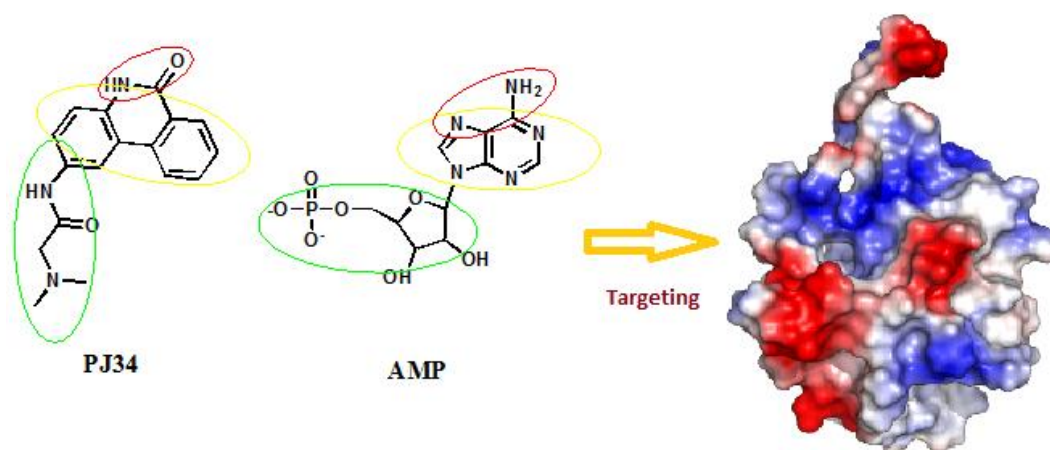


## Supplemental Information

### Structural basis for the identification of the N-terminal domain of coronavirus nucleocapsid protein as an antiviral target

Shing-Yen Lin,<sup>1,2</sup> Chia-Ling Liu,<sup>1,2</sup> Yu-Ming Chang,<sup>3</sup> Jincun Zhao,<sup>4</sup> Stanley Perlman,<sup>4#</sup> and Ming-Hon Hou<sup>1,2,5,\*#</sup>

### TOC



## Figure legends

**Figure S1.** Amino acid sequence alignment (performed using T-coffee) of the N-NTDs from HCoV-OC43 (NP\_937954), SARS-CoV (ABI96968), HCoV-229E (AAG48597), IBV (AAB24054), and MHV (ACO72897); these sequences were retrieved from GenBank. The conserved amino acids in the four sequences are shaded in red, while the similar residues are highlighted in blue.

**Figure S2.** (A) HCoV-OC43 N-NTD sequences aligned with the other N-terminal RNA-binding domains in nucleocapsid proteins from coronaviruses. The conserved amino acids in the four sequences are shaded in black, while the similar residues are highlighted in grey. In addition, the residues marked with an asterisk interact with the RNA using their side chains. (B) Left: Superimposition of the CoV N-NTD of HCoV-OC43-AMP complex (green), SARS (purple), IBV (orange), and MHV (blue). Right: Enlargement of the RNA binding site.

**Figure S3.** (A) The detailed interactions of CMP binding site. The residues in this binding site responsible for interacting with CMP include Ser 64, Phe 66, Arg 122, Tyr 124, Tyr 126 and Arg 164. (B) The detailed interactions of GMP binding site. The residues in this binding site responsible for interacting with GMP include Ser 64, Gly 68, Arg 122, Tyr 124, Tyr 126 and Arg 164. (C) The detailed interactions of UMP binding site. The residues Ser 64, Gly 68, Phe 66, Arg 122, Tyr 124 and Arg 164 form interaction with UMP. The electron density ( $2Fo - Fc$ ) is contoured at 1.0 sigma level. The dotted green lines indicate hydrogen bonds. The red dashed lines indicate ionic interactions. (D) A summary of the amino acids that interact with the N protein of HCoV-OC43 N-NTD.

**Figure S4.** Virus replication is inhibited by presence of mutant N protein. Cells were with plasmids encoding the WT N protein or the mutant (R122A, Y124A or R164A) N protein and then infected with HCoV-OC43 as described in Materials and Methods. Samples were then analyzed for levels of M protein gene transcript. No transfection and no infection controls are also shown.

**Figure S5.** Structural superimposition of the HCoV-OC43 N-NTD-AMP (red) with HCoV-OC43 N-NTD-PJ34 (green) at the residues involved in the ligand binding.

**Figure S6.** Surface charge distribution of N-NTDs from HCoV-OC43(4LI4), SARS-CoV(2OFZ), IBV(2GEC), and MHV(3HD4).

**Figure S7.** (A) Surface charge distribution of the HCoV-OC43 N-NTD-RNA complex in which blue and red indicate positive and negative charges, respectively. (B) A model of the HCoV-OC43 N-NTD-RNA complex. The model was constructed using the crystal structure of an OC43 N-NTD-AMP complex as a template.

# Figure S1

HCoV-OC43 NVVPHYSHFSGITQFQKGKEFEFVEGQGVPITAPGVPATEAKGYMYRHMRR  
HCoV-HKU1 NTIPHYSHFSGITQFQKGRDFKFSGQGVPITAFGVPPSEAKGYMYRHSRR  
MHV SVVPHYSHFSGITQFQKGKEFQFAEGQGVPITANGIPASEQKGYMYRHMRR  
SARS-CoV NTASHFTALQHGK-EELRFPRGQGVPINTNSGPDQIGYRRRATRR  
MERS-CoV NTYSHYTGLQHGK-VPLTFPPGQGVPINANSTPAQNAQGYMRRQDRK  
IBV GSSGNAHFQAIKAKKLNTPPPKFEGSGVPDNENIKPSQHQHGYMRRQAR-  
HCoV-229E YSLYSPLLVDSQ-PMKVIPRNLVPIKDK-NKLIQYMNQKR-  
HCoV-NL63 PSFYMPLLVSSDKAPRYVIPRNLVPIGKGNK-DEQIGYMNQER-

HCoV-OC43 SFKTADGNQRQLLPRHYFYLLGTGPHAKDQYGTIDGVYHVASNQADVNT  
HCoV-HKU1 SFKTADGQKQLLPRHYFYLLGTGPHYANASYGESLEGVHVASNHQADTST  
MHV SFKTPDGQKQLLPRHYFYLLGTGPHAGASYGDSIEGVHVASNQADTNT  
SARS-CoV -VRGGDGKMKELSPRHYFYLLGTGPEASLPYGANKGIVHVAATEGALNTP  
MERS-CoV -INTGNG-IKQLAPRHYFYLLGTGPEAALPFRAYKDGIVHVEDGATDAP  
IBV -FKPGKGGKRPVPAHMYFYLLGTGPAADLNMGDTQDGIHVAARKGADTKS  
HCoV-229E -FRTKKGKRYDLSPKLHFYLLGTGPHKDAKFRERVEGVHVAVDGAKTEP  
HCoV-NL63 -HRMRRGQRVDLPPKVHFYLLGTGPHKDLKFRQRSQGVHVAKEGAKTVN

HCoV-OC43 PADIVDRDPSSDEAIPTRFPPGTVLPQGYIEGS  
HCoV-HKU1 PSDYSSRDPTTQEAIPTRFPPGTILPQGYIEGS  
MHV RSDIYERDPSSHEAIPTRFAPGTVLPQGFYIEGS  
SARS-CoV KDHIQTRNPNNAATVQLPQGTLPKGFYIEGS  
MERS-CoV ST-FGTRNPNNSAIYVQFAPGTLPKGFHIEGT  
IBV RSNQGTDRDPKFDQYPLRFSGG--PDGNFRHDFI  
HCoV-229E TG-YGVRKNSPEIP---H-NQKLPNGVTVYEE  
HCoV-NL63 TS-LGNRKRKQKPLEP---KFSIALPPELSVYEF

