XXIX Symposium on Bioinformatics
and Computer-Aided Drug Discovery

SEARCH FOR INHIBITORS OF SURFACE VIRAL PROTEINS I TYPE BY MOLECULAR MODELLING

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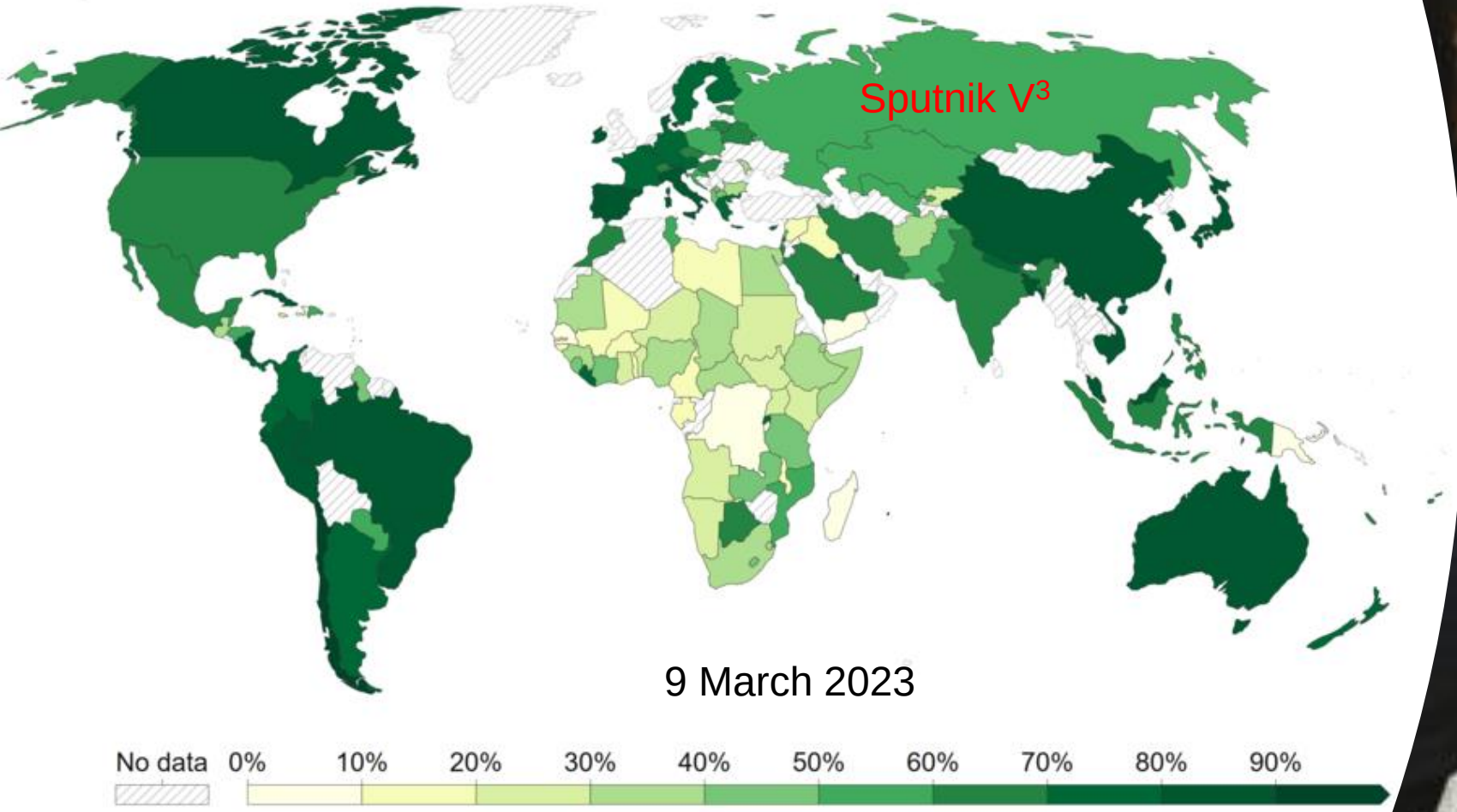
Relevance of research

Virus/Strain	First outbreak	Confirmed cases	Количество смертных случаев
Influenza (A/H1N1)	Spain, May 1918	550 million[1]	17-100 million (0.9-5.3%)
Influenza (A/H3N2)	Hong Kong, 1968	30 million	1-4 million (3-13%)
Influenza (A/H1N1pdm)	North America, 2009	491 382	18 449 (3.7 %)
Coronavirus (SASR-CoV-1)	Guangdong, China, 2003	8096	774 (9.6%)
Coronavirus (MERS-CoV)	Saudi Arabia, 2012	2519	866 (35%)
COVID-19 (SARS-CoV-2)	Wuhan, Hubei, China, 2019	770 436 563[1,2]	6 956 887 (reported) (1.02%)

1. According to WHO
2. Last update **10.03.2023**

Fight against Covid-19

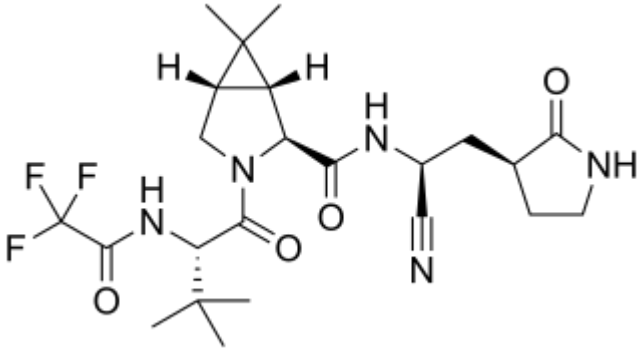
Share of people who completed the initial COVID-19 vaccination protocol[3]



3. Official data collated by Our World in Data
4. Logunov D.Y. at al The Lancet, **2021**, 397 (10275): 671-681.

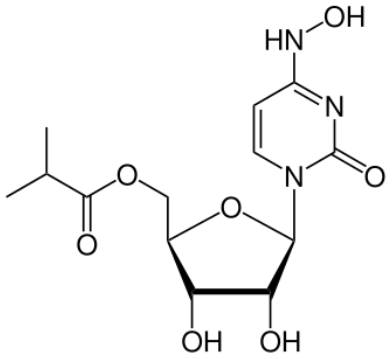
Antiviral drugs

Nirmatrelvir[5]



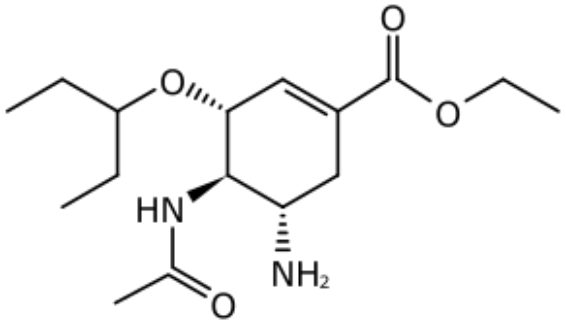
3C-like protease inhibitor (SARS-CoV-2)

Molnupiravir[6]



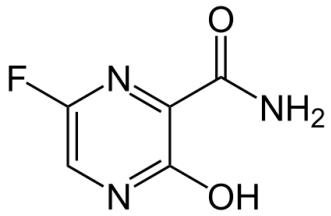
Inhibitor of replication of RNA viruses (SARS-CoV-2)

Oseltamivir[7]



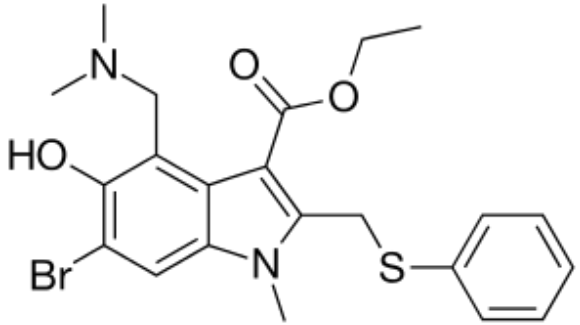
Inhibitor of neuraminidase (Influenza)

Favipiravir[8]



Inhibitor of RNA-polymerase (SARS-CoV-2, Influenza, Ebola, Rabies virus)

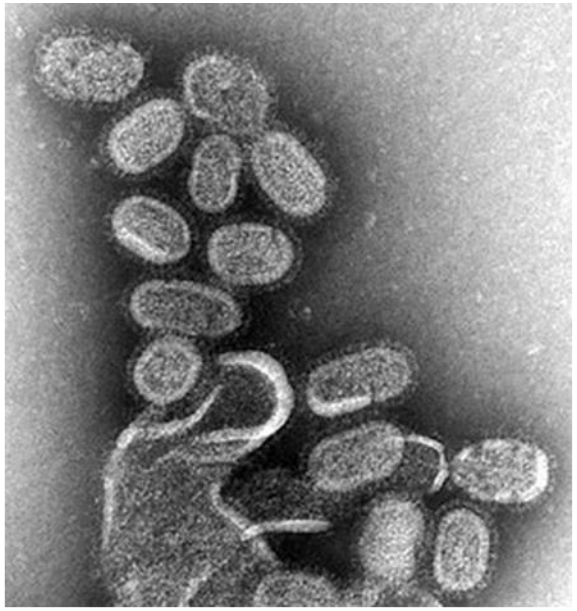
Umifenovirum[9,10,11]



Inhibitor of surface viral proteins (Influenza, SARS-CoV-2, Ebola)

5. Vandyck K., Deval J. Curr. Op. in Virol., **2021**, 49: 36-40.
6. Goldhill D.H. et al. Plos Pathog., **2021**, 17(6): e1008937.
7. Collins P. et al. Nature, **2008**, 453: 1258-1261.
8. Toots M. et al. Translation Research, **2020**, 218: 16-28.
9. Leneva A.I. et al. Antiviral Research, **2009**, 81 (2): 132-440.
10. Hulseberg C.E. et al. J. Virol, **2019**, 93 (8): e02185-18.
11. Amani B. et al. Immun. Inflamm. Dis., **2021**, 9(4): 1197-1208.

