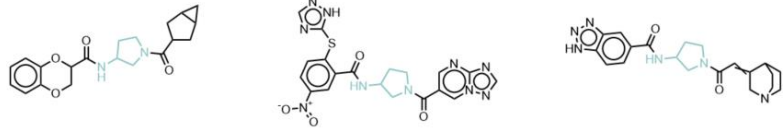


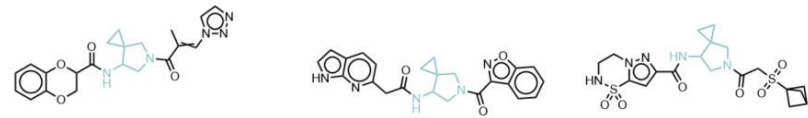
# De-Novo Generated Top 100 Scoring Compounds: *Moving away from Pyridine N-oxides*

- 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.

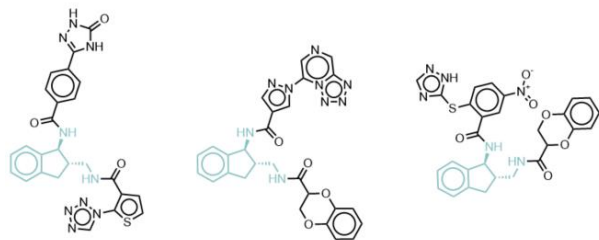
Core 1



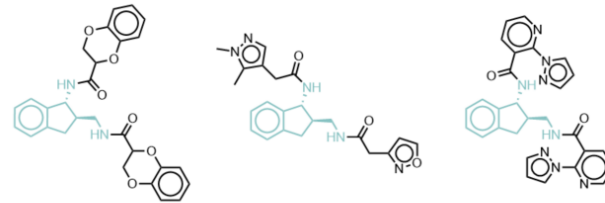
Core 2



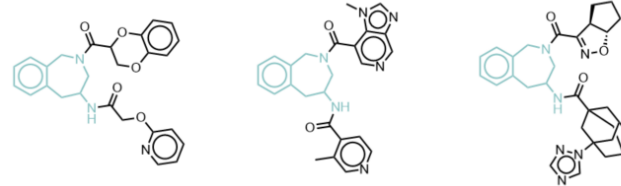
Core 3a



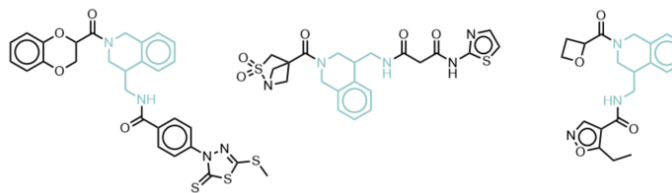
Core 3b



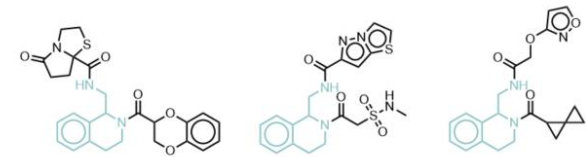
Core 4



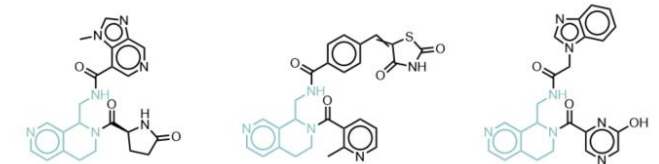
Core 5



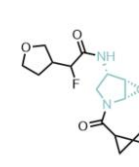
Core 6



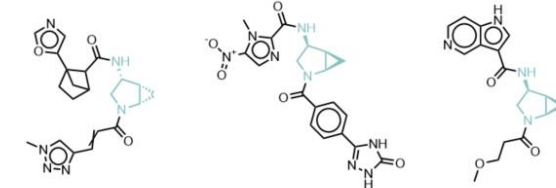
Core 7



Core 8a

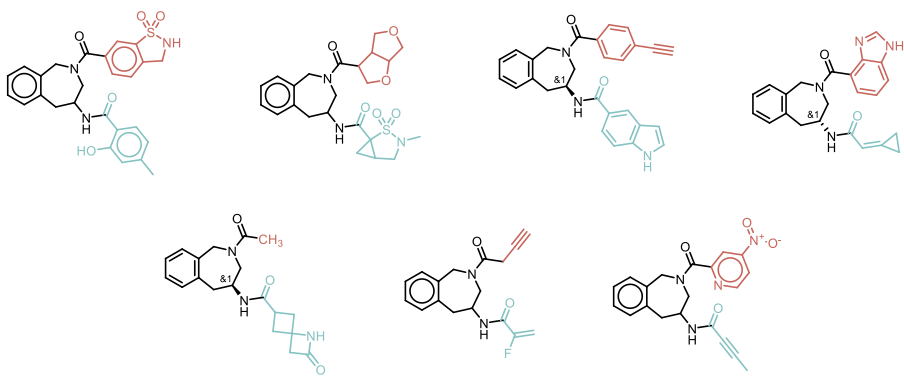


Core 8b



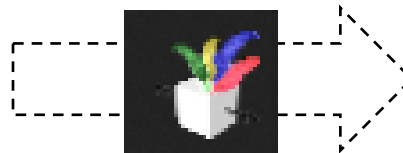
# De-Novo Generated Top 100 Scoring Compounds: Moving away from Pyridine N-oxides whilst retaining cores

**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



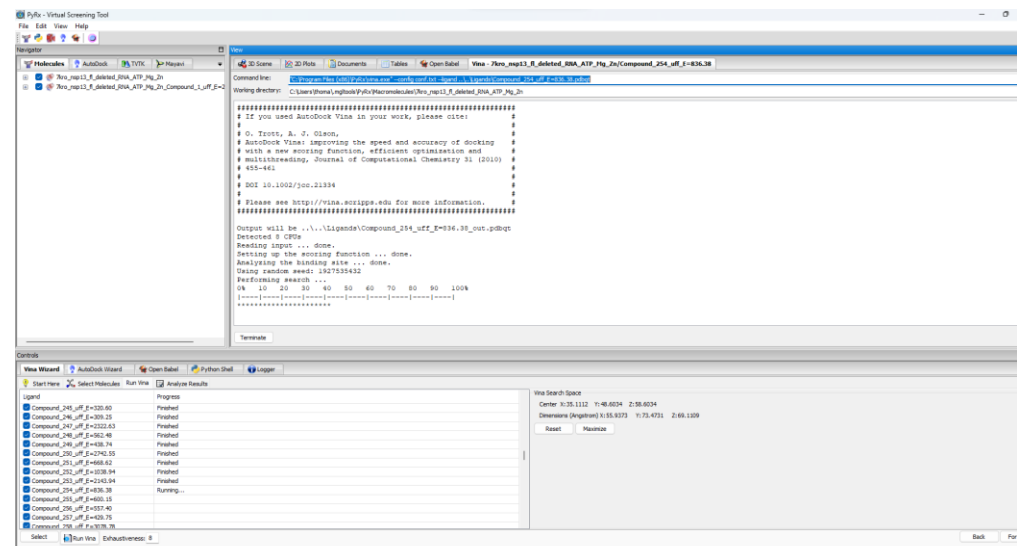
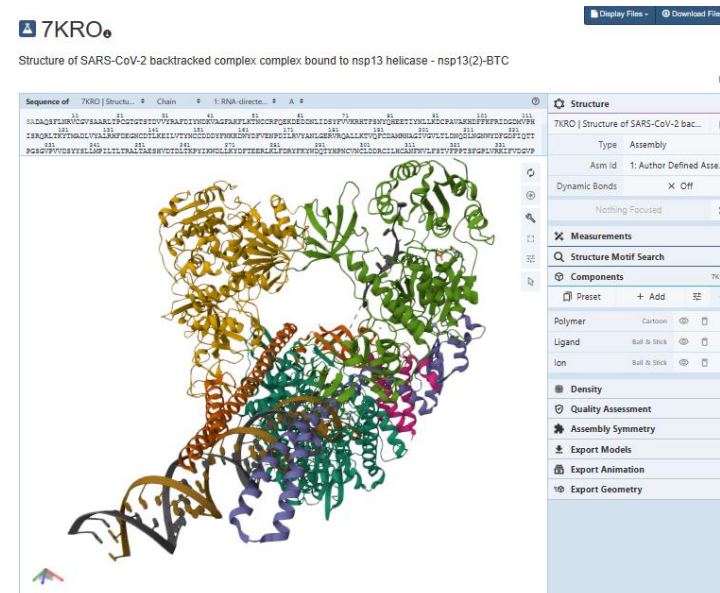
## 3) Virtual Screening with PyRx 0.8 / AutoDock Vina 1.2

- 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](#)

smiles	ID	Total Molweight	cLogP	cLogS	H-Acceptors	H-Donors	Relative PSA	Polar Surface Area	Mutagenic	Tumorigenic	Reproductive Effective	Irritant	Nasty Functions	Rotatable Bonds
...	OT02020000 Core 4 EN000 491 554	2,2975	-5.598	9	2	0.26372	109.28	none	none	none	none	3		
...	OT02020000 Core 4 EN000 493 569	2,8654	-5.023	9	3	0.26693	107.84	none	none	none	none	3		
...	OT02020000 Core 4 EN000 491 554	2,3008	-3.303	9	2	0.26372	109.28	none	none	none	none	3		
...	OT02020000 Core 4 EN000 491 554	2,2975	-5.598	9	2	0.26372	109.28	none	none	none	none	3		
...	OT02020000 Core 4 EN000 493 569	2,3354	-6.202	9	2	0.26372	109.28	none	none	none	none	3		
...	OT02020000 Core 4 EN000 493 526	9,8799	-6.294	10	2	0.28142	109.55	none	none	none	none	3		
...	OT02020000 Core 4 EN000 495 542	2,0829	-5.781	10	1	0.26984	107.17	none	none	none	none	3		
...	OT02020000 Core 4 EN000 493 526	9,8799	-6.294	10	2	0.28142	109.55	none	none	none	none	3		
...	OT02020000 Core 4 EN000 495 542	2,0829	-5.781	10	1	0.26984	107.17	none	none	none	none	3		
...	OT02020000 Core 4 EN000 495 542	1,2854	-4.251	10	1	0.27034	107.17	none	none	none	none	3		
...	OT02020000 Core 4 EN000 495 542	1,2854	-4.251	10	1	0.27034	107.17	none	none	none	none	3		

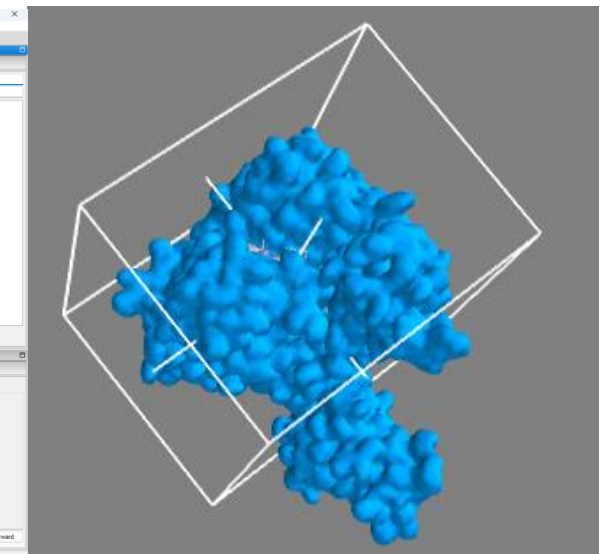
# De-Novo Generated Top 100 Scoring Compounds: Virtual Screen with PyRx 0.8 / AutoDock VINA

- 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.