Figure S2

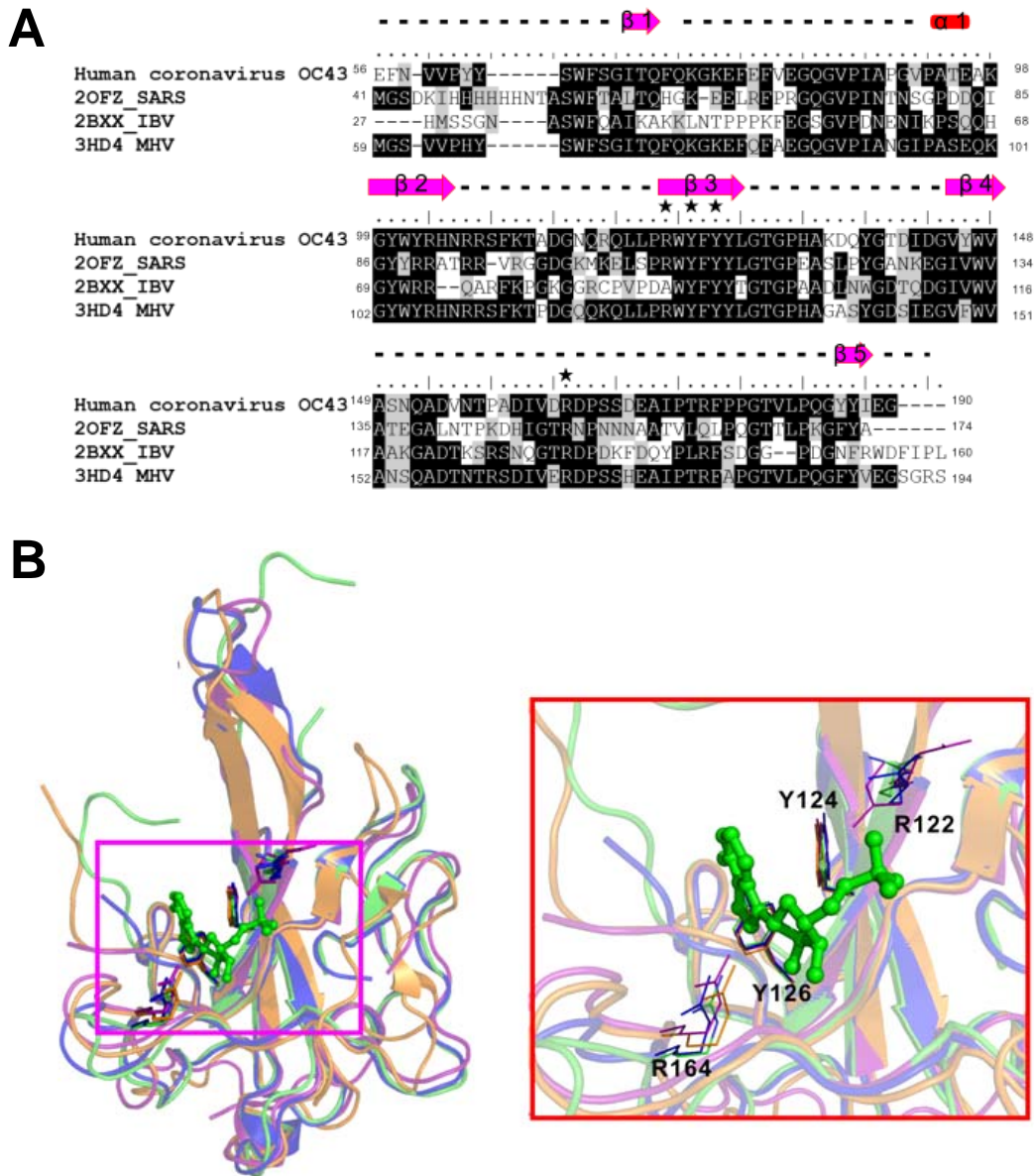
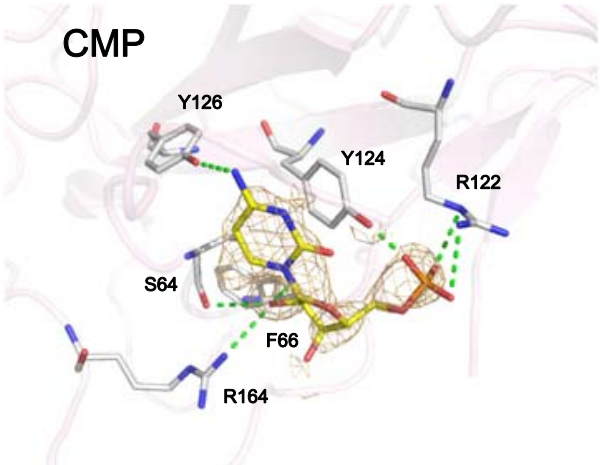
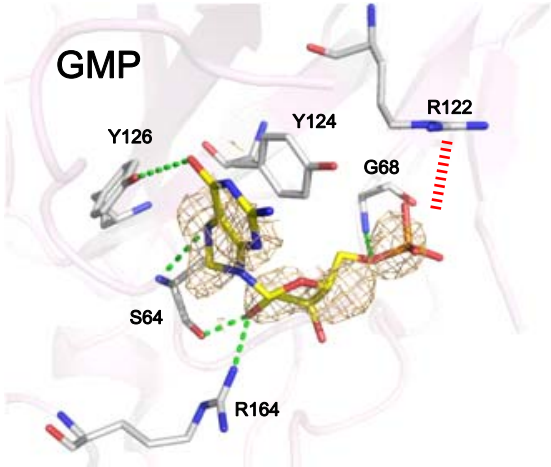


