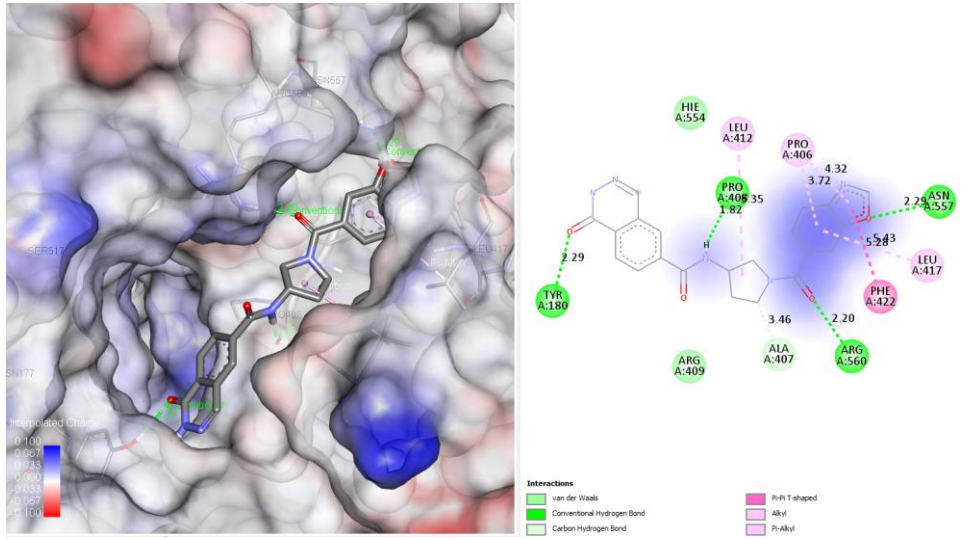


De-Novo Generated Top 100 Scoring Compounds:
Virtual Screen with PyRx 0.8 / AutoDock VINA (Core 1, pdb 7kro)



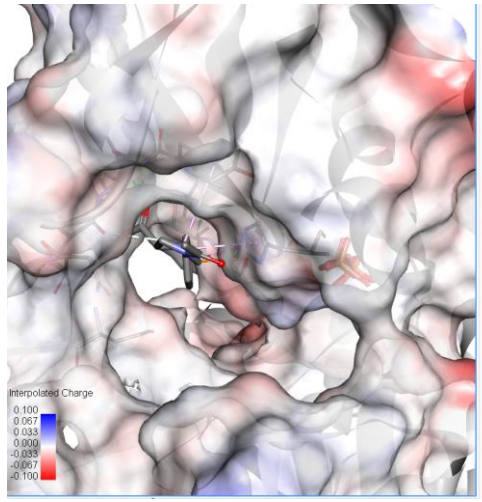


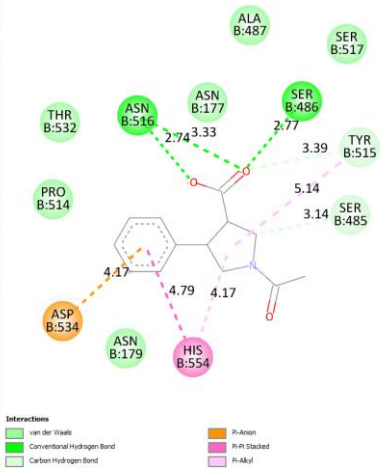


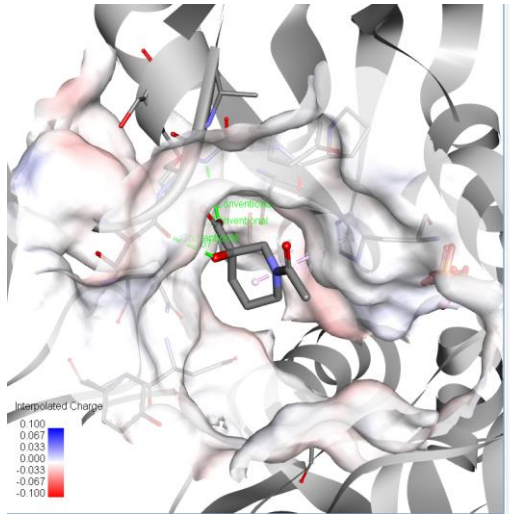


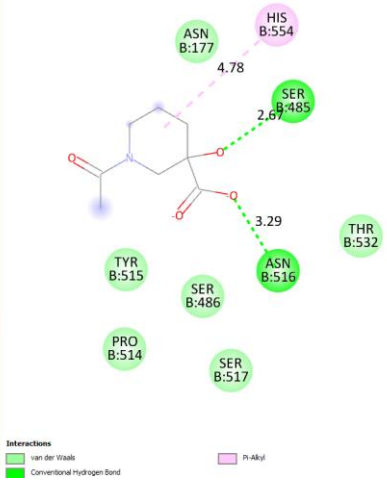
De-Novo Generated Top 100 Scoring Compounds:
Virtual Screen with PyRx 0.8 / AutoDock VINA (Core 1)

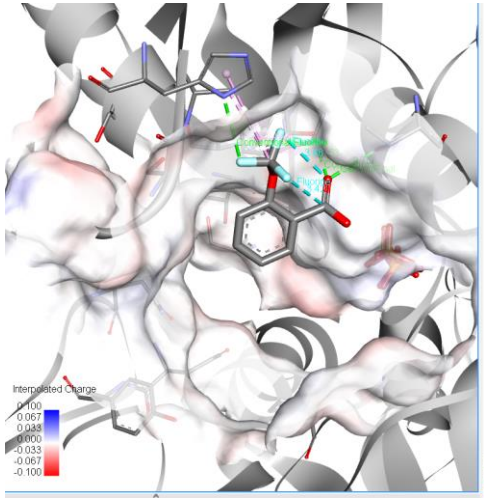
- PANDDA fragment interactions at 5’ RNA site