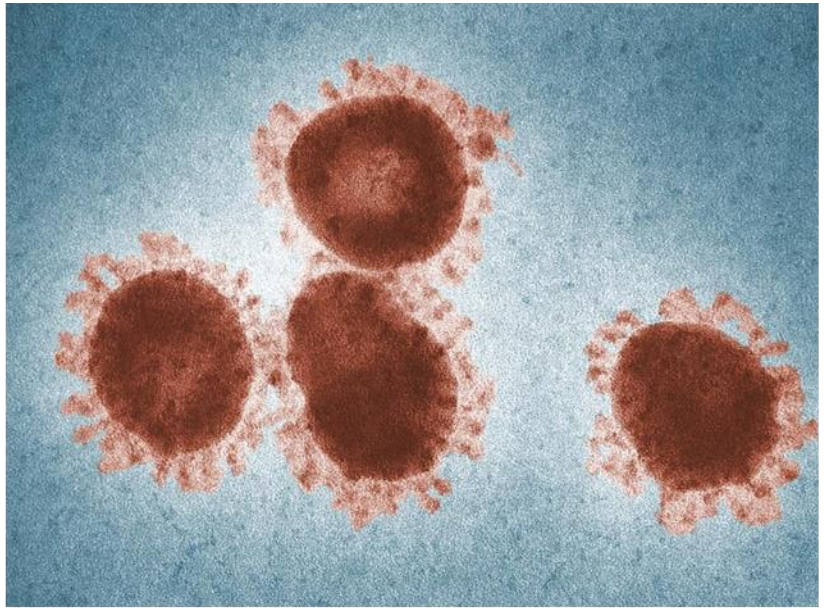
Various viruses



Influenzavirus

Orthomyxoviridae

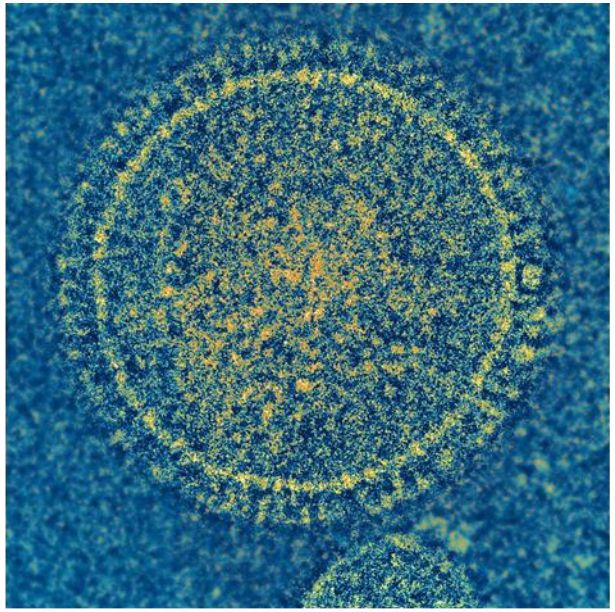
negative-sense RNA viruses



Coronaviruses

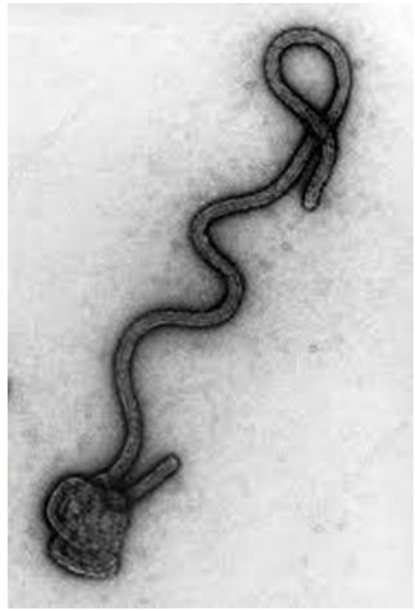
Coronaviridae

positive-sense RNA virus



Human orthopneumovirus

Pneumoviridae



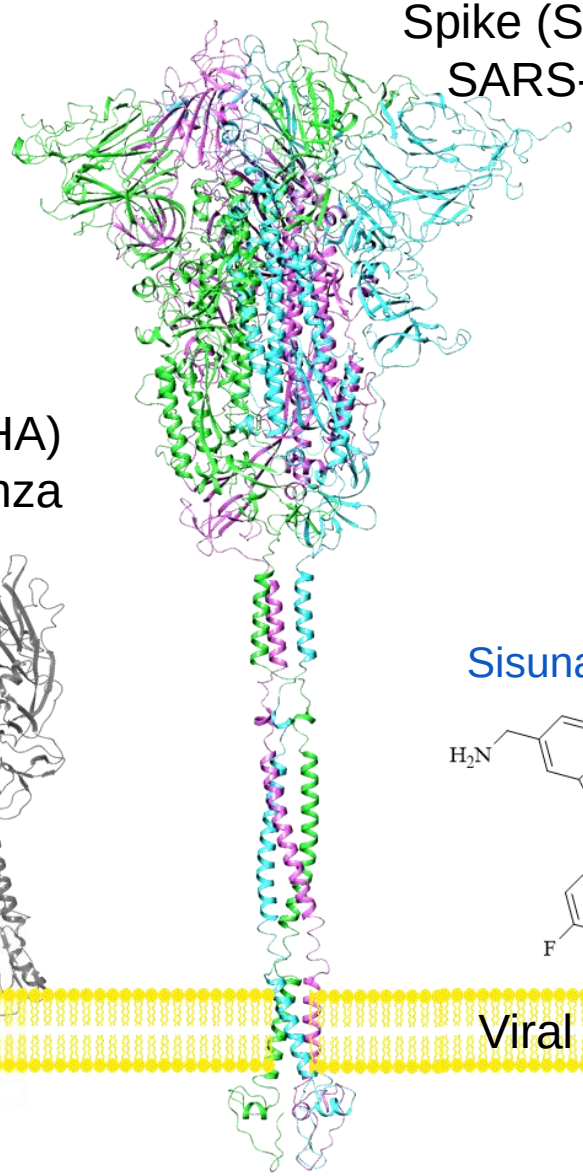
Ebolavirus

Filoviridae

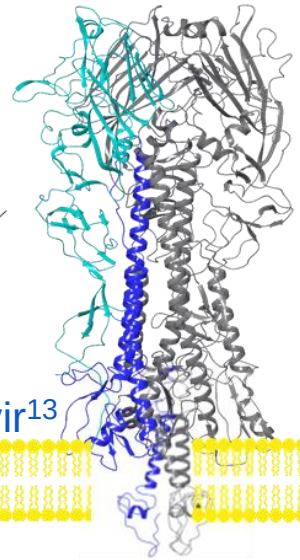
negative-sense, single-stranded RNA virus.

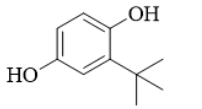
Surface viral proteins

Spike (S)-protein of SARS-CoV-2[14, 15]

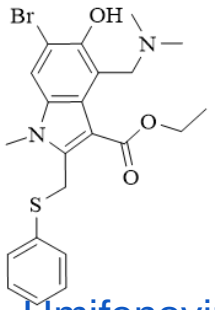


Hemagglutinin (HA) of influenza



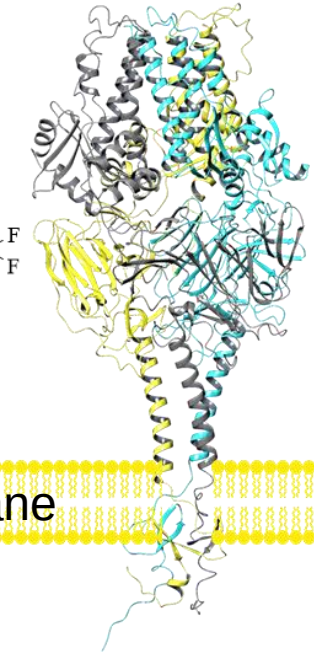


TBHQ[12]

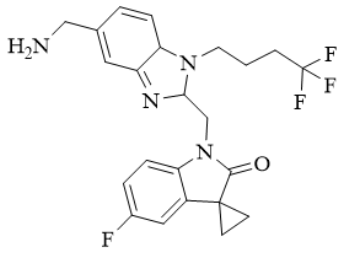


Umifenovir[13]

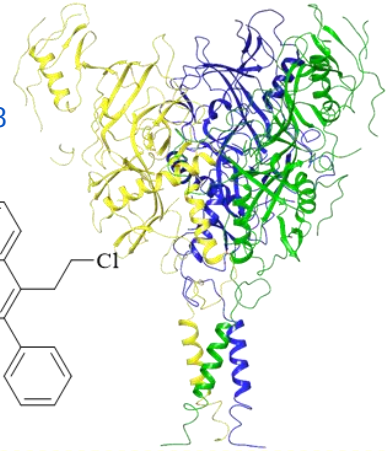
Fusion (F)-protein of RSV



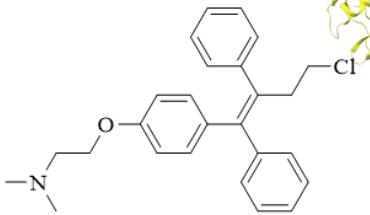
Sisunatovir[16,17]



Glycoprotein (GP) of Ebola virus



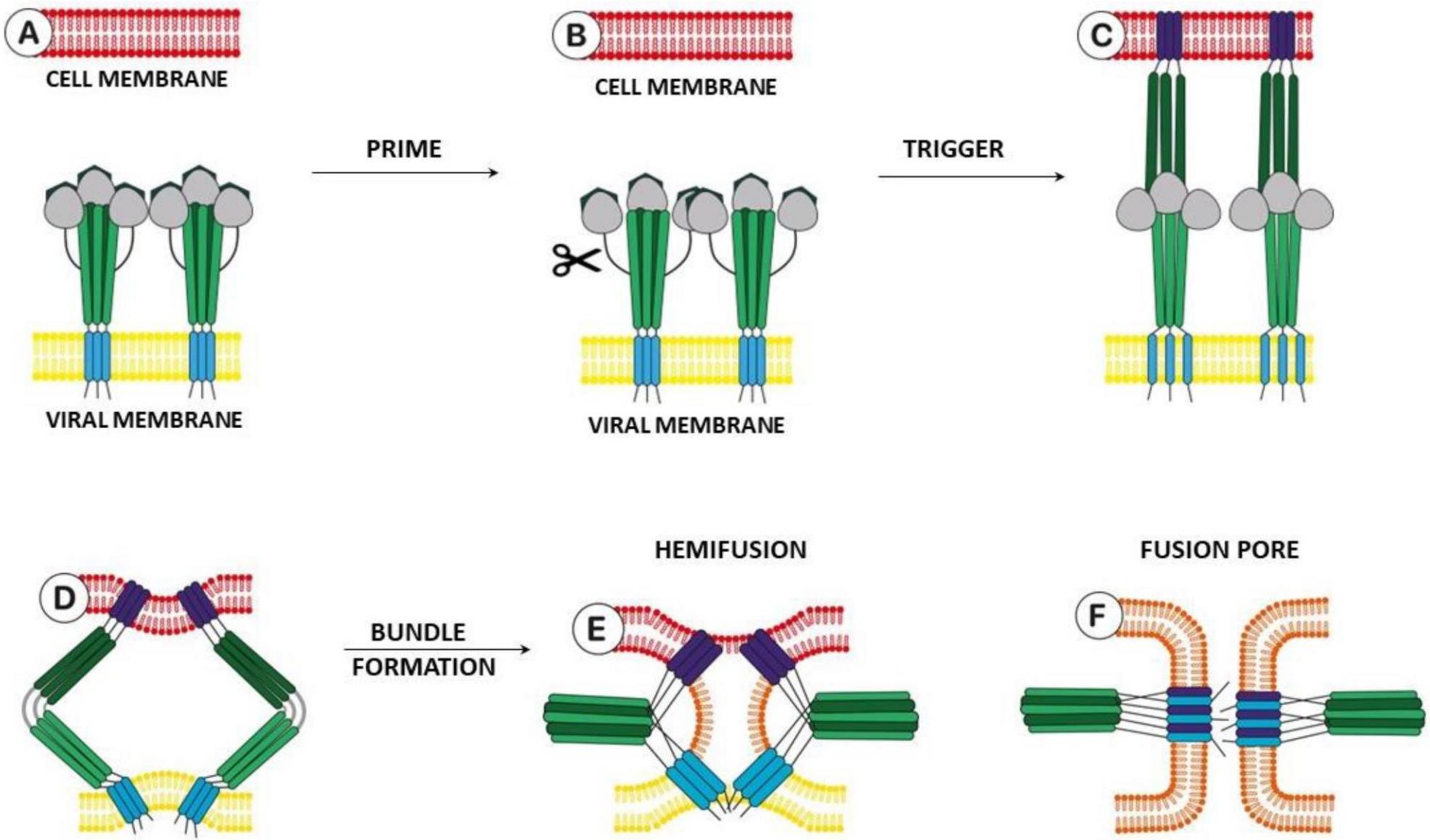
Toremifen[18]



Viral membrane

12. Russell R.J. et al. Proc. Natl. Acad. Sci. **2008**, 105 (46).
13. Kadam R.U., Wilson I.A. Natl. Acad. Sci. **2017**, 114 (2).
14. Huang Y. et al. Acta Pharmacol. Sci. **2020**, 41 (9).
15. Woo H. et al. J. Phys. Chem. **2020**, 20 (2).
16. Rossey I. et al. J. Viro. **2021**, 95 (11).
17. Cockerill G.S. et al. J. Med. Chem. **2021**, 64 (7).
18. Zhao Y. et al. Nature **2016**, 535 (7610).

Model of the fusion process of viral and cellular membranes (adapted from [19])



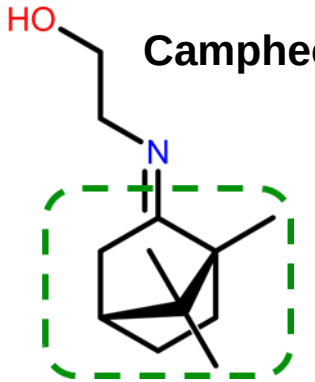
19. Barrett C.T., Dutch R.E. Viruses, **2020**, 12(7): 693.

Thus we will consider

1. Influenza virus hemagglutinin (HA) inhibitors
2. SARS-CoV-2 S-protein inhibitors
3. Respiratory syncytial virus (RSV) F-protein inhibitors
4. Ebola virus glycoprotein (GP) inhibitors
5. Pharmacophore features of type I surface protein inhibitors

1. HA inhibitors: camphecene

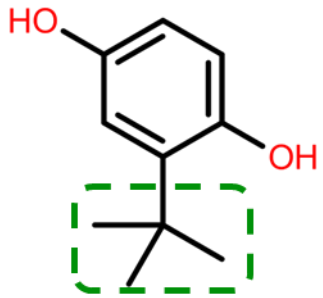
a) Camphecene is active against various strain of influenza[20]
b) Camphecene inhibits the virus in the first hours of its life cycle according to a time-of-addition experiment.
c) Camphecene has hydrophobic group like the TBHQ.
d) Camphecene may bind in the cavity of protein close to fusion peptide, like TBHQ.
e) Energetic parameters of camphecene and TBHQ binding are comparable.



