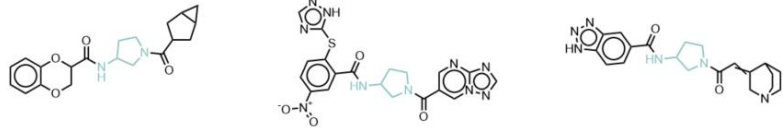


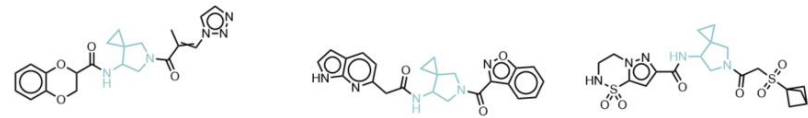
# De-Novo Generated Top 100 Scoring Compounds: *Moving away from Pyridine N-oxides*

- 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.

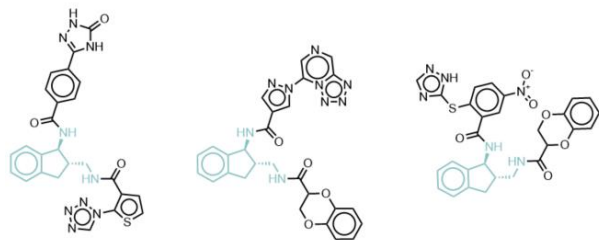
Core 1



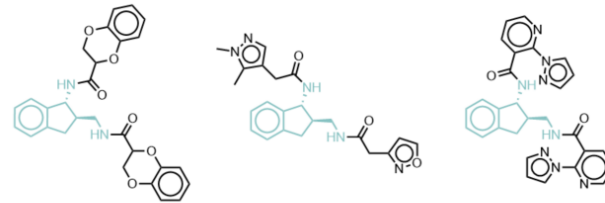
Core 2



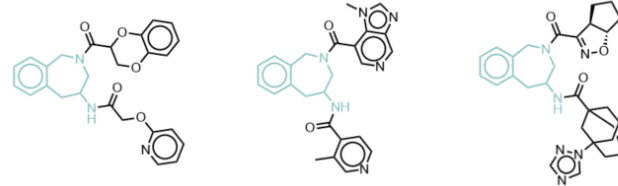
Core 3a



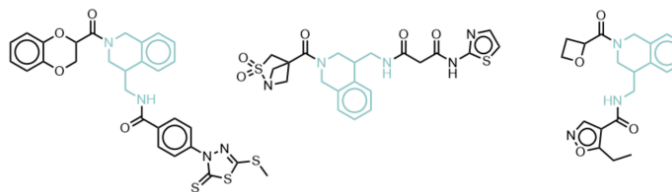
Core 3b



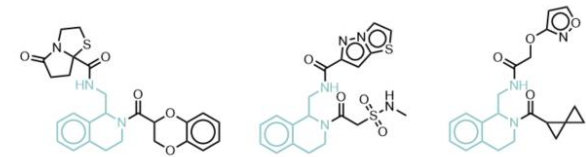
Core 4



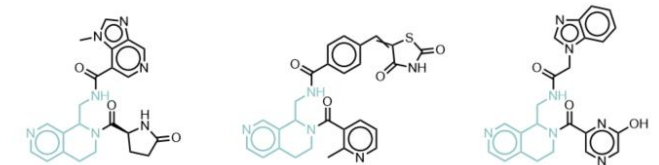
Core 5



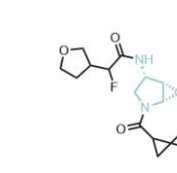
Core 6



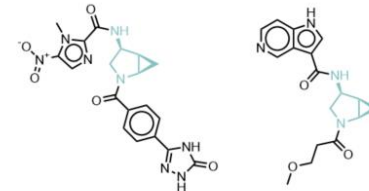
Core 7



Core 8a

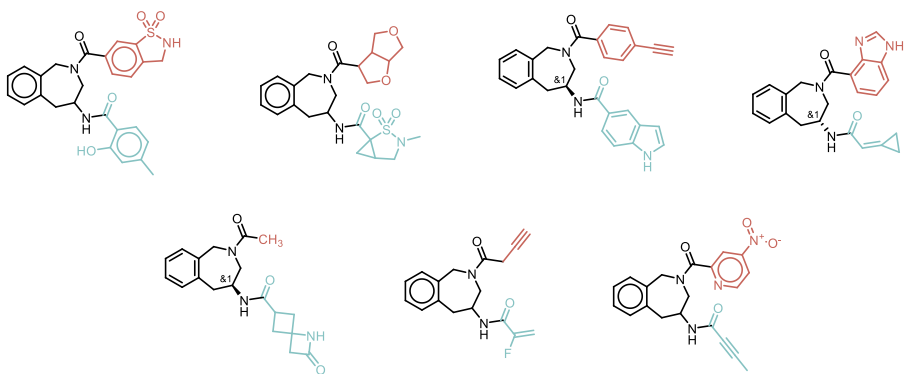


Core 8b



# 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

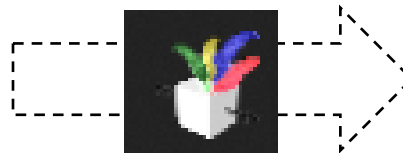
Mutagenic X

Tumorigenic X

Reproductive effective X

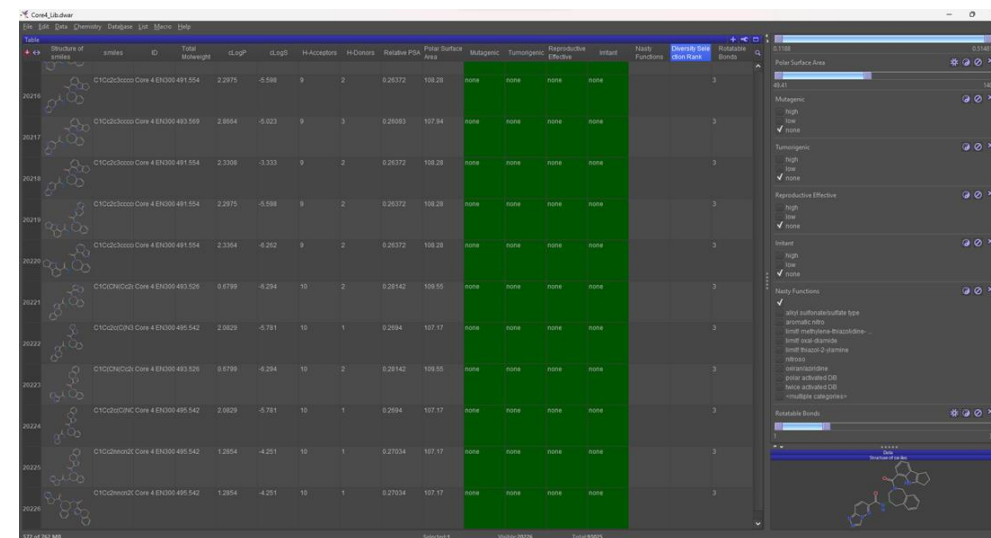
Irritant X

Nasty functions X



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

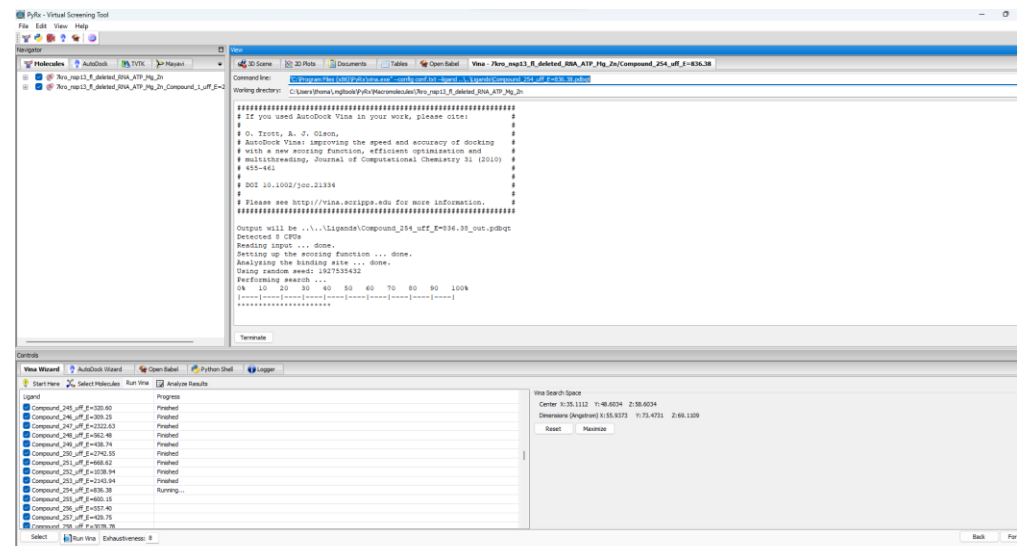
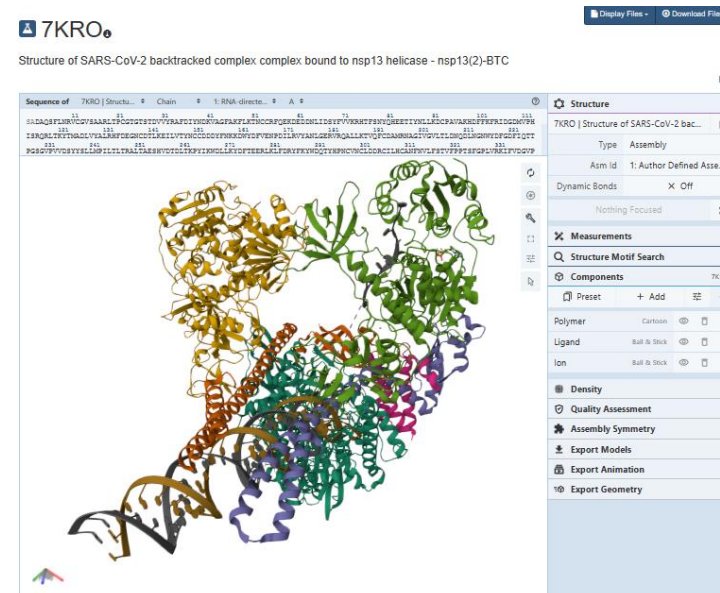
- Reduced to 20'226 molecules.
- DataWarrior's 'diversity selection rank' in order of structural diversity.
- Selected the top 1000 most diverse compounds for virtual screening using PyRx 0.8/AutoDock Vina 1.2. [PyRx - Virtual Screening Tool download | SourceForge.net](#)



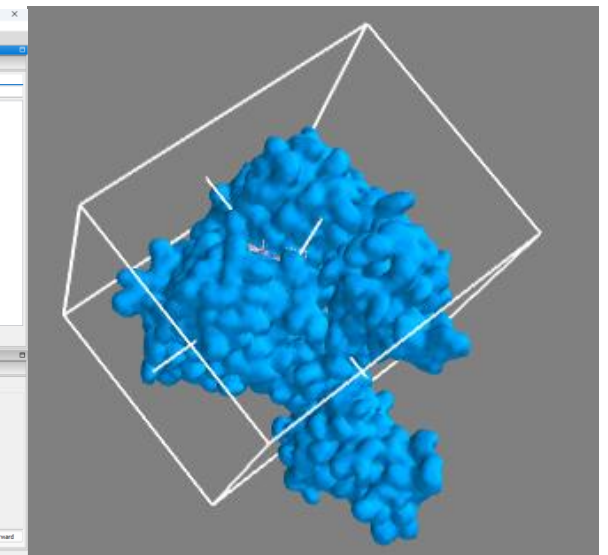
ID	Total Molweight	cLogP	cLogS	H-Acceptors	H-Donors	Relative PSA	Polar Surface Area	Mutagenic	Tumorigenic	Reproductive Effective	Irritant	Nasty Functions	Rotatable Bonds
20216	2,2975	-5.598	9	2	0.26372	108.28	none	none	none	none	3		
20217	2,8654	-5.023	9	3	0.26693	107.94	none	none	none	none	3		
20218	2,3308	-3.303	9	2	0.26372	108.28	none	none	none	none	3		
20219	2,2975	-5.598	9	2	0.26372	108.28	none	none	none	none	3		
20220	2,3354	-6.262	9	2	0.26372	108.28	none	none	none	none	3		
20221	9,8799	-6.294	10	2	0.28142	109.55	none	none	none	none	3		
20222	2,8629	-5.781	10	1	0.2694	107.17	none	none	none	none	3		
20223	9,8799	-6.294	10	2	0.28142	109.55	none	none	none	none	3		
20224	2,8629	-5.781	10	1	0.2694	107.17	none	none	none	none	3		
20225	1,2854	-4.251	10	1	0.27034	107.17	none	none	none	none	3		
20226	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.