Figure S3

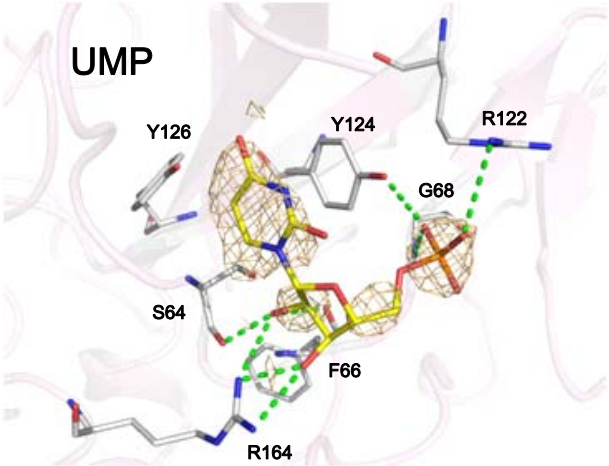
A



B



C



D

	AMP	CMP	GMP	UMP
S64	●		●	●
F66		●		●
G68	●		●	●
R122	●	●	●	●
Y124	●	●	●	●
Y126	●	●	●	
R164	●	●	●	●

Figure S4

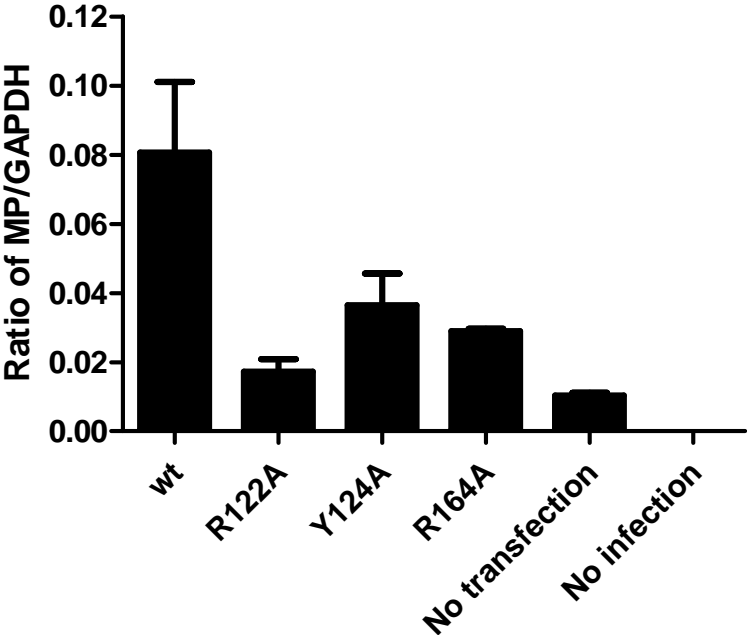


Figure S5

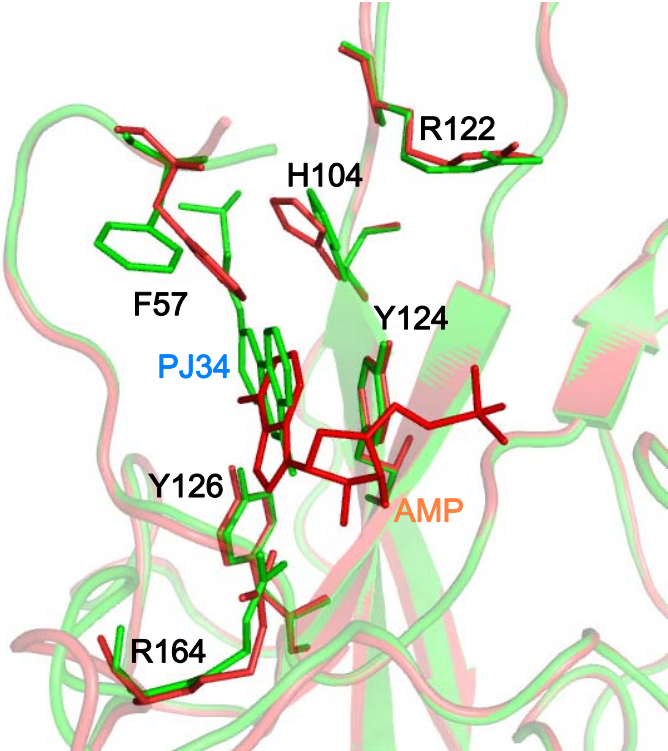




Figure S6

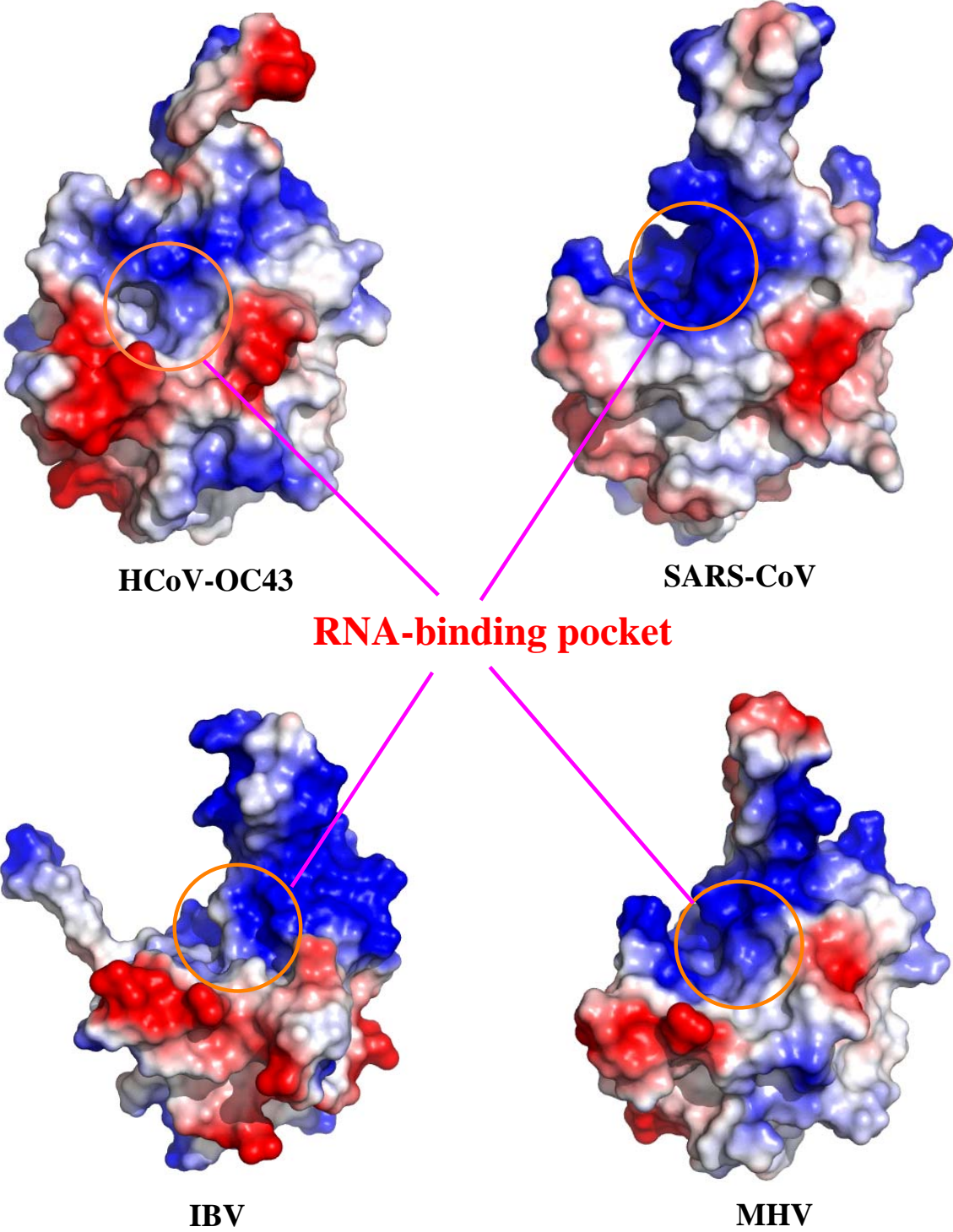
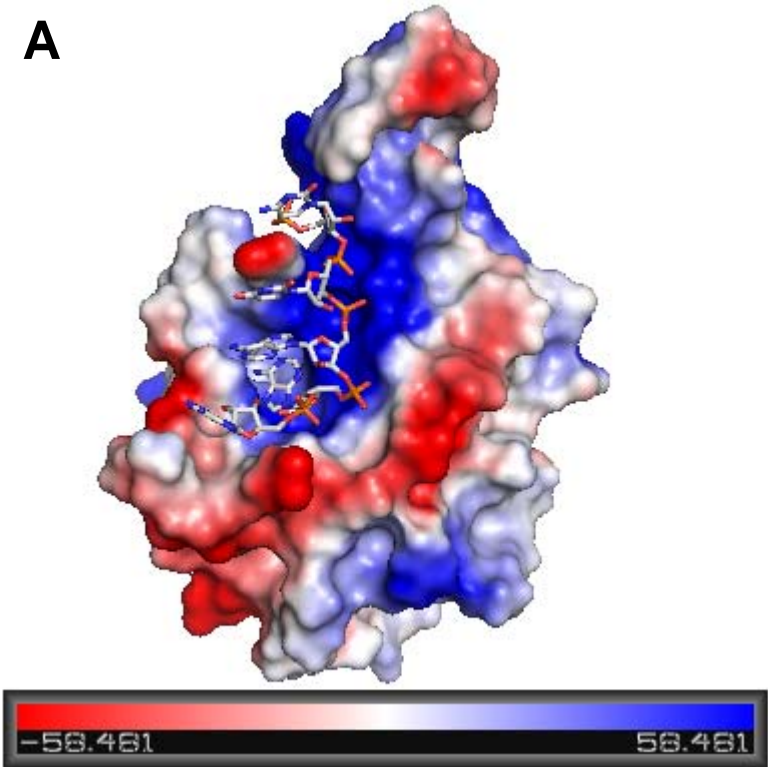
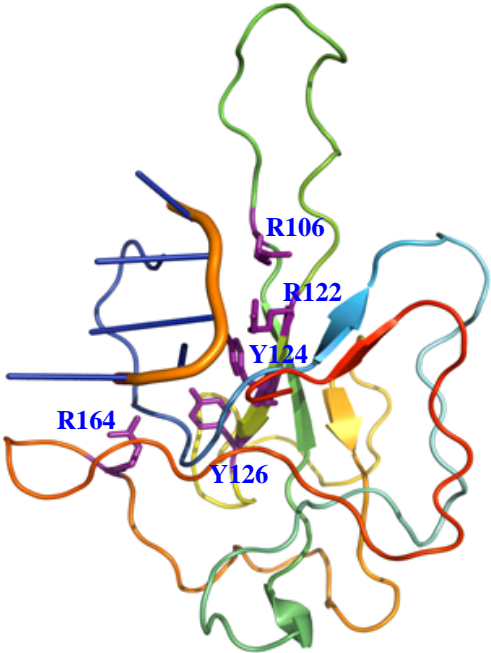


Figure S7

A



B

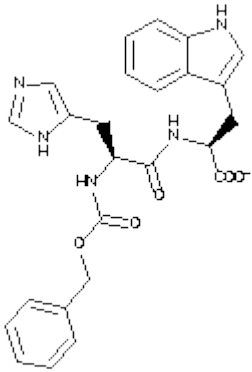
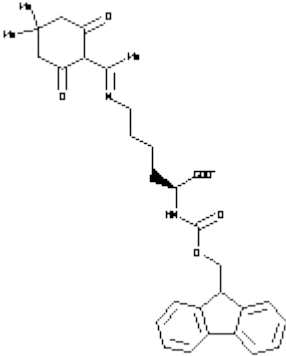
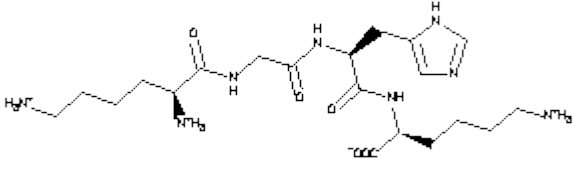
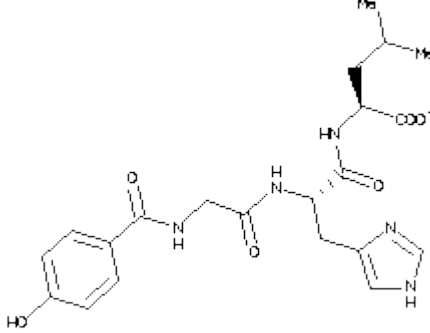


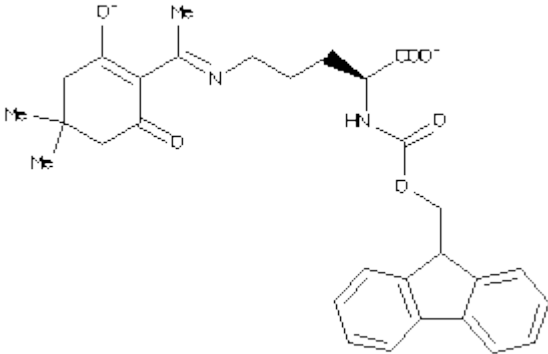
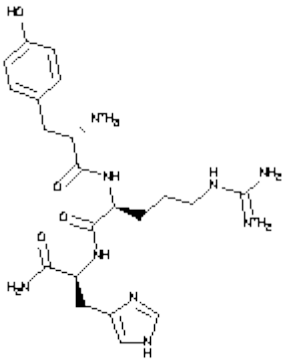
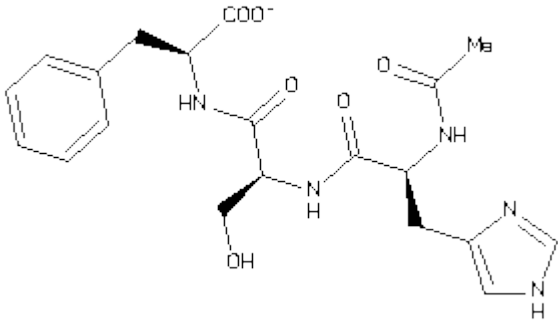
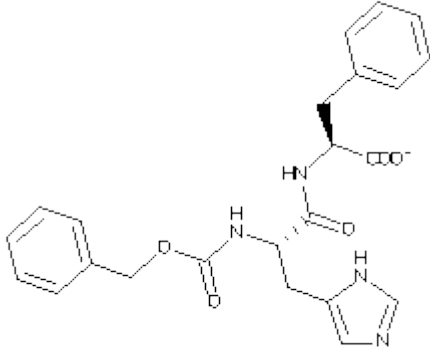
**Table S1.** Data collection and refinement statistics for HCoV-OC43 N-NTD-CMP, HCoV-OC43 N-NTD-GMP and HCoV-OC43 N-NTD-UMP crystals