Camphecene (CPH)

Hydrophobic part

TBHQ

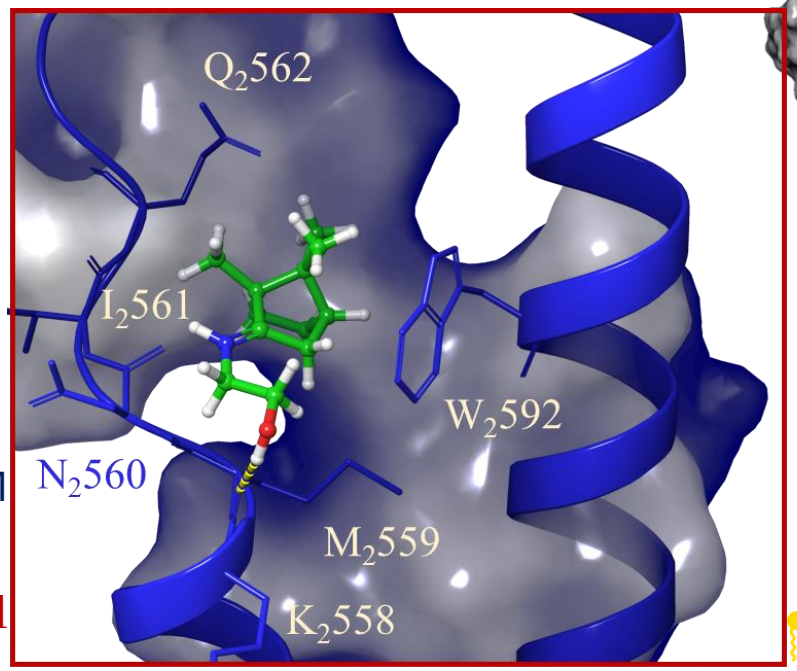


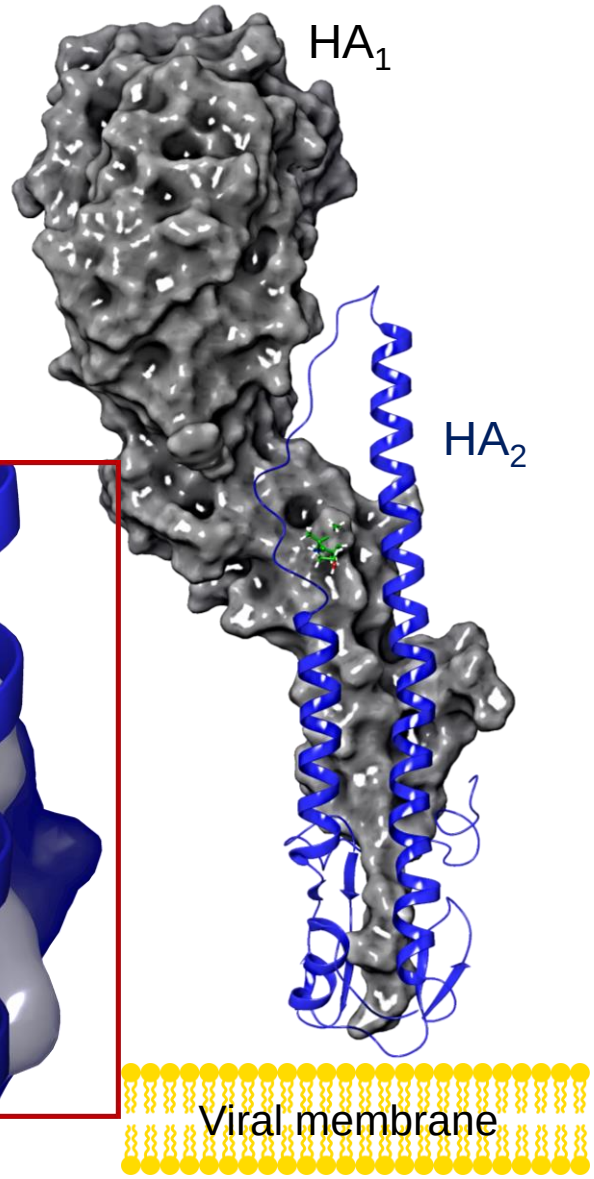
IC_{50}(H1N1, H3N2, H5N1) = 1.2 – 10.3 μM
SI = 75 - 645

E_{bind} : -7.0 ± 0.2 kcal/mol[21]

IC_{50} (H3N2) ~ 6.0μM
SI ~ 8

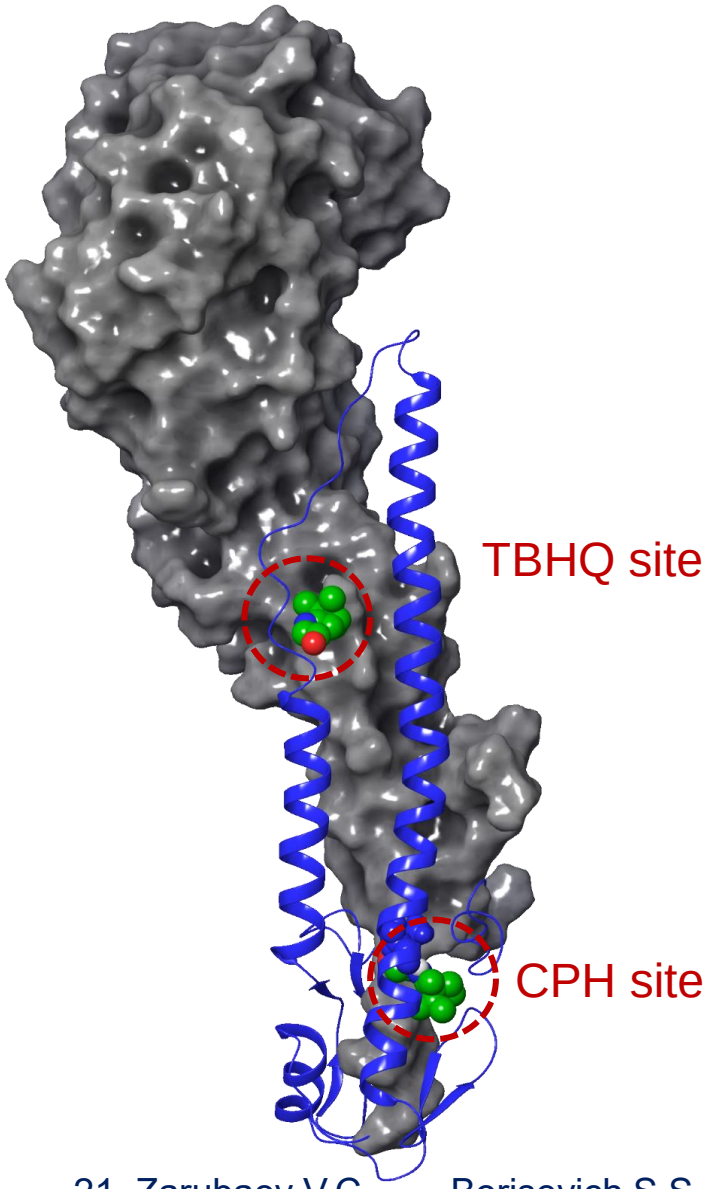
E_{bind} : -7.1 ± 0.3 kcal/mol



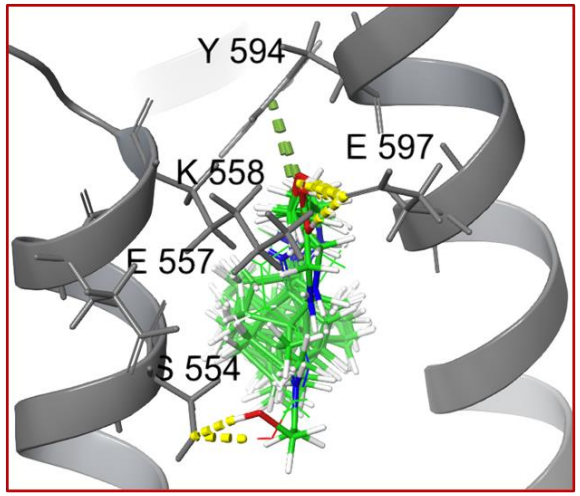


20. Sokolova A.S. et al. Eur. J. Med. Chem. **2015**, 105 (13): 263-273
21. Zarubaev V.C., …, Borisevich S.S. et. Al Virology, **2018**, 524: 69-77

1. HA inhibitors: camphecene[21]

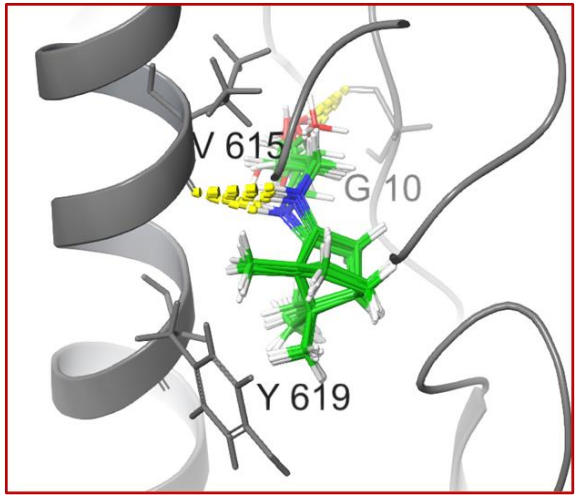


Camphecene in TBHQ site

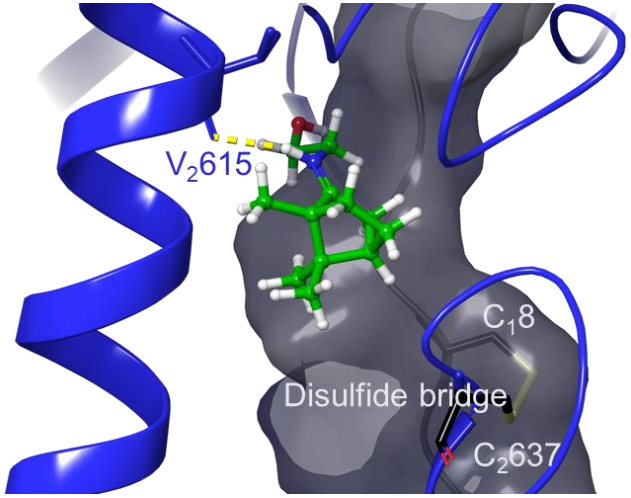


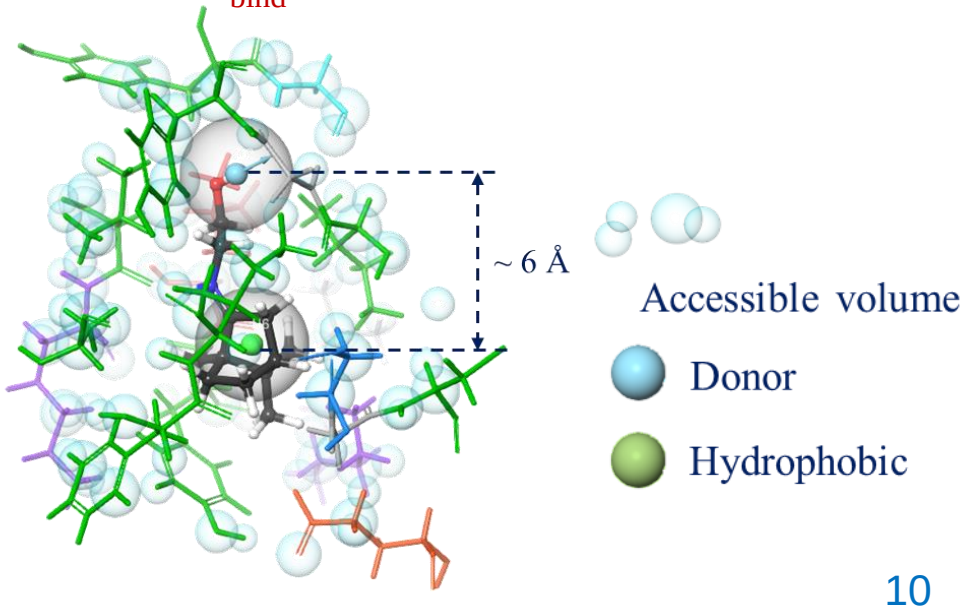
E_{bind} : -7.0 ± 0.2 kcal/mol

Camphecene in CPH site



E_{bind} : -7.1 ± 0.3 kcal/mol





21. Zarubaev V.C., …, Borisevich S.S. et. Al Virology, **2018**, 524: 69-77

1. HA inhibitors: camphecene

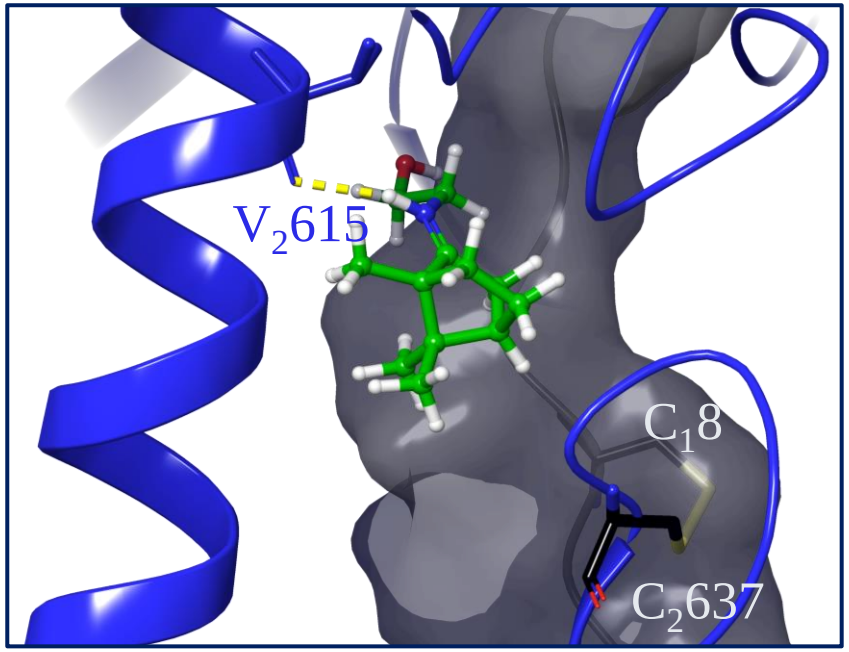
HA_2	517	…	524	…	614	615	616	617	618	619	620	…	622	623	…	632	633	…	635
H1	M	…	Y	…	N	V	K	N	L	Y	E	…	V	K	…	E	I	…	N
H1-pdm	M	…	Y	…	N	V	K	N	L	Y	E	…	V	R	…	E	I	…	N
H3	M	…	F	…	E	M	N	K	L	F	E	…	T	K	…	E	M	…	N
H5	M	…	Y	…	N	V	K	N	L	Y	D	…	V	K	…	E	I	…	N

Штамм	IC_{50}, μM	SI	QM-docking score	QM-Emodel
A/H1N1/PR/8/34	1.2±1.2	645	-7.07±0.30	-71.40±1.70
A/H1N1/Cal/07/09-pdm	3.8±1.1	204	-7.03±0.51	-68.40±1.15
A/H3N2/Aichi/2/68	10.3±1.1	75	No docking results	
A/H5N2/Mallard/	8.0±1.0	97	-7.95±0.27	-52.81±2.38