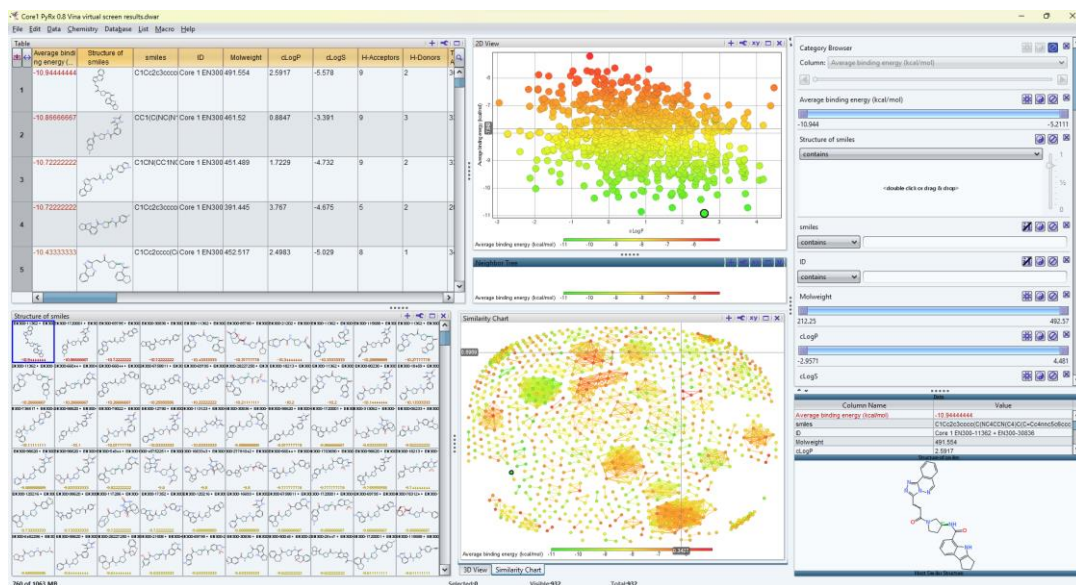


Ligand	Progress	Exhaustiveness
Compound_248_nif_#=232.00	Finished	8
Compound_246_nif_#=232.25	Finished	8
Compound_247_nif_#=232.43	Finished	8
Compound_249_nif_#=242.46	Finished	8
Compound_240_nif_#=438.74	Finished	8
Compound_239_nif_#=2742.55	Finished	8
Compound_231_nif_#=688.62	Finished	8
Compound_232_nif_#=2238.94	Finished	8
Compound_233_nif_#=2143.54	Finished	8
Compound_234_nif_#=4336.38	Running...	8
Compound_235_nif_#=4682.35	Running...	8
Compound_236_nif_#=557.40	Running...	8
Compound_237_nif_#=429.75	Running...	8
Compound_238_nif_#=410.76	Running...	8

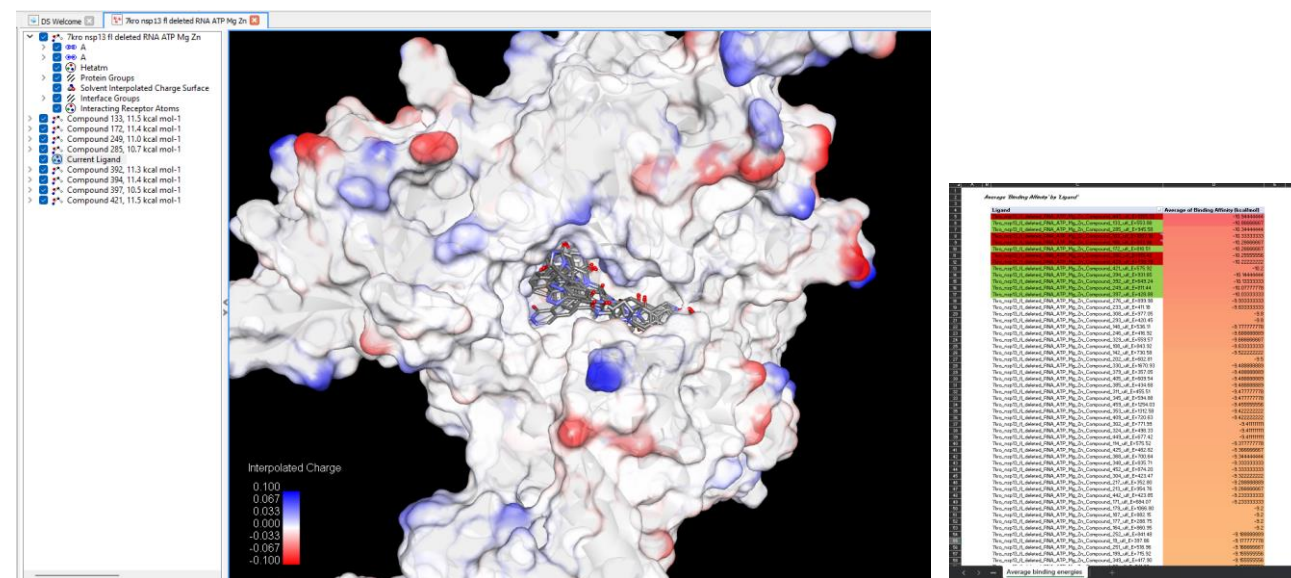


# De-Novo Generated Top 100 Scoring Compounds: Virtual Screen with PyRx 0.8 / AutoDock VINA (Core 1, pdb 7kro)

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

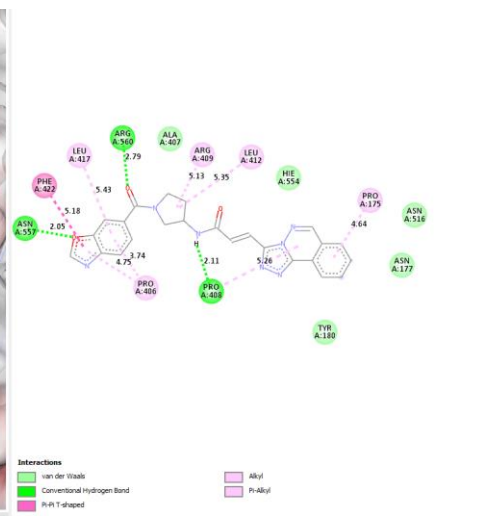
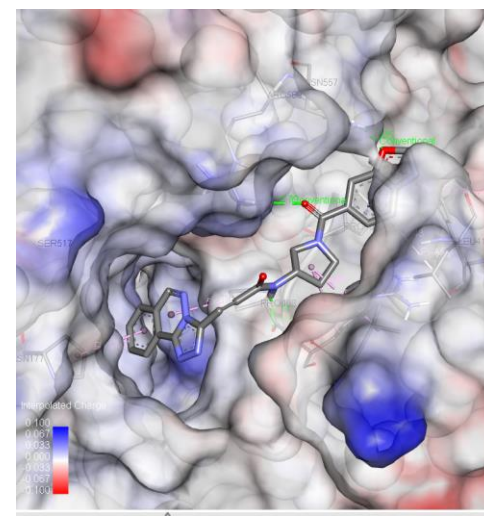
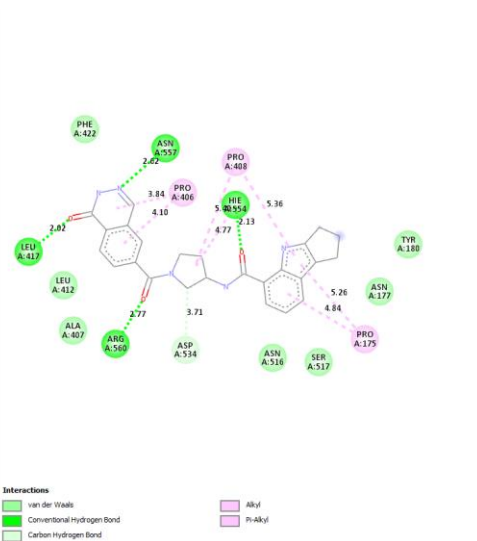
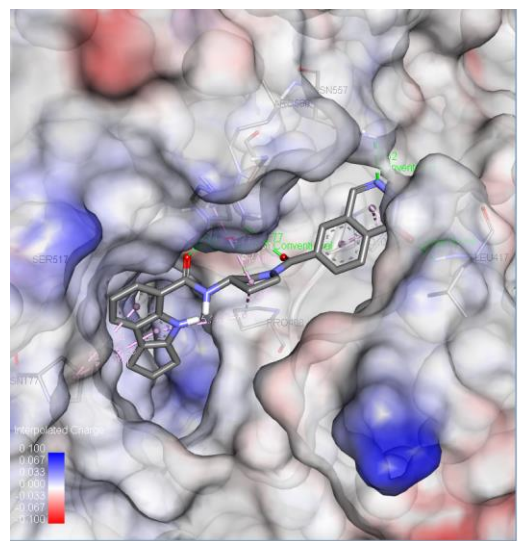
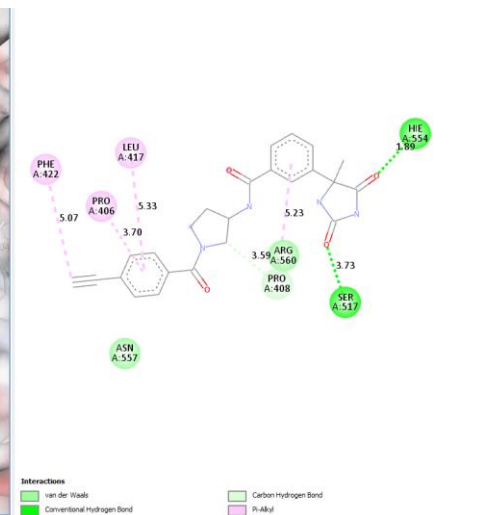
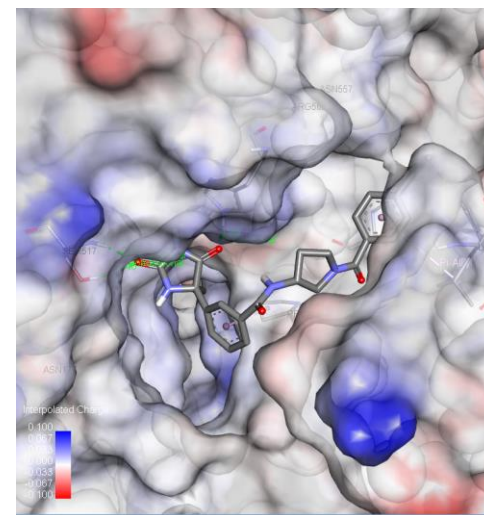
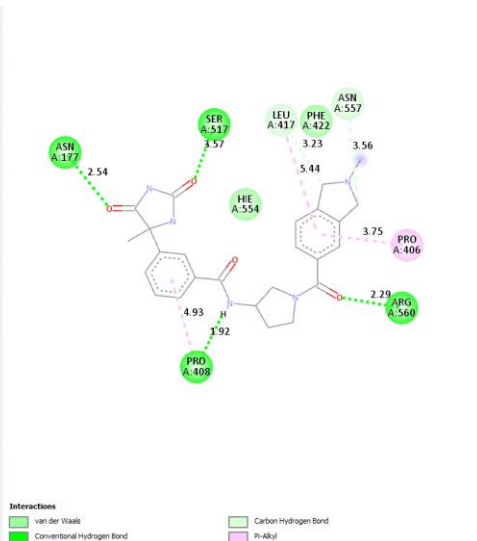
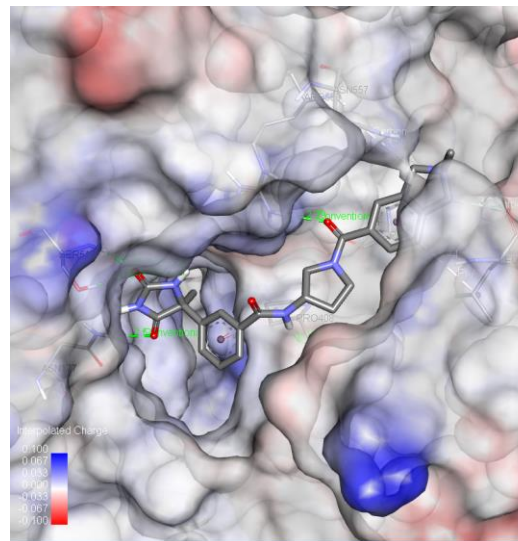


- Visually inspected** minimum energy poses to validate, eliminating incorrect geometries (cis-amides).