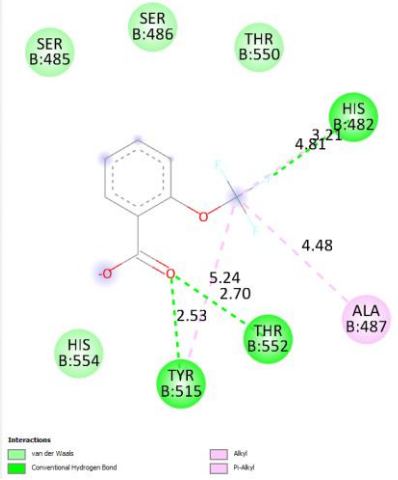






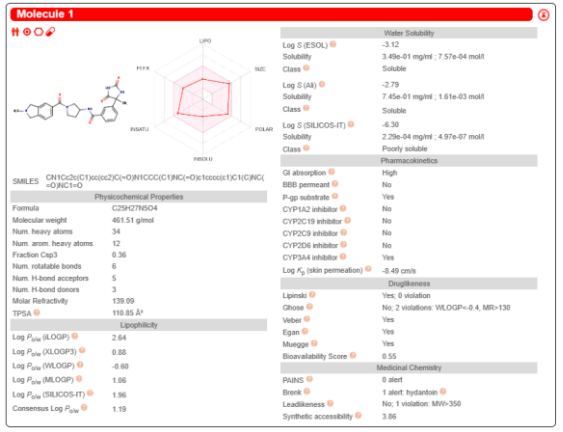


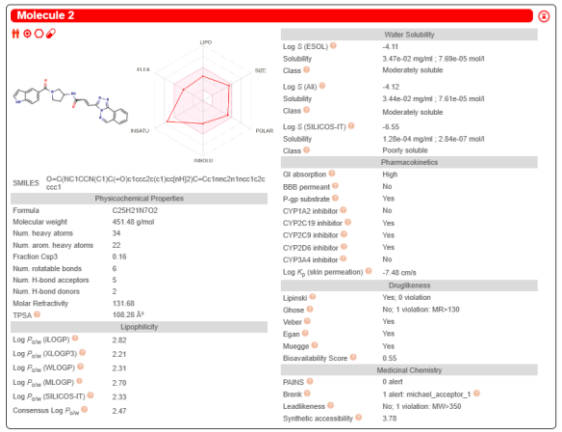


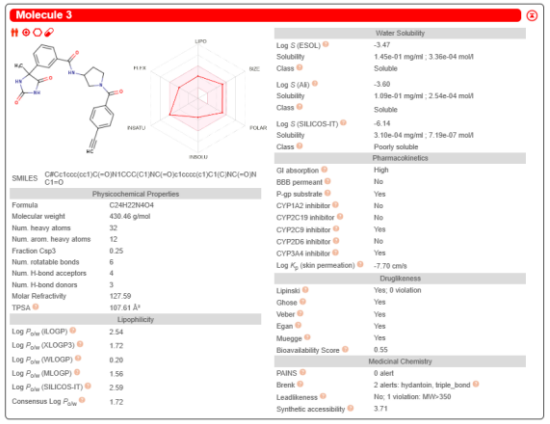


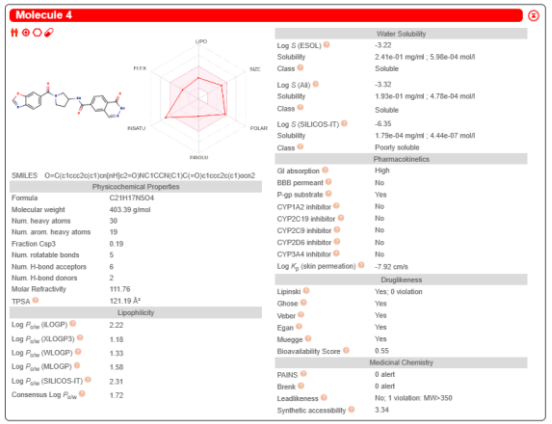
De-Novo Generated Top 100 Scoring Compounds:
Core1 compounds

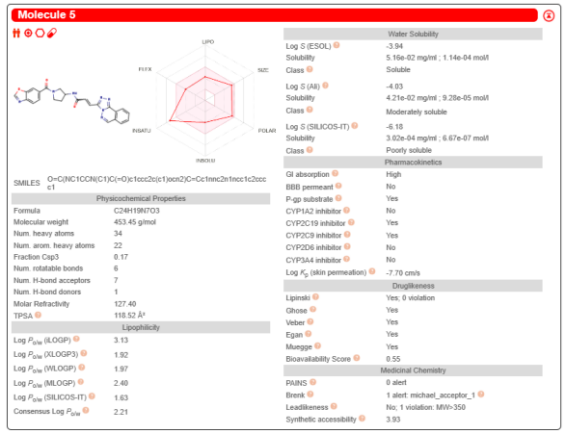
- All compounds PAINS filtering (SwissADME) cleared.