HA_1	8	9	10	11
H1	C	I	G	Y
H1-pdm	C	I	G	Y
H3	C	L	G	H
H5	C	I	G	Y

1. HA inhibitors: camphecene[21]

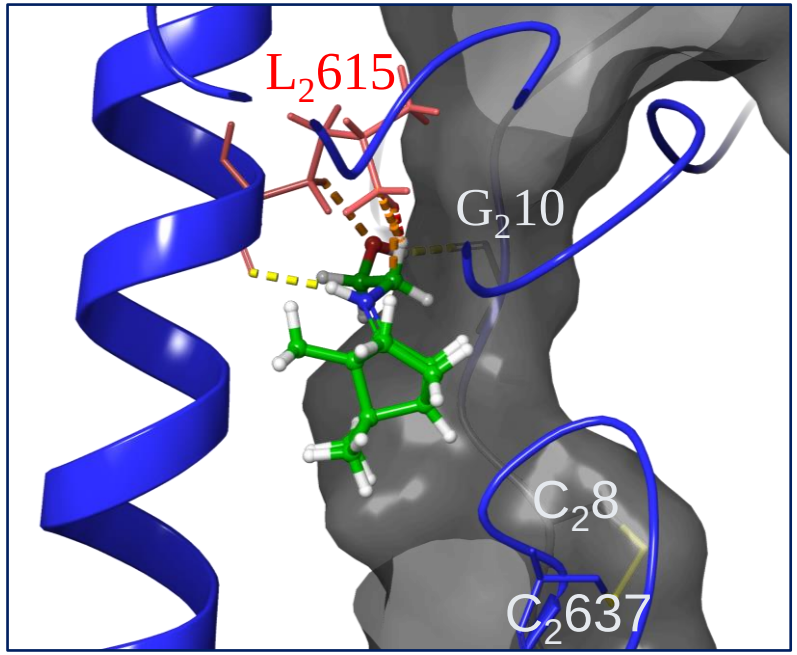
CPH-site V615



E_{bind} : -7.1 ± 0.3 kcal/mol

IC_{50} (A/H1N/PR/8/34) = 3.0±0.5 μM

CPH-site V615L

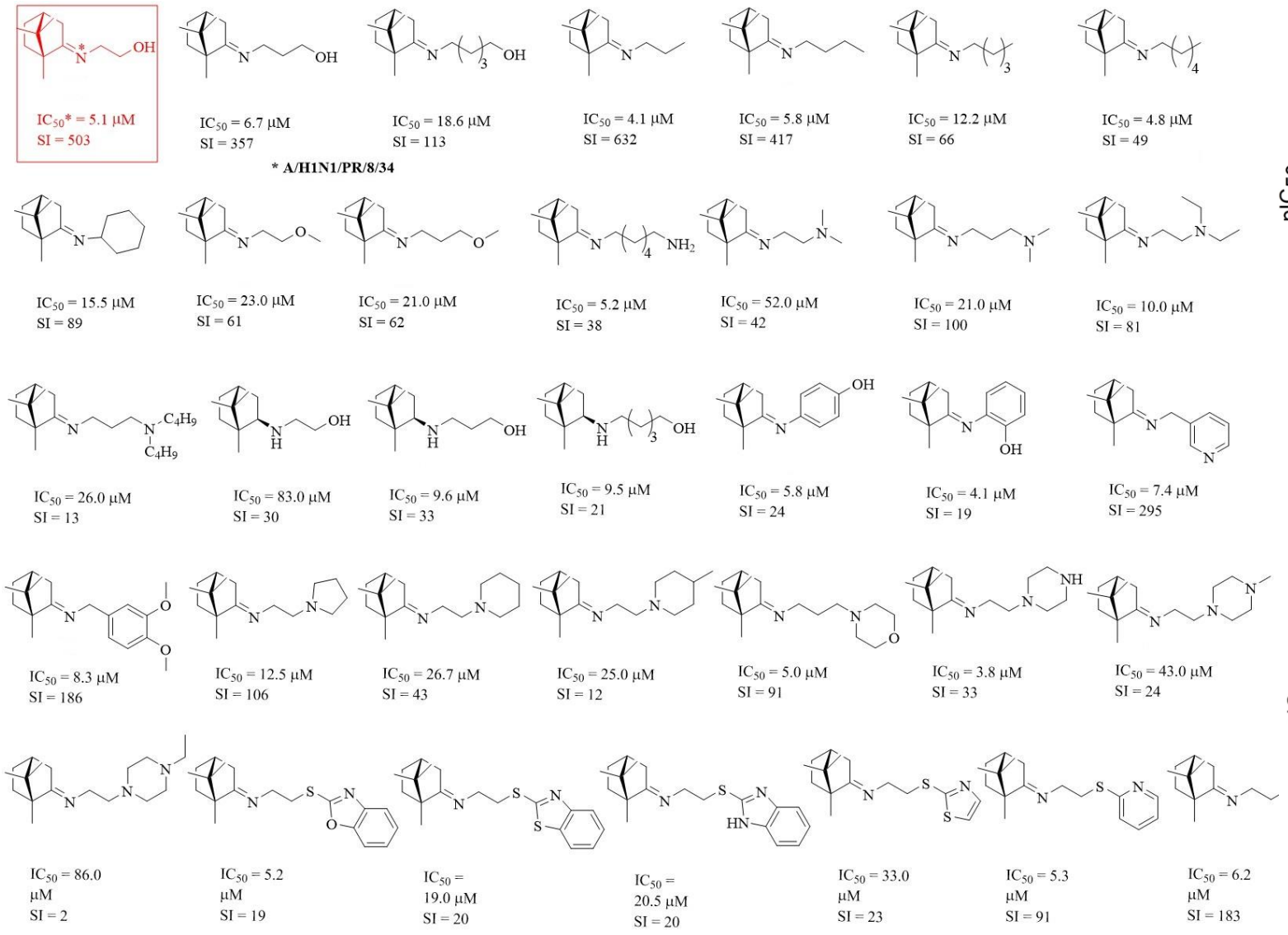


E_{bind} : -5.7 ± 0.5 kcal/mol

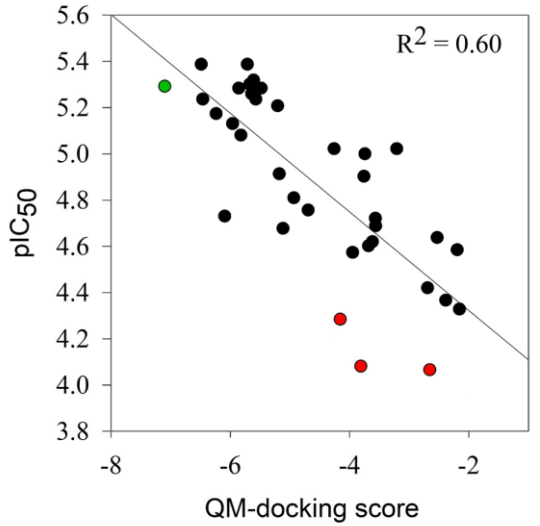
IC_{50} (A/H1N/PR/8/34-mutant) = 477.4±44.2 μM

21. Zarubaev V.C., …, Borisevich S.S. et. Al Virology, **2018**, 524: 69-77

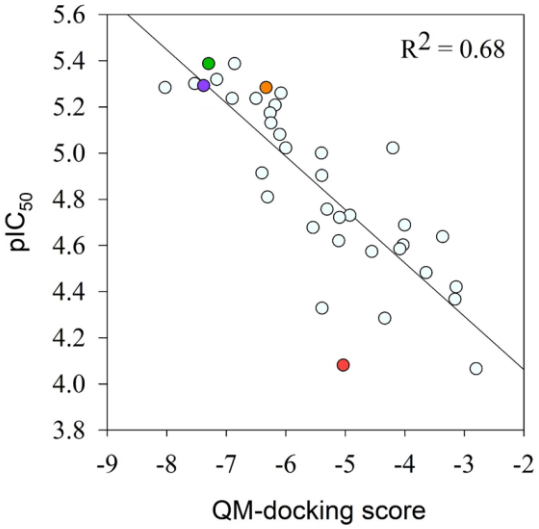
1. HA inhibitors: camphecene analogues[22]



TBHQ site

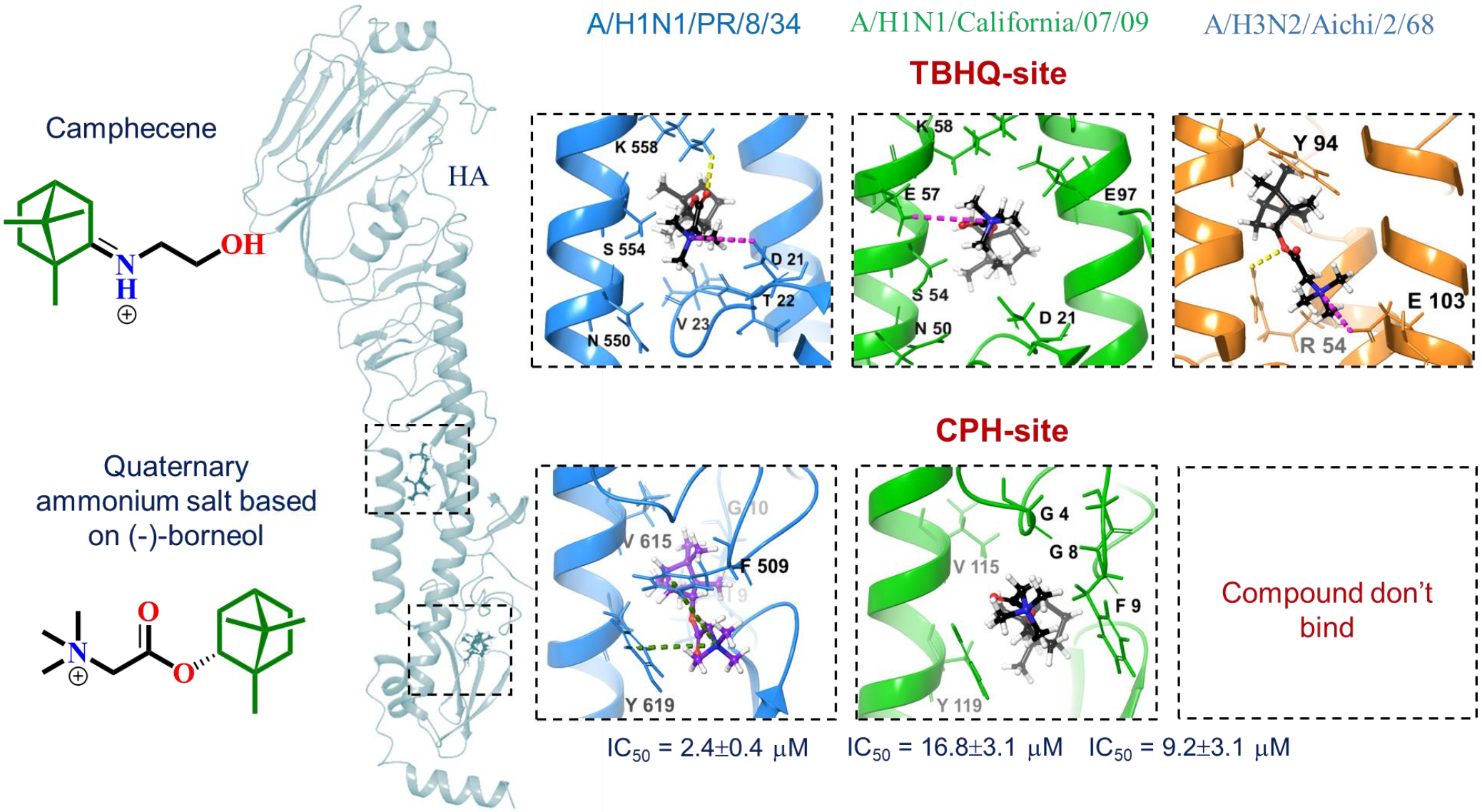


CPH site



22. Borisevich S.S. et al JBSD, **2020**, 5481-5492.

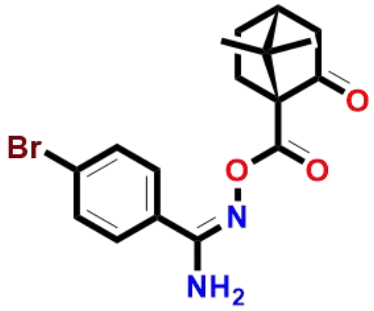
1. HA inhibitors: compound with rigid hydrophobic part[23]