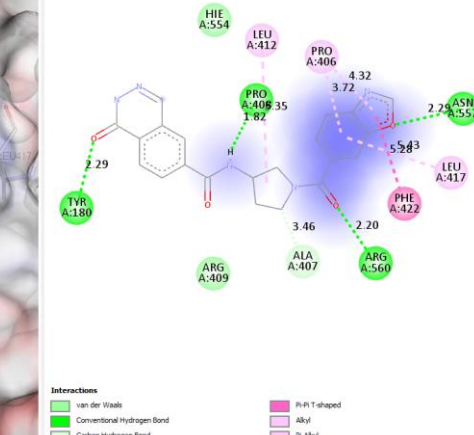
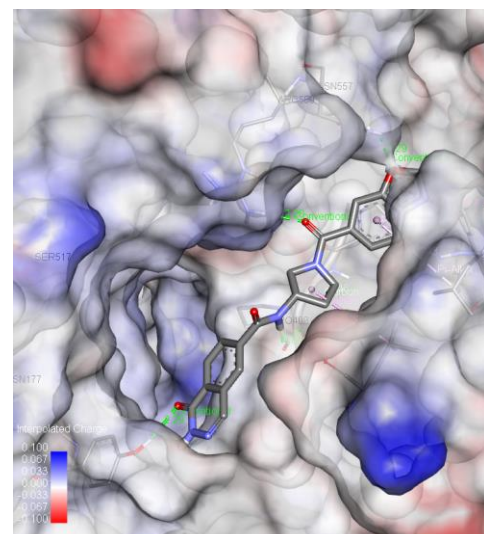
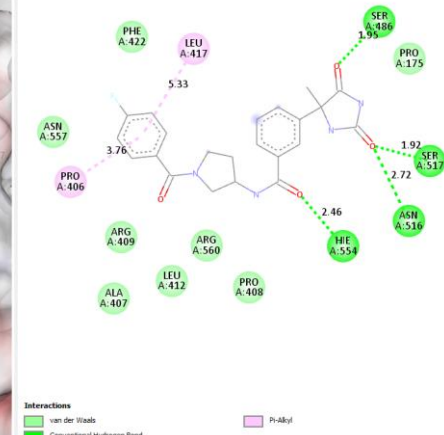
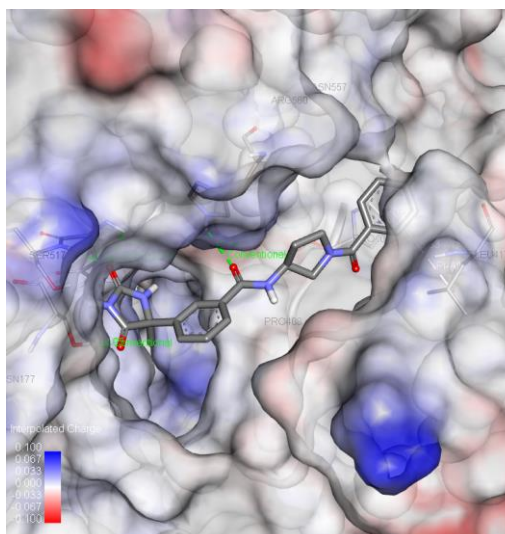
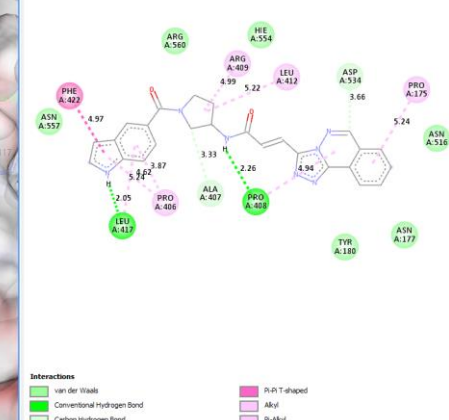
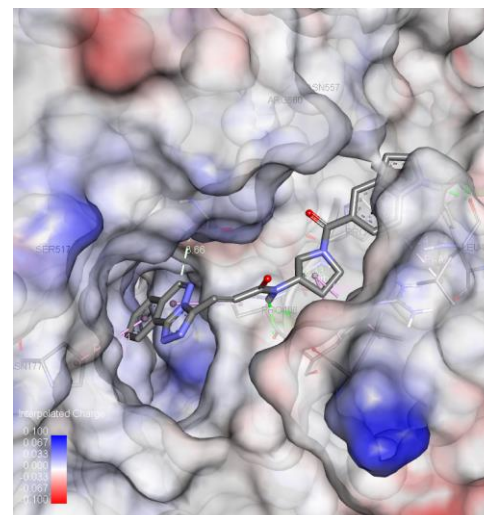
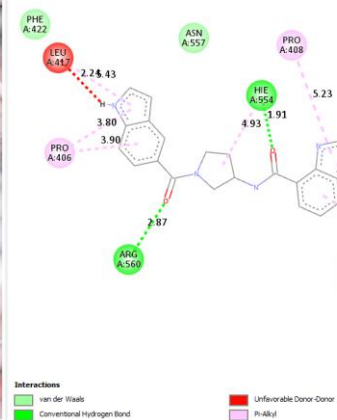
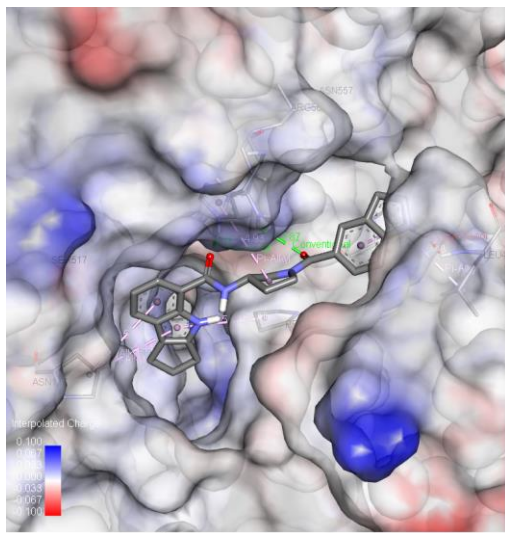




# De-Novo Generated Top 100 Scoring Compounds: Virtual Screen with PyRx 0.8 / AutoDock VINA (Core 1 , pdb 7kro)



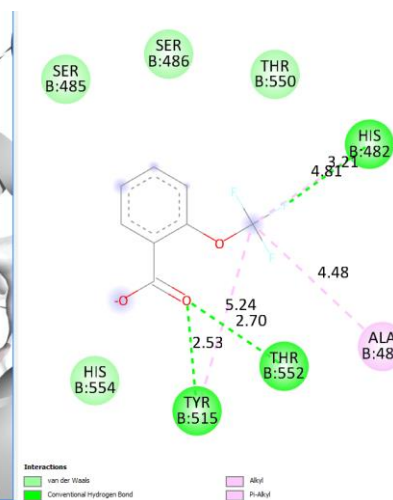
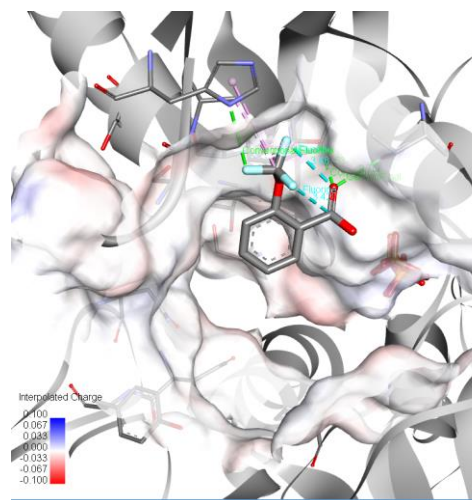
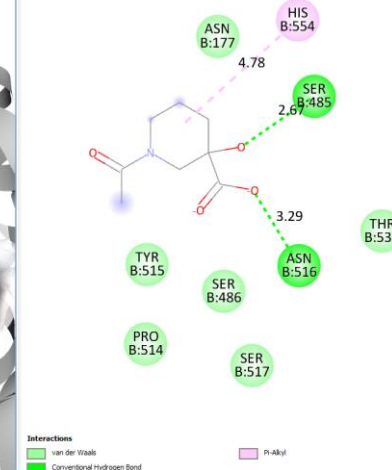
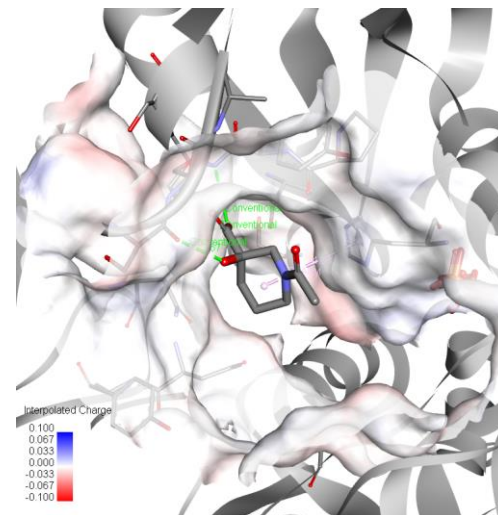
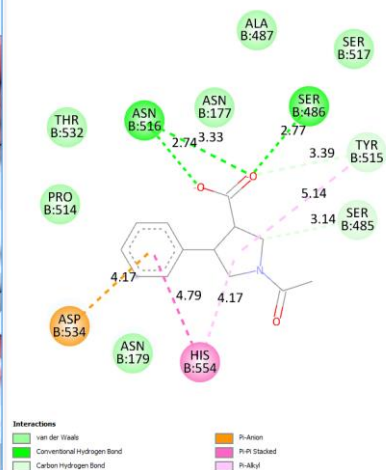
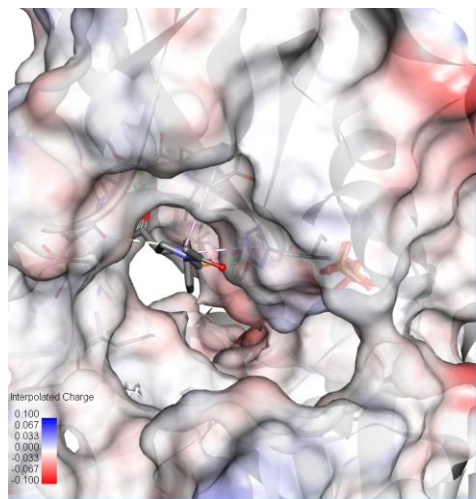
# De-Novo Generated Top 100 Scoring Compounds: Virtual Screen with PyRx 0.8 / AutoDock VINA (Core 1, pdb 7kro)