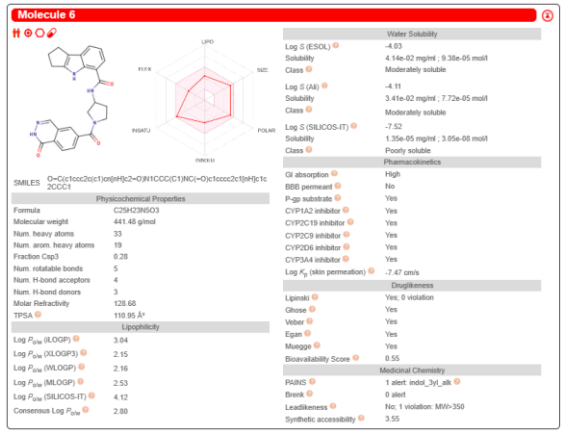


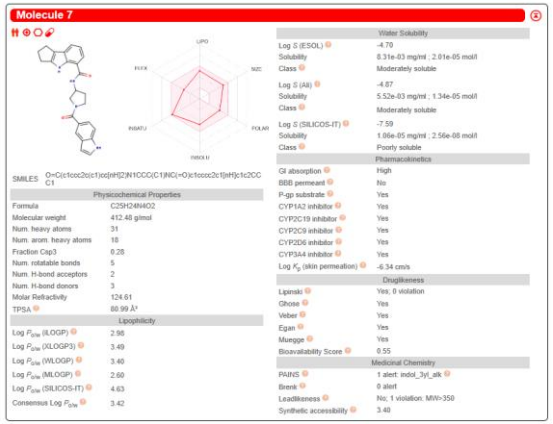


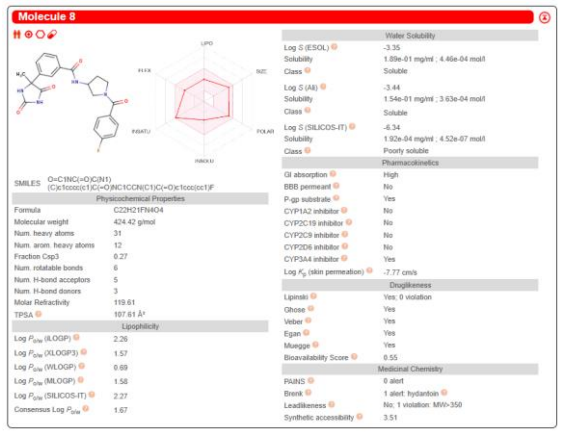








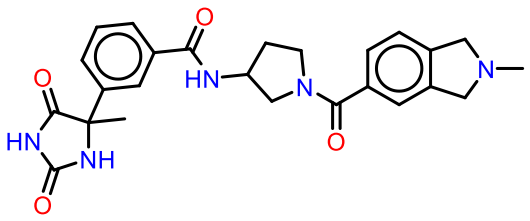




De-Novo Generated Top 100 Scoring Compounds:
Core1 compounds

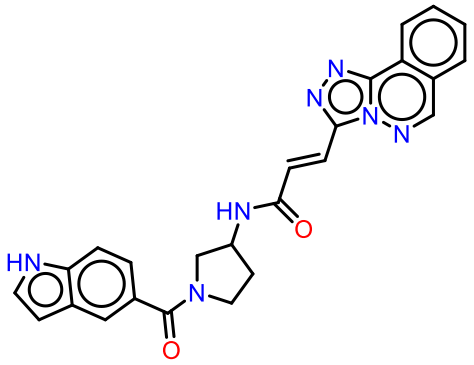
Compound 133

Core 1 EN300-1720001 + EN300-98620



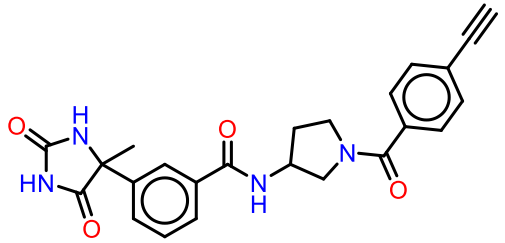
Compound 285

Core 1 EN300-69795 + EN300-11362



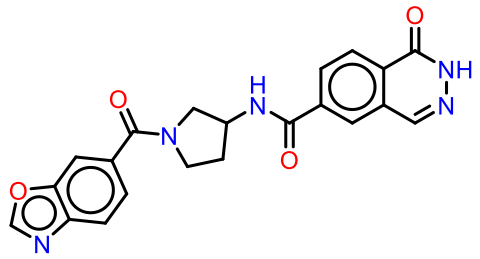
Compound 172

Core 1 EN300-115688 + EN300-98620



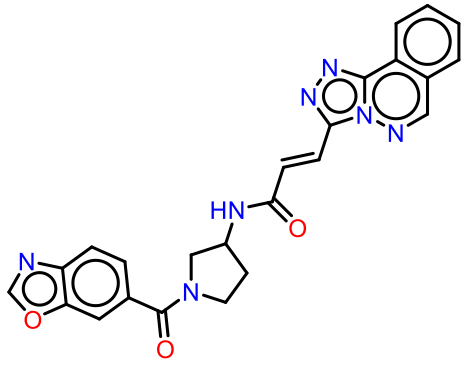
Compound 421

Core 1 EN300-66044 + EN300-6758811



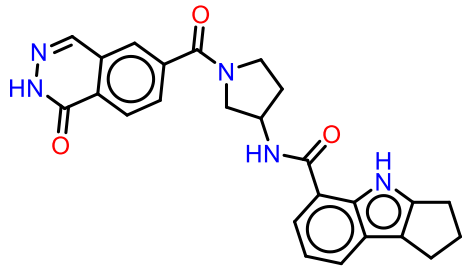
Compound 394

Core 1 EN300-66044 + EN300-11362



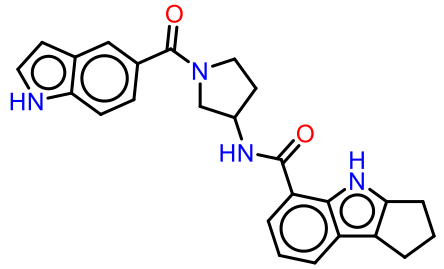
Compound 392

Core 1 EN300-6758811 + EN300-30836



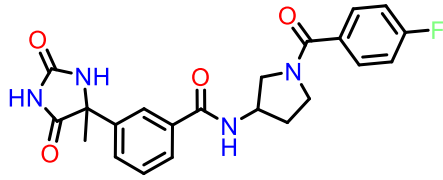
Compound 249

Core 1 EN300-69795 + EN300-30836



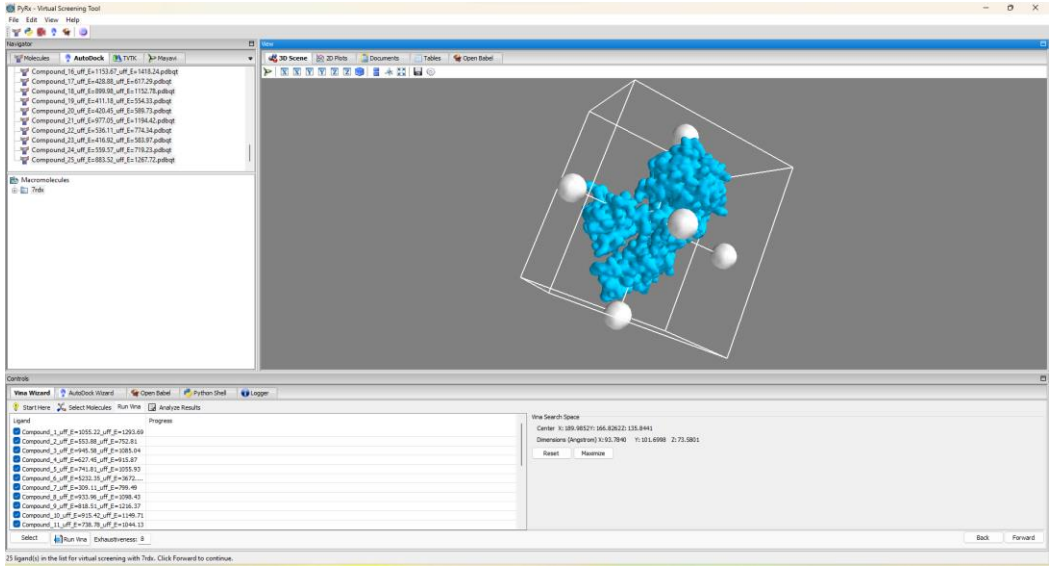
Compound 397

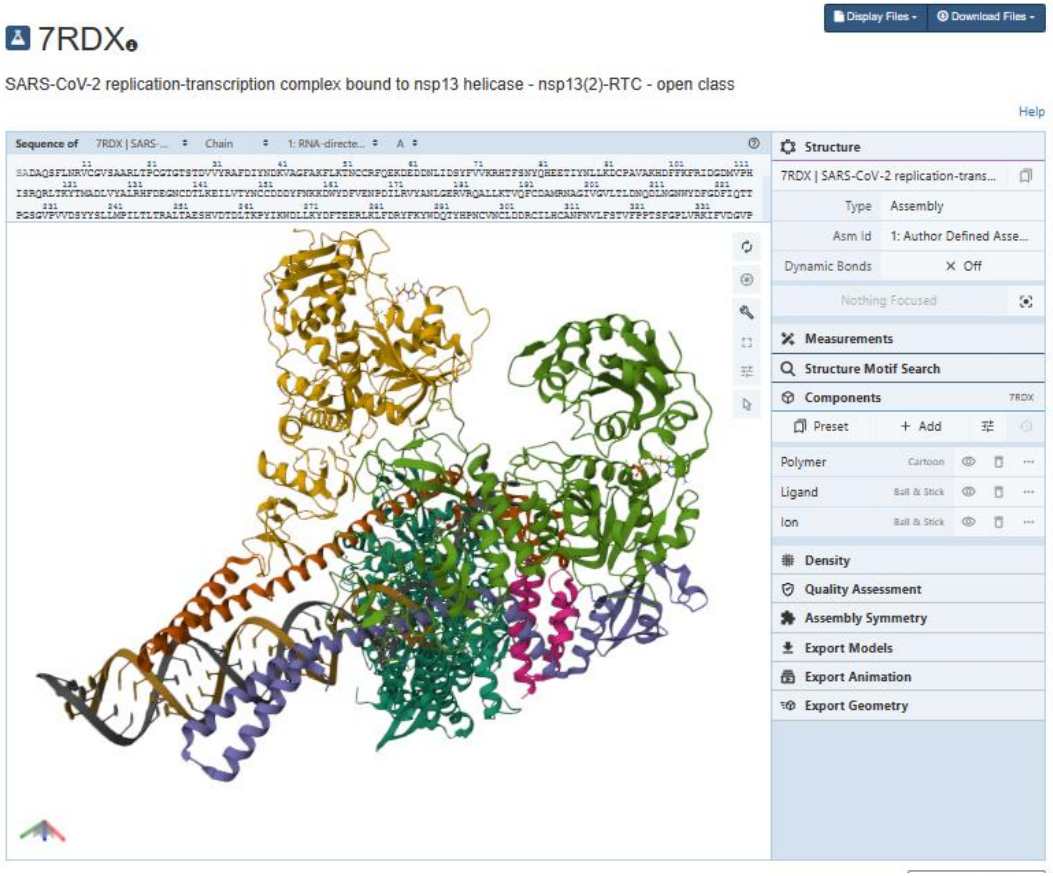
Core 1 EN300-18213 + EN300-98620



De-Novo Generated Top 100 Scoring Compounds:
Virtual Screen with PyRx 0.8 / AutoDock VINA (pdb 7rdx)