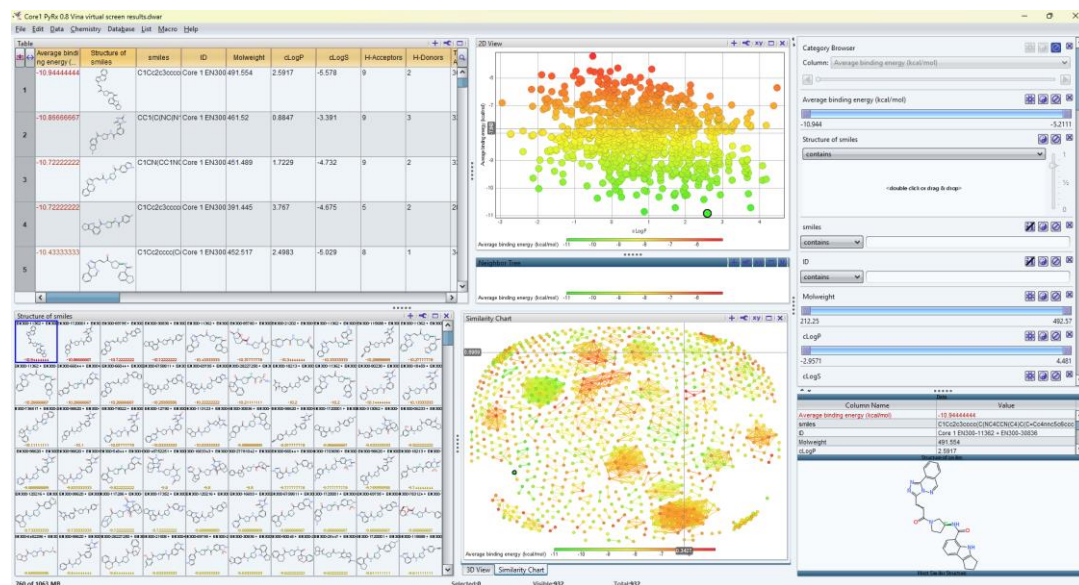
```
Command line: C:\Users\shana\ngithub\PyRx\Macromolecules\Nsp_nsp13_F_deleted_RNA_ATP_Mg_Zn\Compound_254_off_E=836.38
Working directory: C:\Users\shana\ngithub\PyRx\Macromolecules\Nsp_nsp13_F_deleted_RNA_ATP_Mg_Zn
=====
# If you used AutoDock Vina in your work, please cite:
#
# O. Trott, A. J. Olson,
# AutoDock Vina: improving the speed and accuracy of docking
# with a new scoring function, efficient optimization and
# multithreading. Journal of Computational Chemistry 31 (2010)
# 455-461
# DOI 10.1002/jcc.21334
#
# Please see http://vina.scripps.edu for more information.
=====
Output will be ...\\Ligands\Compound_254_off_E=836.38_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 132733932
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
[=====]
=====
Terminate
```

Ligand	Progress
Compound_248_off_E=230.80	Finished
Compound_246_off_E=230.25	Finished
Compound_247_off_E=232.43	Finished
Compound_249_off_E=262.46	Finished
Compound_240_off_E=436.74	Finished
Compound_250_off_E=2742.55	Finished
Compound_251_off_E=668.62	Finished
Compound_252_off_E=2238.94	Finished
Compound_253_off_E=2145.54	Finished
Compound_254_off_E=836.38	Running...
Compound_255_off_E=660.25	Finished
Compound_256_off_E=557.40	Finished
Compound_257_off_E=429.75	Finished
Compound_258_off_E=610.76	Finished

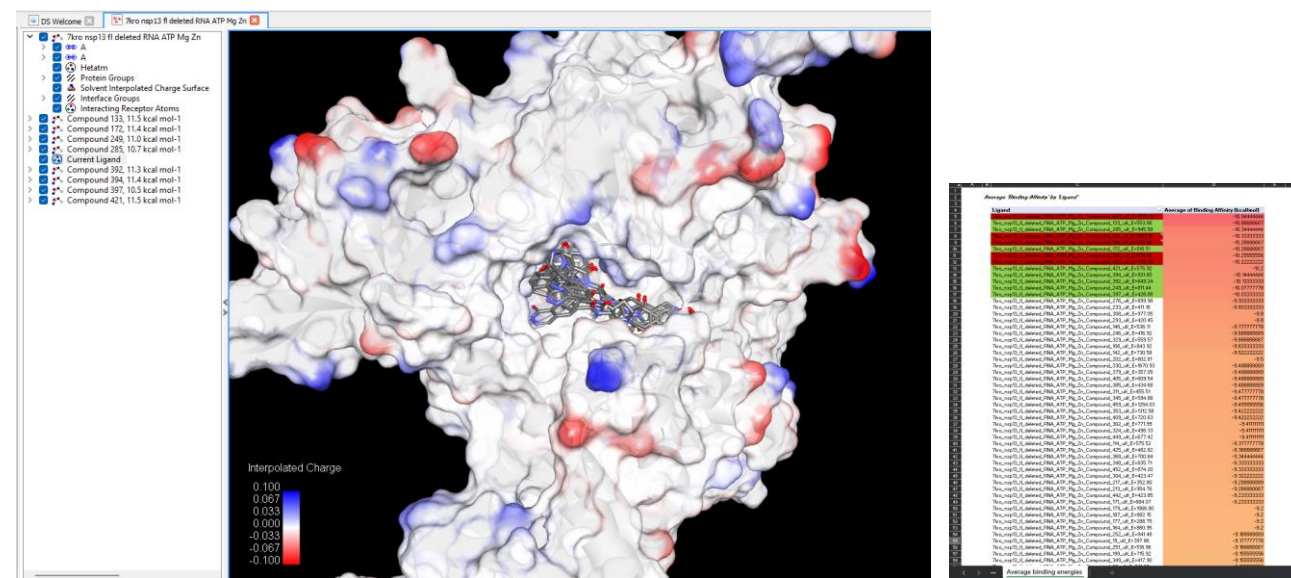


# De-Novo Generated Top 100 Scoring Compounds: Virtual Screen with PyRx 0.8 / AutoDock VINA (Core 1, pdb 7kro)

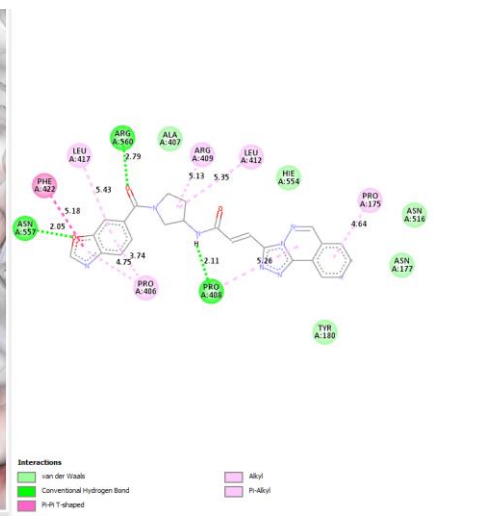
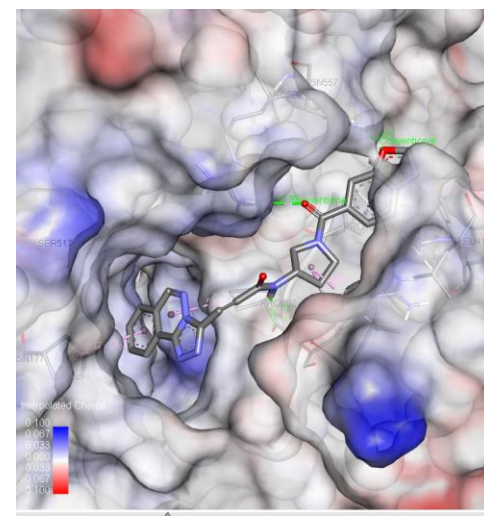
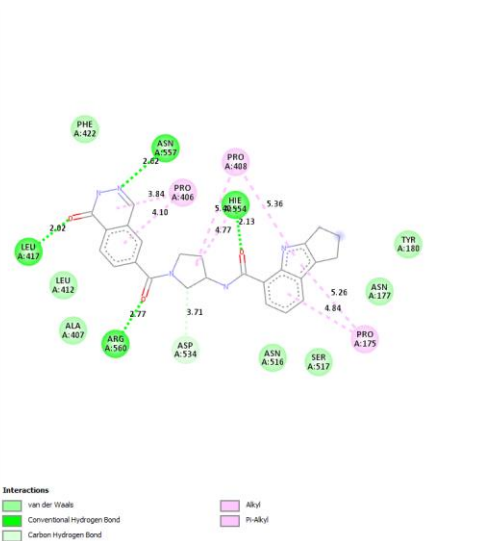
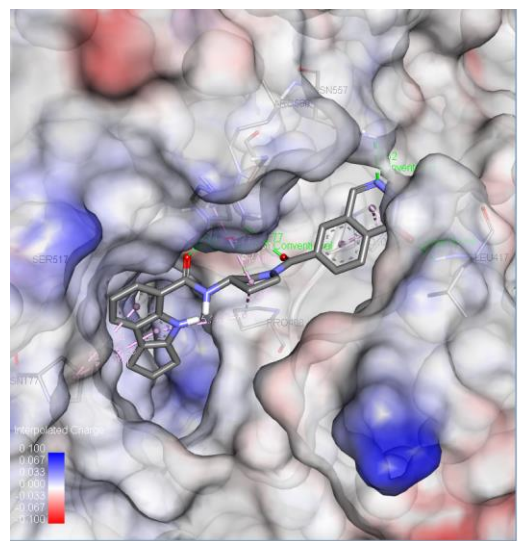
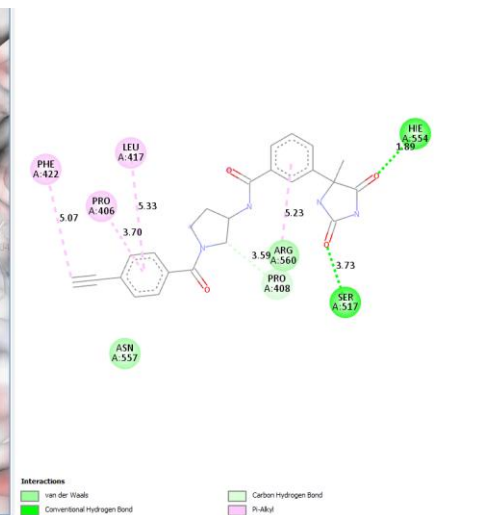
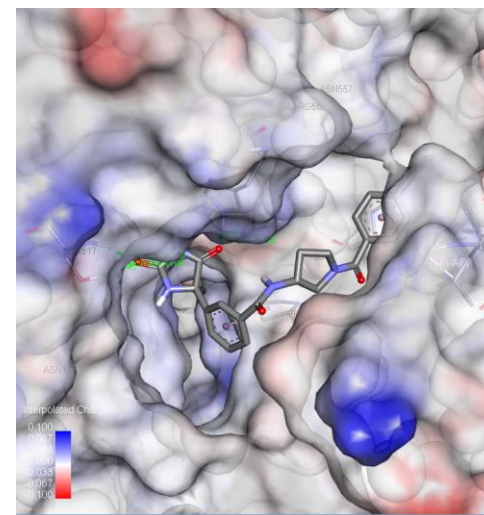
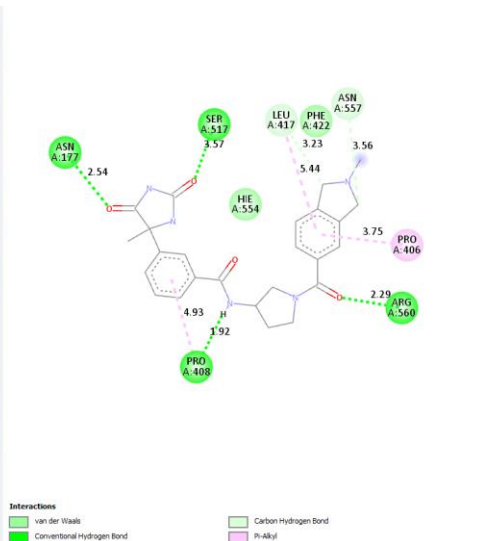
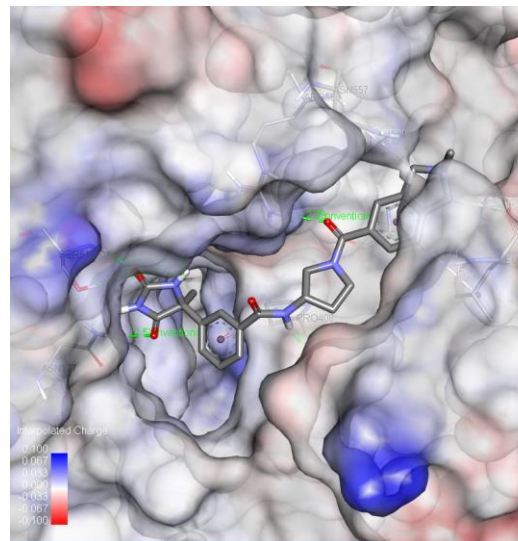
- Ranked average binding free energy scores, then applied cutoff at **-10.0 kcal/mol** (lead-like binding energy)