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

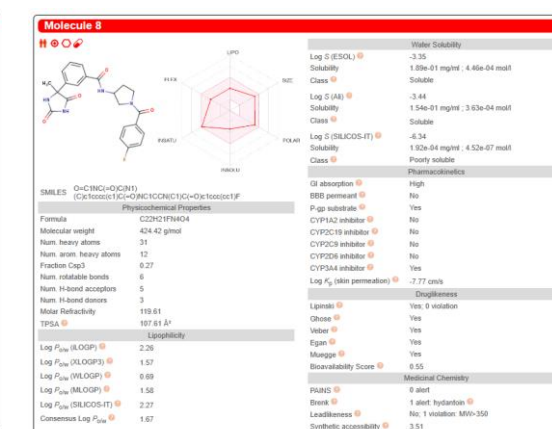
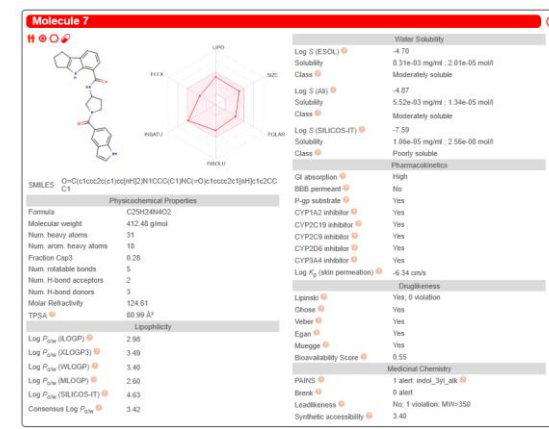
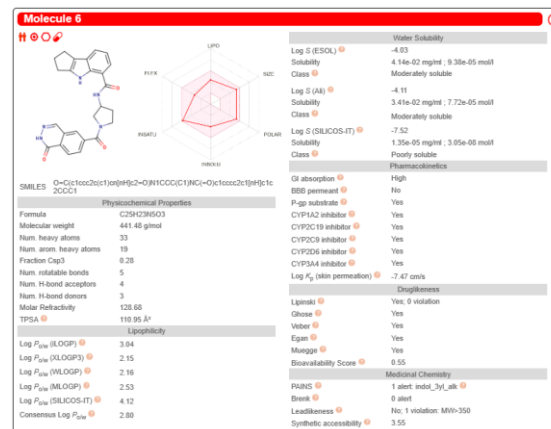
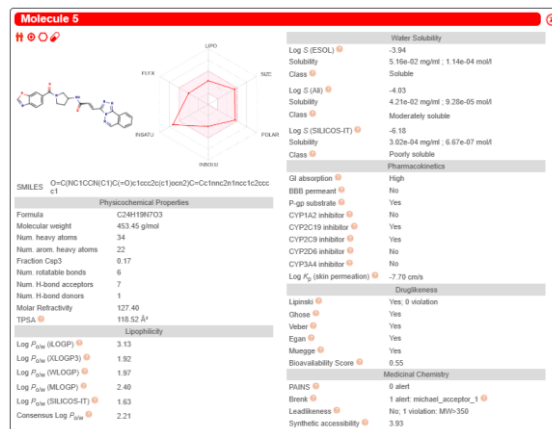
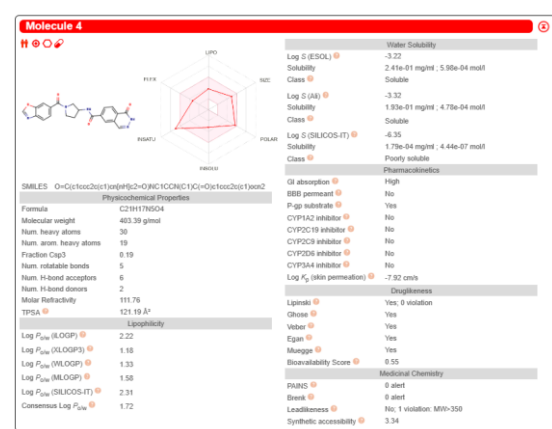
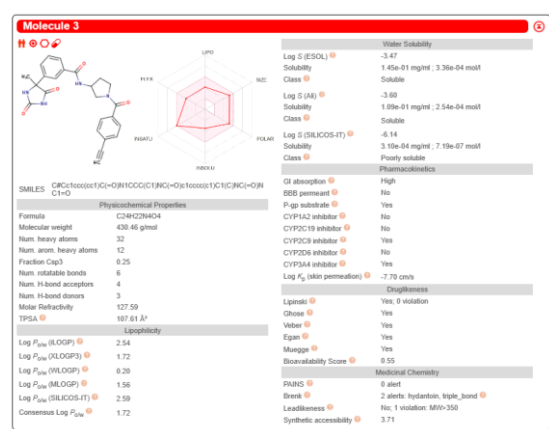
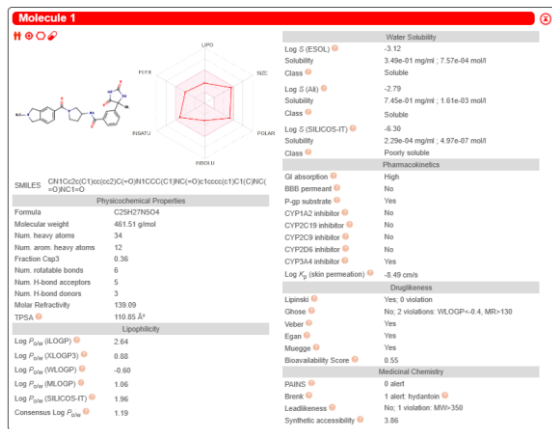
- PANDDA fragment interactions at 5' RNA site



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



- All compounds PAINS filtering (SwissADME) cleared.