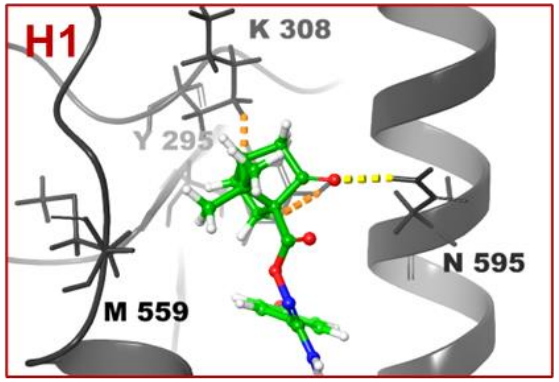


23. Sokolova A.S., …, Borisevich S.S. et al Arch. Virol, **2021**, 166 (7): 1965-1976.

1. HA inhibitors: compound with rigid hydrophobic part[24]

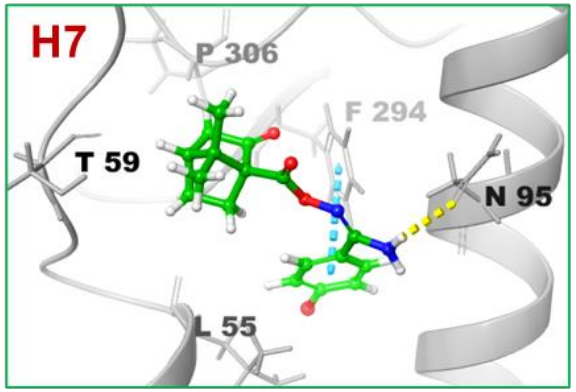
TBHQ-site





-42.1 kJ/mol

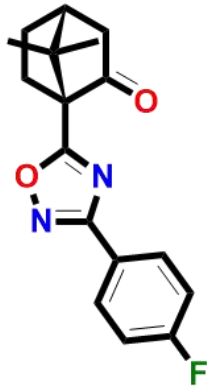
IC_{50} (H1N1) = 13.0±2.0 μM

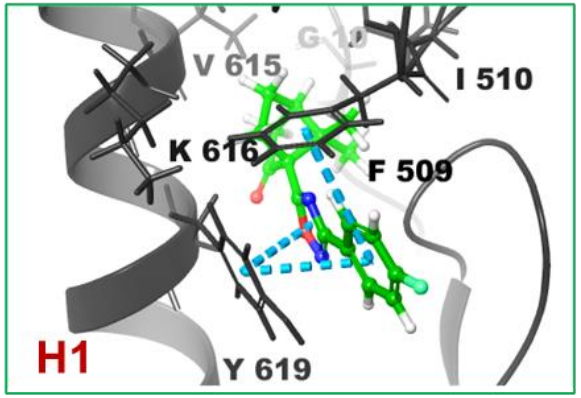


-51.6 kJ/mol

IC_{50} (H7N9) = 3.0±0.4 μM

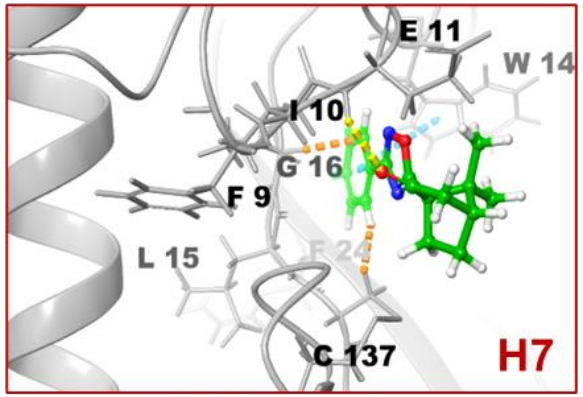
CPH-site





-50.8 kJ/mol

IC_{50} (H1N1) = 4.0±0.5 μM

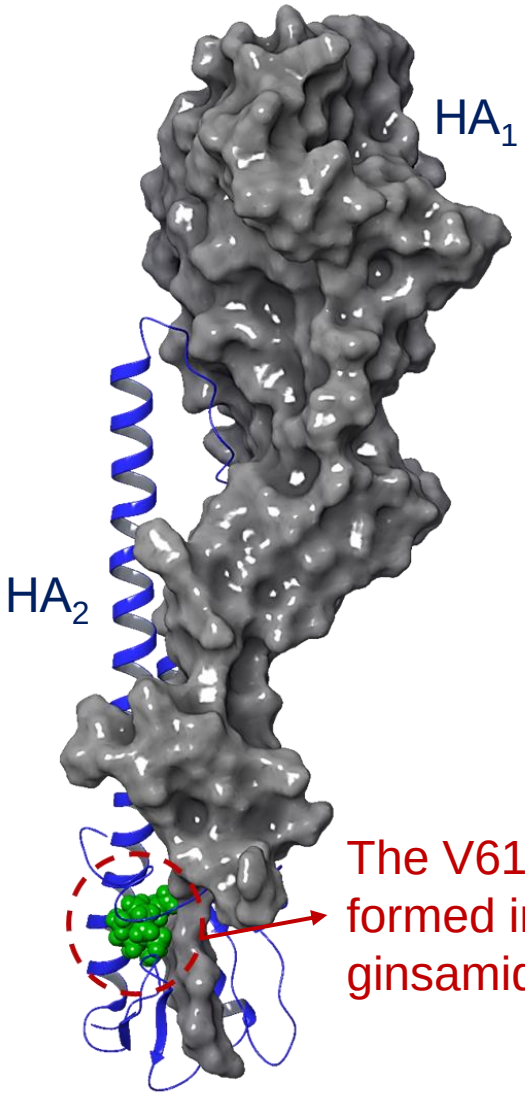


-41.0 kJ/mol

IC_{50} (H7N9) = 30.0±5.0 μM

24. Chernyshov V.V., …, Borisevich S.S. et al BMCL, **2021**, 55: 128465.

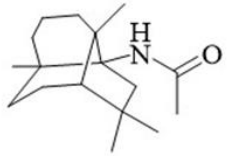
1. HA inhibitors: compound with rigid hydrophobic part[25-26]



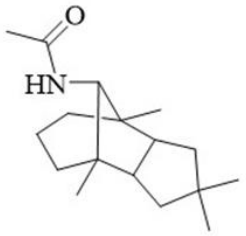
The V615L mutation was formed in the HA of the ginsamide-resistant virus strain.

These compounds can bind in CPH-site

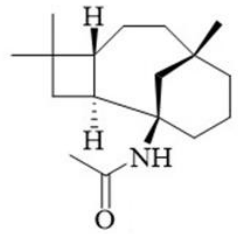
Ginsamid



IC_{50} (A/H1N1/PR/8/34) = 0.152 μM
SI = 7500
IC_{50} (A/H1N1/California/7/09) = 10.7 μM
SI = 107
IC_{50} (A/H1N1/Vladivostok/2/09) = 0.38 μM
SI = 3000
IC_{50} (A/H3N2/Aichi/2/68) = 789.0 μM
SI =9
IC_{50} (A/H7N3/Anhui/1/13) = 125.5 μM
SI =9

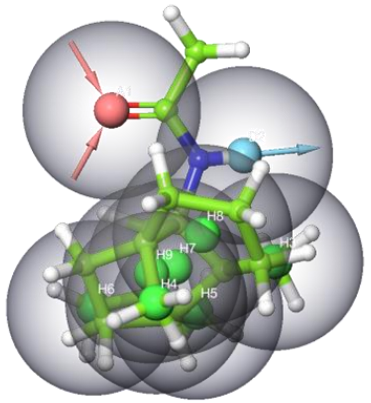


IC_{50} (A/H1N1/PR/8/34) = 19.0 μM
SI = 60

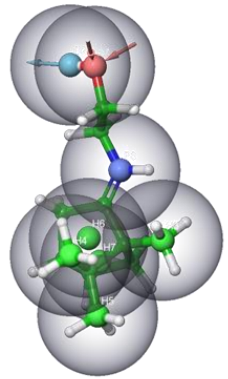


IC_{50} (A/H1N1/PR/8/34) = 6.46 μM
SI = 109

Similar pharmacophore profile



Ginsamid



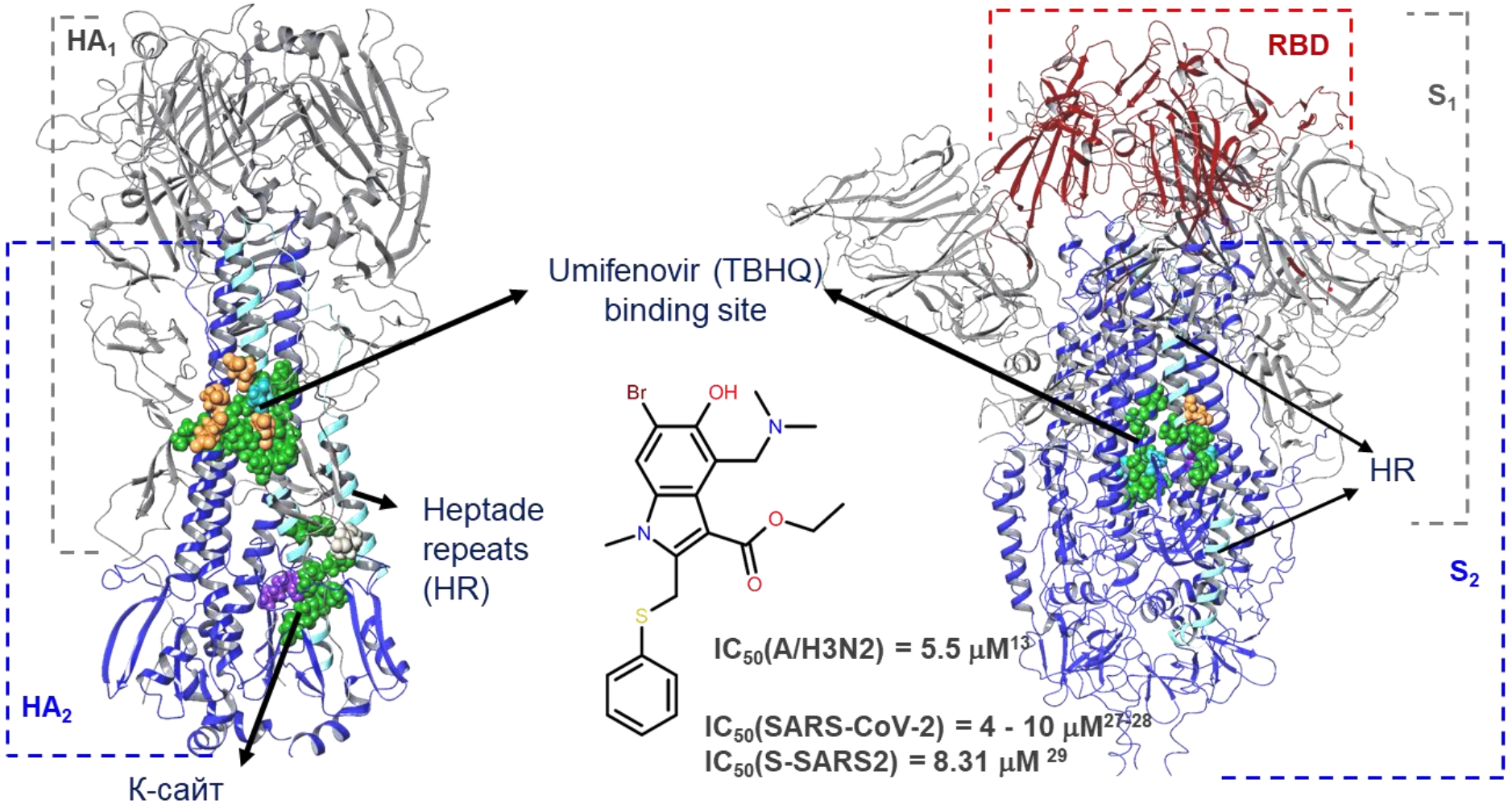
Camphecene

25. Volubueva A.S., …, Borisevich S.S. et al Molucules, **2021**, 26(22): 6794.
26. Yarovay O.I.,…Borisevich S.S. et al. Mend.Comm, **2022**, 32 (5): 609-611.

1. HA: alternative binding site

So, we described the pharmacophore profile of an alternative binding site located in the region of the **fusion peptide** of influenza virus haemagglutinin. The site is saturated with hydrophobic residues, including **valine** 615. It has been shown that this cavity is preferable for the binding of small molecules with a volume of no **more than 300** Å^3, containing a rigid **hydrophobic** group and a polar substituent. The binding of small molecules at the site of proteolysis energetically stabilizes the protein conformation, which complicates its subsequent conformational rearrangements and prevents the fusion of viral and cell membranes.

2. S-protein inhibitors: Umifenovir

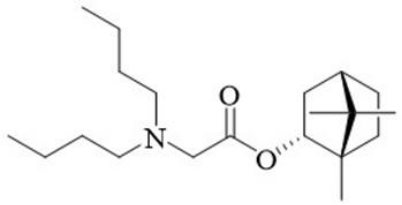


Amino acids features:

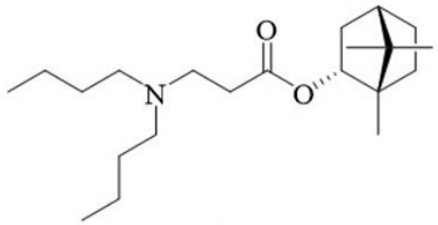
- Hydrophobic
- Polar
- Positive charged
- Negative charged
- Glycine

13. Kadam R.U., Wilson I.A. Natl. Acad. Sci. **2017**, 114 (2).
27. Wang X. et al Cell Discov. **2020**, 6(1): 28.
28. Cai L. et al Int. J. Mol. Med. **2021**, 47(4): 43.
29. Borisevich S.S. at al Viruses, **2022**, 14(1): 119.

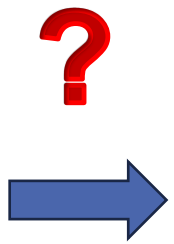
2. S-protein inhibitors: compound[30] with rigid hydrophobic part



IC_{50} (Wuhan Lineages B) = 9.6 μM
SI = 32
IC_{50} (Delta Lineage B.1.617.2) = 17.6 μM
SI = 17
IC_{50} (Omicron Lineage B.1.1.529) = 7.7 μM
SI = 40



IC_{50} (Wuhan Lineages B) =4.7 μM
SI = 71
IC_{50} (Delta Lineage B.1.617.2) = 3.5 μM
SI = 96
IC_{50} (Omicron Lineage B.1.1.529) = 3.3 μM
SI = 102



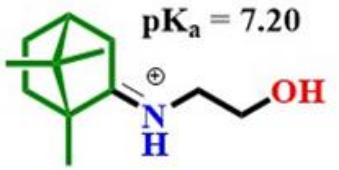
Which is biological target?