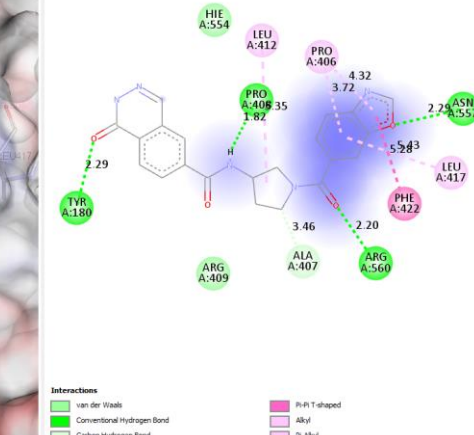
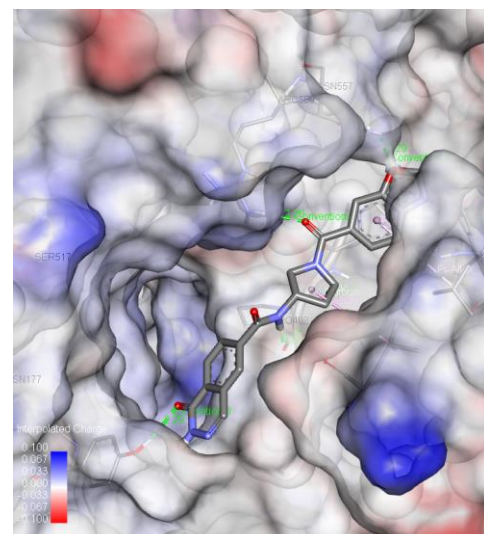
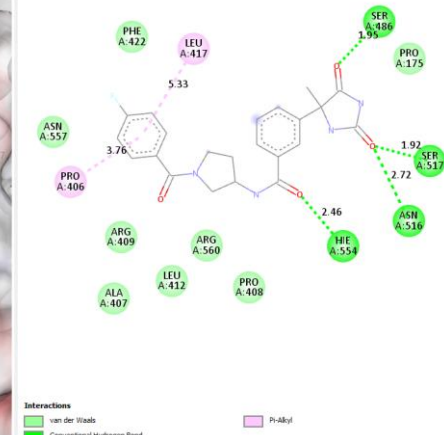
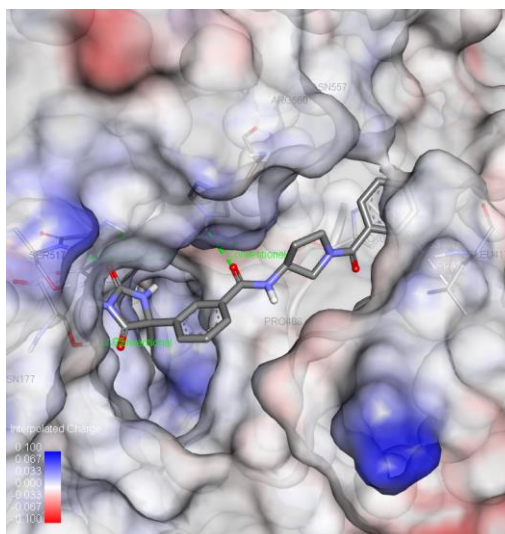
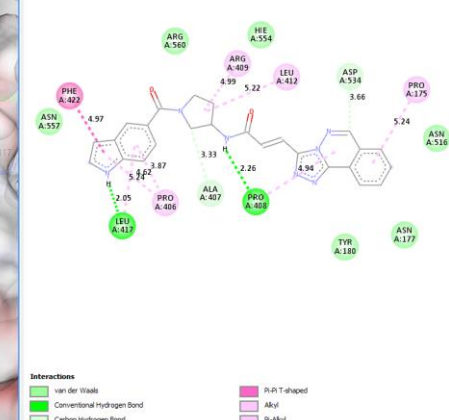
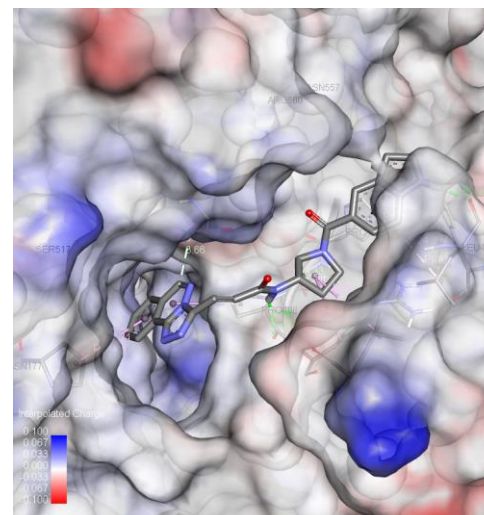
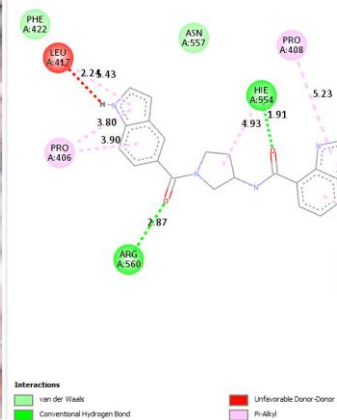
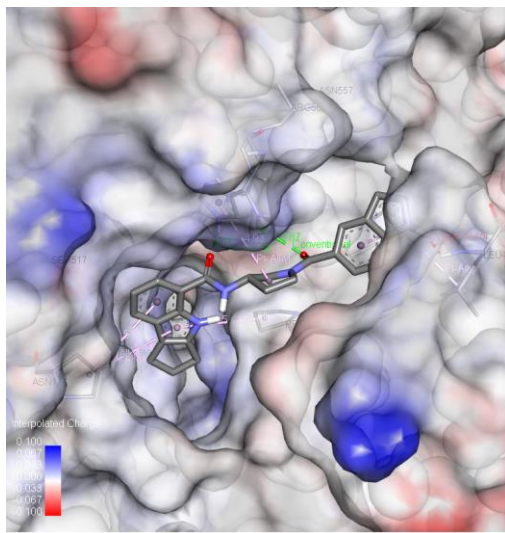
- Visually inspected** minimum energy poses to validate, eliminating incorrect geometries (cis-amides).



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

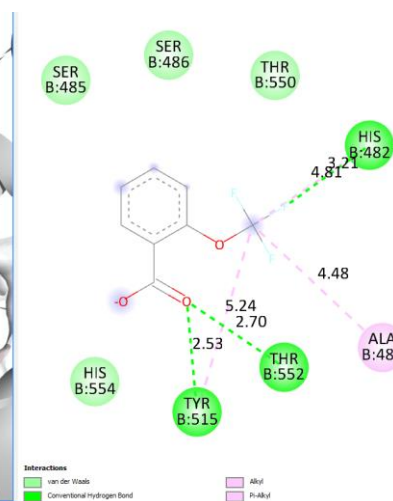
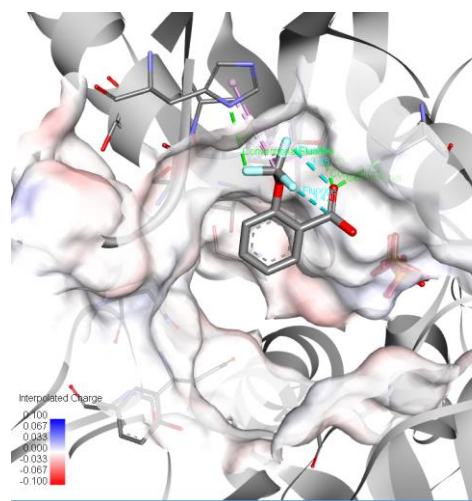
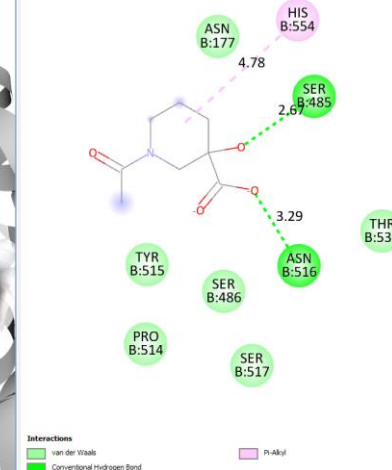
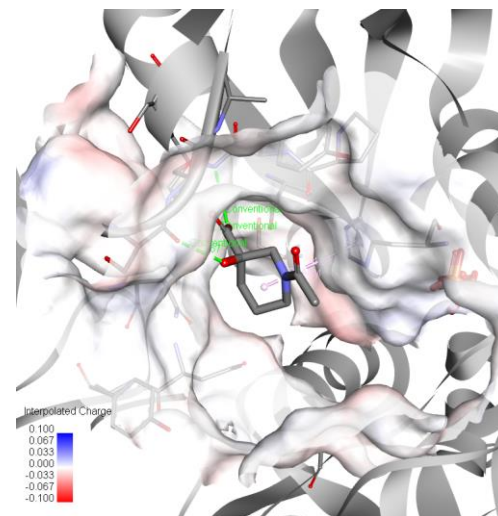
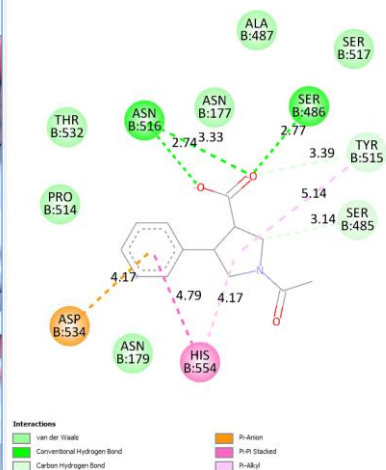
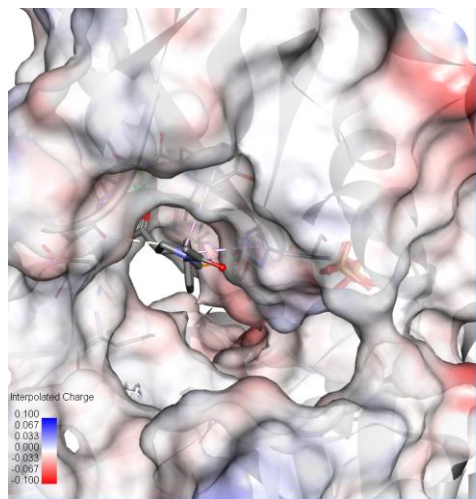