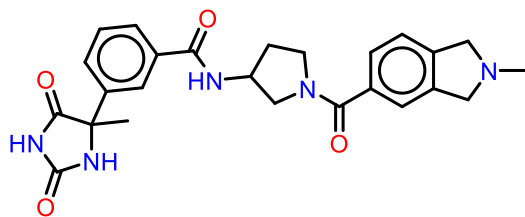




# De-Novo Generated Top 100 Scoring Compounds: Core1 compounds

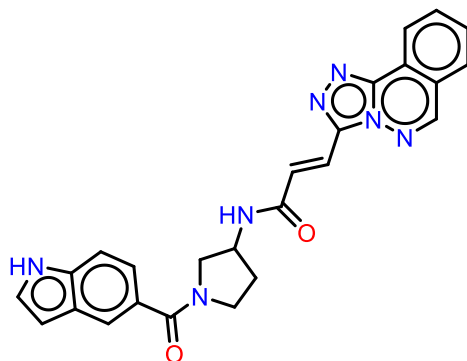
**Compound 133**

Core 1 EN300-1720001 + EN300-98620



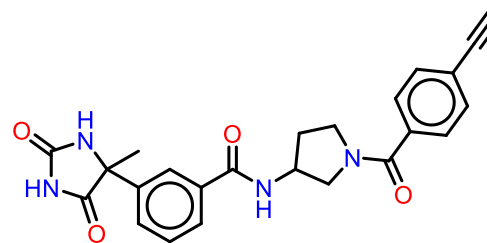
**Compound 285**

Core 1 EN300-69795 + EN300-11362



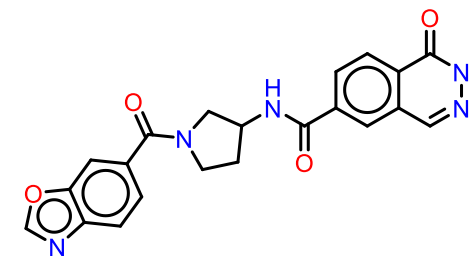
**Compound 172**

Core 1 EN300-115688 + EN300-98620



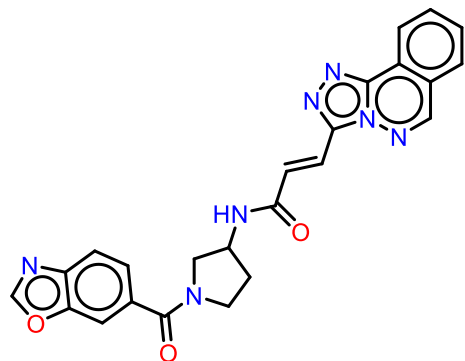
**Compound 421**

Core 1 EN300-66044 + EN300-6758811



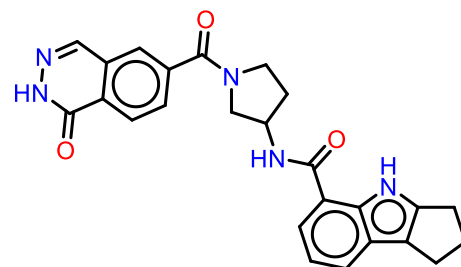
**Compound 394**

Core 1 EN300-66044 + EN300-11362



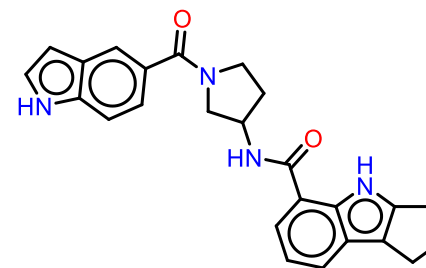
**Compound 392**

Core 1 EN300-6758811 + EN300-30836



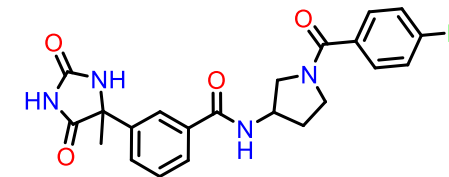
**Compound 249**

Core 1 EN300-69795 + EN300-30836



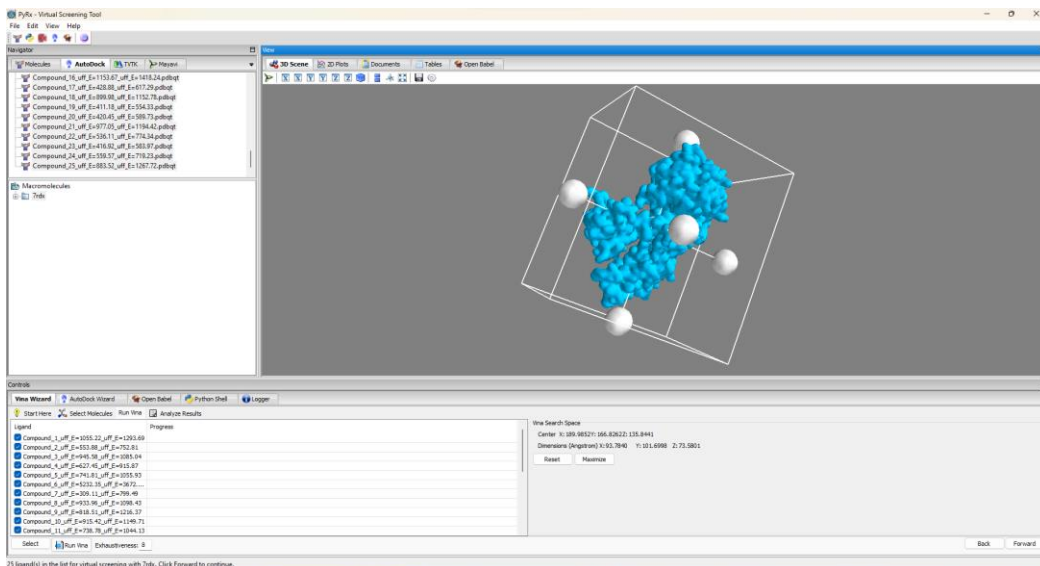
**Compound 397**

Core 1 EN300-18213 + EN300-98620



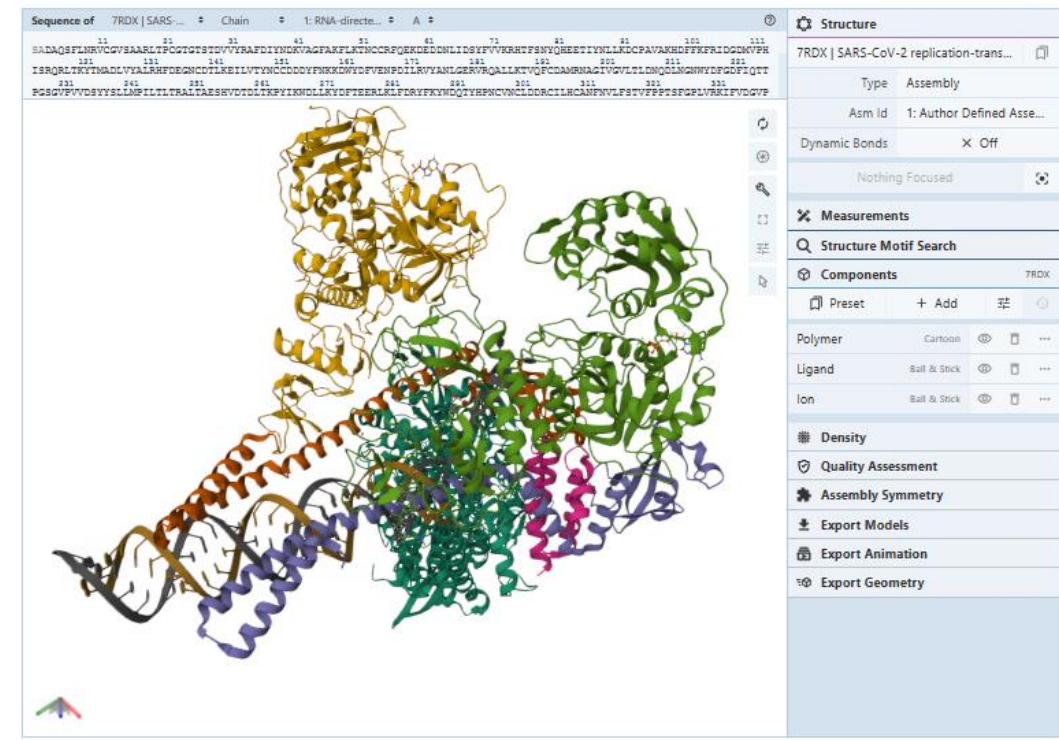
# De-Novo Generated Top 100 Scoring Compounds: Virtual Screen with PyRx 0.8 / AutoDock VINA (pdb 7kro)

- 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.



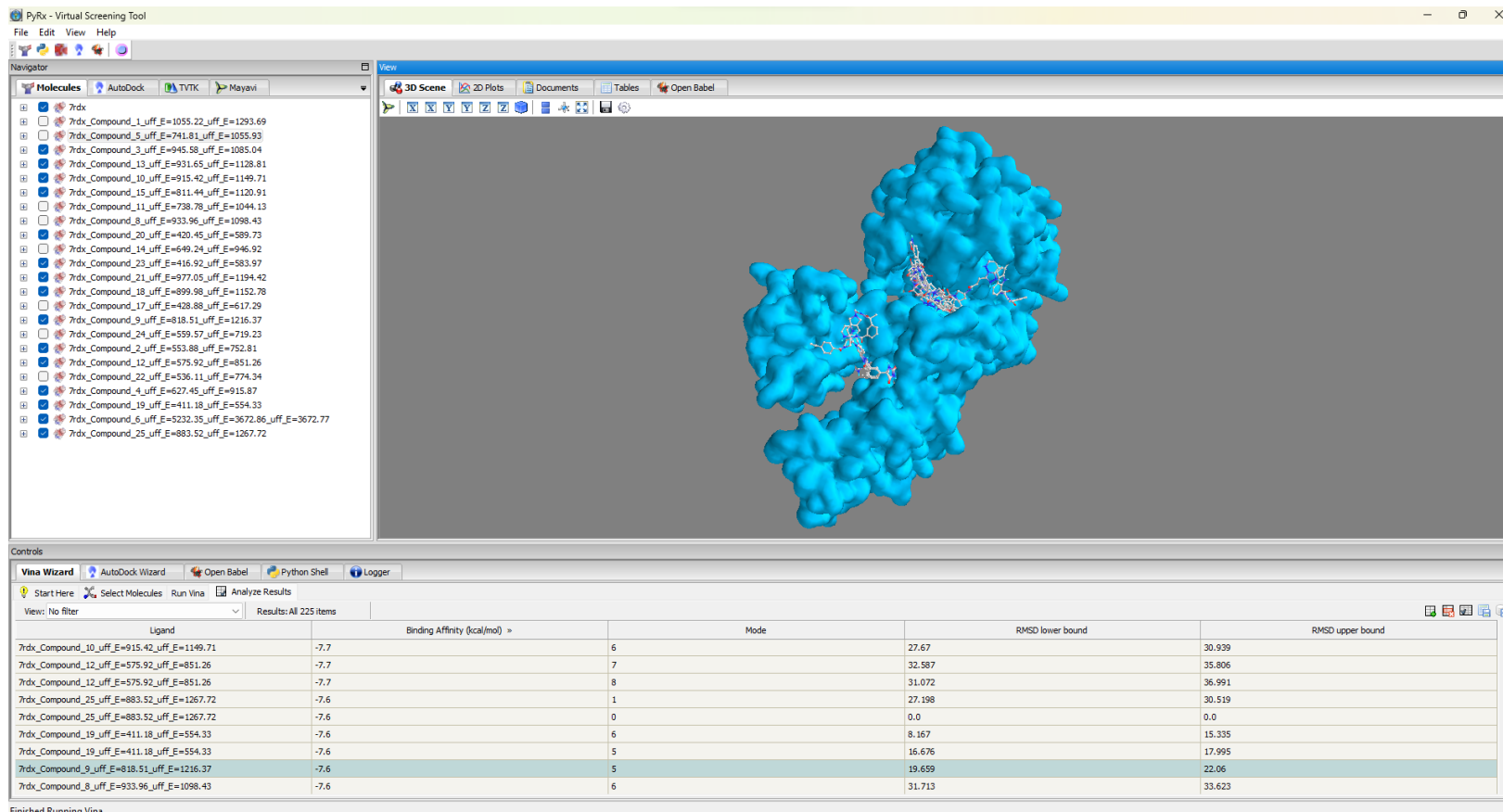
## 7RDX

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).

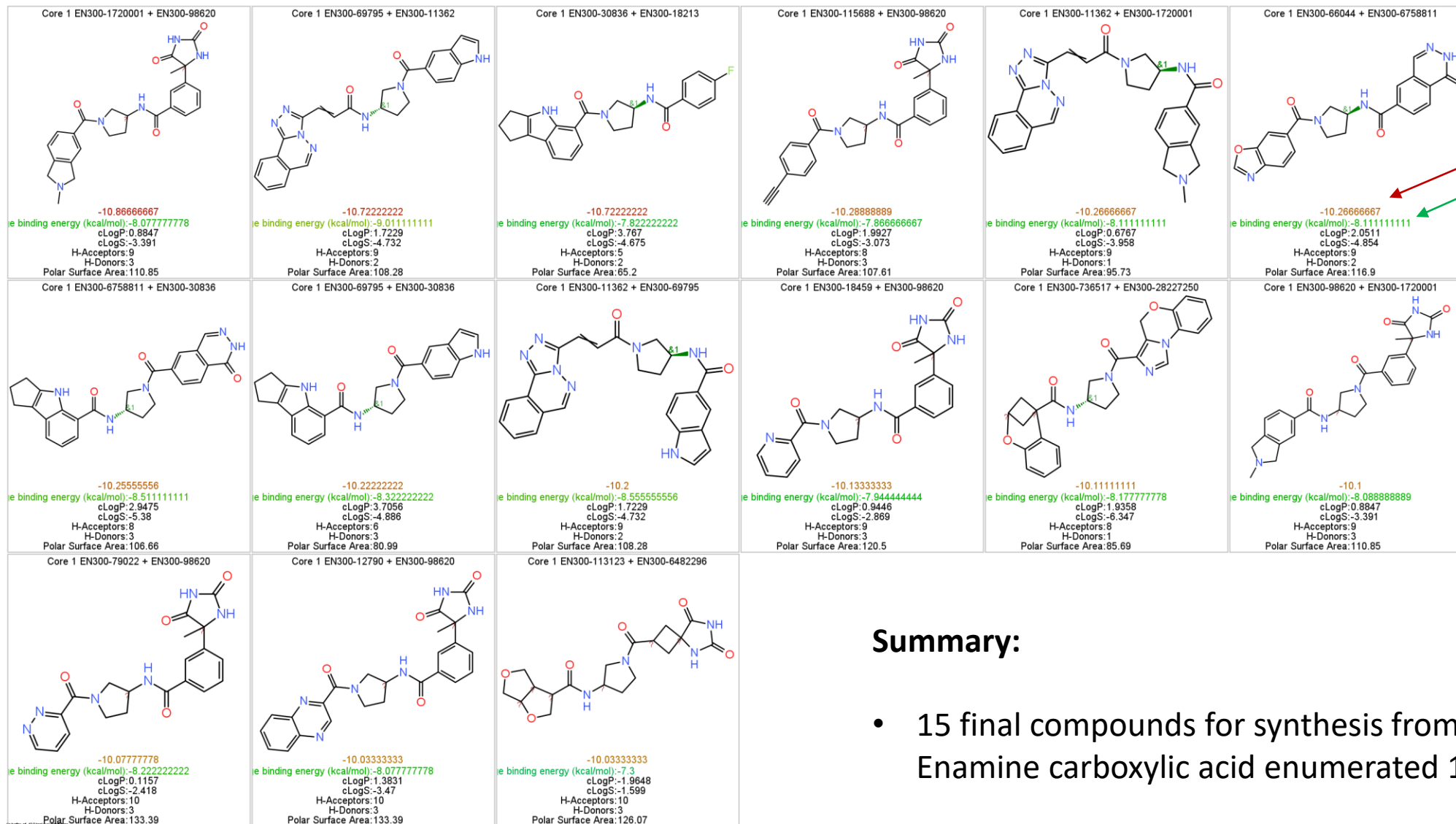


Average 'Binding Affinity' by 'Ligand'

Ligand	Average of Binding Affinity
7rdx_Compound_5_uhf_E=741.81_uhf_E=1055.93	-9.12222222
7rdx_Compound_3_uhf_E=945.58_uhf_E=1085.04	-9.01111111
7rdx_Compound_1_uhf_E=1055.22_uhf_E=1293.69	-8.74444444
7rdx_Compound_13_uhf_E=931.65_uhf_E=1128.81	-8.62222222
7rdx_Compound_18_uhf_E=899.98_uhf_E=1152.78	-8.55555556
7rdx_Compound_14_uhf_E=649.24_uhf_E=946.92	-8.51111111
7rdx_Compound_15_uhf_E=811.44_uhf_E=1120.91	-8.32222222
7rdx_Compound_17_uhf_E=428.88_uhf_E=617.29	-8.28888889
7rdx_Compound_23_uhf_E=416.92_uhf_E=583.97	-8.22222222
7rdx_Compound_21_uhf_E=977.05_uhf_E=1194.42	-8.17777778
7rdx_Compound_10_uhf_E=915.42_uhf_E=1149.71	-8.13333333
7rdx_Compound_12_uhf_E=575.92_uhf_E=851.26	-8.11111111
7rdx_Compound_11_uhf_E=738.78_uhf_E=1044.13	-8.11111111
7rdx_Compound_22_uhf_E=536.11_uhf_E=774.34	-8.08888889
7rdx_Compound_24_uhf_E=559.57_uhf_E=719.23	-8.07777778
7rdx_Compound_2_uhf_E=553.88_uhf_E=752.81	-8.07777778
7rdx_Compound_8_uhf_E=933.96_uhf_E=1098.43	-8.02222222
7rdx_Compound_20_uhf_E=420.45_uhf_E=589.73	-7.94444444
7rdx_Compound_9_uhf_E=818.51_uhf_E=1216.37	-7.86666667
7rdx_Compound_4_uhf_E=627.45_uhf_E=915.87	-7.82222222
7rdx_Compound_19_uhf_E=411.18_uhf_E=554.33	-7.75555556
7rdx_Compound_25_uhf_E=883.52_uhf_E=1267.72	-7.3
7rdx_Compound_16_uhf_E=1153.67_uhf_E=1418.24	-6.91111111
7rdx_Compound_6_uhf_E=5232.35_uhf_E=3672.86_uhf_E=3672.77	-6.61111111
7rdx_Compound_7_uhf_E=309.11_uhf_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)