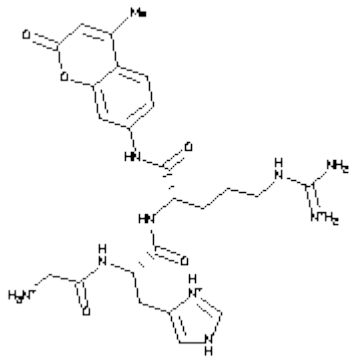
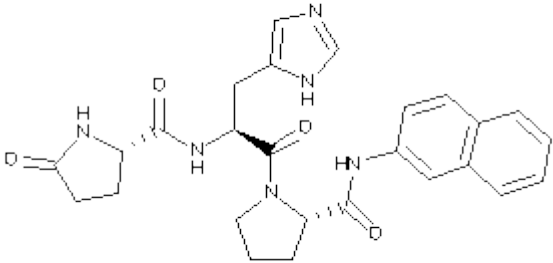
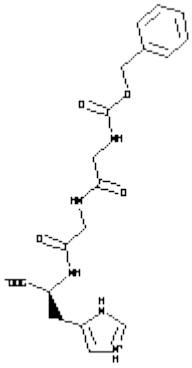
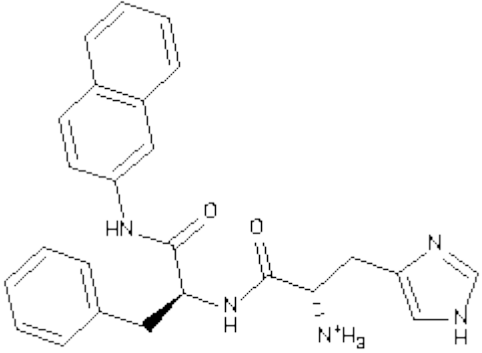
Names	HCoV-OC43 N-NTD-CMP	HCoV-OC43 N-NTD-GMP	HCoV-OC43 N-NTD-UMP
PDB number	4LMC	4LM9	4LM7
Data Collection	NSRRC BL13B1		
Space group	P6 <sub>5</sub>	P6 <sub>5</sub>	P6 <sub>5</sub>
Resolution (Å) <sup>a</sup>	30-1.74 (1.80-1.74) <sup>a</sup>	30-1.60 (1.63-1.60) <sup>a</sup>	30-1.72 (2.59-1.72) <sup>a</sup>
Wavelength (Å)	1.00000	1.00000	1.00000
Unit Cell Dimensions			
<i>a</i> = <i>b</i> (Å)	81.897	81.971	81.938
<i>c</i> (Å)	42.889	42.732	42.891
No. of reflections			
Observed	82635	144518	122776
Unique	17034	21252	17712
Completeness (%)	94.8(94.8) <sup>a</sup>	97.3(98.9) <sup>a</sup>	98.8(100) <sup>a</sup>
<i>R</i> <sub>merge</sub> (%)	4.0(16.1) <sup>a</sup>	3.1(16.1) <sup>a</sup>	3.9(15.0) <sup>a</sup>
<i>I</i> / $\sigma$ ( <i>I</i> )	34.06(9.59) <sup>a</sup>	51.97 (2.86) <sup>a</sup>	37.4(13.0) <sup>a</sup>
Refinement			
No. of reflections	16138	20167	17394
<i>R</i> <sub>work</sub> (95% data)	0.21	0.22	0.20
<i>R</i> <sub>free</sub> (5% data)	0.25	0.26	0.23
Geometry deviations			
Bond lengths (Å)	0.014	0.013	0.016
Bond angles (°)	1.783	1.565	1.690
No. of all protein atoms			
Mean B-values (Å <sup>2</sup> )	34.19	19.49	29.30
No. of ligand atoms			
Mean B-values (Å <sup>2</sup> )	50.53	33.54	41.94
No. of water molecules			
Mean B-values (Å <sup>2</sup> )	36.81	23.97	44.46
Ramachandran plot (%)			
Most favored	96.0	96.0	95.3
Generously allowed	0.8	1.6	3.1
Others	3.2	2.4	1.6

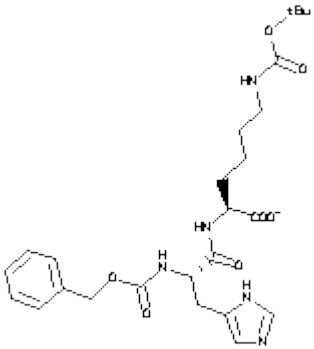
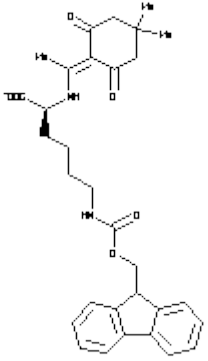
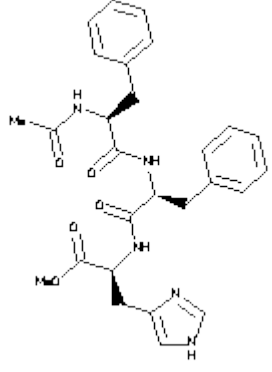
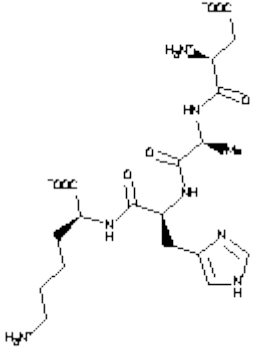
<sup>a</sup> Values in the parenthesis are for the highest resolution

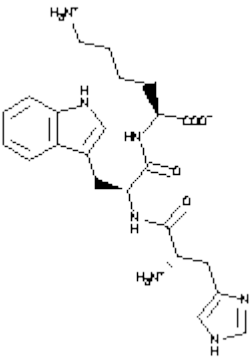
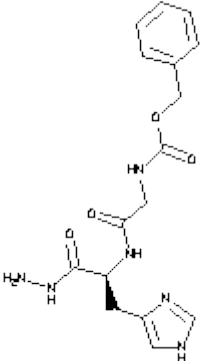
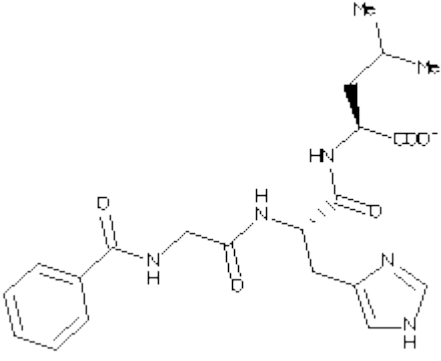
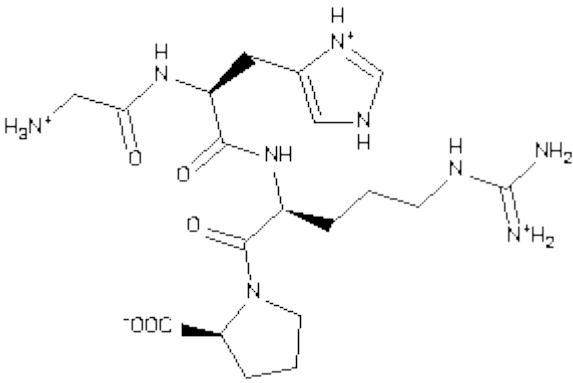
**Table S2.** The chemical structures and docking scores of 87 potential hits.

 <p>The structure shows a central amide backbone. One end is linked to a benzimidazole ring system. The other end is linked to a benzimidazole ring system via a methylene bridge. A phenyl group is attached to the amide nitrogen.</p>	<p><b>ZINC ID :</b> ZINC15721543 <b>Score :</b> 135.925</p>
 <p>The structure features a pyridinone ring system with two methyl groups. It is connected via a propyl chain to a chiral center with a methyl group and a carboxylate group. This is further linked to an amide group connected to a benzimidazole ring system.</p>	<p><b>ZINC ID :</b> ZINC08781153 <b>Score :</b> 129.222</p>
 <p>The structure is a complex peptide-like molecule with multiple amide bonds. It includes a benzimidazole ring system and several long alkyl chains, some of which are terminated with primary amine groups.</p>	<p><b>ZINC ID :</b> ZINC15722196 <b>Score :</b> 127.518</p>
 <p>The structure consists of a central amide backbone. One end is linked to a 4-hydroxyphenyl group. The other end is linked to a benzimidazole ring system. A chiral center with a methyl group and a carboxylate group is also present.</p>	<p><b>ZINC ID :</b> ZINC15721227 <b>Score :</b> 123.882</p>