# De-Novo Generated Top 100 Scoring Compounds: Virtual Screen with PyRx 0.8 / AutoDock VINA (Core 1, pdb 7kro)



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

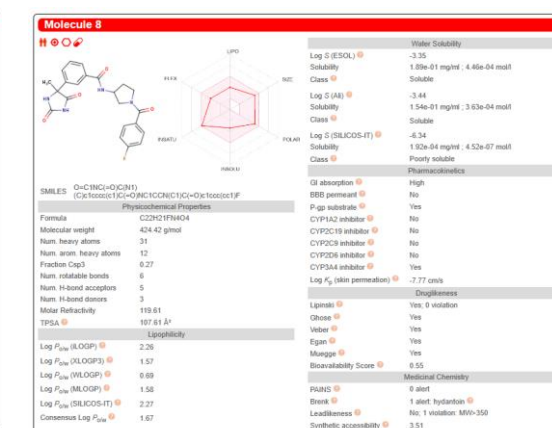
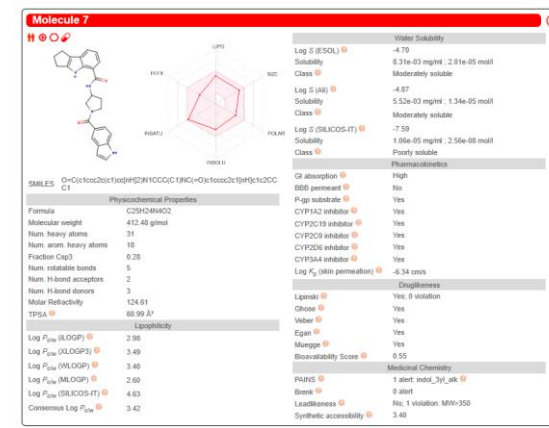
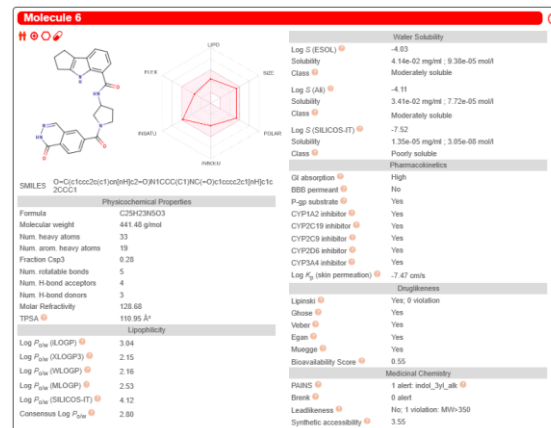
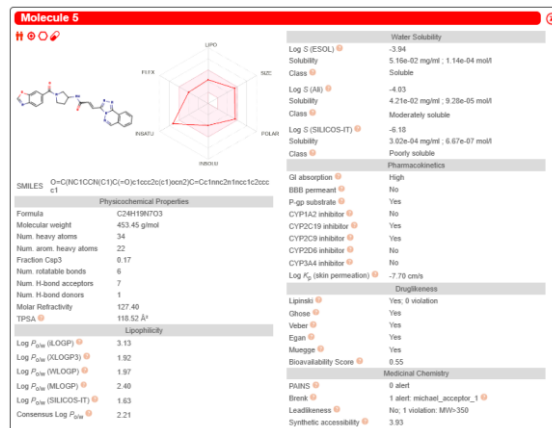
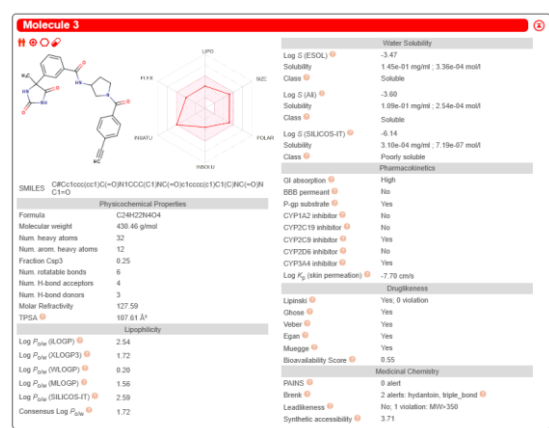
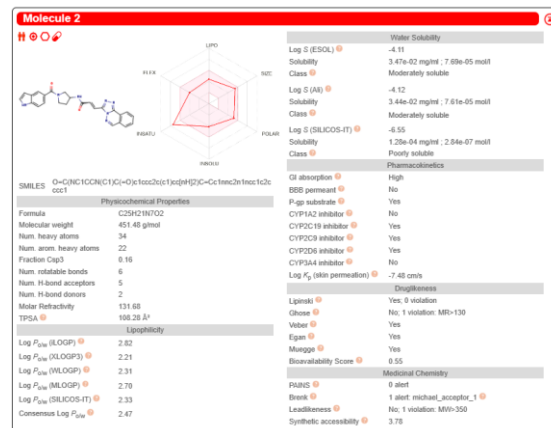
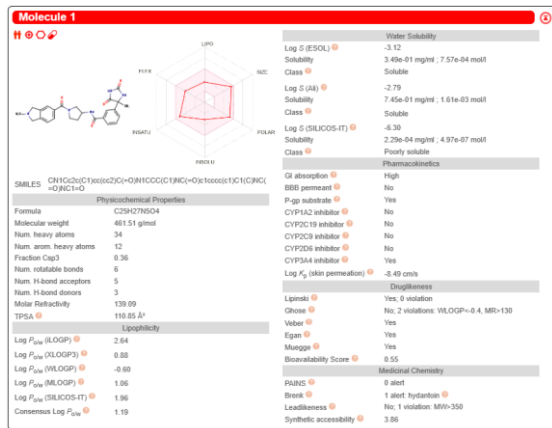
- PANDDA fragment interactions at 5' RNA site



# De-Novo Generated Top 100 Scoring Compounds: Core1 compounds



- All compounds PAINS filtering (SwissADME) cleared.