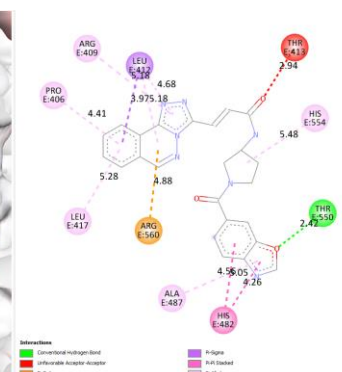
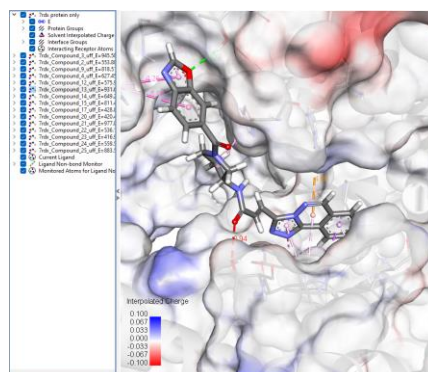
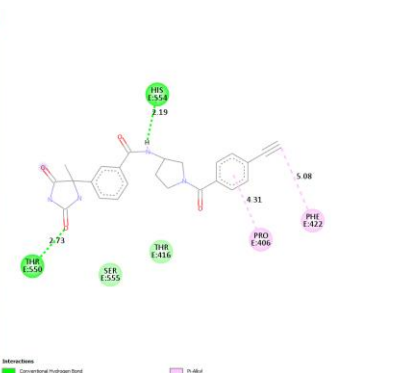
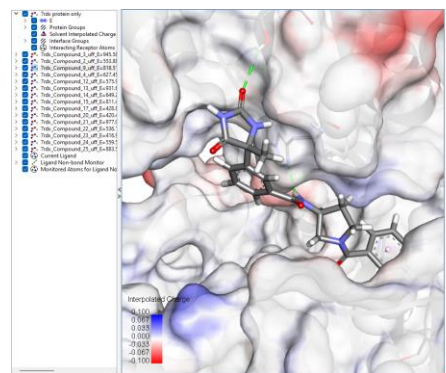
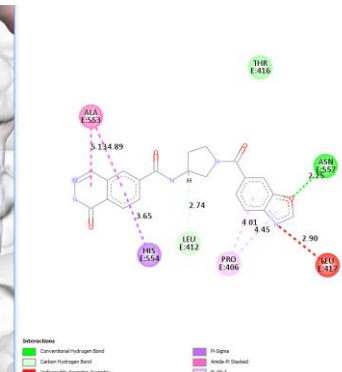
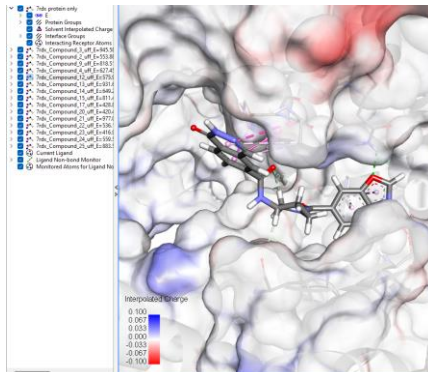
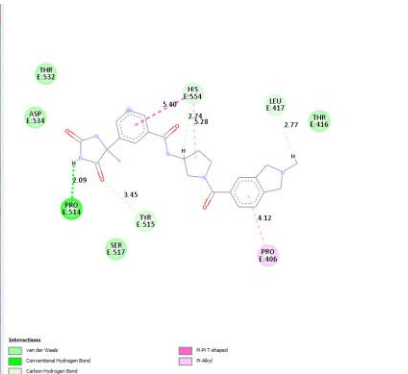
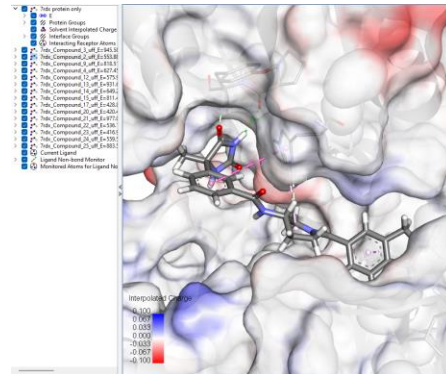
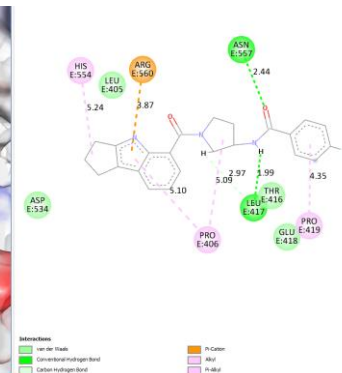
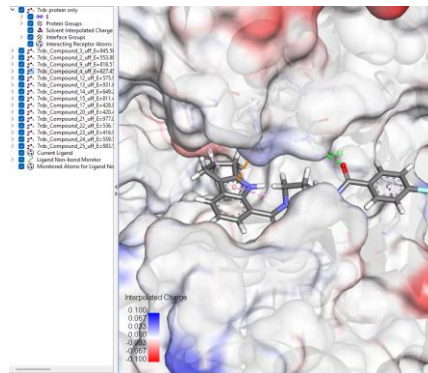
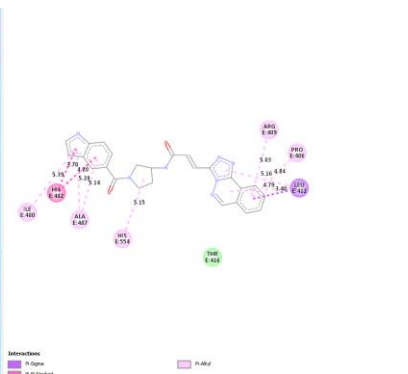
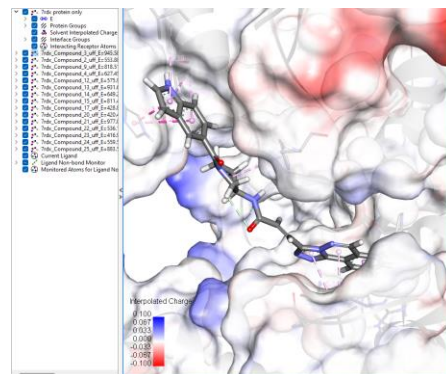
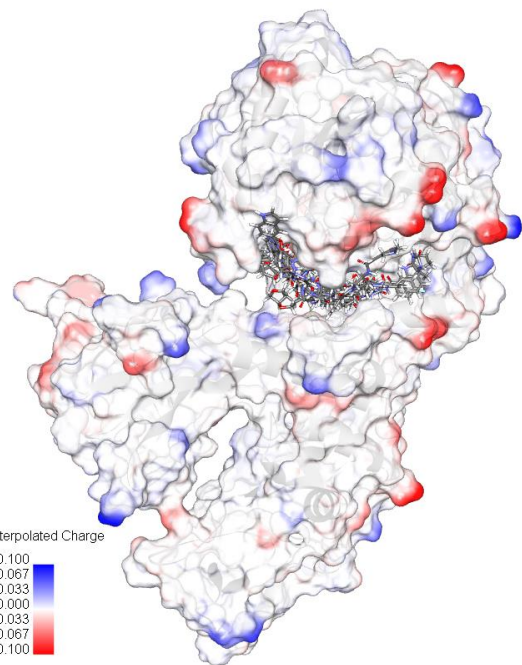
Average binding free energies that cleared visual pose inspection (kcal/mol):

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

## Summary:

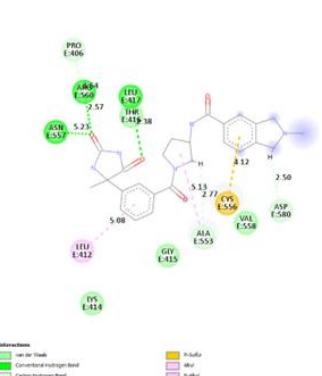
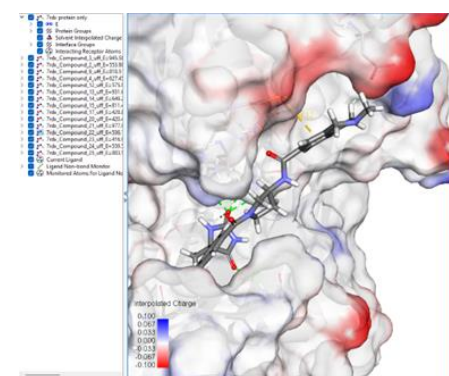
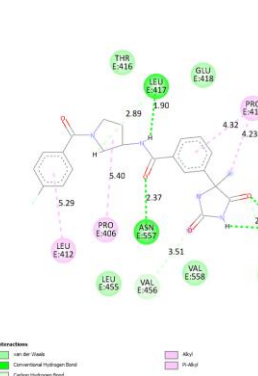
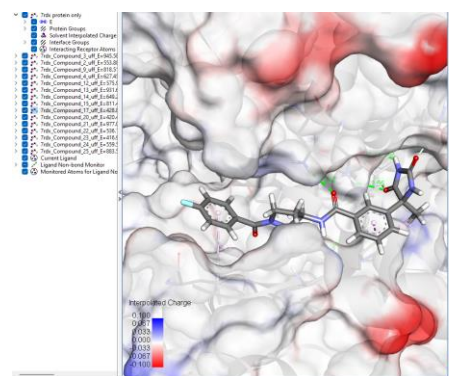
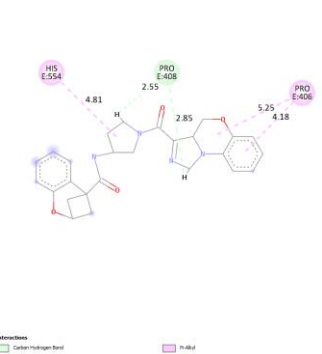
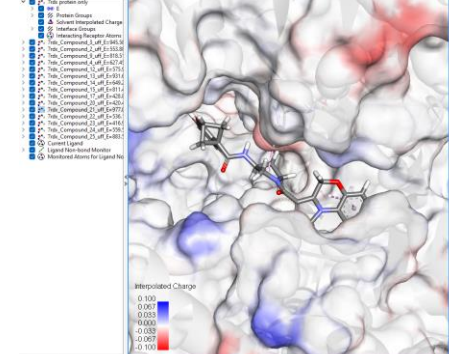
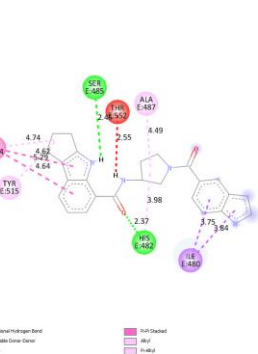
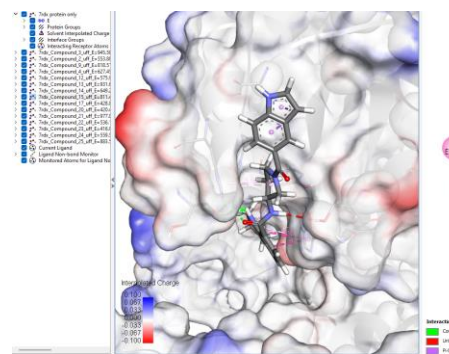
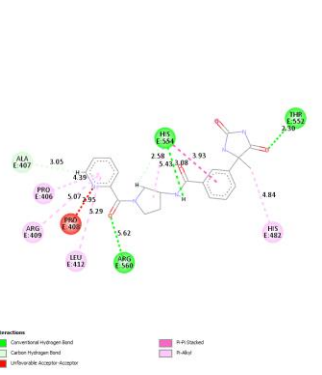
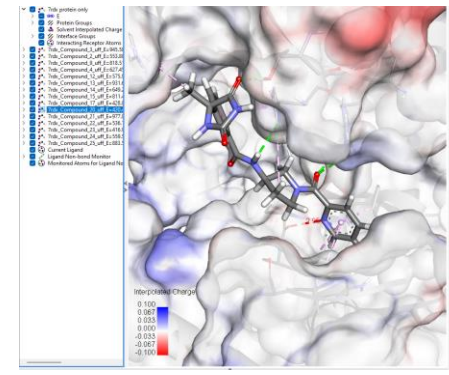
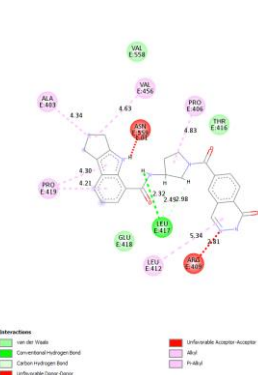
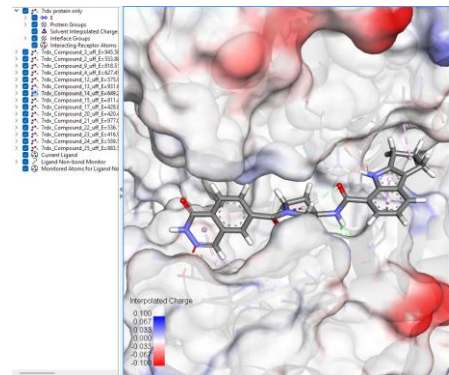
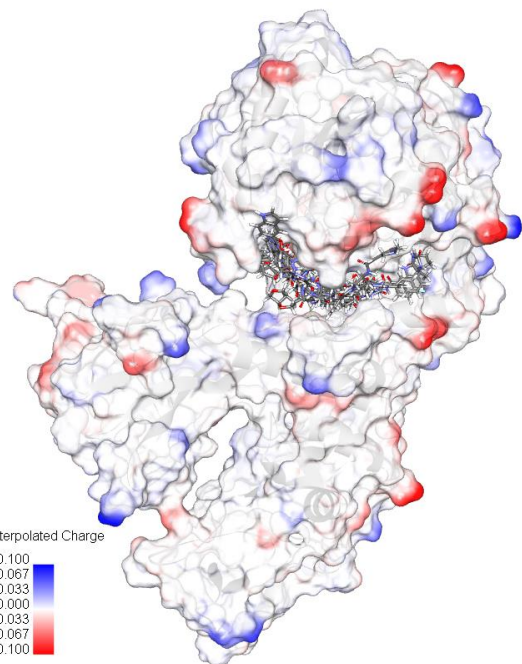
- 15 final compounds for synthesis from >93'000 Core Enamine carboxylic acid enumerated 1 library.