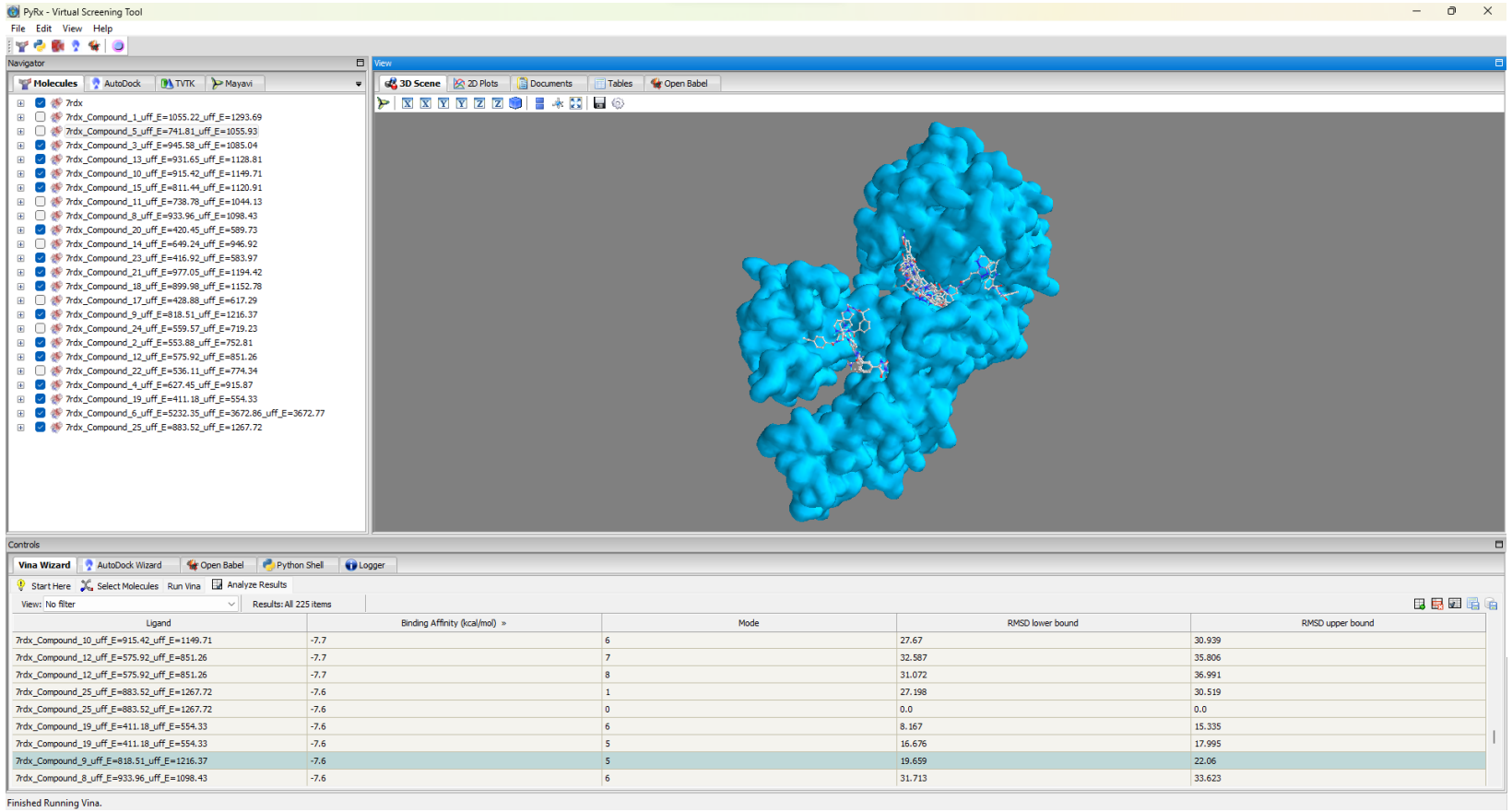
- Next, I factored in the helicase's flexibility by docking to two structural extremes, pdb 7kro (1B domain closed) and 7rdx (1B domain open).
- Selected ligands with average binding scores of **-10 kcal/mol or below** from the first docking run (7kro)**.**
- Re-docked these to **pdb 7rdx (open state)** as part of replicase-transcriptase complex (RTC) cryo-EM structure.
- 1B domain fully open; RNA unbound, ATP bound.
- Unbiased virtual screen (full protein) using energy-minimised ligands and exhaustiveness = 8 conformers.





De-Novo Generated Top 100 Scoring Compounds:
Virtual Screen with PyRx 0.8 / AutoDock VINA (pdb 7rdx)

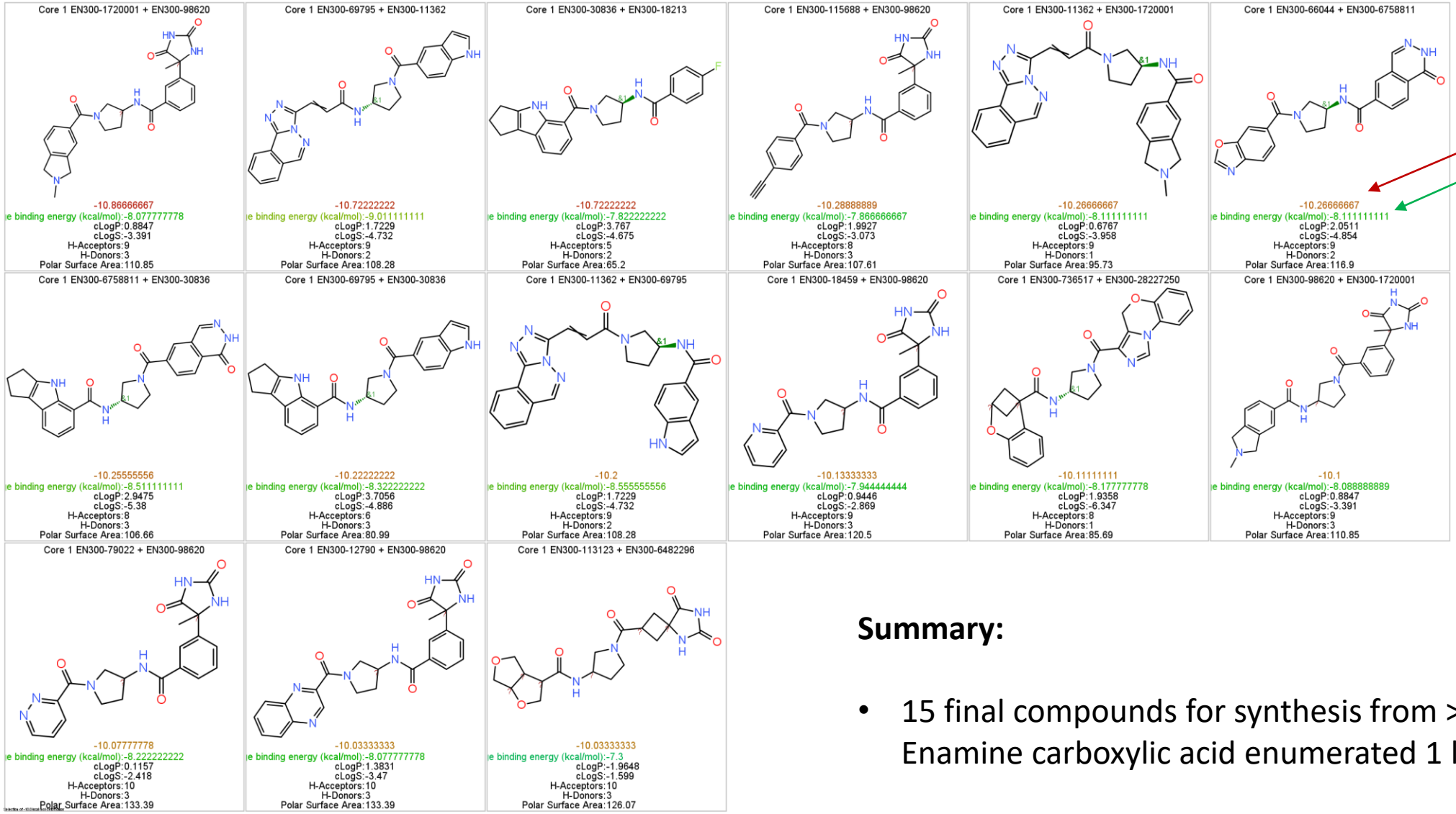
- Visually inspected poses again; selected compounds that remained bound at 5’ RNA site in both structures (pdb 7kro and 7rdx).