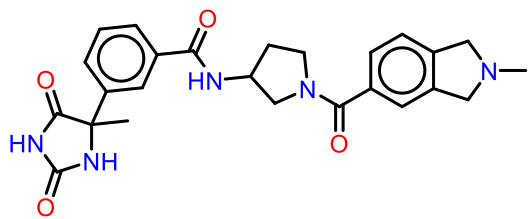




# De-Novo Generated Top 100 Scoring Compounds: *Core1 compounds*

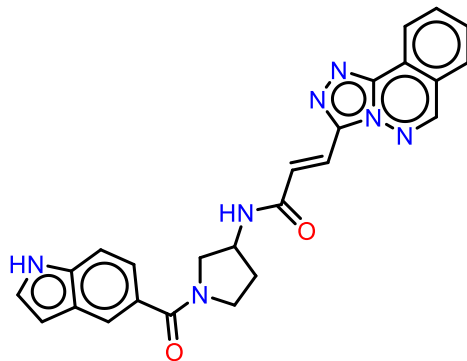
**Compound 133**

Core 1 EN300-1720001 + EN300-98620



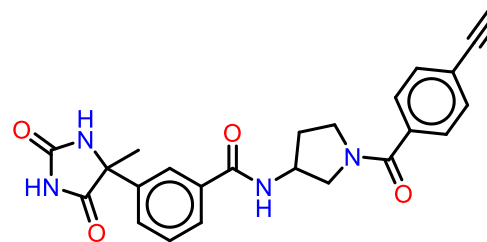
**Compound 285**

Core 1 EN300-69795 + EN300-11362



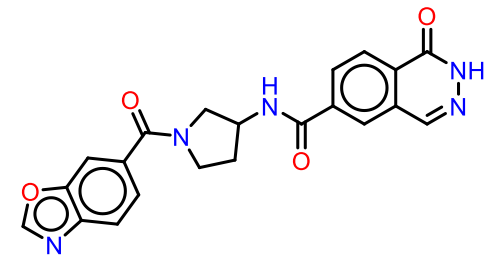
**Compound 172**

Core 1 EN300-115688 + EN300-98620



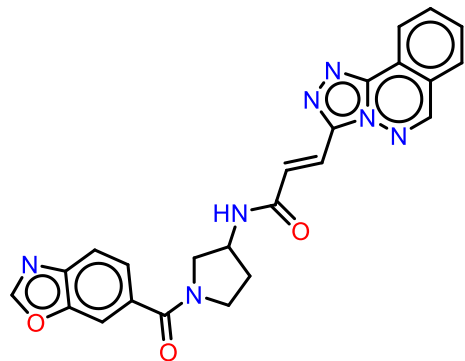
**Compound 421**

Core 1 EN300-66044 + EN300-6758811



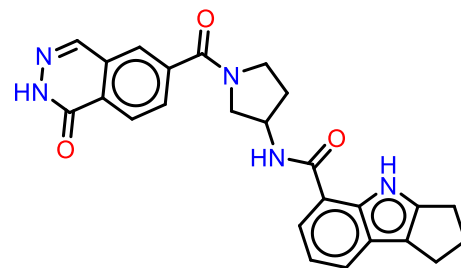
**Compound 394**

Core 1 EN300-66044 + EN300-11362



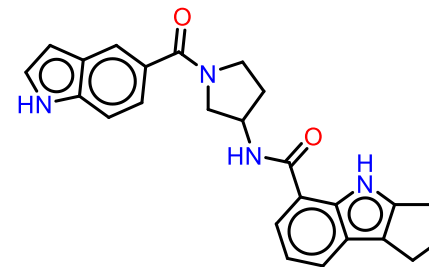
**Compound 392**

Core 1 EN300-6758811 + EN300-30836



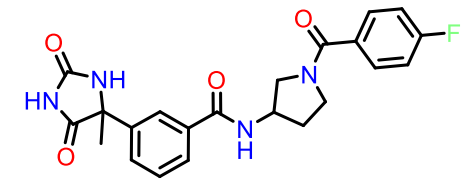
**Compound 249**

Core 1 EN300-69795 + EN300-30836



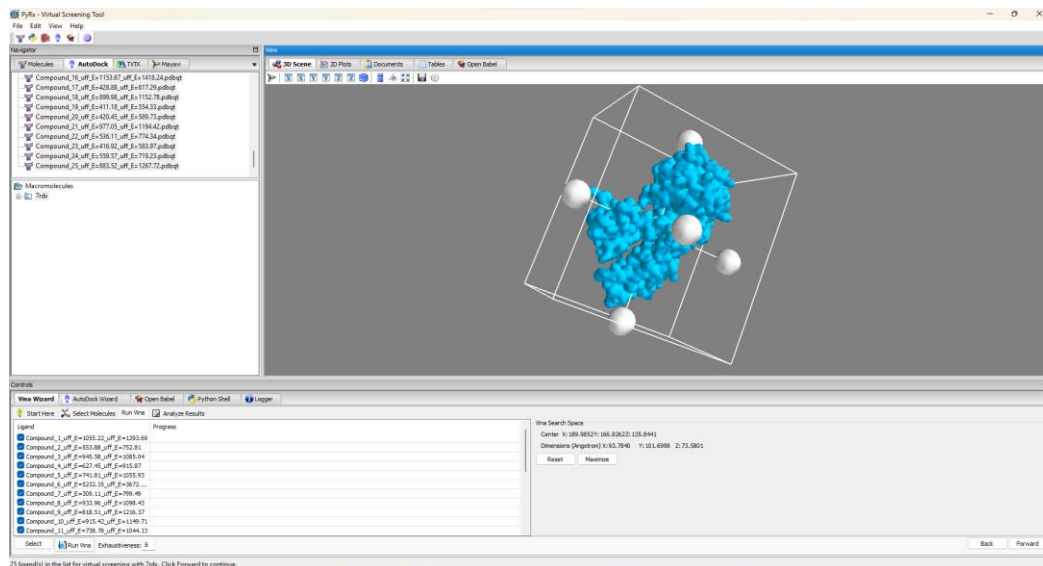
**Compound 397**

Core 1 EN300-18213 + EN300-98620



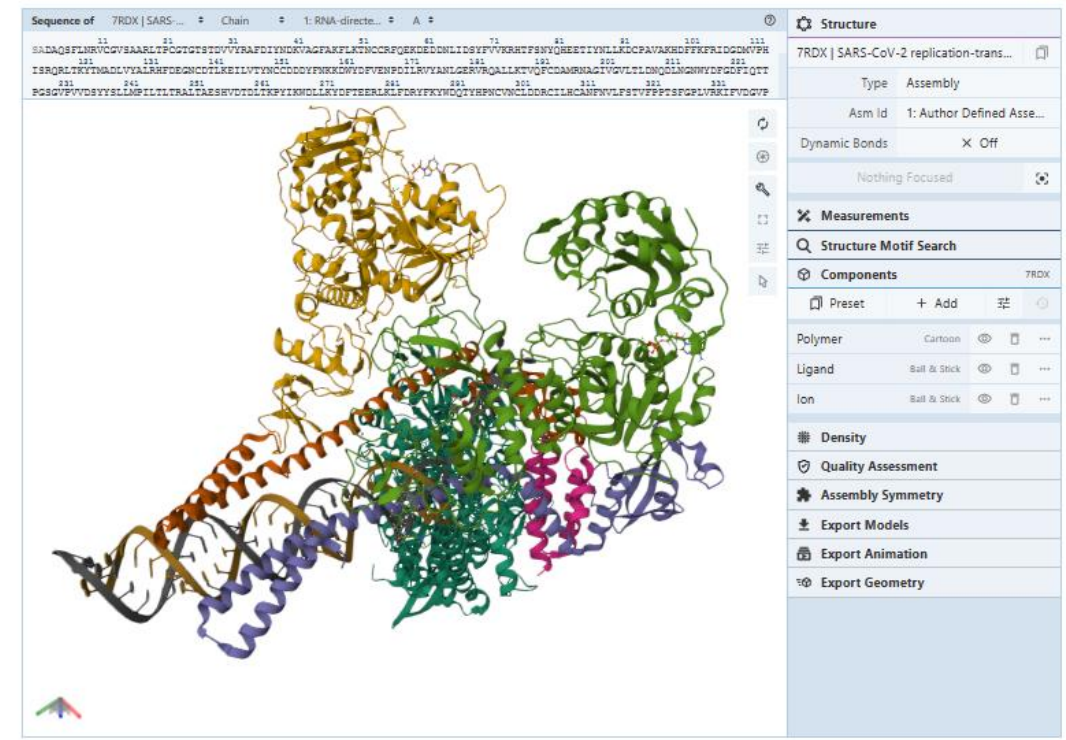
# De-Novo Generated Top 100 Scoring Compounds: Virtual Screen with PyRx 0.8 / AutoDock VINA (pdb 7kro)

- 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.



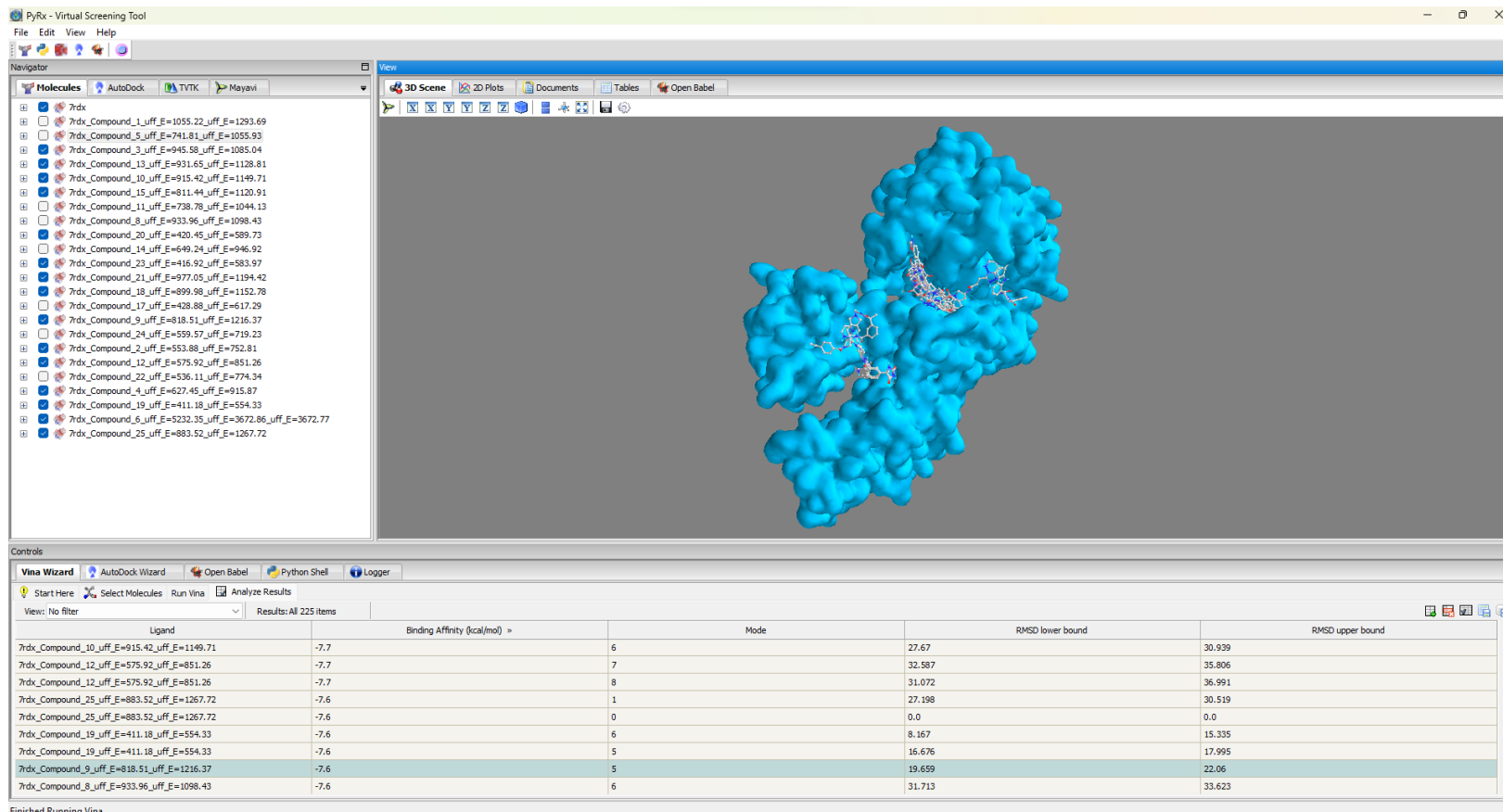
## 7RDX

SARS-CoV-2 replication-transcription complex bound to nsp13 helicase - nsp13(2)-RTC - open class



# 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).



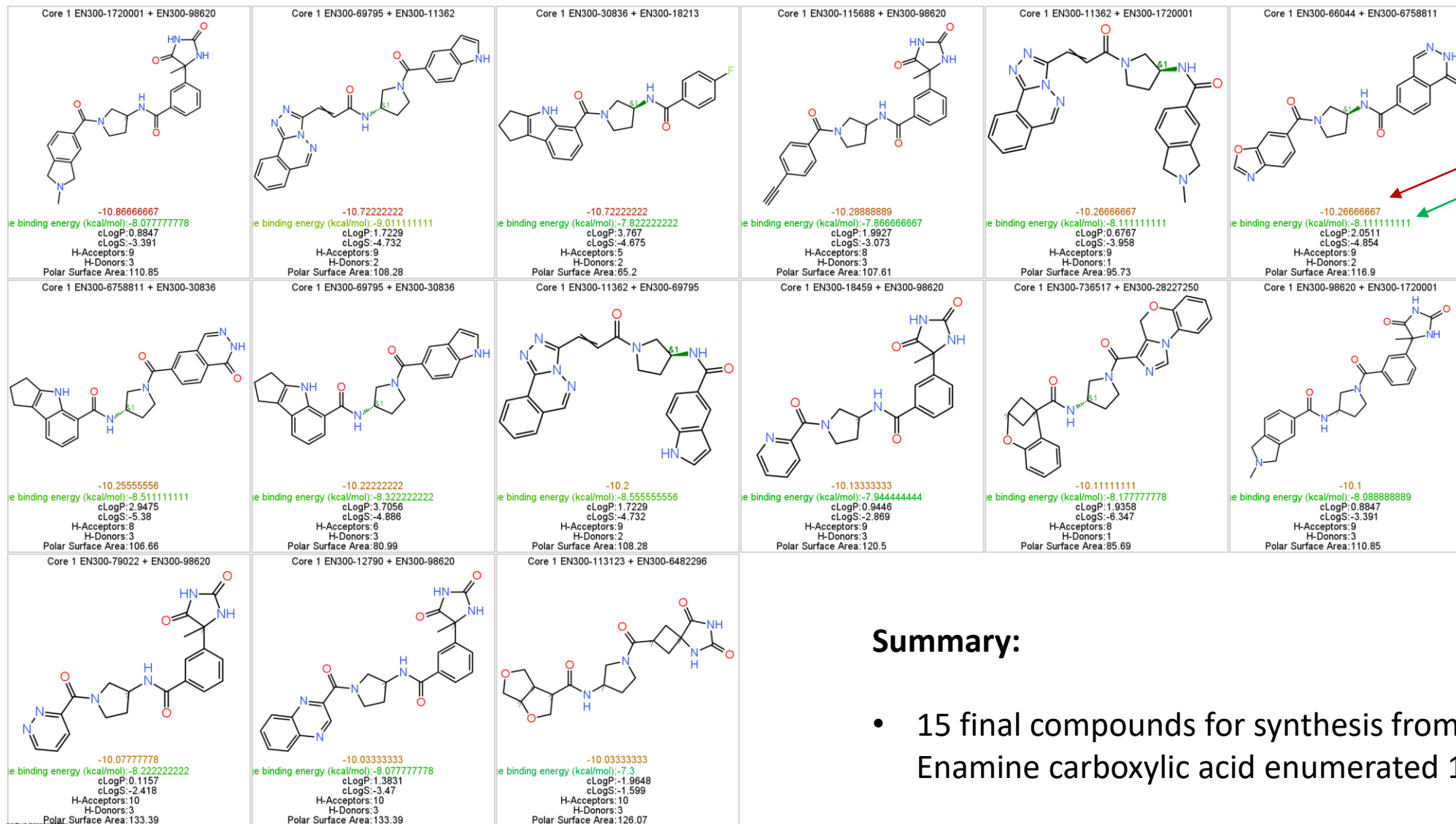
The screenshot shows the PyRx - Virtual Screening Tool interface. The main window displays a 3D molecular model of a protein (blue) with a ligand (red) bound to it. The left sidebar shows a list of molecules with their respective binding affinities. The bottom panel shows the VINA Wizard interface with a table of results.