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



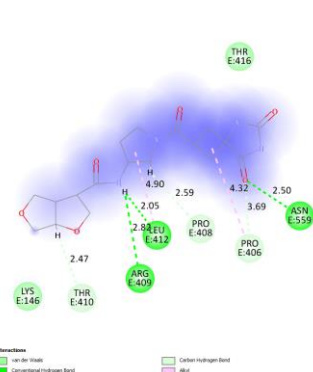
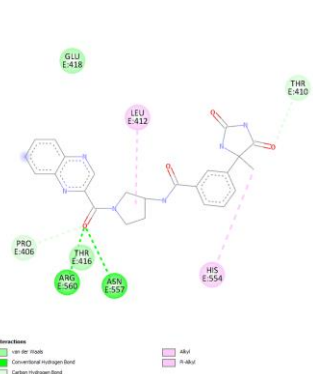
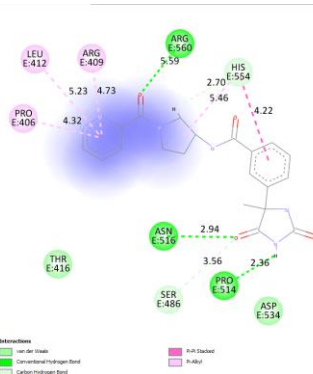
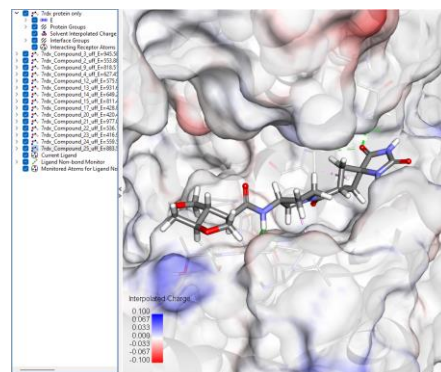
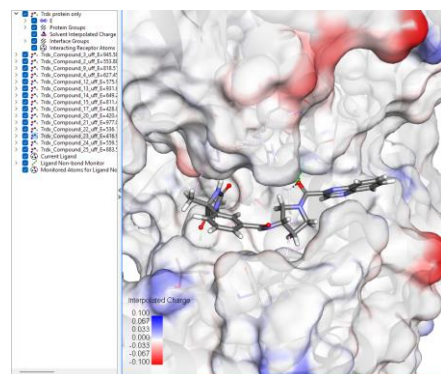
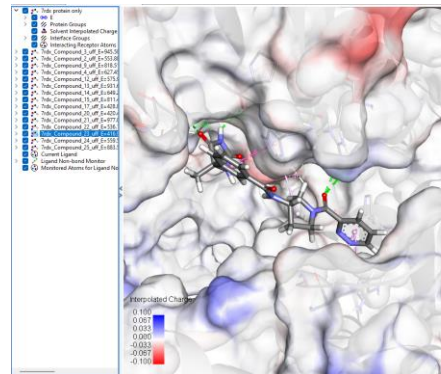
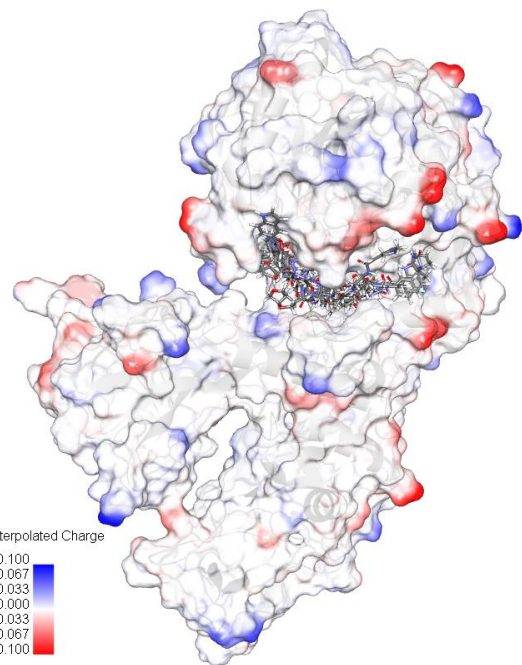