Average 'Binding Affinity' by 'Ligand'

Ligand	Average of Binding Affinity
7rdx_Compound_5_uff_E=741.81_uff_E=1055.93	-9.122222222
7rdx_Compound_3_uff_E=945.58_uff_E=1085.04	-9.011111111
7rdx_Compound_1_uff_E=1055.22_uff_E=1293.69	-8.744444444
7rdx_Compound_13_uff_E=931.65_uff_E=1128.81	-8.622222222
7rdx_Compound_18_uff_E=899.98_uff_E=1152.78	-8.555555556
7rdx_Compound_14_uff_E=649.24_uff_E=946.92	-8.511111111
7rdx_Compound_15_uff_E=811.44_uff_E=1120.91	-8.322222222
7rdx_Compound_17_uff_E=428.88_uff_E=617.29	-8.288888889
7rdx_Compound_23_uff_E=416.92_uff_E=583.97	-8.222222222
7rdx_Compound_21_uff_E=977.05_uff_E=1194.42	-8.177777778
7rdx_Compound_10_uff_E=915.42_uff_E=1149.71	-8.133333333
7rdx_Compound_12_uff_E=575.92_uff_E=851.26	-8.111111111
7rdx_Compound_11_uff_E=738.78_uff_E=1044.13	-8.111111111
7rdx_Compound_22_uff_E=536.11_uff_E=774.34	-8.088888889
7rdx_Compound_24_uff_E=559.57_uff_E=719.23	-8.077777778
7rdx_Compound_2_uff_E=553.88_uff_E=752.81	-8.077777778
7rdx_Compound_8_uff_E=933.96_uff_E=1098.43	-8.022222222
7rdx_Compound_20_uff_E=420.45_uff_E=589.73	-7.944444444
7rdx_Compound_9_uff_E=818.51_uff_E=1216.37	-7.866666667
7rdx_Compound_4_uff_E=627.45_uff_E=915.87	-7.822222222
7rdx_Compound_19_uff_E=411.18_uff_E=554.33	-7.755555556
7rdx_Compound_25_uff_E=883.52_uff_E=1267.72	-7.3
7rdx_Compound_16_uff_E=1153.67_uff_E=1418.24	-6.911111111
7rdx_Compound_6_uff_E=5232.35_uff_E=3672.86_uff_E=3672.77	-6.611111111
7rdx_Compound_7_uff_E=309.11_uff_E=799.49	-5.444444444
Grand Total	**-7.994222222**

De-Novo Generated Top 100 Scoring Compounds:
Virtual Screen with PyRx 0.8 / AutoDock VINA (pdb 7rdx and 7kro)



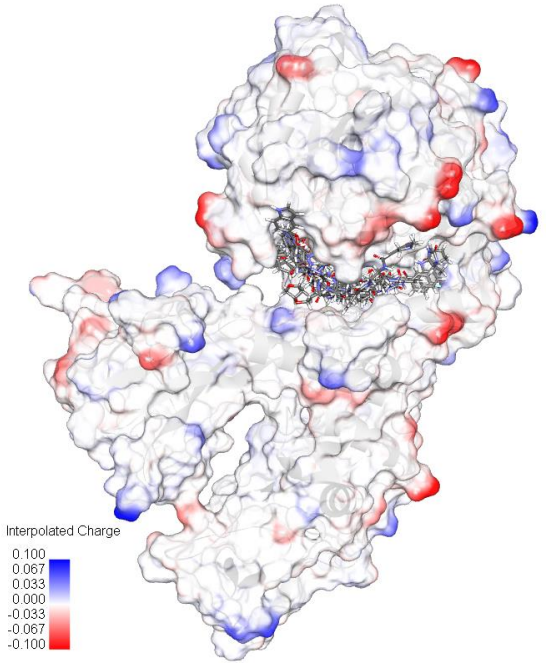
Average binding free energies that cleared visual pose inspection (kcal/mol:

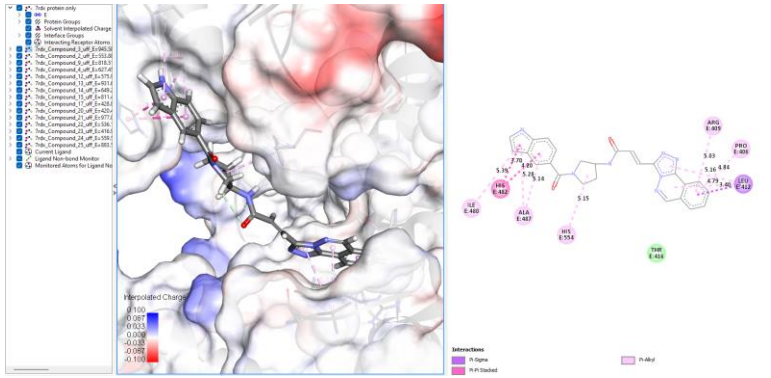
7kro (red/top)
7rdx (green/bottom)

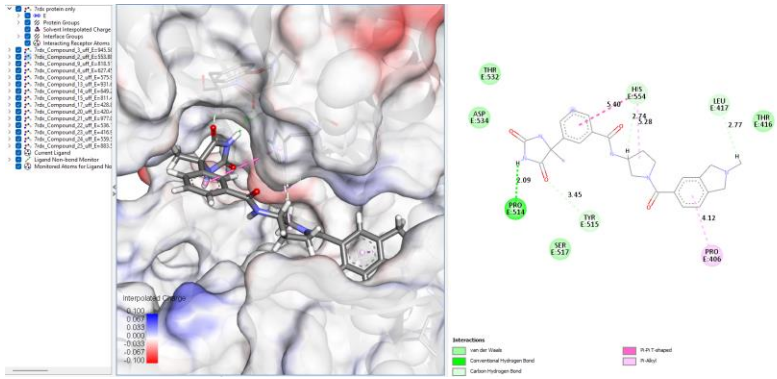
Summary:

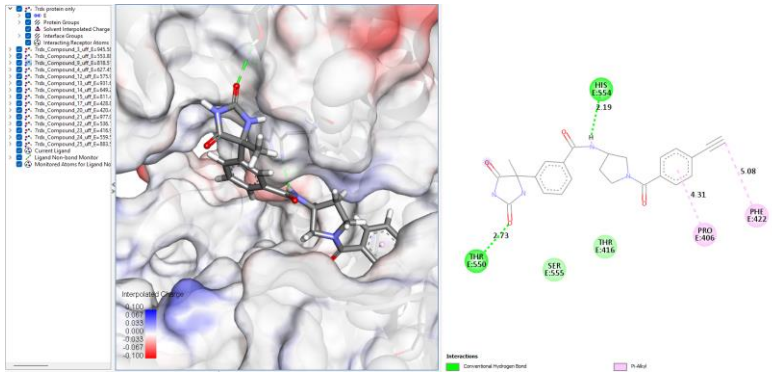
- 15 final compounds for synthesis from >93’000 Core Enamine carboxylic acid enumerated 1 library.

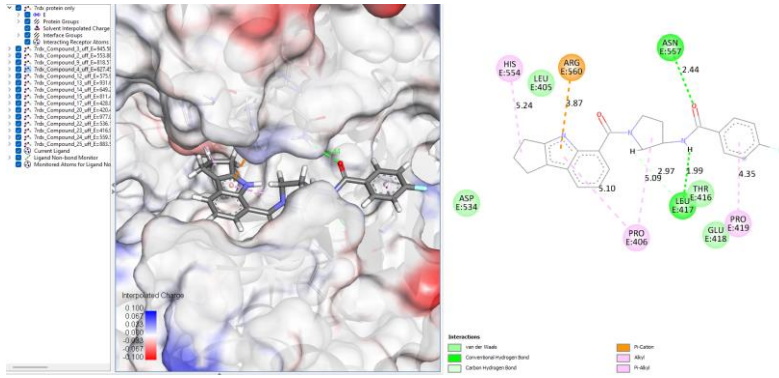
De-Novo Generated Top 100 Scoring Compounds:
Virtual Screen with PyRx 0.8 / AutoDock VINA (pdb 7rdx)