Ligand	Binding Affinity (kcal/mol)	Mode	RMSD lower bound	RMSD upper bound
7rdx_Compound_10_uff_E=915.42_uff_E=1149.71	-7.7	6	27.67	30.939
7rdx_Compound_12_uff_E=575.92_uff_E=851.26	-7.7	7	32.587	35.806
7rdx_Compound_12_uff_E=575.92_uff_E=851.26	-7.7	8	31.072	36.991
7rdx_Compound_25_uff_E=883.52_uff_E=1267.72	-7.6	1	27.198	30.519
7rdx_Compound_25_uff_E=883.52_uff_E=1267.72	-7.6	0	0.0	0.0
7rdx_Compound_19_uff_E=411.18_uff_E=554.33	-7.6	6	8.167	15.335
7rdx_Compound_19_uff_E=411.18_uff_E=554.33	-7.6	5	16.676	17.995
7rdx_Compound_9_uff_E=818.51_uff_E=1216.37	-7.6	5	19.659	22.06
7rdx_Compound_8_uff_E=933.96_uff_E=1098.43	-7.6	6	31.713	33.623

Average 'Binding Affinity' by 'Ligand'

Ligand	Average of Binding Affinity
7rdx_Compound_5_uff_E=741.81_uff_E=1055.93	-9.12222222
7rdx_Compound_3_uff_E=945.58_uff_E=1085.04	-9.01111111
7rdx_Compound_1_uff_E=1055.22_uff_E=1293.69	-8.74444444
7rdx_Compound_13_uff_E=931.65_uff_E=1128.81	-8.62222222
7rdx_Compound_18_uff_E=899.98_uff_E=1152.78	-8.55555556
7rdx_Compound_14_uff_E=649.24_uff_E=946.92	-8.51111111
7rdx_Compound_15_uff_E=811.44_uff_E=1120.91	-8.32222222
7rdx_Compound_17_uff_E=428.88_uff_E=617.29	-8.28888889
7rdx_Compound_23_uff_E=416.92_uff_E=583.97	-8.22222222
7rdx_Compound_21_uff_E=977.05_uff_E=1194.42	-8.17777778
7rdx_Compound_10_uff_E=915.42_uff_E=1149.71	-8.13333333
7rdx_Compound_12_uff_E=575.92_uff_E=851.26	-8.11111111
7rdx_Compound_11_uff_E=738.78_uff_E=1044.13	-8.11111111
7rdx_Compound_22_uff_E=536.11_uff_E=774.34	-8.08888889
7rdx_Compound_24_uff_E=559.57_uff_E=719.23	-8.07777778
7rdx_Compound_2_uff_E=553.88_uff_E=752.81	-8.07777778
7rdx_Compound_8_uff_E=933.96_uff_E=1098.43	-8.02222222
7rdx_Compound_20_uff_E=420.45_uff_E=589.73	-7.94444444
7rdx_Compound_9_uff_E=818.51_uff_E=1216.37	-7.86666667
7rdx_Compound_4_uff_E=627.45_uff_E=915.87	-7.82222222
7rdx_Compound_19_uff_E=411.18_uff_E=554.33	-7.75555556
7rdx_Compound_25_uff_E=883.52_uff_E=1267.72	-7.3
7rdx_Compound_16_uff_E=1153.67_uff_E=1418.24	-6.91111111
7rdx_Compound_6_uff_E=5232.35_uff_E=3672.86_uff_E=3672.77	-6.61111111
7rdx_Compound_7_uff_E=309.11_uff_E=799.49	-5.44444444
<b>Grand Total</b>	<b>-7.99422222</b>

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



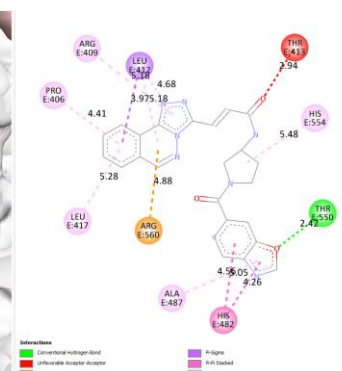
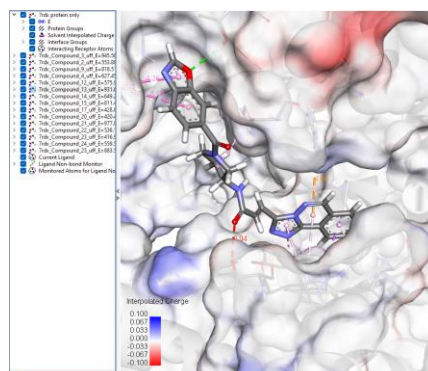
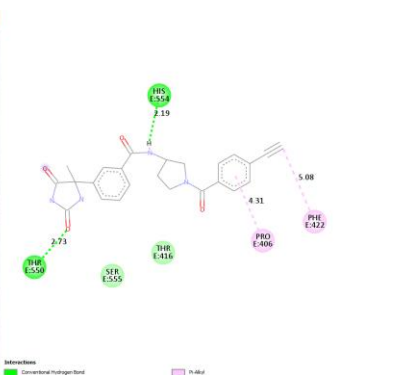
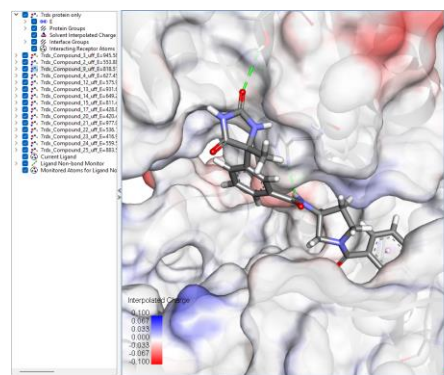
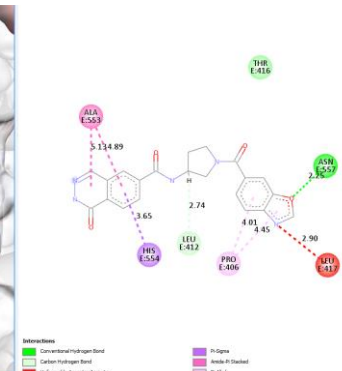
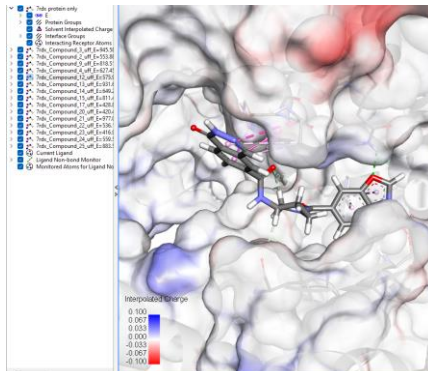
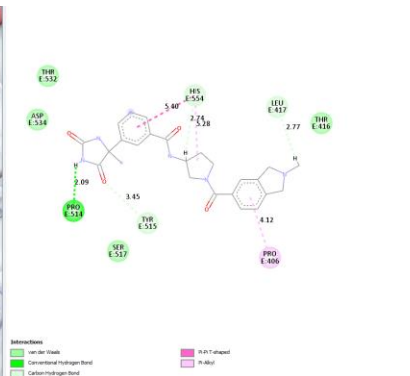
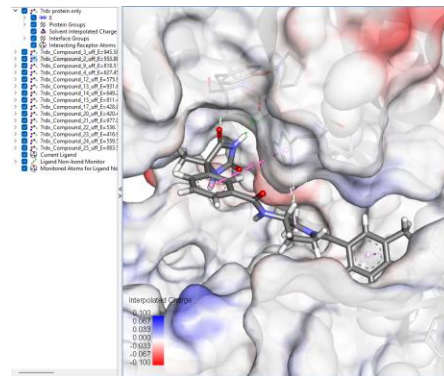
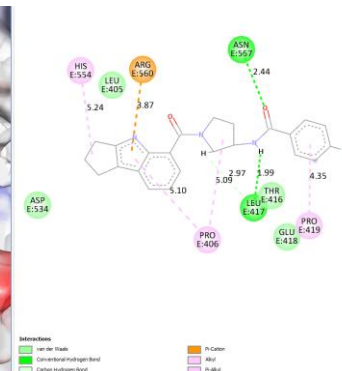
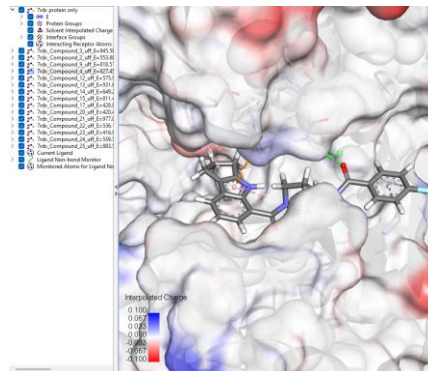
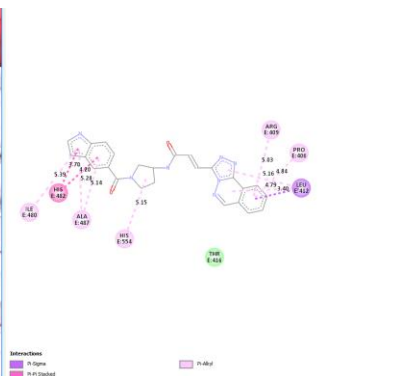
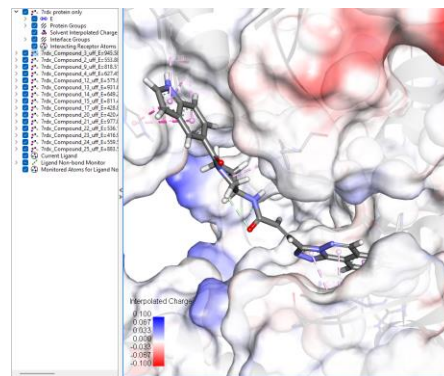
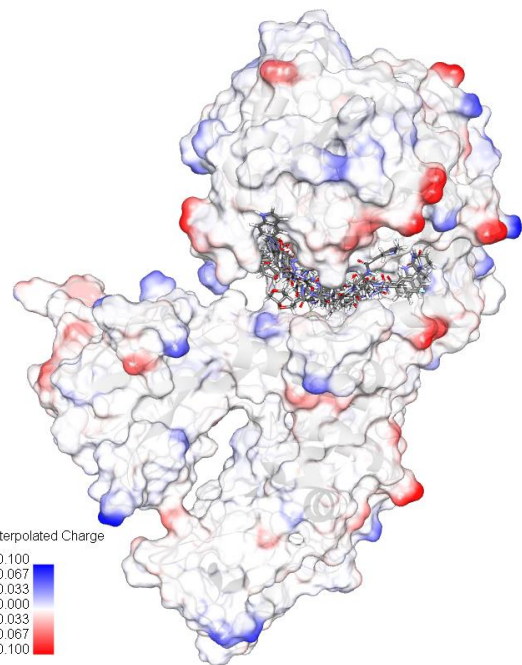
Average binding free energies that cleared visual pose inspection (kcal/mol):