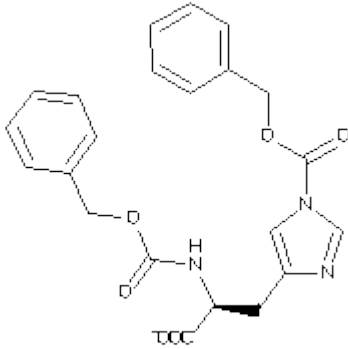
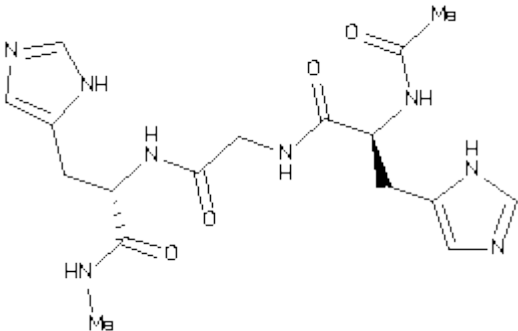
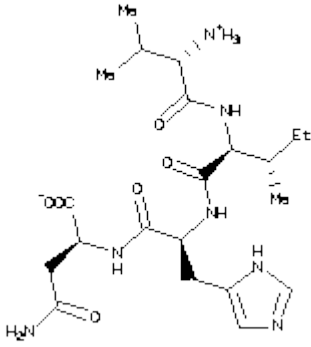
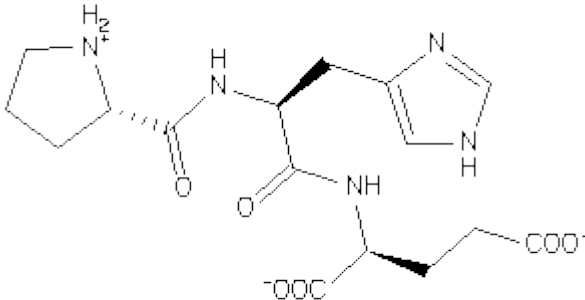
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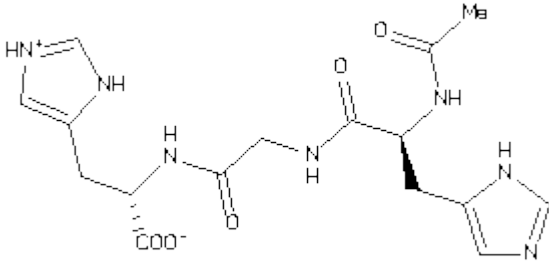
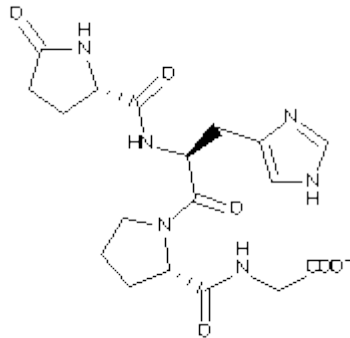
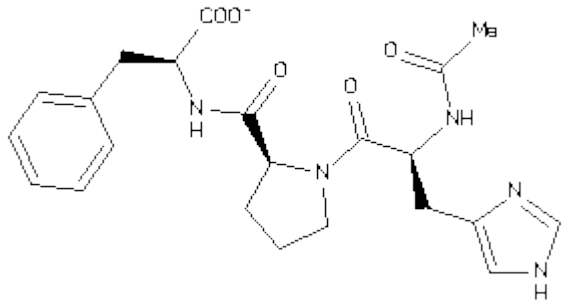
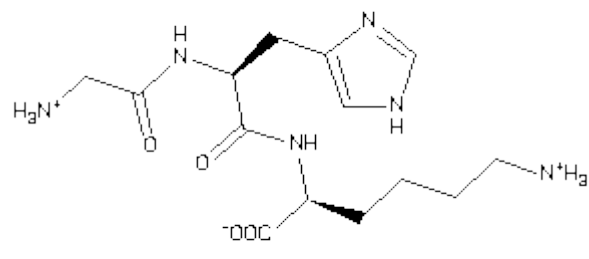
 <p>The structure shows a central chain of amide bonds. On the left, there is a 6-methyl-2,3-dihydroquinolin-4(1H)-one ring system. The chain continues through a secondary amide, a primary amide, and another secondary amide. On the right, it ends with a primary amide group.</p>	<p><b>ZINC ID :</b> ZINC15722313  <b>Score :</b> 118.923</p>
 <p>The structure features a central piperidine ring. It is substituted with a 2,3-dihydro-1H-imidazole-4-carboxamide group, a 2,3-dihydro-1H-imidazole-4-carboxamide group, and a 2,3-dihydro-1H-imidazole-4-carboxamide group. Additionally, there is a 2,3-dihydro-1H-imidazole-4-carboxamide group and a 2,3-dihydro-1H-imidazole-4-carboxamide group.</p>	<p><b>ZINC ID :</b> ZINC15721220  <b>Score :</b> 117.611</p>
 <p>The structure shows a central chain of amide bonds. It starts with a 2,3-dihydro-1H-imidazole-4-carboxamide group, followed by a secondary amide, a primary amide, and another secondary amide. The chain ends with a primary amide group.</p>	<p><b>ZINC ID :</b> ZINC15722043  <b>Score :</b> 117.377</p>
 <p>The structure features a central chain of amide bonds. It starts with a 2,3-dihydro-1H-imidazole-4-carboxamide group, followed by a secondary amide, a primary amide, and another secondary amide. The chain ends with a primary amide group.</p>	<p><b>ZINC ID :</b> ZINC15721913  <b>Score :</b> 117.319</p>

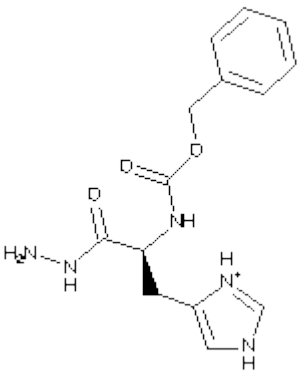
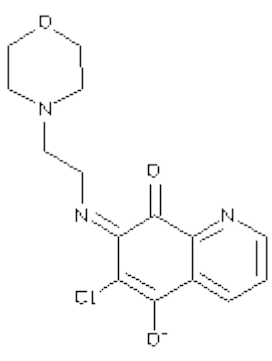
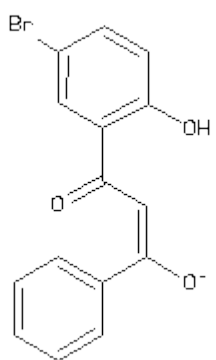
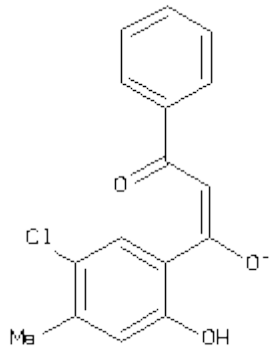
 <p>Chemical structure of a peptide derivative. It features a benzyl group attached to a carbonyl group, which is linked to a peptide backbone. The backbone includes a proline ring and a terminal tert-butyl ester group.</p>	<p><b>ZINC ID :</b> ZINC15722049  <b>Score :</b> 115.862</p>
 <p>Chemical structure of a peptide derivative. It features a fluorenyl group attached to a carbonyl group, which is linked to a peptide backbone. The backbone includes a proline ring and a terminal tert-butyl ester group.</p>	<p><b>ZINC ID :</b> ZINC15722084  <b>Score :</b> 115.498</p>
 <p>Chemical structure of a peptide derivative. It features multiple phenyl groups attached to the peptide backbone, which includes a proline ring and a terminal tert-butyl ester group.</p>	<p><b>ZINC ID :</b> ZINC15721523  <b>Score :</b> 115.449</p>
 <p>Chemical structure of a peptide derivative. It features a proline ring and a terminal primary amine group attached to the peptide backbone. The backbone also includes a tert-butyl ester group.</p>	<p><b>ZINC ID :</b> ZINC15721853  <b>Score :</b> 113.235</p>

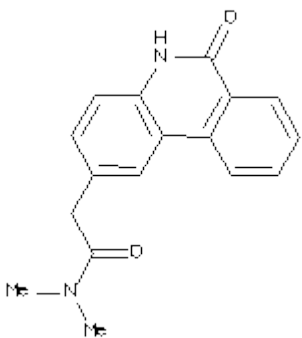
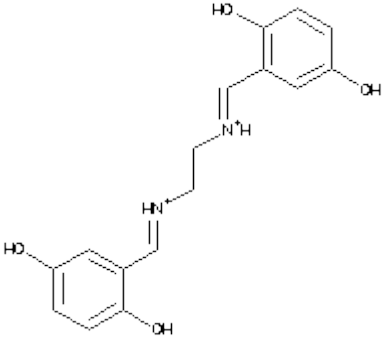
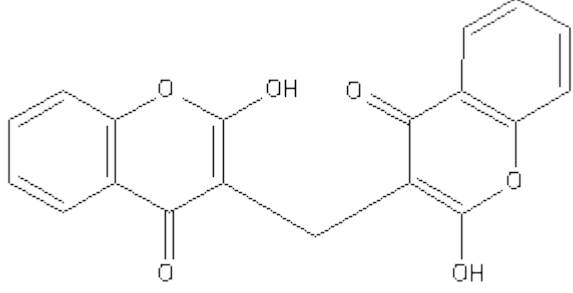
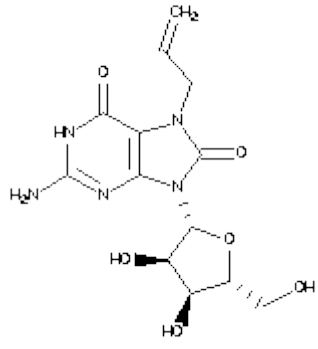
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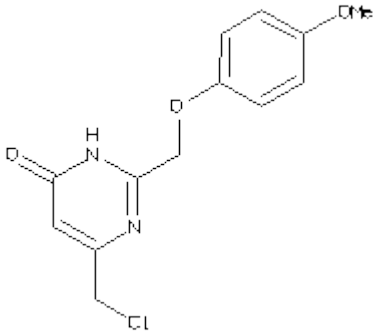
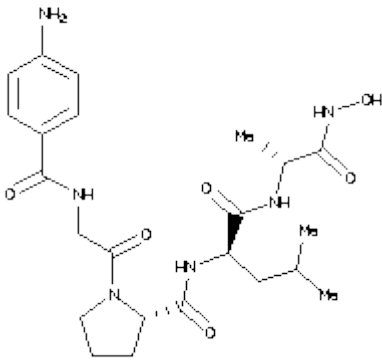
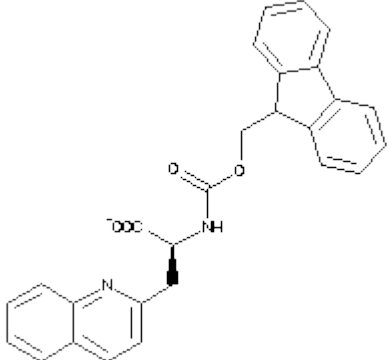
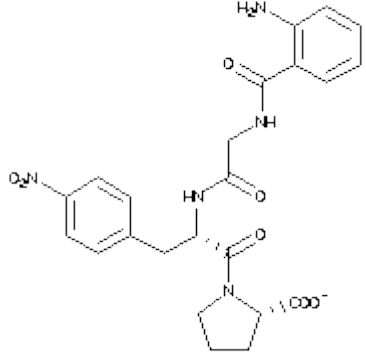