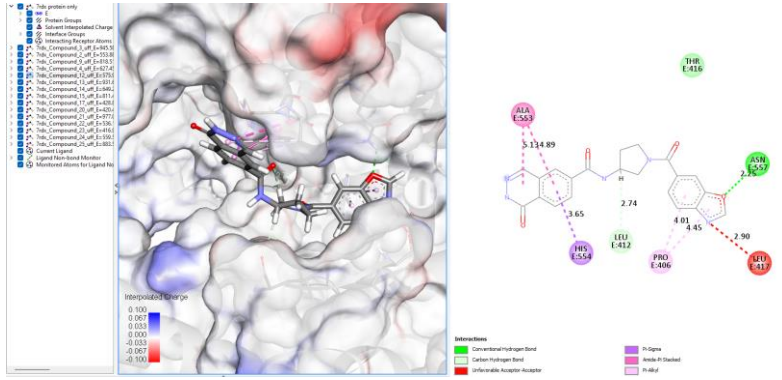


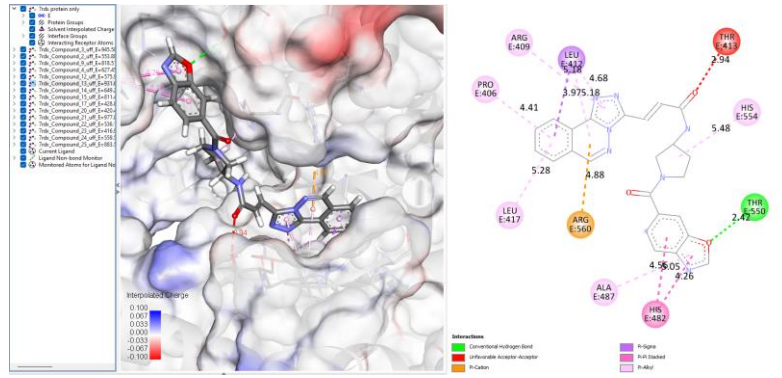




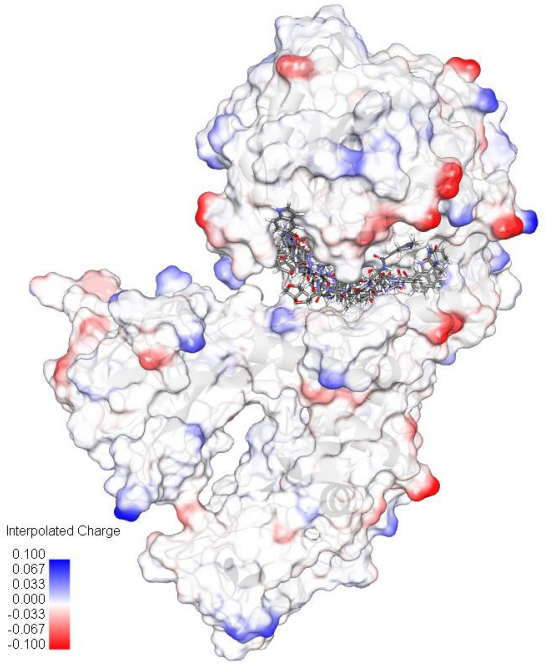


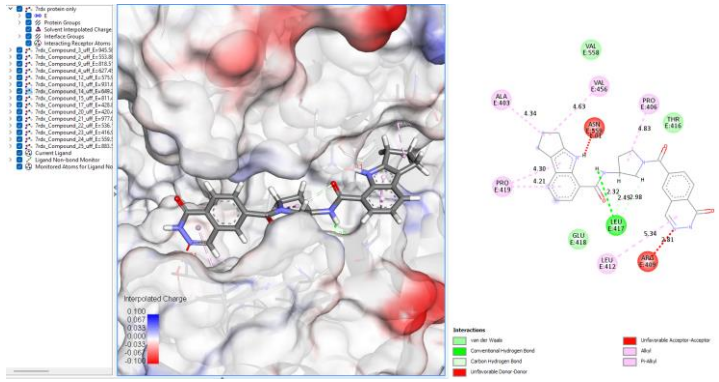


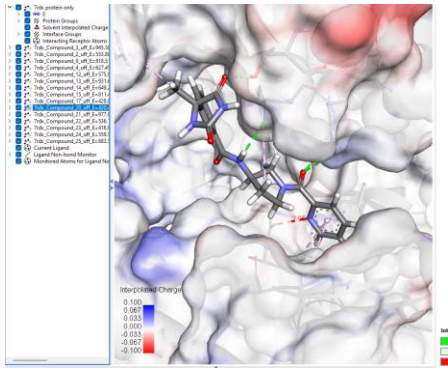


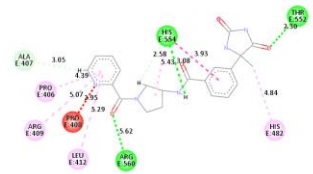


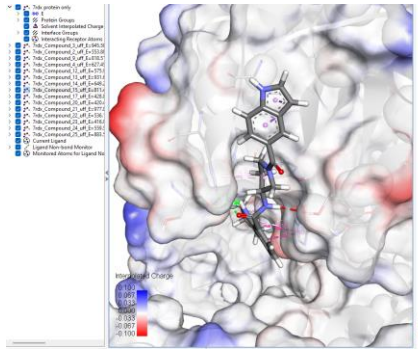
De-Novo Generated Top 100 Scoring Compounds:
Virtual Screen with PyRx 0.8 / AutoDock VINA (pdb 7rdx)