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

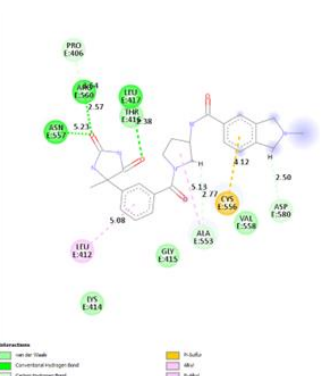
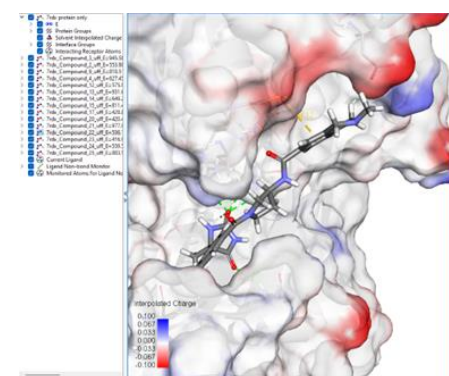
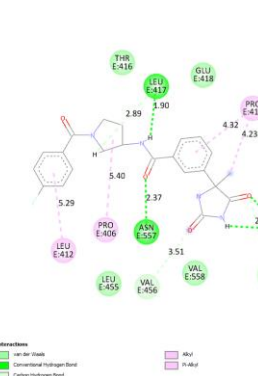
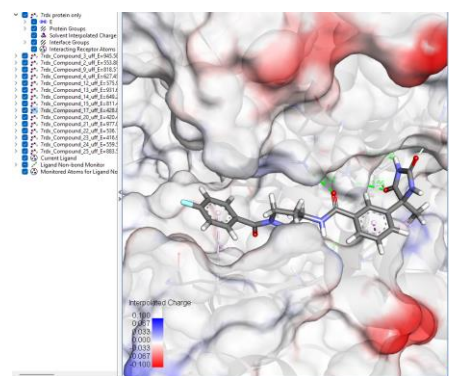
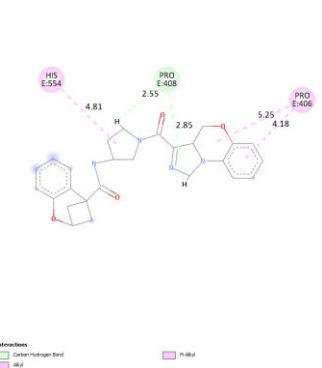
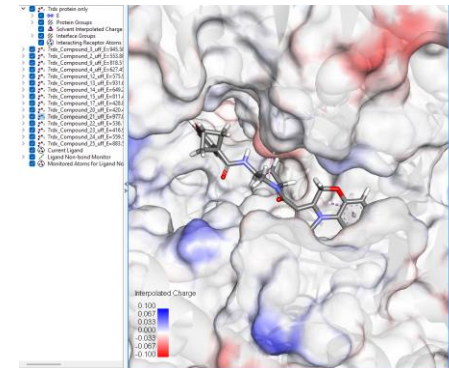
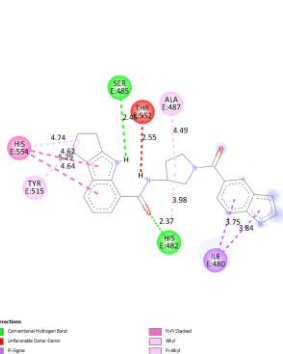
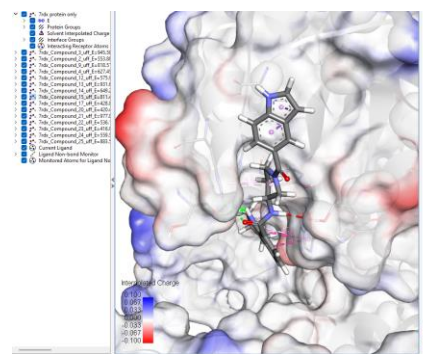
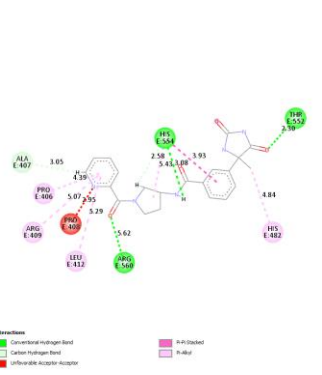
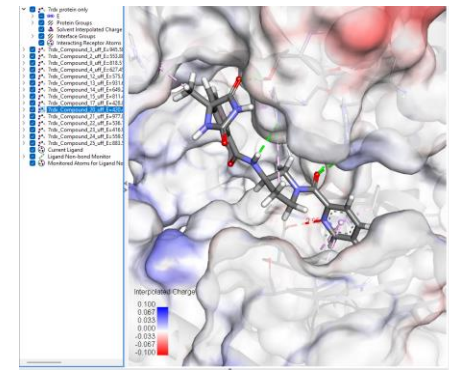
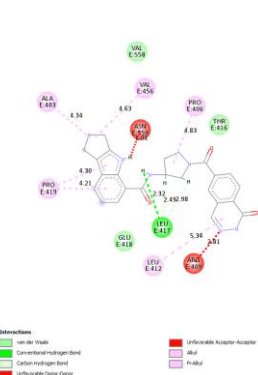
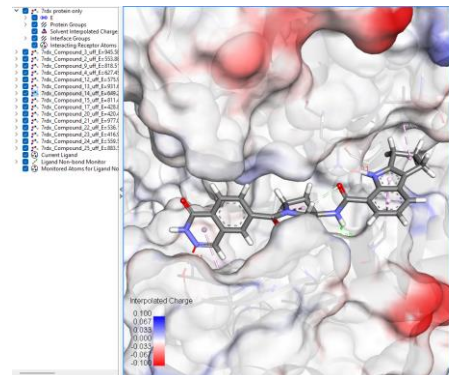
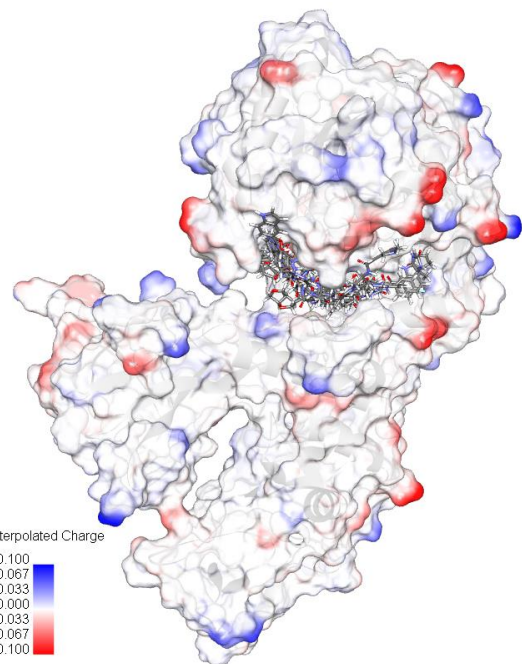
## Summary:

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

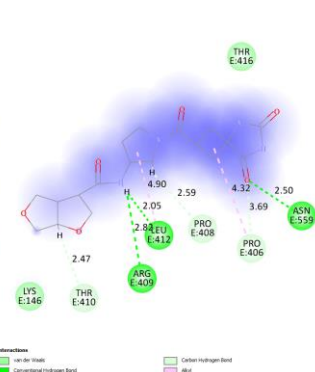
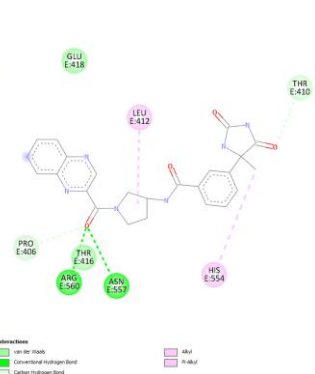
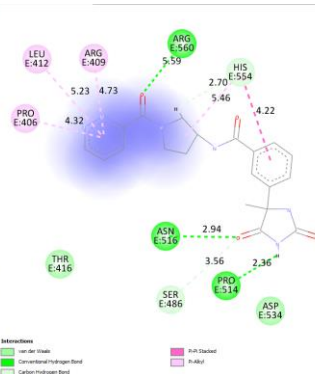
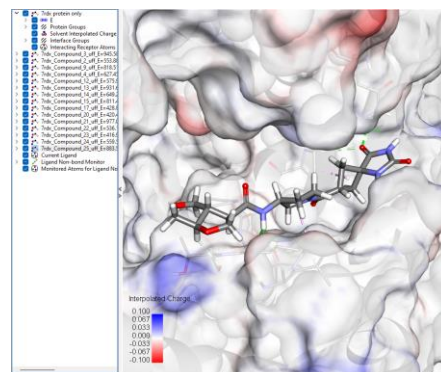
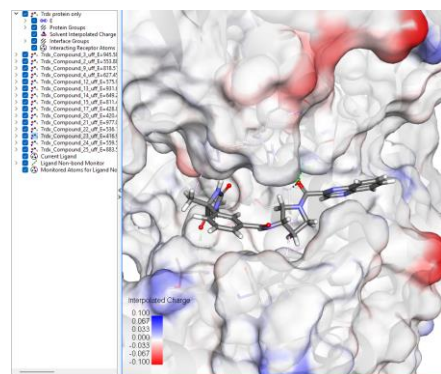
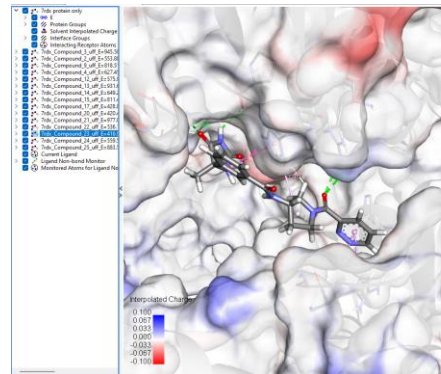
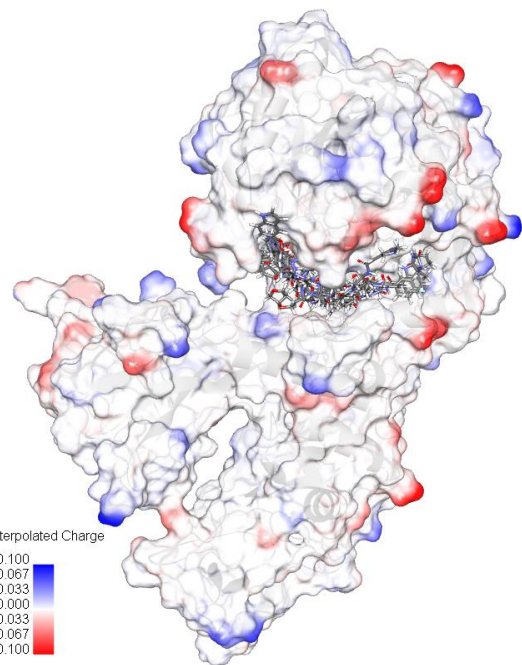
# De-Novo Generated Top 100 Scoring Compounds: Virtual Screen with PyRx 0.8 / AutoDock VINA (pdb 7rdx)