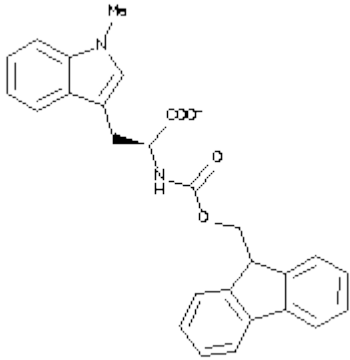
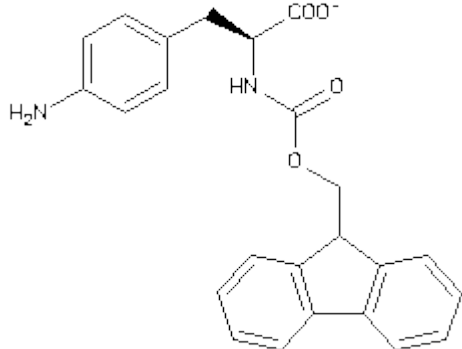
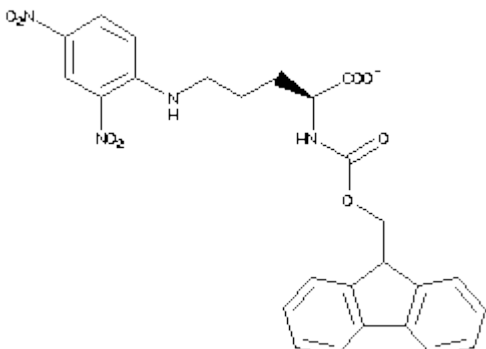
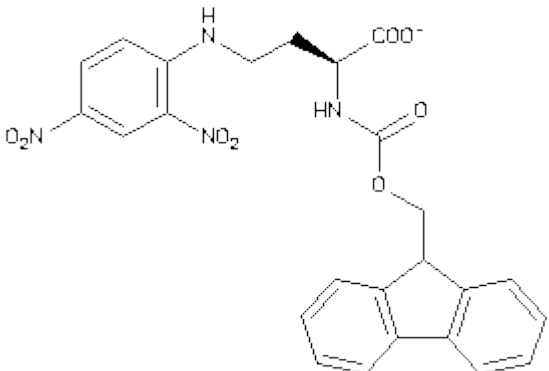
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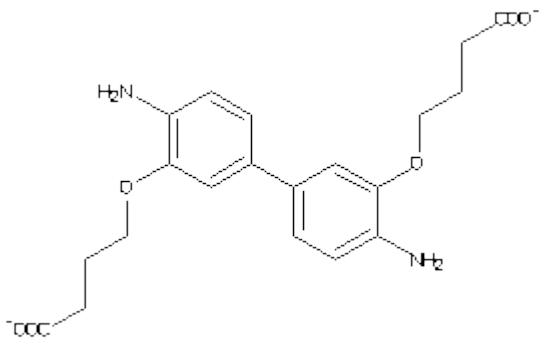
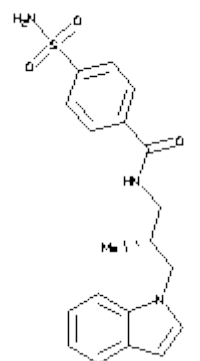
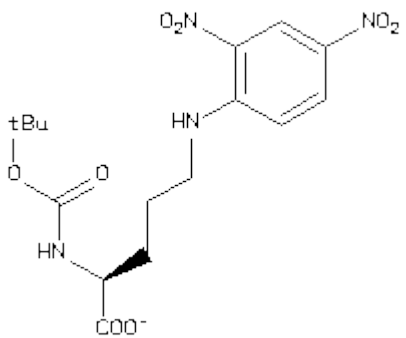
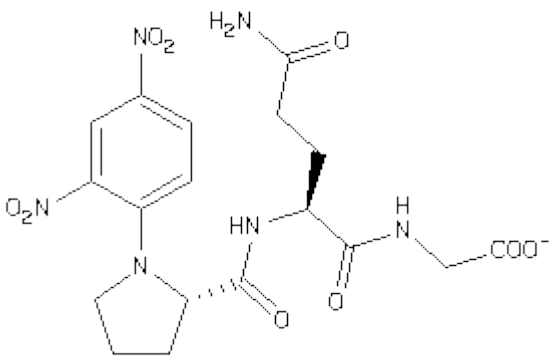
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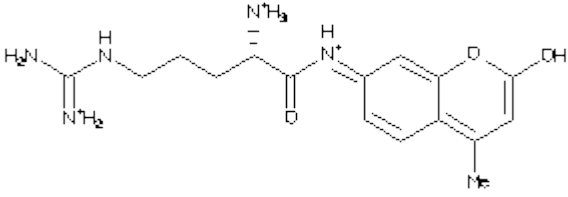
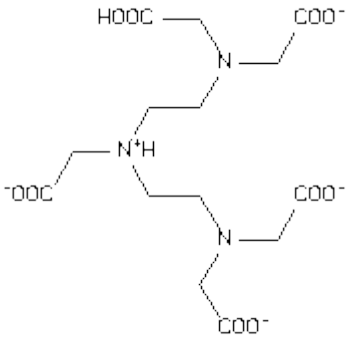
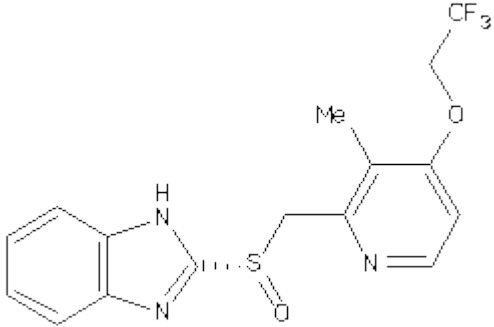
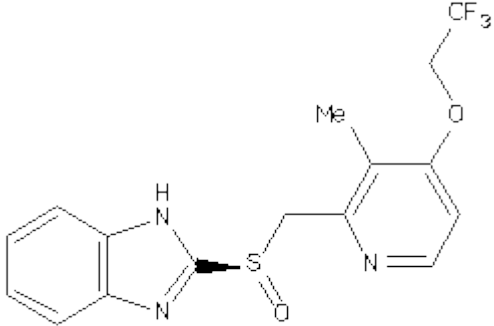
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	<p><b>ZINC ID :</b> ZINC00155947  <b>Score :</b> 82.894</p>
	<p><b>ZINC ID :</b> ZINC00155949  <b>Score :</b> 100.623</p>

 <p>Chemical structure of a benzimidazole derivative. It features a benzimidazole core with a dimethylaminoethyl group (-CH<sub>2</sub>-CH<sub>2</sub>-N(CH<sub>3</sub>)<sub>2</sub>) attached to the 2-position of the benzimidazole ring.</p>	<p><b>ZINC ID :</b> ZINC02507482  <b>Score :</b> 102.854</p>
 <p>Chemical structure of a bis-phenolamine derivative. It consists of two 3,4-dihydroxyphenyl groups connected via a bis-phenolamine bridge (-NH-CH<sub>2</sub>-CH<sub>2</sub>-NH-).</p>	<p><b>ZINC ID :</b> ZINC02526442  <b>Score :</b> 128.009</p>
 <p>Chemical structure of a bis-phenol ether derivative. It features two 2,6-dihydroxyphenyl groups connected via a bis-phenol ether bridge (-O-CH<sub>2</sub>-CH<sub>2</sub>-O-).</p>	<p><b>ZINC ID :</b> ZINC03869855  <b>Score :</b> 109.043</p>
 <p>Chemical structure of a nucleoside derivative. It features a purine base (adenine) attached to a ribose sugar moiety via a glycosidic bond.</p>	<p><b>ZINC ID :</b> ZINC04674533  <b>Score :</b> 113.00</p>

