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

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## Relevance of research

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

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. Official data collated by Our World in Data
Antiviral drugs

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

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

**Oseltamivir**
Inhibitor of neuraminidase (Influenza)

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

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

Various viruses

**Influenzavirus**  
*Orthomyxoviridae*  
negative-sense RNA viruses

**Coronaviruses**  
*Coronaviridae*  
positive-sense RNA virus

**Human orthopneumovirus**  
*Pneumoviridae*  
negative-sense, single-stranded RNA virus.

**Ebolavirus**  
*Filoviridae*
Surface viral proteins

Hemagglutinin (HA) of influenza

Spike (S)-protein of SARS-CoV-2\(^{14, 15}\)

Fusion (F)-protein of RSV

Glycoprotein (GP) of Ebola virus

Umifenovir\(^{13}\)

TBHQ\(^{12}\)

Sisunatovir\(^{16, 17}\)

Toremifen\(^{18}\)

Viral membrane

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

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\textsuperscript{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.

\textbf{IC}_{50}(\text{H1N1, H3N2, H5N1}) = 1.2 - 10.3 \ \mu\text{M} \\
\text{SI} = 75 - 645 \\
E_{\text{bind}}: -7.0 \pm 0.2 \ \text{kcal/mol}\textsuperscript{21} \\

\textbf{IC}_{50}(\text{H3N2}) \sim 6.0 \mu\text{M} \\
\text{SI} \sim 8 \\
E_{\text{bind}}: -7.1 \pm 0.3 \ \text{kcal/mol} \\

1. HA inhibitors: camphecene

Camphecene in TBHQ site

\[ E_{\text{bind}} : -7.0 \pm 0.2 \text{ kcal/mol} \]

Camphecene in CPH site

\[ E_{\text{bind}} : -7.1 \pm 0.3 \text{ kcal/mol} \]

1. HA inhibitors: camphecene

<table>
<thead>
<tr>
<th>Штамм</th>
<th>IC$_{50}$, µM</th>
<th>SI</th>
<th>QM-docking score</th>
<th>QM-Emodel</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/H1N1/PR/8/34</td>
<td>1.2±1.2</td>
<td>645</td>
<td>-7.07±0.30</td>
<td>-71.40±1.70</td>
</tr>
<tr>
<td>A/H1N1/Cal/07/09-pdm</td>
<td>3.8±1.1</td>
<td>204</td>
<td>-7.03±0.51</td>
<td>-68.40±1.15</td>
</tr>
<tr>
<td>A/H3N2/Aichi/2/68</td>
<td>10.3±1.1</td>
<td>75</td>
<td>No docking results</td>
<td></td>
</tr>
<tr>
<td>A/H5N2/Mallard/</td>
<td>8.0±1.0</td>
<td>97</td>
<td>-7.95±0.27</td>
<td>-52.81±2.38</td>
</tr>
</tbody>
</table>
1. HA inhibitors: camphecene

**CPH-site V615**

$$E_{\text{bind}} = -7.1 \pm 0.3 \text{ kcal/mol}$$

$$IC_{50} (A/H1N/PR/8/34) = 3.0\pm0.5 \mu M$$

**CPH-site V615L**

$$E_{\text{bind}} = -5.7 \pm 0.5 \text{ kcal/mol}$$

$$IC_{50} (A/H1N/PR/8/34\text{-mutant}) = 477.4\pm44.2 \mu M$$

1. HA inhibitors: camphecene analogues\textsuperscript{22}
1. HA inhibitors: compound with rigid hydrophobic part

A/H1N1/PR/8/34 A/H1N1/California/07/09 A/H3N2/Aichi/2/68

**TBHQ-site**

- K 558
- S 554
- D 21
- N 550
- V 23
- T 22

**CPH-site**

- G 4
- G 8
- V 115
- Y 119
- Y 619
- F 509
- V 615
- Y 619

**IC₅₀ values**

- TBHQ-site: 2.4±0.4 μM
- CPH-site: 16.8±3.1 μM; 9.2±3.1 μM

1. HA inhibitors: compound with rigid hydrophobic part

**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

1. HA inhibitors: compound with rigid hydrophobic part

These compounds can bind in CPH-site

IC₅₀ (A/H1N1/PR/8/34) = 0.152 μM
SI = 7500
IC₅₀ (A/H1N1/California/7/09) = 10.7 μM
SI = 107
IC₅₀ (A/H1N1/Vladivostok/2/09) = 0.38 μM
SI = 3000
IC₅₀ (A/H3N2/Aichi/2/68) = 789.0 μM
SI = 9
IC₅₀ (A/H7N3/Anhui/1/13) = 125.5 μM
SI = 9

IC₅₀ (A/H1N1/PR/8/34) = 19.0 μM
SI = 60
IC₅₀ (A/H1N1/PR/8/34) = 6.46 μM
SI = 109

Similar pharmacophore profile

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

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 Å³, 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:
- Positive charged
- Hydrophobic
- Negative charged
- Polar
- Glycine