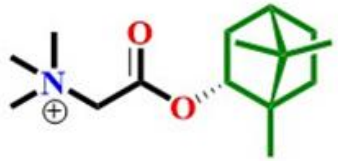
S-protein

Which is a binding place?

Region of HR

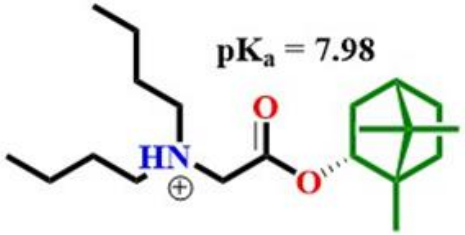
Active against influenza A/H1N1[20, 23, 31]





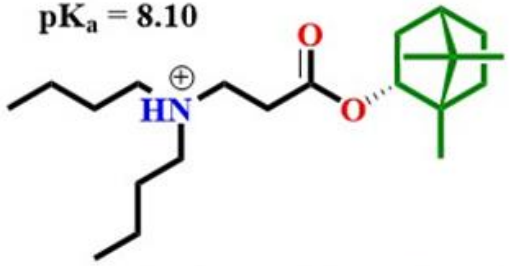
HA inhibitors[21, 23]

IC_{50} (A/H1N1/PR/8/34) = 5.1 μM



IC_{50} (A/H1N1/PR/8/34) = 2.4 μM

IC_{50} (A/H1N1/PR/8/34) > 34 μM

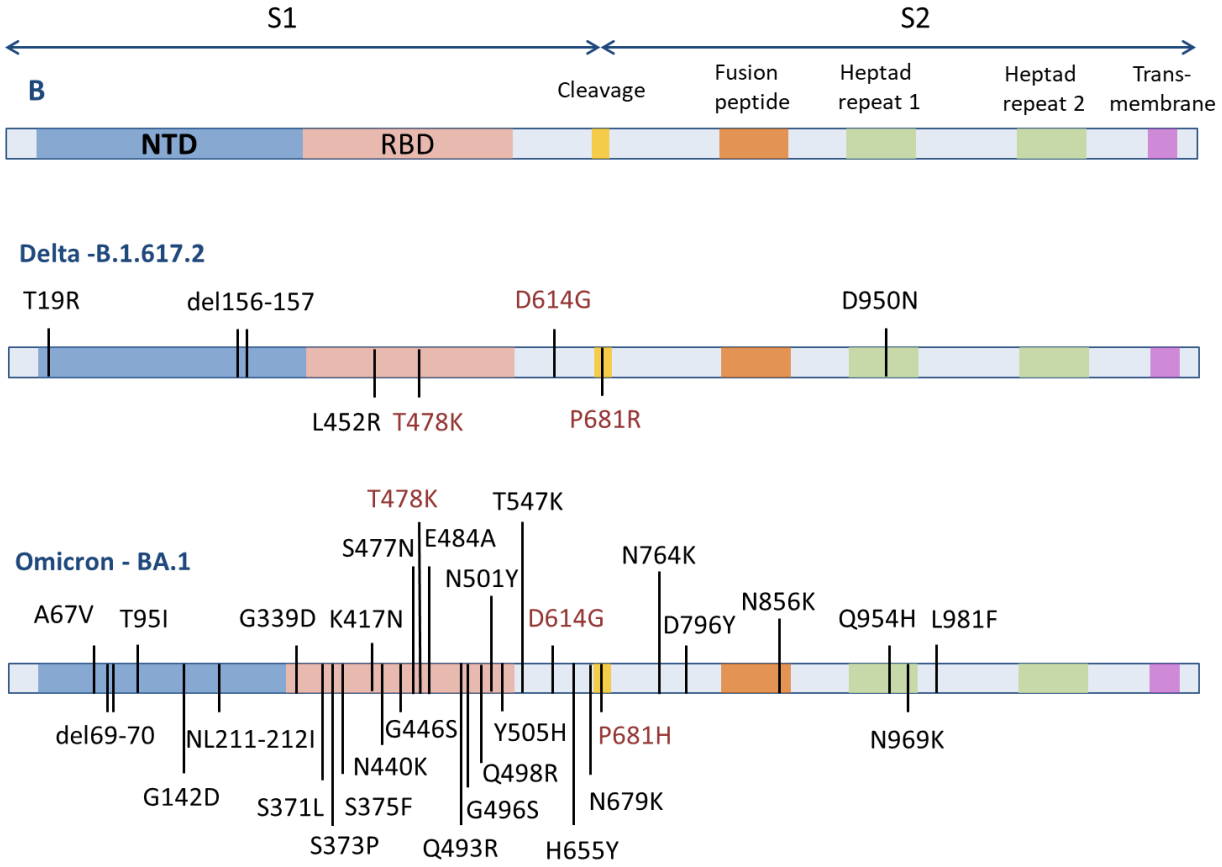


IC_{50} (A/H1N1/PR/8/34) = 7.4 μM

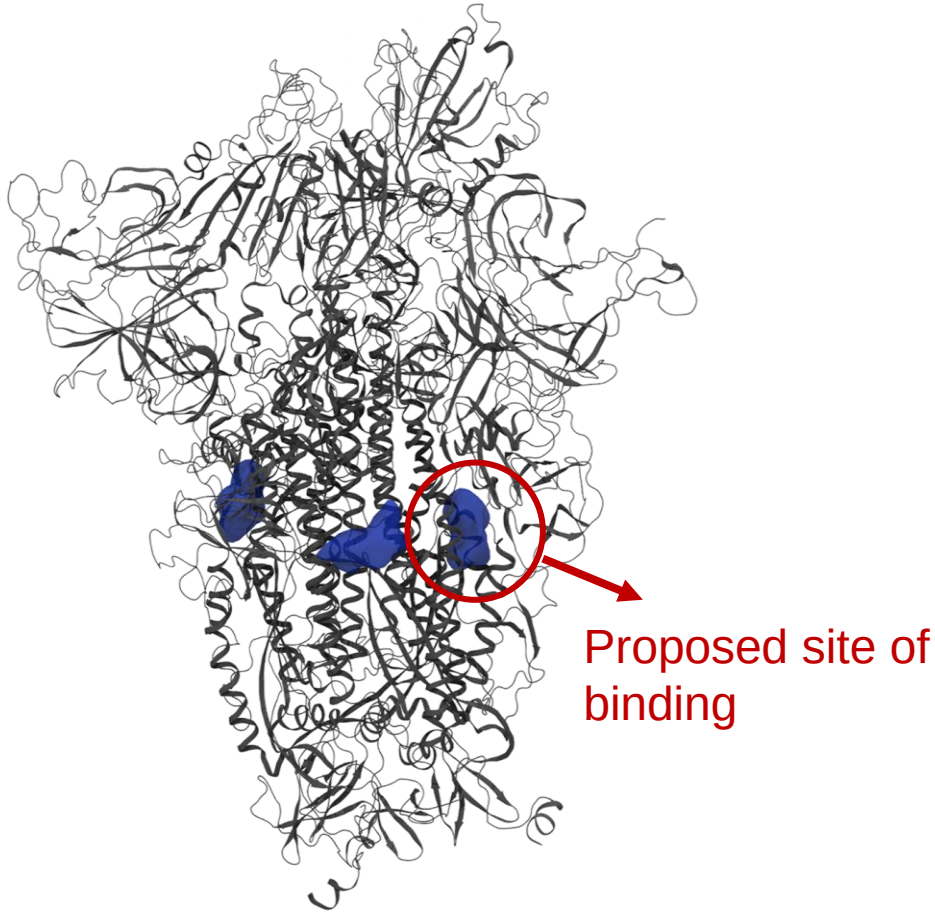
20. Sokolova A.S. et al. Eur. J. Med. Chem. **2015**, 105 (13): 263-273
21. Zarubaev V.C., …, Borisevich S.S. et. Al Virology, **2018**, 524: 69-77
23. Sokolova A.S., …, Borisevich S.S. et al Arch. Virol, **2021**, 166 (7): 1965-1976.
30. Yarovaya O.I.,.. Borisevich S.S.. et al. Viruses **2022**, 14 (6): 1295
31. Sokolova A.S. et. MedComChem, **2017**, 8(5): 960-963

2. S-protein inhibitors: compound[30] with rigid hydrophobic part

Mutations of amino acid residues in different strains of the SARS-CoV-2 virus[32]



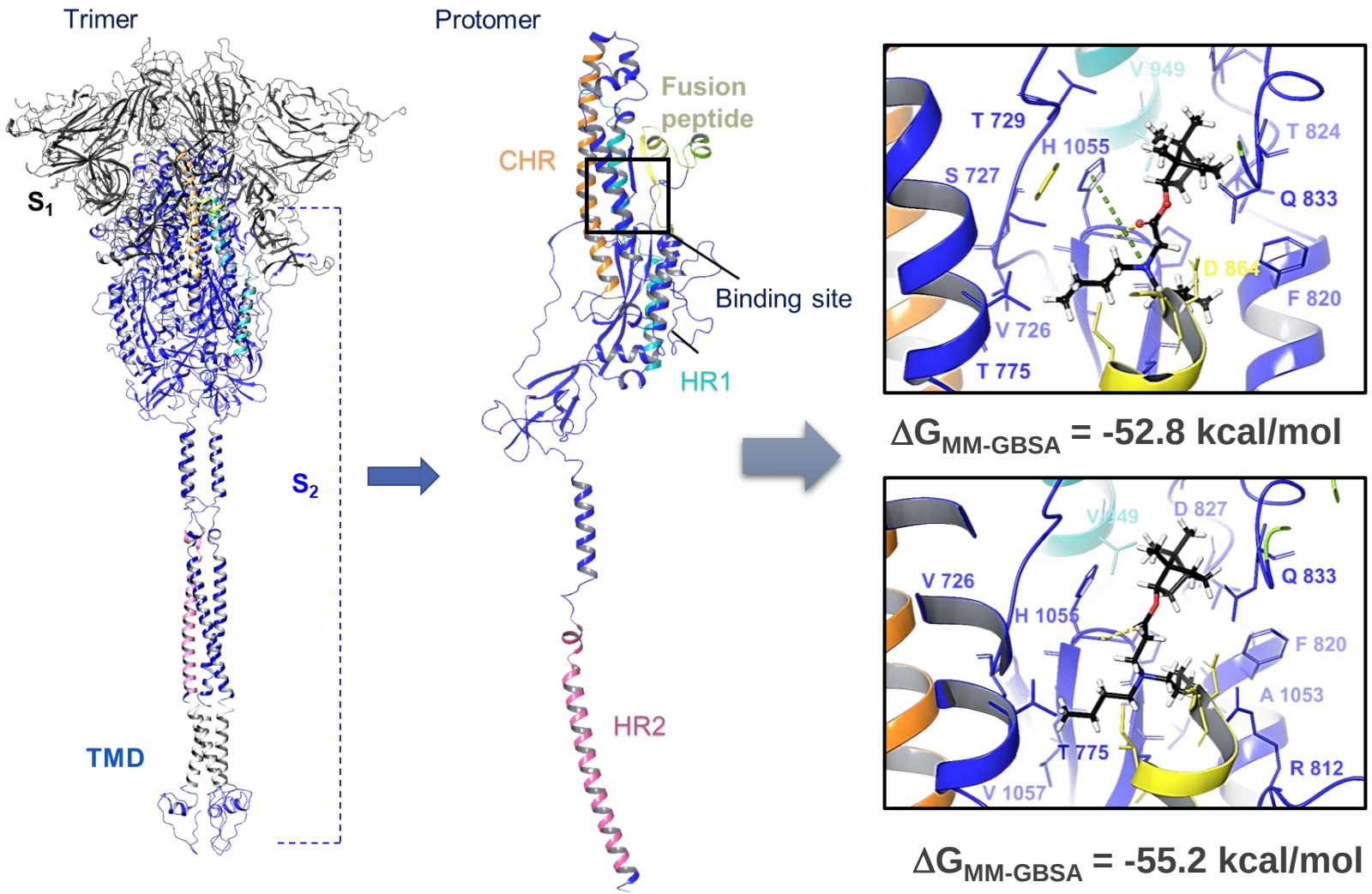
Molecular dynamic results[30]



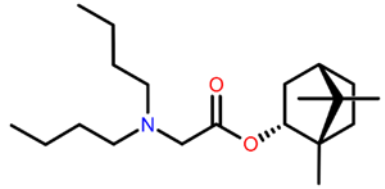
30. Yarovaya O.I.,.. Borisevich S.S.. et al. Viruses **2022**, 14 (6): 1295
32. Jackson C.B. et. Nat. Rev. Mol. Cell Biol, **2022**, 23 (1): 3-20