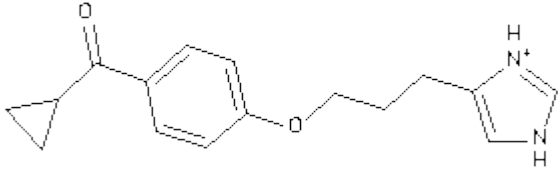
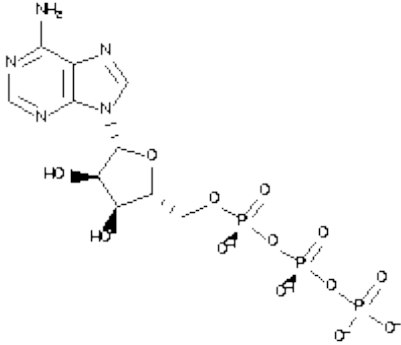
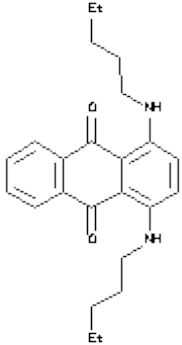
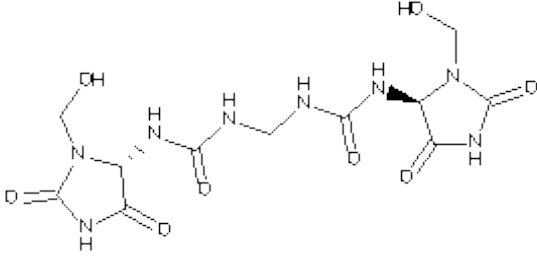
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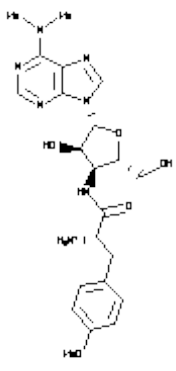
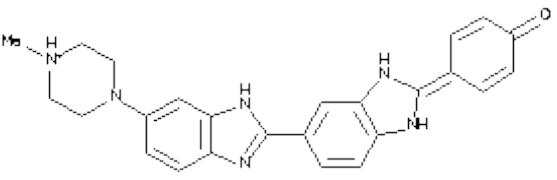
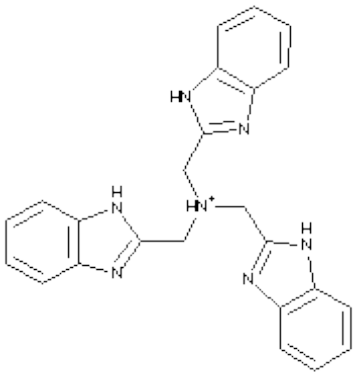
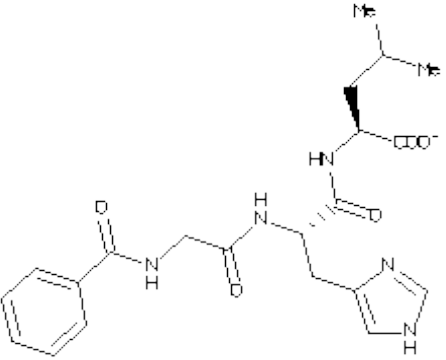
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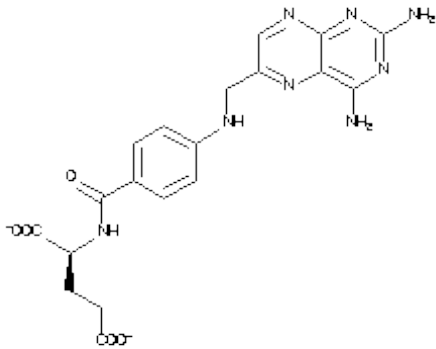
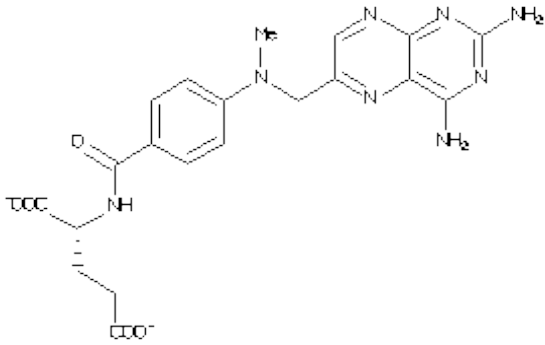
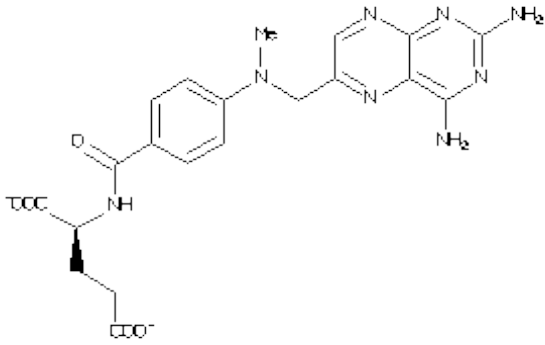
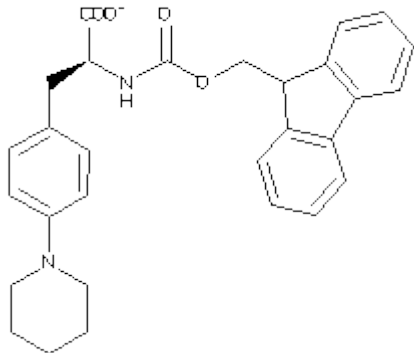
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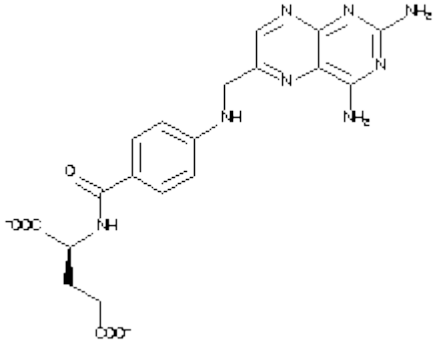
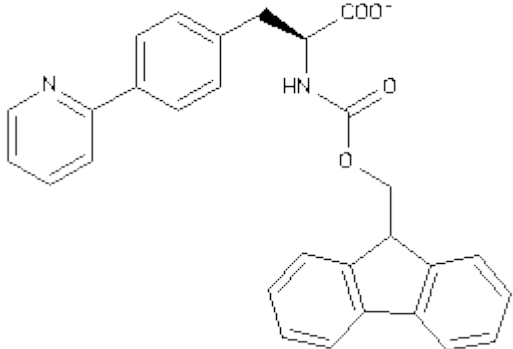
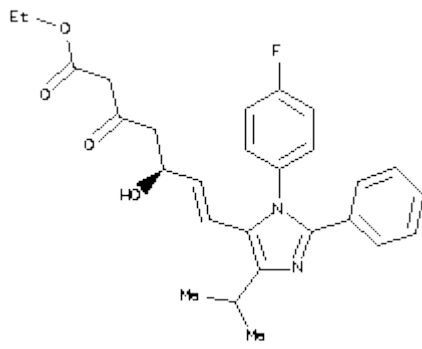
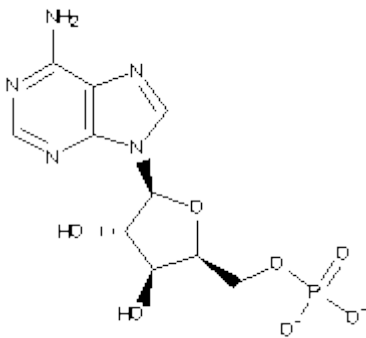
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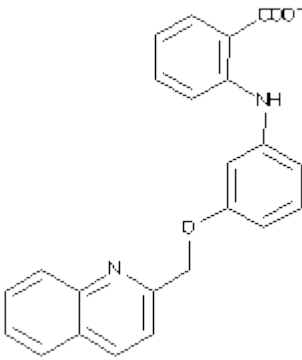
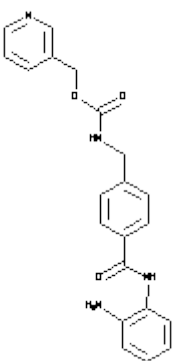
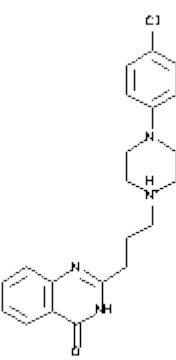
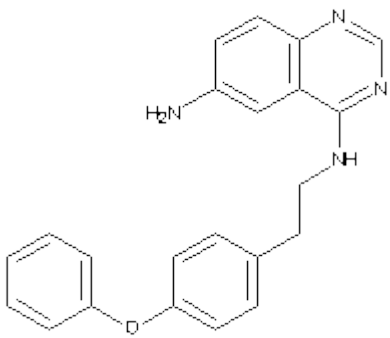


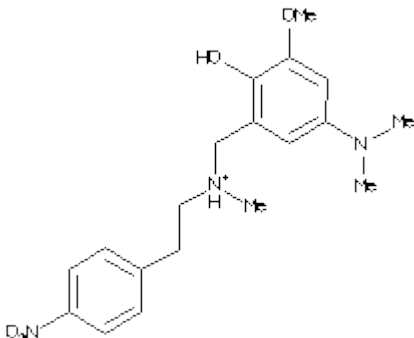
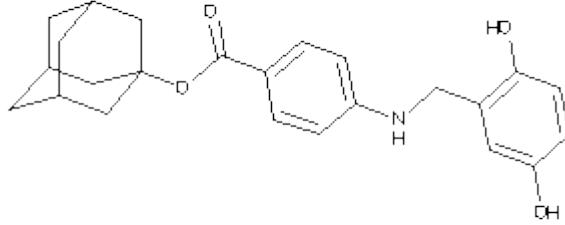
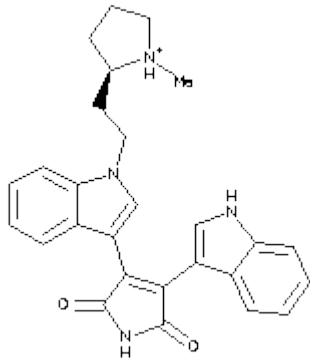
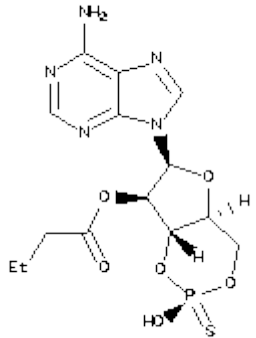
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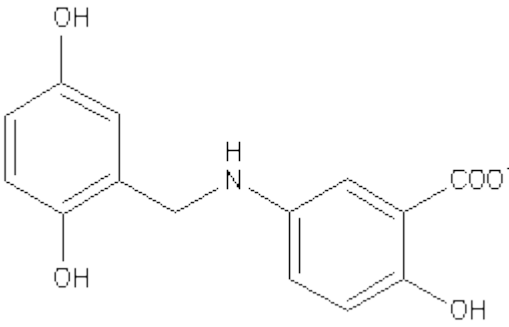
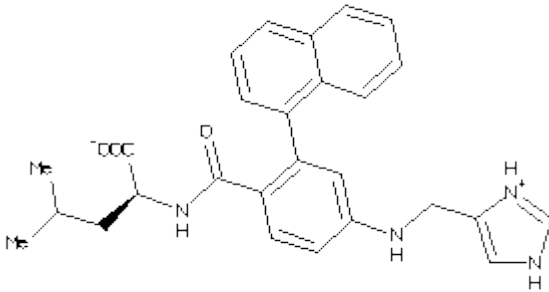
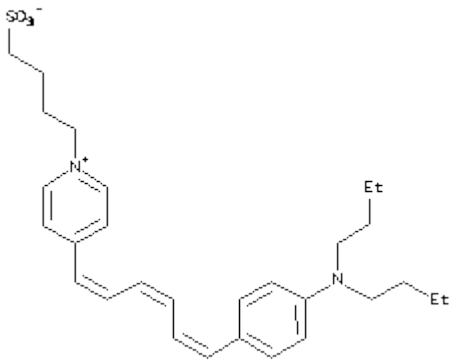
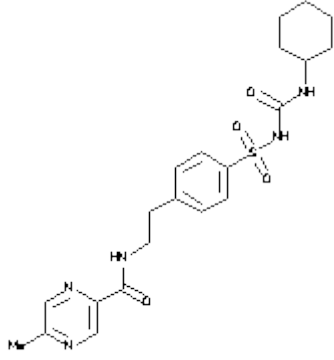
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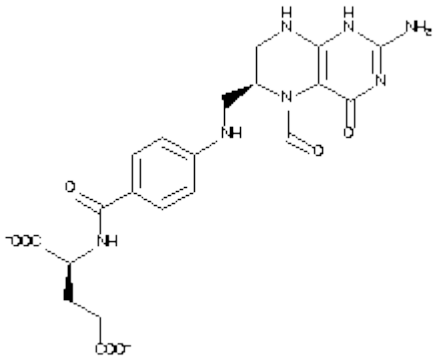
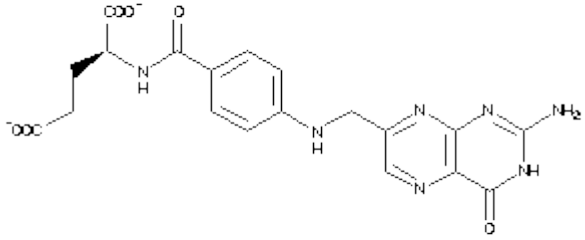
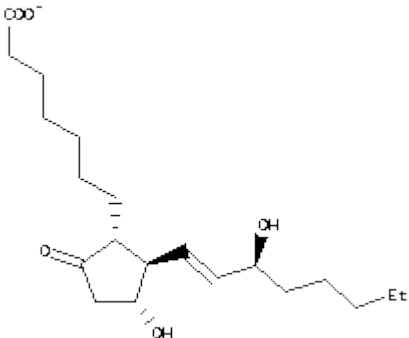
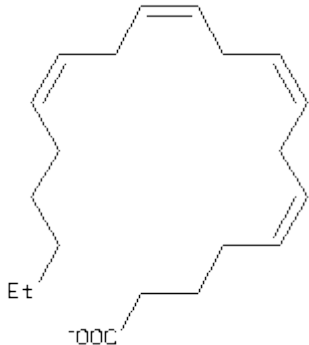
 <p>Chemical structure showing a piperidine ring connected to a p-aminophenyl group, which is further connected to a 2,4,6-triaminopyrimidin-5-ylmethyl group.</p>	<p><b>ZINC ID:</b> ZINC02036915  <b>Score:</b> 114.859</p>
 <p>Chemical structure showing a piperidine ring connected to a p-aminophenyl group, which is further connected to a 2,4,6-triaminopyrimidin-5-ylmethyl group. A methyl group is attached to the nitrogen atom of the piperidine ring.</p>	<p><b>ZINC ID:</b> ZINC06920406  <b>Score:</b> 120.634</p>
 <p>Chemical structure showing a piperidine ring connected to a p-aminophenyl group, which is further connected to a 2,4,6-triaminopyrimidin-5-ylmethyl group. A methyl group is attached to the nitrogen atom of the piperidine ring.</p>	<p><b>ZINC ID:</b> ZINC01529323  <b>Score:</b> 122.052</p>
 <p>Chemical structure showing a piperidine ring connected to a p-aminophenyl group, which is further connected to a 2,4,6-triaminopyrimidin-5-ylmethyl group. A methyl group is attached to the nitrogen atom of the piperidine ring.</p>	<p><b>ZINC ID:</b> ZINC12958249  <b>Score:</b> 119.59</p>