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



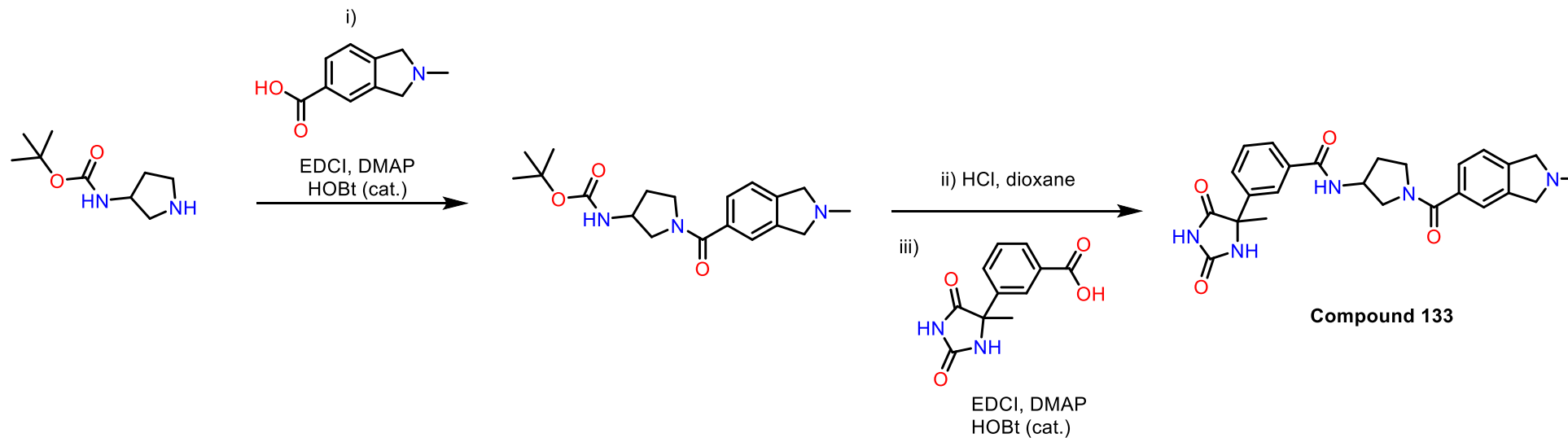
# De-Novo Generated Top 100 Scoring Compounds: Virtual Screen with PyRx 0.8 / AutoDock VINA (pdb 7kro and 7rdx)



## SMILES String:

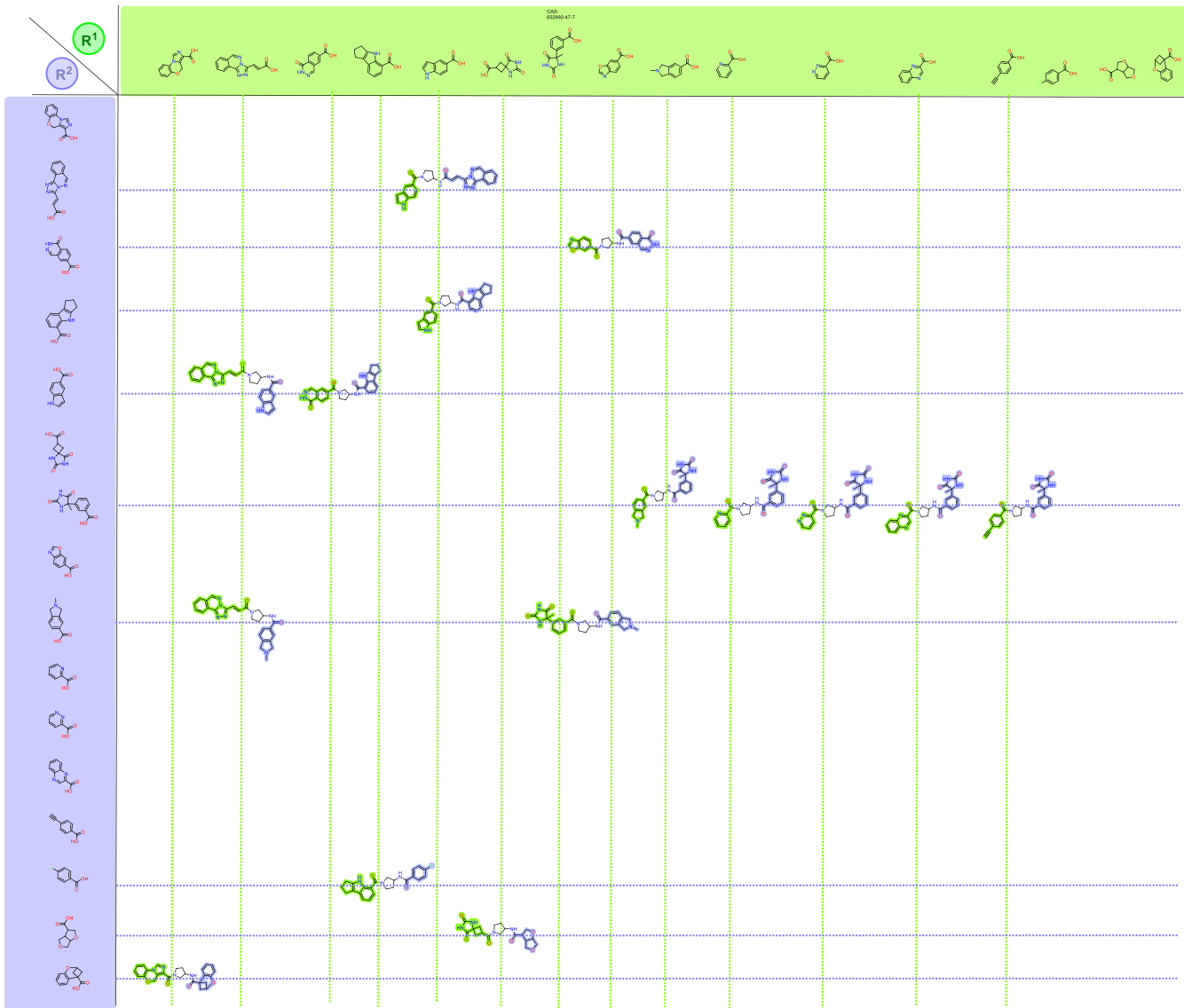
1. CC1(C(NC(N1)=O)=O)c1cccc(c1)C(NC1CCN(C1)C(c1ccc2CN(C)Cc2c1)=O)=O
2. CC1(C(NC(N1)=O)=O)c1cccc(c1)C(N1CCC(C1)NC(c1ccc2CN(C)Cc2c1)=O)=O
3. C1CN(CC1NC(C=Cc1nnc2c3ccccc3cnn12)=O)C(c1ccc2c(cc[nH]2)c1)=O
4. C1Cc2c3cccc(C(N4CCC(C4)NC(c4ccc(cc4)F)=O)=O)c3[nH]c2C1
5. CC1(C(NC(N1)=O)=O)c1cccc(c1)C(NC1CCN(C1)C(c1ccc(C#C)cc1)=O)=O
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)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(c1cccn1)=O)=O
12. CC1(C(NC(N1)=O)=O)c1cccc(c1)C(NC1CCN(C1)C(c1cccn1)=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

- Simple 3-step diamide synthesis.

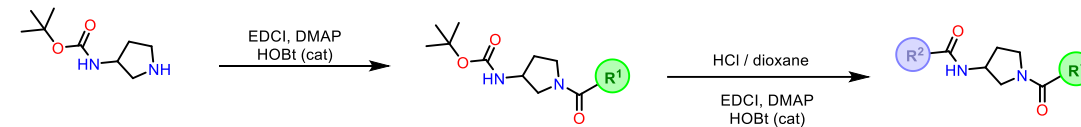




# De-Novo Generated Top 100 Scoring Compounds: Virtual Screen with PyRx 0.8 / AutoDock VINA



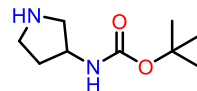
## Synthesis:



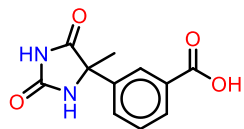
- 13x R1 reactions.
- 15x Boc-deprotections reactions.
- 9x R2 reactions.
- Total reactions = 24
- Total compounds = 15

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

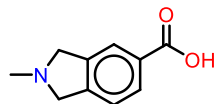
CAS:  
122536-76-9  
SMILES:  
CC(C)(C)OC(=O)NC1CCNC1



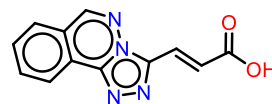
CAS:  
652992-47-7  
SMILES:  
CC1(c2cccc(C(=O)O)c2)NC(=O)NC1=O



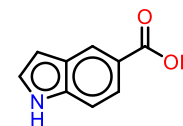
CAS:  
1065065-38-4  
SMILES:  
CN1Cc2ccc(C(=O)O)cc2C1



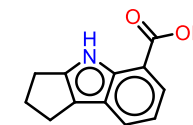
CAS:  
793716-18-4  
SMILES:  
O=C(O)C=Cc1nnc2c3ccccc3cnn12



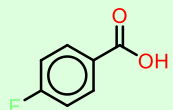
CAS:  
1670-81-1  
SMILES:  
O=C(O)c1ccc2[nH]ccc2c1



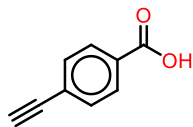
CAS:  
422312-00-3  
SMILES:  
O=C(O)c1cccc2c3c([nH]c12)CCC3



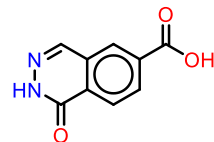
CAS:  
456-22-4  
SMILES:  
O=C(O)c1ccc(F)cc1



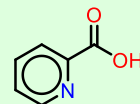
CAS:  
10602-00-3  
SMILES:  
C#Cc1ccc(C(=O)O)cc1



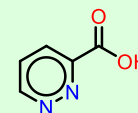
CAS:  
102126-65-8  
SMILES:  
O=C(O)c1ccc2c(=O)[nH]ncc2c1



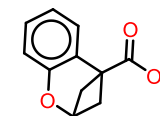
CAS:  
98-98-6  
SMILES:  
O=C(O)c1ccccc1



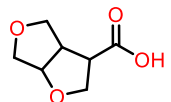
CAS:  
2164-61-6  
SMILES:  
O=C(O)c1ccccc1



CAS:  
2680543-50-2  
SMILES:  
O=C(O)C12CC(C1)Oc1cccc12



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



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