IC$_{50}$(A/H3N2) = 5.5 µM$^{13}$
IC$_{50}$(SARS-CoV-2) = 4 - 10 µM$^{27,28}$
IC$_{50}$(S-SARS2) = 8.31 µM$^{20}$

2. S-protein inhibitors: compound\textsuperscript{30} with rigid hydrophobic part

\begin{align*}
\text{IC}_{50} \text{ (Wuhan Lineages B)} &= 9.6 \text{ } \mu\text{M} \\
\text{SI} &= 32 \\
\text{IC}_{50} \text{ (Delta Lineage B.1.617.2)} &= 17.6 \text{ } \mu\text{M} \\
\text{SI} &= 17 \\
\text{IC}_{50} \text{ (Omicron Lineage B.1.1.529)} &= 7.7 \text{ } \mu\text{M} \\
\text{SI} &= 40
\end{align*}

\begin{align*}
\text{IC}_{50} \text{ (Wuhan Lineages B)} &= 4.7 \text{ } \mu\text{M} \\
\text{SI} &= 71 \\
\text{IC}_{50} \text{ (Delta Lineage B.1.617.2)} &= 3.5 \text{ } \mu\text{M} \\
\text{SI} &= 96 \\
\text{IC}_{50} \text{ (Omicron Lineage B.1.1.529)} &= 3.3 \text{ } \mu\text{M} \\
\text{SI} &= 102
\end{align*}

**Which is biological target?**
S-protein

**Which is a binding place?**
Region of HR

Active against influenza A/H1N1\textsuperscript{20, 23, 31}

\begin{align*}
pK_a &= 7.20 \\
\text{HA inhibitors}^{21, 23} \\
\text{IC}_{50} \text{ (A/H1N1/PR/8/34)} &= 5.1 \text{ } \mu\text{M} \\
\text{IC}_{50} \text{ (A/H1N1/PR/8/34)} &= 2.4 \text{ } \mu\text{M}
\end{align*}


2. S-protein inhibitors: compound\textsuperscript{30} with rigid hydrophobic part

Mutations of amino acid residues in different strains of the SARS-CoV-2 virus\textsuperscript{32}

Molecular dynamic results\textsuperscript{30}

2. S-protein inhibitors: compound\textsuperscript{30} with rigid hydrophobic part

**Hypothesis**

\[ \Delta G_{\text{MM-GBSA}} = -52.8 \text{ kcal/mol} \]

**Evidence**

IC\textsubscript{50} (SARS2-S*) = 17.4 \text{ \(\mu\)M}  
SI = 10  
IC\textsubscript{50} (SARS2-S**) = 16.0 \text{ \(\mu\)M}  
SI = 11

IC\textsubscript{50} (SARS2-S*') = 25.8 \text{ \(\mu\)M}  
SI = 29  
IC\textsubscript{50} (SARS2-S**') = 14.2 \text{ \(\mu\)M}  
SI = 53

* Wuhan Lineages B  
** Delta Lineage B.1.617.2

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

IC$_{50}$ (A/RSV) = 8.9 $\mu$M
SI = 111

IC$_{50}$ (A/H1N1/PR8/34) > 34 $\mu$M

IC$_{50}$ (A/RSV) = 5.0 $\mu$M
SI = 83

IC$_{50}$ (A/H1N1/PR8/34) = 7.1 $\mu$M
SI = 82

3. F-protein inhibitors: molecular dynamic results

Interaction between amino acids and ligands

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

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

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

4. GP-protein inhibitors: binding site

$\Delta G_{\text{bind}} = -43.8$ ккал/моль

IC$_{50}$ = 45.3 $\mu$M

Protomer HA

Protomer GP

$\Delta G_{\text{bind}} = -44.5$ kcal/mol

IC$_{50}$ = 18.3 $\mu$M
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**

![GP-Zaire Ebola virus](image)

**Binding site in HA**

**Binding site in S-protein**

**Inhibitors of HA and GP**

**Inhibitors of HA and S-protein**

**Inhibitors of HA and F-protein**

### Compounds with broad antiviral activity

<table>
<thead>
<tr>
<th><strong>Descriptions</strong></th>
<th><strong>Values</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>$V$, Å$^3$</td>
<td>264 $-$ 376</td>
</tr>
<tr>
<td>$MW$, Da</td>
<td>251 $-$ 337</td>
</tr>
<tr>
<td>$N$ (атомов)</td>
<td>45 $-$ 63 (18 $-$ 24)</td>
</tr>
<tr>
<td>$-(\text{CH}_2)_n-$</td>
<td>1 $-$ 2</td>
</tr>
</tbody>
</table>
Main conclusion

It has been shown that small molecules with a volume of up to 350 Å³ 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.
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