	<p><b>ZINC ID:</b> ZINC02036915  <b>Score:</b> 114.859</p>
	<p><b>ZINC ID:</b> ZINC12958628  <b>Score:</b> 113.199</p>
	<p><b>ZINC ID:</b> ZINC12957897  <b>Score:</b> 100.462</p>
	<p><b>ZINC ID:</b> ZINC03201893  <b>Score:</b> 99.1148</p>

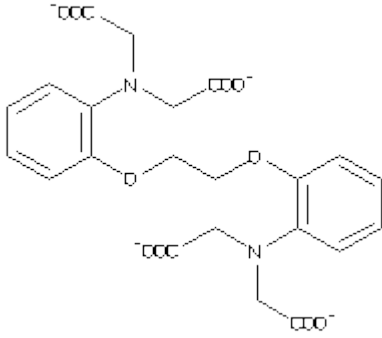
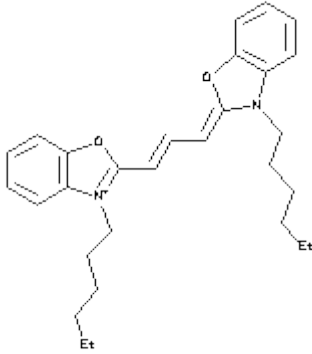
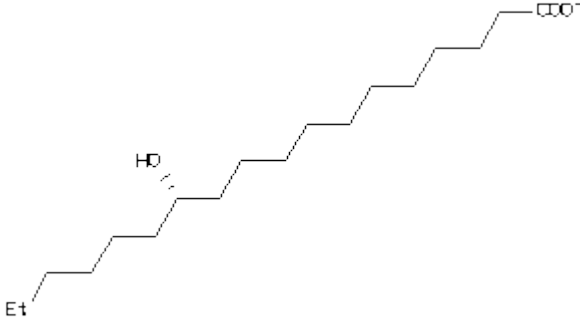
 <p>Chemical structure of a quinoline derivative. It features a quinoline ring system connected via a methylene group to a benzamide group. The benzamide group has a carboxylate group (-COO<sup>-</sup>) at the ortho position relative to the amide nitrogen.</p>	<p><b>ZINC ID:</b> ZINC00596951 <b>Score:</b> 98.7548</p>
 <p>Chemical structure of a pyridine derivative. It features a pyridine ring connected via a methylene group to a benzamide group. The benzamide group is further connected to another benzamide group, which is in turn connected to a benzimidazole ring system.</p>	<p><b>ZINC ID:</b> ZINC01488870 <b>Score:</b> 105.73</p>
 <p>Chemical structure of a quinoline derivative. It features a quinoline ring system connected via a propyl chain to a piperazine ring. The piperazine ring is further connected to a chlorophenyl group (a benzene ring with a chlorine atom at the para position).</p>	<p><b>ZINC ID:</b> ZINC03820039 <b>Score:</b> 98.2651</p>
 <p>Chemical structure of a quinoline derivative. It features a quinoline ring system connected via a propyl chain to a benzimidazole ring system. The benzimidazole ring system is further connected to another benzimidazole ring system, which has an amino group (-NH<sub>2</sub>) at the 2-position.</p>	<p><b>ZINC ID:</b> ZINC01489490 <b>Score:</b> 97.5368</p>

	<p><b>ZINC ID:</b> ZINC03938697  <b>Score:</b> 95.7617</p>
	<p><b>ZINC ID:</b> ZINC03941698  <b>Score:</b> 95.3101</p>
	<p><b>ZINC ID:</b> ZINC02560260  <b>Score:</b> 92.8234</p>
	<p><b>ZINC ID:</b> ZINC14806350  <b>Score:</b> 92.7004</p>

	<p><b>ZINC ID:</b> ZINC00388549  <b>Score:</b> 91.4878</p>
	<p><b>ZINC ID:</b> ZINC13796731  <b>Score:</b> 91.0907</p>
	<p><b>ZINC ID:</b> ZINC12405163  <b>Score:</b> 116.753</p>
	<p><b>ZINC ID:</b> ZINC00537795  <b>Score:</b> 114.478</p>

	<p><b>ZINC ID:</b> ZINC18456279  <b>Score:</b> 113.363</p>
	<p><b>ZINC ID:</b> ZINC04261891  <b>Score:</b> 109.313</p>
	<p><b>ZINC ID:</b> ZINC03813088  <b>Score:</b> 105.863</p>
	<p><b>ZINC ID:</b> ZINC04474696  <b>Score:</b> 103.915</p>



 <p>The structure shows two phenol rings connected by a 1,3-bis(2-hydroxyethyl)oxy linker. Each phenol ring is substituted with an N-(2-carboxymethyl)amino group (-NH-CH<sub>2</sub>-COO<sup>-</sup>).</p>	<p><b>ZINC ID:</b> ZINC03860554  <b>Score:</b> 93.4135</p>
 <p>The structure features two indole rings connected by a trans-alkene bridge. Each indole ring has an N-ethyl group (-NH-Et) and a methyl group (-CH<sub>3</sub>) at the 3-position.</p>	<p><b>ZINC ID:</b> ZINC02167083  <b>Score:</b> 92.2656</p>
 <p>The structure shows a long, zigzag hydrocarbon chain. One end is terminated with an ethyl group (-Et) and the other with a carboxylate group (-COO<sup>-</sup>). A hydroxyl group (-OH) is attached to the chain via a dashed bond.</p>	<p><b>ZINC ID:</b> ZINC03861566  <b>Score:</b> 90.5868</p>

**Table S3.** The compounds obtained from virtual screening that decreased the RNA-binding capacity of N protein by more than 10%.

No.	Compound	Inhibition of RNA-binding activity
O1	3,3'-Methylenebis(4-hydroxycoumarin)	- <sup>a</sup>
O2	1-(5-Chloro-2-hydroxy-4-methylphenyl)-3-phenyl-1,3-propanedione	-
O3	6-chloro-7-(2-morpholin-4-yl-ethylamino)quinoline-5,8dione	+
O4	1-(5-Bromo-2-hydroxyphenyl)-3-phenyl-1,3-propanedione	-
O5	N-(6-Oxo-5,6-dihydrophenanthridin-2-yl)-(N,N-dimethylamino)acetamide hydrochloride (PJ34)	+
O6	7-Allyl-7,8-dihydro-8-oxoguanosine	-
O7	N,N-Bis(2,5-dihydroxybenzylidene)ethylenediamine	-
O8	6-(chloromethyl)-2-[(4-methoxyphenoxy)methyl]pyrimidin-4-ol	-

<sup>a</sup>No significant inhibition was observed.