2. S-protein inhibitors: compound[30] with rigid hydrophobic part

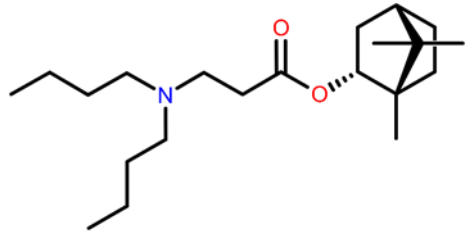
Hypothesis



Evidence



IC_{50} (SARS2-S*) = 17.4 μM
SI = 10
IC_{50} (SARS2-S**) = 16.0 μM
SI = 11



IC_{50} (SARS2-S*) = 25.8 μM
SI = 29
IC_{50} (SARS2-S**) = 14.2 μM
SI = 53

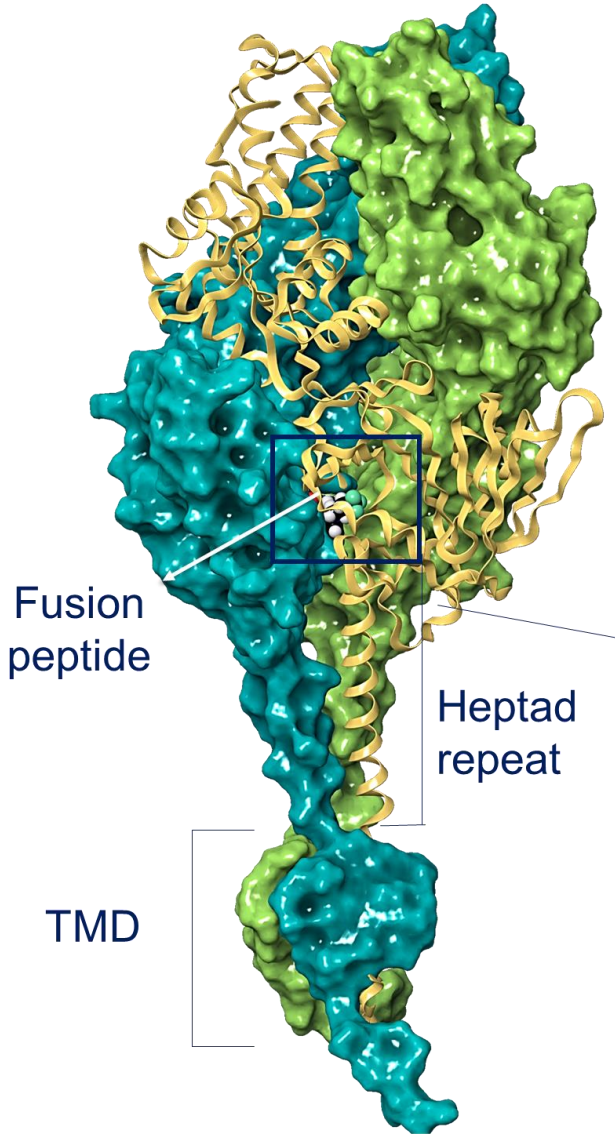
* Wuhan Lineages B
** Delta Lineage B.1.617.2

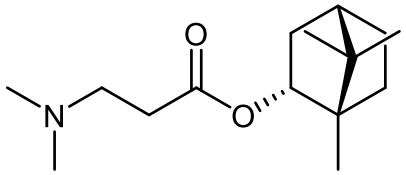
30. Yarovaya O.I.,.. Borisevich S.S.. et al. Viruses **2022**, 14 (6): 1295

2. S-protein: binding site

S protein inhibitors can bind to the heptad repeat region of the S protein of SARS-CoV-2 in a manner like binding to the HA stem. The pharmacophore profile of the binding site of S-protein inhibitors is like the profile of the binding region of HA inhibitors: **hydrophobic** amino acid residues**: alanine, leucine**, **isoleucine** and **phenylalanine**, positively charged **lysine** and negatively charged **glutamic acid**, with atoms of which hydrogen and salt bridges can be formed by analogy with binding in HA. The location of inhibitors in the HR region may affect the secondary structure of the S protein, stabilizing it.

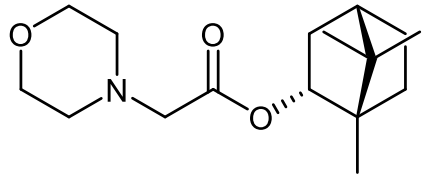
3. F-protein inhibitors: binding site[33]





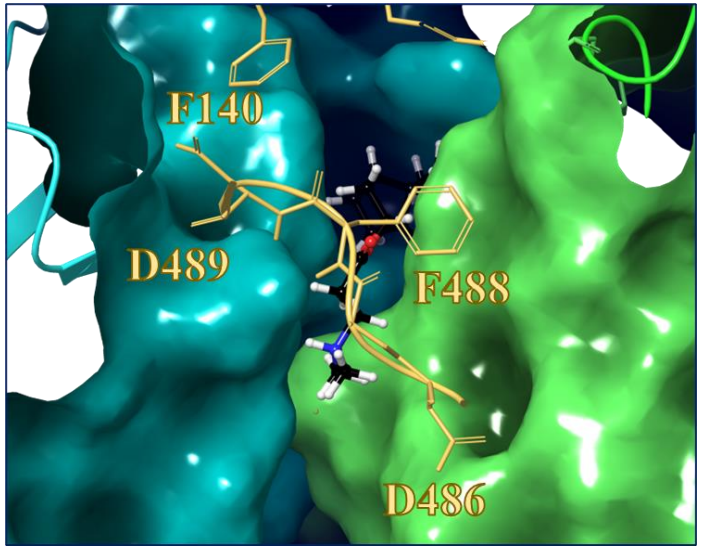
IC_{50} (A/RSV) = 8.9 μM
SI = 111

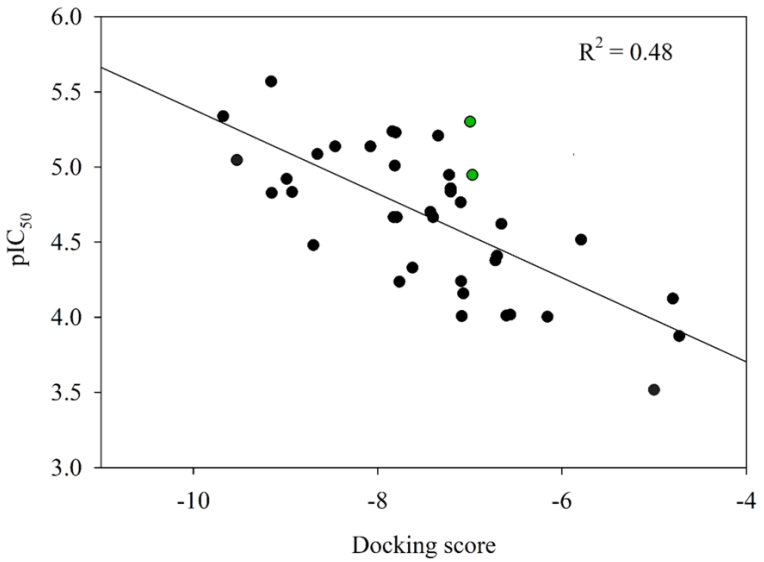
IC_{50} (A/H1N1/PR8/34) > 34 μM



IC_{50} (A/RSV) = 5.0 μM
SI = 83

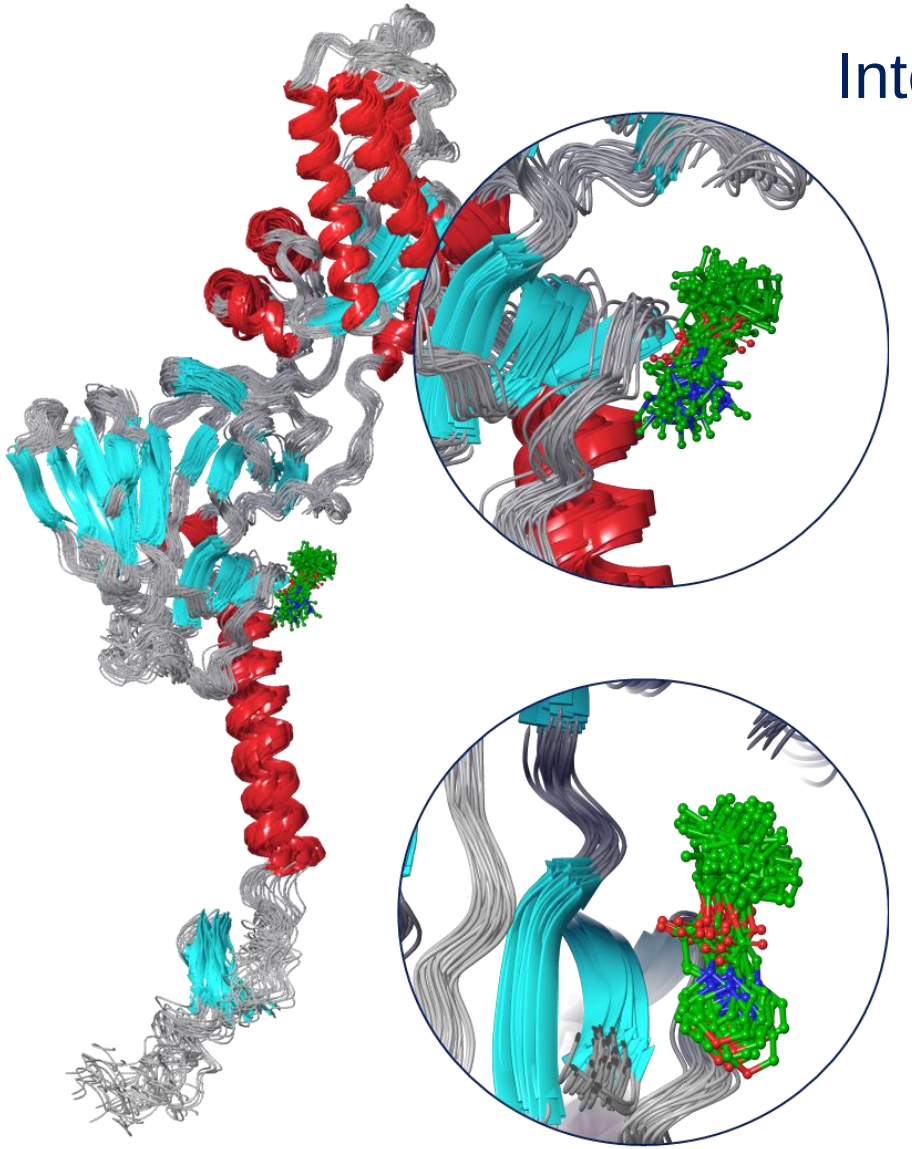
IC_{50} (A/H1N1/PR8/34) = 7.1 μM
SI = 82



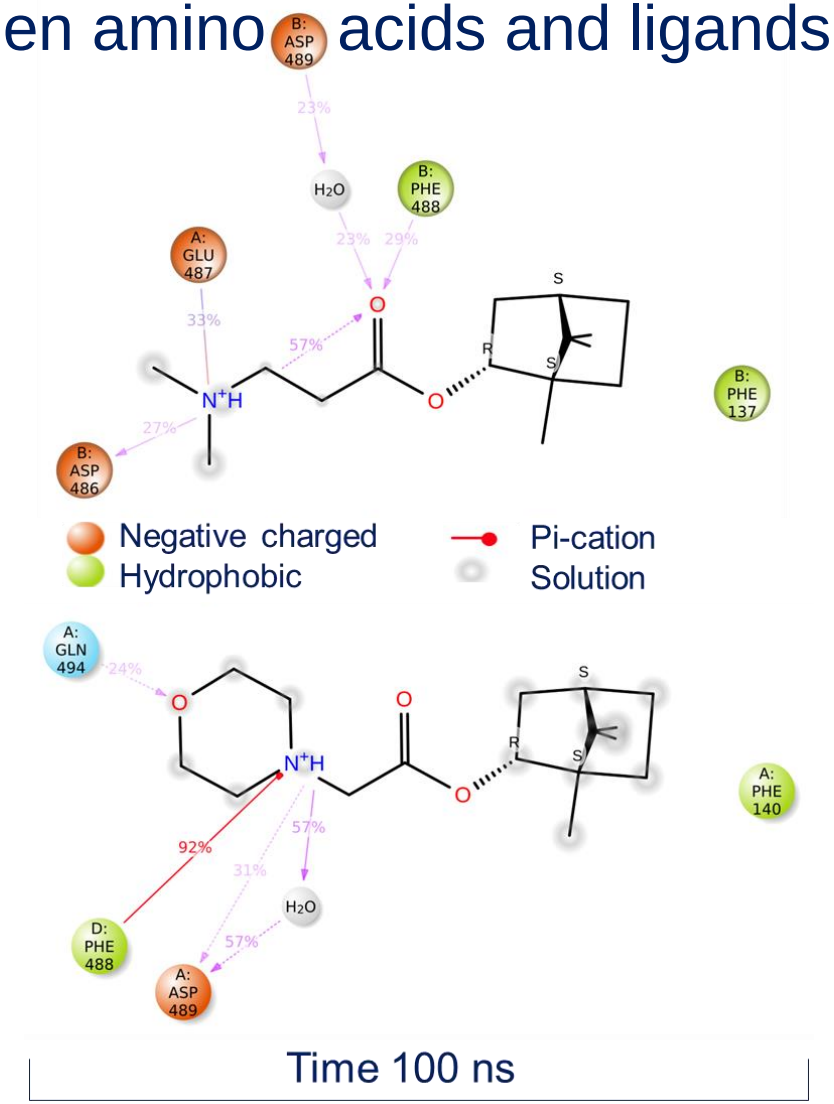


33. Sokolova A.S.,… Borisevich S.S. et al Pharmaceuticals, **2022**, 15 (11).

3. F-protein inhibitors: molecular dynamic results[33]



Interaction between amino acids and ligands



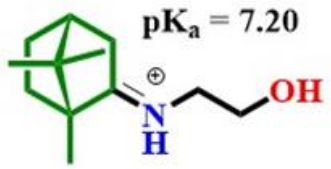
33. Sokolova A.S.,… Borisevich S.S. et al Pharmaceuticals, **2022**, 15 (11).