# De-Novo Generated Top 100 Scoring Compounds: Virtual Screen with PyRx 0.8 / AutoDock VINA (pdb 7rdx)





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

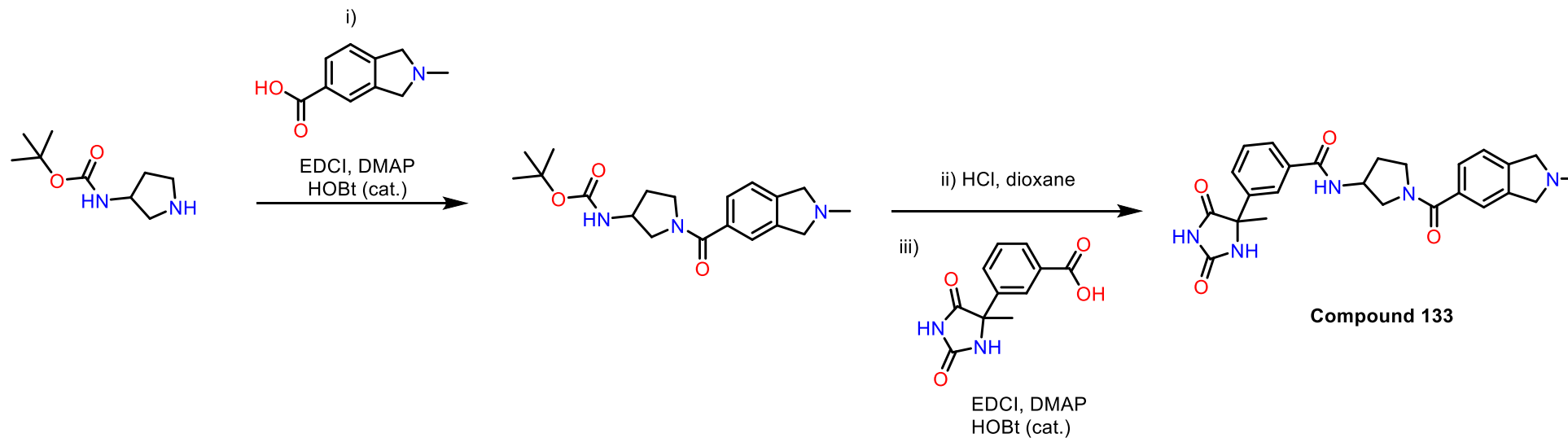


## SMILES String:

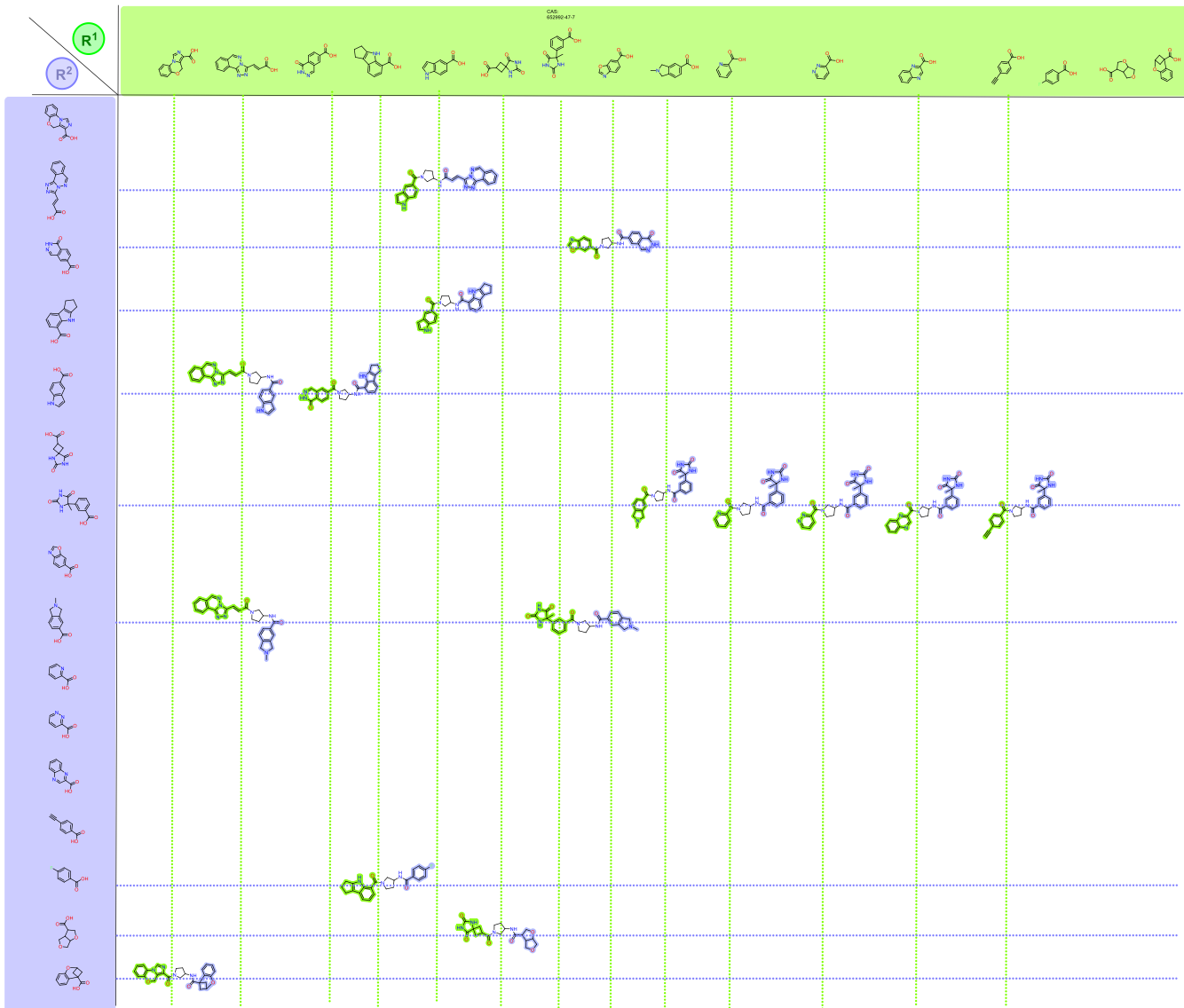
1. CC1(C(NC(N1)=O)=O)c1cccc(c1)C(NC1CCN(C1)C(c1ccc2CN(C)Cc2c1)=O)=O
2. CC1(C(NC(N1)=O)=O)c1cccc(c1)C(N1CCC(C1)NC(c1ccc2CN(C)Cc2c1)=O)=O
3. C1CN(CC1NC(C=Cc1nnc2c3ccccc3cnn12)=O)C(c1ccc2c(cc[nH]2)c1)=O
4. C1Cc2c3cccc(C(N4CCC(C4)NC(c4ccc(cc4)F)=O)=O)c3[nH]c2C1
5. CC1(C(NC(N1)=O)=O)c1cccc(c1)C(NC1CCN(C1)C(c1ccc(C#C)cc1)=O)=O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

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

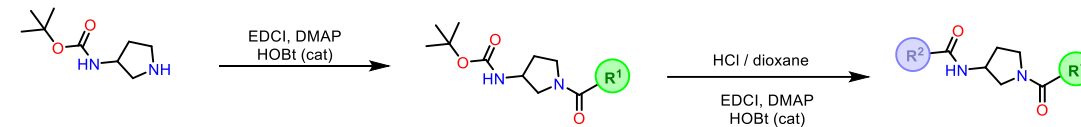
- Simple 3-step diamide synthesis.



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



## Synthesis:

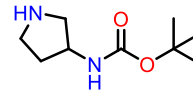


- 13x R1 reactions.
- 15x Boc-deprotections reactions.
- 9x R2 reactions.
- Total reactions = 24
- Total compounds = 15

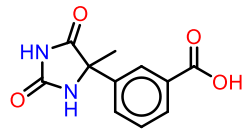


# De-Novo Generated Top 100 Scoring Compounds: Virtual Screen with PyRx 0.8 / AutoDock VINA

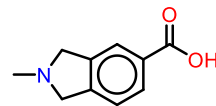
CAS:  
122536-76-9  
SMILES:  
CC(C)(C)OC(=O)NC1CCNC1



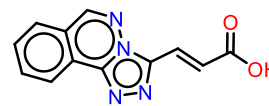
CAS:  
652992-47-7  
SMILES:  
CC1(c2cccc(C(=O)O)c2)NC(=O)NC1=O



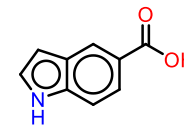
CAS:  
1065065-38-4  
SMILES:  
CN1Cc2ccc(C(=O)O)cc2C1



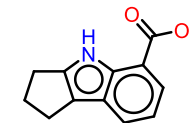
CAS:  
793716-18-4  
SMILES:  
O=C(O)C=Cc1nnc2c3ccccc3cnn12



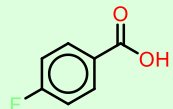
CAS:  
1670-81-1  
SMILES:  
O=C(O)c1ccc2[nH]ccc2c1



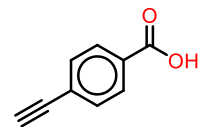
CAS:  
422312-00-3  
SMILES:  
O=C(O)c1cccc2c3c([nH]c12)CCC3



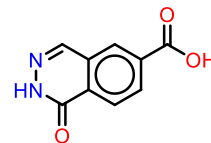
CAS:  
456-22-4  
SMILES:  
O=C(O)c1ccc(F)cc1



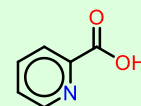
CAS:  
10602-00-3  
SMILES:  
C#Cc1ccc(C(=O)O)cc1



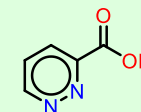
CAS:  
102126-65-8  
SMILES:  
O=C(O)c1ccc2c(=O)[nH]ncc2c1



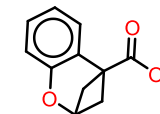
CAS:  
98-98-6  
SMILES:  
O=C(O)c1ccccn1



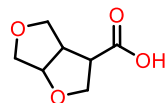
CAS:  
2164-61-6  
SMILES:  
O=C(O)c1cccnn1



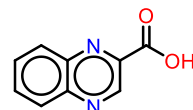
CAS:  
2680543-50-2  
SMILES:  
O=C(O)C12CC(C1)Oc1ccccc12



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



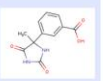
CAS:  
879-65-2  
SMILES:  
O=C(O)c1cnc2ccccc2n1



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



## [3-\(4-methyl-2,5-dioximidazolidin-4-yl\)benzoic acid | EnamineStore](#)



5.0 g

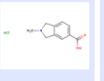
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Catalog ID	EN300-58620
Product class	Building Block
Name	3-(4-methyl-2,5-dioximidazolidin-4-yl)benzoic acid
Formula	C <sub>14</sub> H <sub>14</sub> N <sub>2</sub> O <sub>4</sub>
Class	Carboxylic Acids
Subclass	Aromatic Acids
ACD	MFC02329274
number	652962-47-7
CAS number	0.789
CllogP	234.2
MW	96%
Purity	

## [2-methyl-2,3-dihydro-1H-isoindole-5-carboxylic acid hydrochloride | EnamineStore](#)



1.0 g

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Catalog ID	EN300-172001
Product class	Building Block
Name	2-methyl-2,3-dihydro-1H-isoindole-5-carboxylic acid hydrochloride
Formula	C <sub>9</sub> H <sub>11</sub> ClN <sub>2</sub> O <sub>2</sub>
Class	Carboxylic Acids
Subclass	Aromatic Acids
ACD	MFC020927758
number	1365065-36-4
CAS number	-1.045
CllogP	213.7
MW	96%
Purity	

## [3-\[\[1,2,4\]triazolo\[3,4-a\]phthalazin-3-yl\]prop-2-enoic acid | EnamineStore](#)



1.0 g


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Download SDS

Catalog ID	EN300-11362
Product class	Building Block
Name	3-[[1,2,4]triazolo[3,4-a]phthalazin-3-yl]prop-2-enoic acid
Formula	C <sub>17</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub>
Class	LAB Reagents, Carboxylic Acids
Subclass	Aliphatic Carboxylic Acids,
ACD	MFC006364483
number	752716-18-4
CAS number	0.89
CllogP	240.2
MW	96%
Purity	

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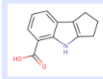
United Kingdom

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### Indole-5-carboxylic acid.

Pack Size	Price	Availability
1 g	£12.00	5+ In Stock - Ships Immediately
5 g	£30.00	5+ In Stock - Ships Immediately
10 g	£34.00	2 In Stock - Ships Immediately
10 g	£73.00	1 In Stock - Ships Immediately

## [1H,2H,3H,4H-cyclopenta\[b\]indole-5-carboxylic acid | EnamineStore](#)



1.0 g


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Download SDS

Catalog ID	EN300-30836
Product class	Building Block
Name	1H,2H,3H,4H-cyclopenta[b]indole-5-carboxylic acid
Formula	C <sub>12</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub>
Class	Carboxylic Acids
Subclass	Aromatic Acids
ACD	MFC009863427
number	422312-00-3
CAS number	3.08
CllogP	201.2
MW	96%
Purity	

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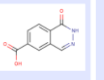
United Kingdom

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### 4-Ethynylbenzoic acid

Pack Size	Price	Availability
1 g	£12.00	2 In Stock - Ships Immediately
5 g	£34.00	5+ In Stock Germany - Ships 2 - 4 Days
10 g	£59.00	2 In Stock Germany - Ships 2 - 4 Days
10 g	£132.00	In Stock China - 10-14 Days
100 g	£504.00	In Stock China - 10-14 Days
500 g	£2016.00	In Stock China - 10-14 Days

## [1-oxo-1,2-dihydrophthalazine-6-carboxylic acid | EnamineStore](#)



1.0 g

In Stock [Add to cart](#)

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Catalog ID	EN300-678811
Product class	Building Block
Name	1-oxo-1,2-dihydrophthalazine-6-carboxylic acid
Formula	C <sub>8</sub> H <sub>6</sub> N <sub>2</sub> O <sub>3</sub>
Class	Carboxylic Acids
Subclass	Aromatic Acids
ACD	MFC028679116
number	102126-65-8
CAS number	0.814
CllogP	190.2
MW	95%
Purity	

## [8-oxatricyclo\[7.1.1.0,2,7\]undeca-2,4,6-triene-1-carboxylic acid | EnamineStore](#)



0.25 g

In Stock [Add to cart](#)

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Catalog ID	EN300-2827280
Product class	Building Block
Name	8-oxatricyclo[7.1.1.0,2,7]undeca-2,4,6-triene-1-carboxylic acid
Formula	C <sub>11</sub> H <sub>10</sub> O <sub>2</sub>
Class	Carboxylic Acids
Subclass	Aromatic Acids
ACD	MFC034186604
number	2480543-50-2
CAS number	1.279
CllogP	190.2
MW	96%
Purity	

## [hexahydrofuro\[2,3-c\]furan-3-carboxylic acid | EnamineStore](#)



0.5 g

In Stock [Add to cart](#)

Find Analogs

Download SDS

Catalog ID	EN300-4482296
Product class	Building Block
Name	hexahydrofuro[2,3-c]furan-3-carboxylic acid
Formula	C <sub>7</sub> H <sub>10</sub> O <sub>4</sub>
Class	Carboxylic Acids
Subclass	Aliphatic Carboxylic Acids, Polycyclic Carboxylic Acids
ACD	MFC031719399
number	225548-84-3
CAS number	-0.147
CllogP	155.2
MW	96%
Purity	

## [879-65-2 Cas No. | Quinoxaline-2-carboxylic acid | Apollo \(apolloscientific.co.uk\)](#)



QUINOXALINE-2-CARBOXYLIC ACID

CATALOGUE NUMBER: OR3730

Synonym(s): 2-Carboxyquinoxaline; 2-Carboxyl-1,4-benzodiazine

CAS Number: 879-65-2

Commodity Code: 2933990090

MDL Number: MFC000012334

PRICE	CHEMICAL DATA	HEALTH & SAFETY	DOCUMENTATION		
Size	UK Stock <input type="checkbox"/>	US Stock <input type="checkbox"/>	Price	Quantity	Add to Basket
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