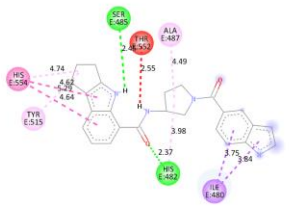


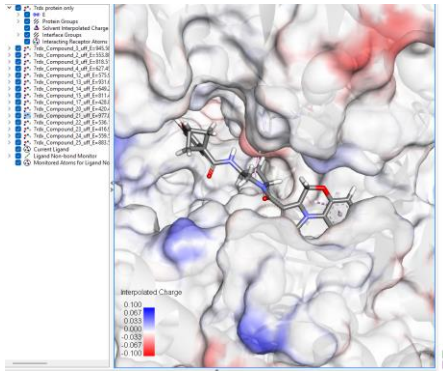


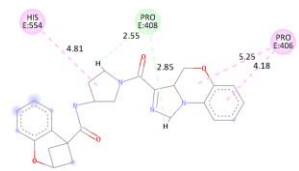


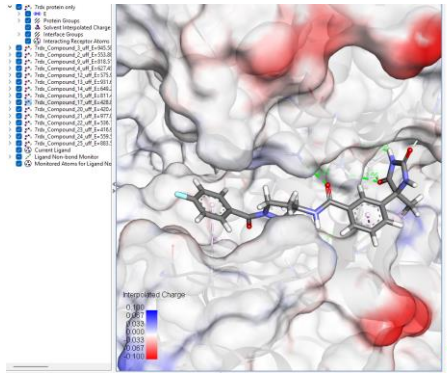


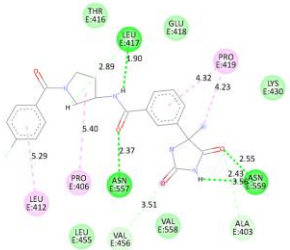


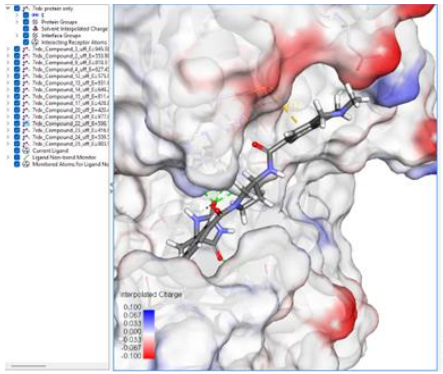


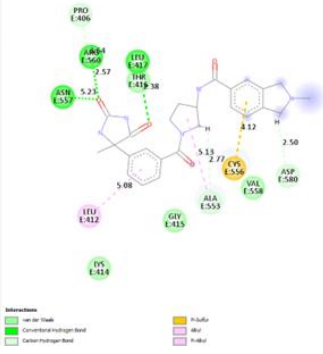




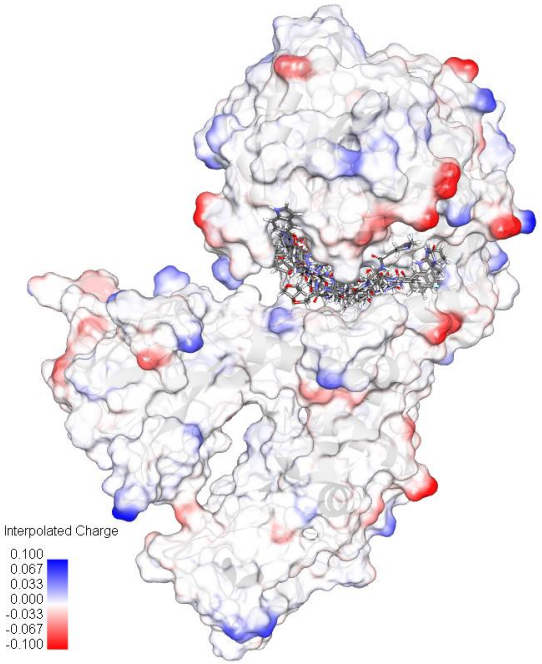


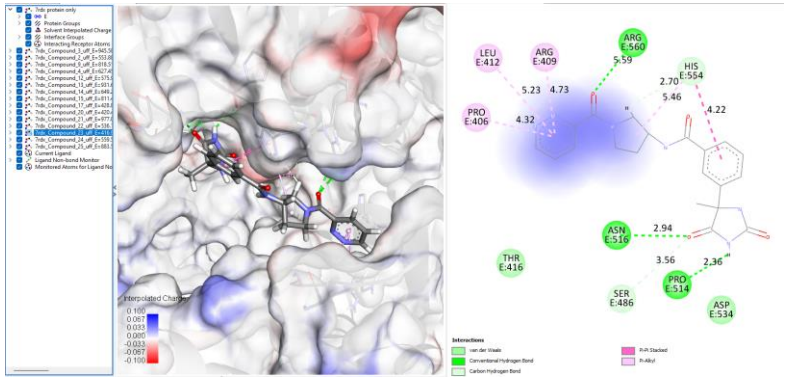


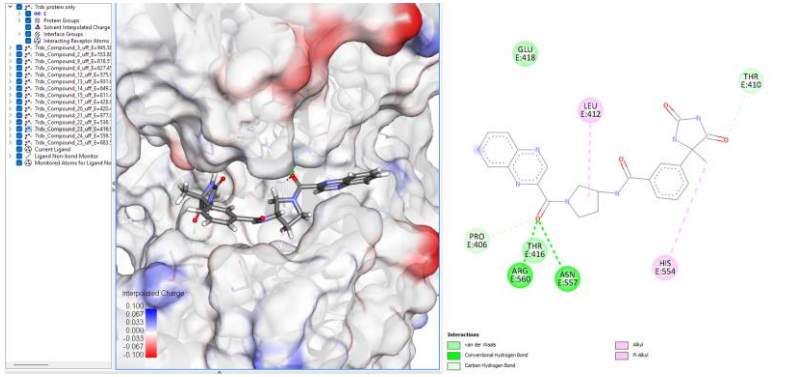


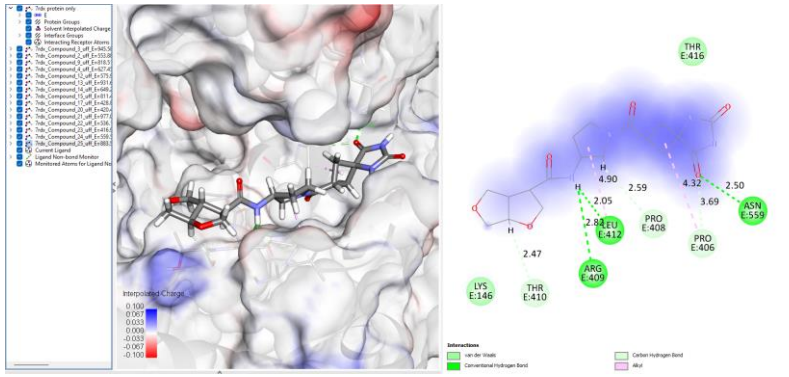


De-Novo Generated Top 100 Scoring Compounds:
Virtual Screen with PyRx 0.8 / AutoDock VINA (pdb 7kro and 7rdx)







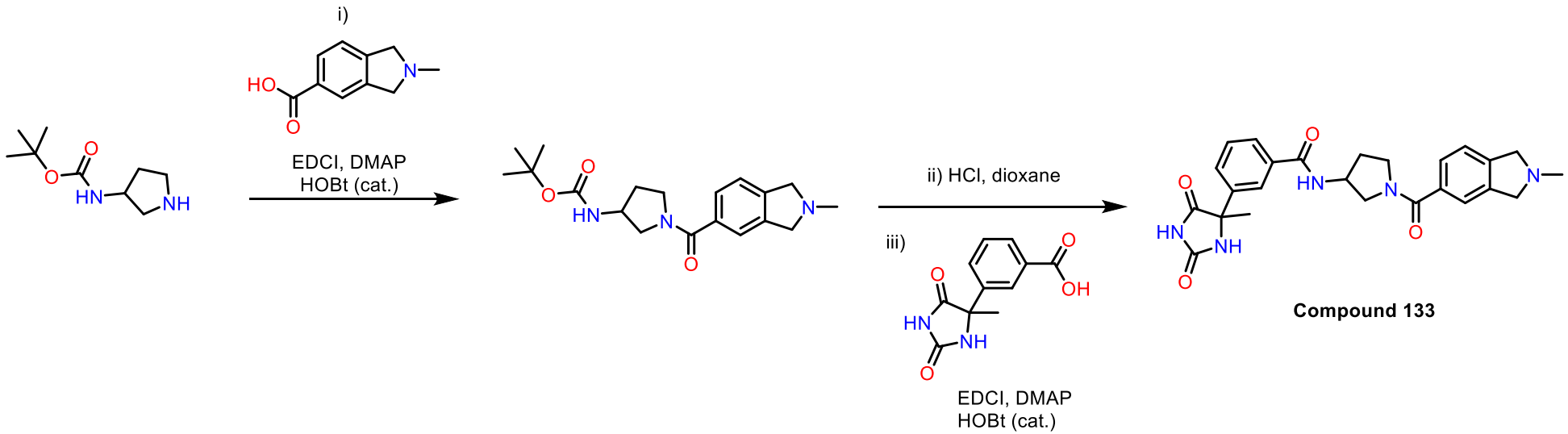


SMILES String:

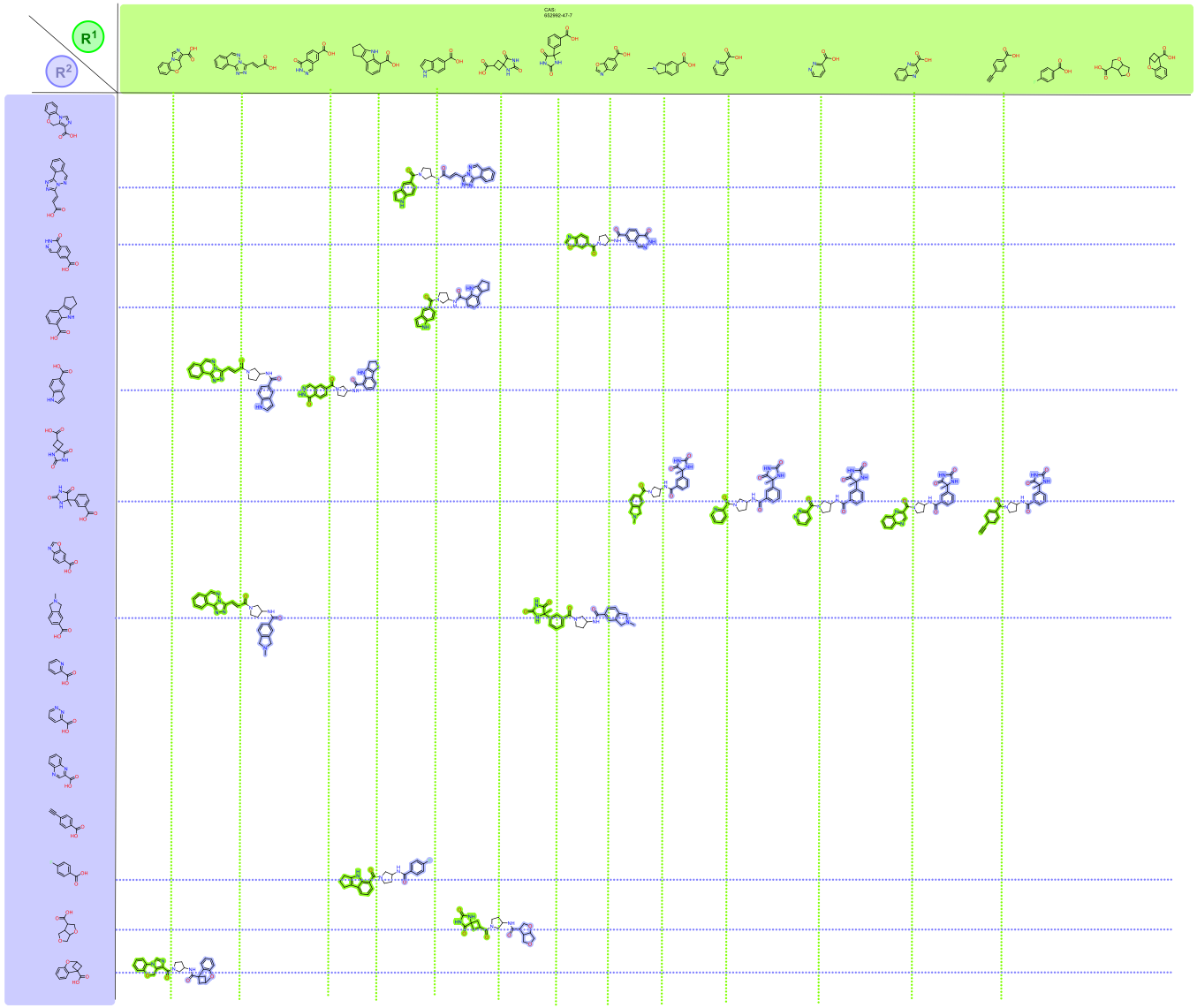
1. CC1(C(NC(N1)=O)=O)c1cccc(c1)C(NC1CCN(C1)C(c1ccc2CN(C)Cc2c1)=O)=O
2. CC1(C(NC(N1)=O)=O)c1cccc(c1)C(N1CCC(C1)NC(c1ccc2CN(C)Cc2c1)=O)=O
3. C1CN(CC1NC(C=Cc1nnc2c3ccccc3cnn12)=O)C(c1ccc2c(cc[nH]2)c1)=O
4. C1Cc2c3cccc(C(N4CCC(C4)NC(c4ccc(cc4)F)=O)=O)c3[nH]c2C1
5. CC1(C(NC(N1)=O)=O)c1cccc(c1)C(NC1CCN(C1)C(c1ccc(C#C)cc1)=O)=O
6. CN1Cc2ccc(cc2C1)C(NC1CCN(C1)C(C=Cc1nnc2c3ccccc3cnn12)=O)=O
7. C1CN(CC1NC(c1ccc2C(NN=Cc2c1)=O)=O)C(c1ccc2c(c1)ocn2)=O
8. C1Cc2c3cccc(C(NC4CCN(C4)C(c4ccc5C(NN=Cc5c4)=O)=O)=O)c3[nH]c2C1
9. C1Cc2c3cccc(C(NC4CCN(C4)C(c4ccc5c(cc[nH]5)c4)=O)=O)c3[nH]c2C1
10. C1CN(CC1NC(c1ccc2c(cc[nH]2)c1)=O)C(C=Cc1nnc2c3ccccc3cnn12)=O
11. CC1(C(NC(N1)=O)=O)c1cccc(c1)C(NC1CCN(C1)C(c1ccccn1)=O)=O
12. CC1(C(NC(N1)=O)=O)c1cccc(c1)C(NC1CCN(C1)C(c1cccnn1)=O)=O
13. C1CN(CC1NC(C12CC(C1)Oc1ccccc12)=O)C(c1c2COc3ccccc3n2cn1)=O
14. C1CN(CC1NC(C1COC2COCC12)=O)C(C1CC2(C1)C(NC(N2)=O)=O)=O
15. CC1(C(NC(N1)=O)=O)c1cccc(c1)C(NC1CCN(C1)C(c1cnc2ccccc2n1)=O)=O

De-Novo Generated Top 100 Scoring Compounds:
Virtual Screen with PyRx 0.8 / AutoDock VINA (pdb 7kro and 7rdx)

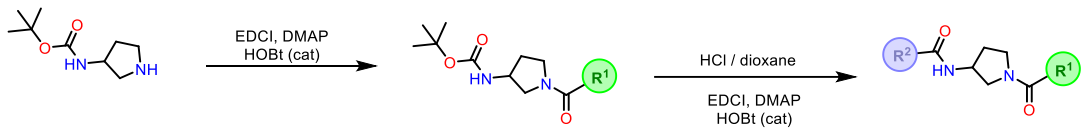
- Simple 3-step diamide synthesis.



De-Novo Generated Top 100 Scoring Compounds:
Virtual Screen with PyRx 0.8 / AutoDock VINA



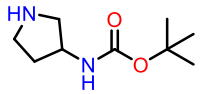
Synthesis:



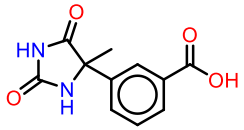
- 13x R1 reactions.
- 15x Boc-deprotections reactions.
- 9x R2 reactions.
- Total reactions = 24
- Total compounds = 15

De-Novo Generated Top 100 Scoring Compounds:
Virtual Screen with PyRx 0.8 / AutoDock VINA

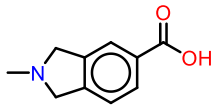
CAS:
122536-76-9
SMILES:
CC(C)(C)OC(=O)NC1CCNC1



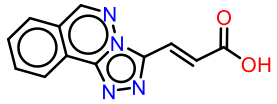
CAS:
652992-47-7
SMILES:
CC1(c2cccc(C(=O)O)c2)NC(=O)NC1=O



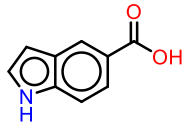
CAS:
1065065-38-4
SMILES:
CN1Cc2ccc(C(=O)O)cc2C1



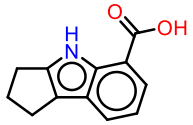
CAS:
793716-18-4
SMILES:
O=C(O)C=Cc1nnc2c3ccccc3cnn12



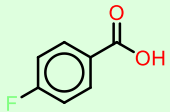
CAS:
1670-81-1
SMILES:
O=C(O)c1ccc2[nH]ccc2c1



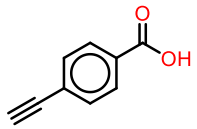
CAS:
422312-00-3
SMILES:
O=C(O)c1cccc2c3c([nH]c12)CCC3



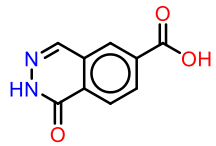
CAS:
456-22-4
SMILES:
O=C(O)c1ccc(F)cc1



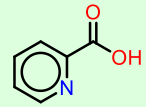
CAS:
10602-00-3
SMILES:
C#Cc1ccc(C(=O)O)cc1



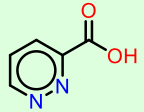
CAS:
102126-65-8
SMILES:
O=C(O)c1ccc2c(=O)[nH]ncc2c1



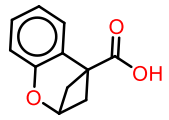
CAS:
98-98-6
SMILES:
O=C(O)c1ccccn1



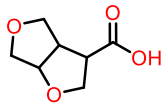
CAS:
2164-61-6
SMILES:
O=C(O)c1cccnn1



CAS:
2680543-50-2
SMILES:
O=C(O)C12CC(C1)Oc1ccccc12



CAS:
2253640-84-3
SMILES:
O=C(O)C1COC2COCC21



CAS:
879-65-2
SMILES:
O=C(O)c1cnc2ccccc2n1