3. F-protein inhibitors

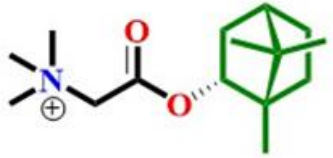
The analysis of the pharmacophore profile of the binding site of inhibitors of the F protein of respiratory syncytial virus made it possible to explain the antiviral activity of N-containing derivatives of (-)-borneol esters, which may be associated with the effect of small molecules on the F protein. The binding site is located inside the F protein trimer and is rich in hydrophobic residues, including **phenylalanine**, **leucine**, and **isoleucine**. Hydrogen and salt bridges are registered between the inhibitor atoms and the negatively charged **asparagine**.

4. GP-protein inhibitors

Active against influenza

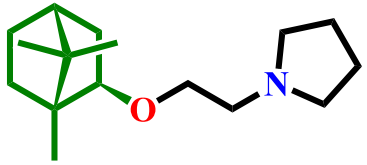


IC_{50} (A/H1N1/PR/8/34) = 5.1 μM



IC_{50} (A/H1N1/PR/8/34) = 2.4 μM

Inhibit HA



IC_{50} (A/H1N1)* = 45.3 μM
SI = 26

IC_{50} (EboV-GP)** = 0.12 μM
SI = 4166

IC_{50} (EBOV)*** = 18.3 μM
SI = 12

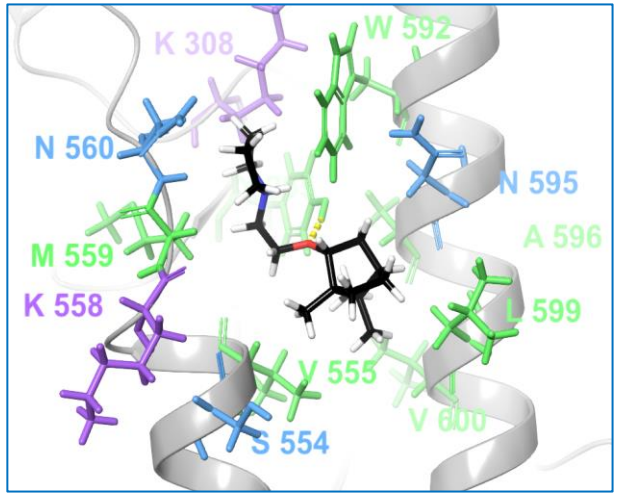
Inhibit GP[34]

Are the pharmacophore profiles of the binding site of HA inhibitors and glycoprotein of Ebola virus similar?

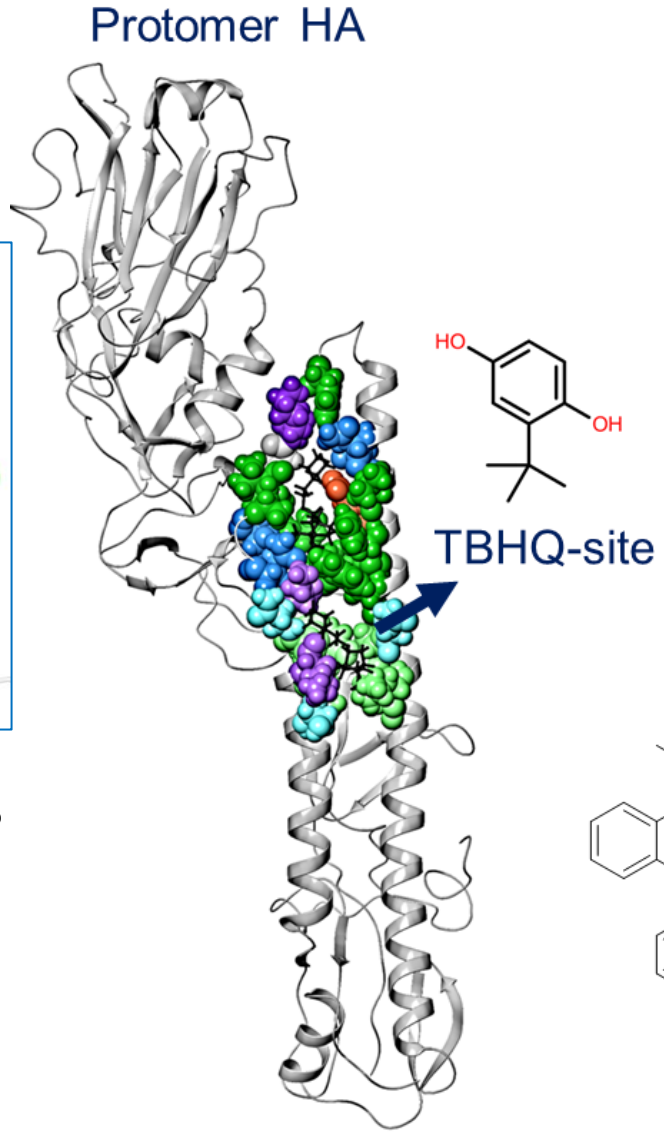
34. Sokolova A.S.,… Borisevich S.S. et al Molecules, **2021**, 26 (8).

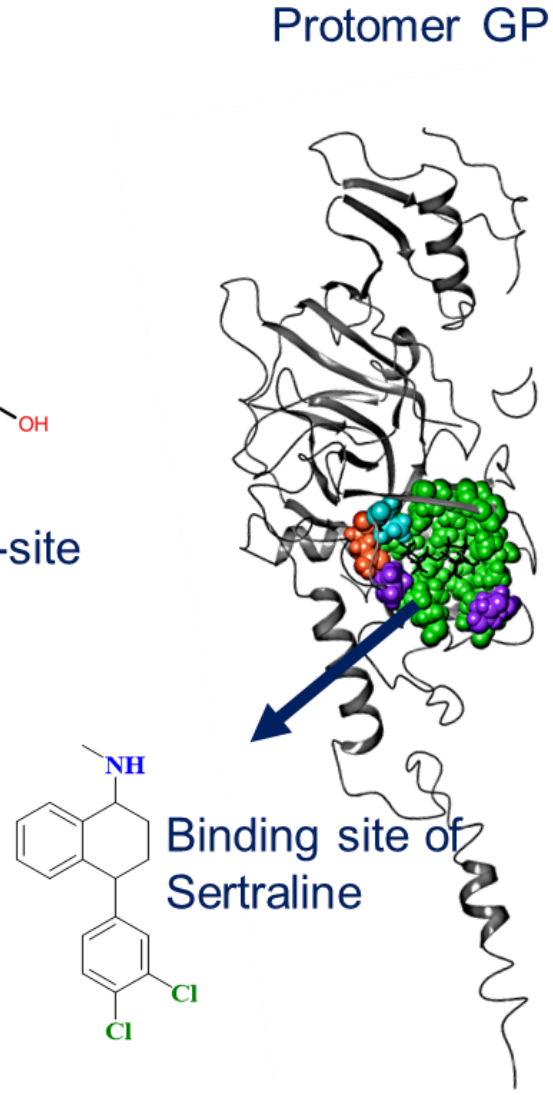
4. GP-protein inhibitors: binding site

IC_{50} = 45.3 μM

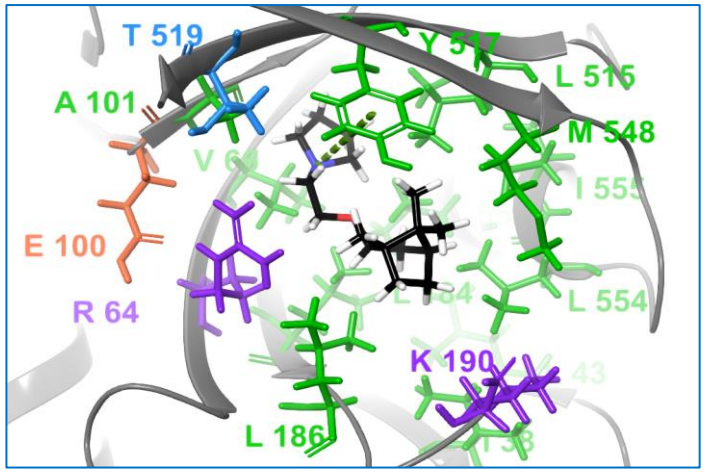


ΔG_{bind} = -43.8 ккал/моль





IC_{50} = 18.3 μM



ΔG_{bind} = -44.5 kcal/mol

4. GP-protein inhibitors

According to the results of molecular modeling, the bicyclic framework of camphene derivatives ensures effective binding to the **hydrophobic** cavities of the binding sites in HA and GP. Activation of both surface proteins occurs at low pH values. Further search for **new analogues**, including these two structural fragments, may lead to the discovery of a new inhibitor that targets the membrane fusion stage and has a **broad** spectrum of antiviral activity.

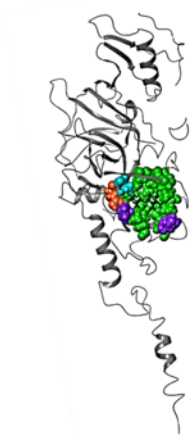
5. Compounds with broad antiviral activity

Ligand-protein complex

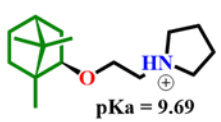
Binding site in GP

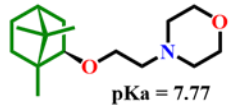
Binding site in HA

Binding site in S-protein

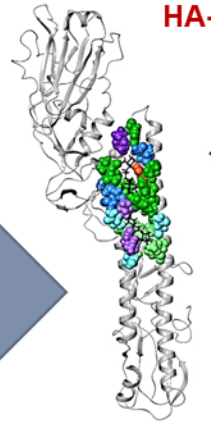


GP-Zaire Ebola virus

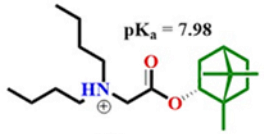


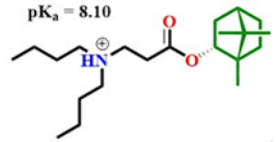


Inhibitors of HA and GP



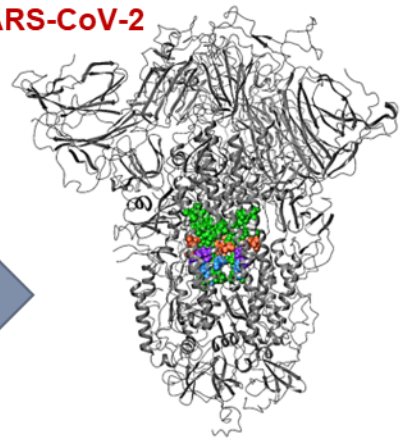
HA-A/Puerto Rico/8/34 (H1N1)



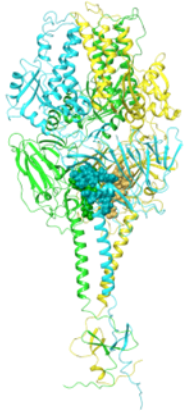


Inhibitors of HA and S-protein

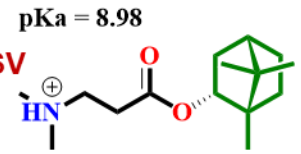
S-белок SARS-CoV-2

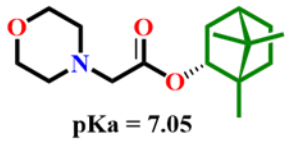


Binding site in F-protein



F-A/RSV





Inhibitors of HA and F-protein

Compounds with broad antiviral activity

Descriptions			
V, Å^3	264 – 376	HBA	2 – 3
MW, Da	251 – 337	HBD	0
N (атомов)	45 – 63 (18 – 24)	Positive	–NH$^+$–
–(CH$_2$)$_n$–	1 – 2	logP	2.60 – 5.78

Main conclusion

It has been shown that small molecules with a volume of up **to 350 Å^3** and a few **about 70 atoms**, containing a rigid hydrophobic fragment, an acceptor group and a protonated nitrogen atom can **simultaneously** bind to the binding sites of inhibitors of type I surface proteins: namely, hemagglutinin of influenza virus, S-protein of SARS-CoV-2, F protein of respiratory syncytial virus and glycoprotein GP of Ebola virus. The studies carried out allow us to conclude that the mechanism of the antiviral activity of these compounds probably lies in the suppression of the fusogenic activity of the mentioned viral proteins

My team

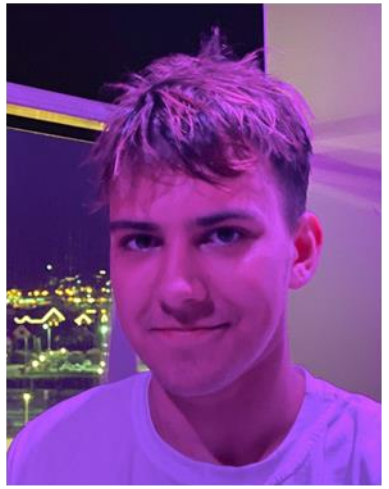
«Кванты и динамика»
«Quanta and dynamics»

Edward (Ufa)

Rita (Ufa)

Alfia (Ufa)

Evgeniy (Novosibirsk)



Egor (Novosibirsk)

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Thanks for your attention ☺