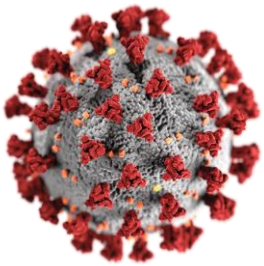
University of Baghdad
College of Pharmacy



Structural Basis of SARS-CoV-2 Receptor Binding and the Small Molecule Compounds Available as Potential Therapeutics

Presented by: Maryam Rasheed Abd
Assistant Lecturer
Department of Pharmacology and Toxicology

Thursday, March 10, 2022

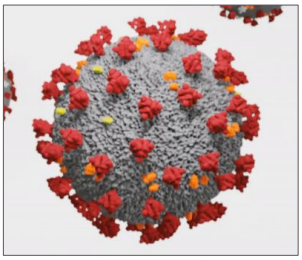
Outline

- COVID-19 Pandemic
- Structure of SARS-CoV-2
- Entry and Lifecycle of SARS-CoV-2
- Potential small-molecule compounds available against SARS-CoV-2
- Conclusions

COVID-19 Pandemic

- CoVs caused three outbreaks :

Virus	SARS-CoV	MERS-CoV	SARS-CoV-2
Disease	Severe acute respiratory syndrome	Middle eastern respiratory syndrome	Coronavirus infectious disease 2019 (COVID-19)



- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
- Declared a pandemic by the WHO in March 2020.
- Evidence for nonsymptomatic/presymptomatic spread.

https://doi.org/10.1016/j.it.2020.10.004

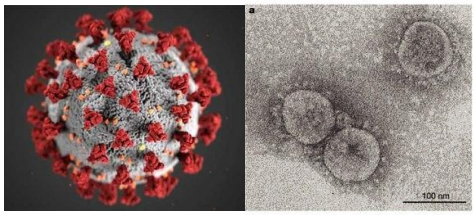
COVID-19 Pandemic

- Since its emergence, it has infected millions of people, killed close to 3 million people worldwide, and costs world economies trillions of dollars.
- Several vaccines are being administered to people for protective immunity.
- Additional COVID-19 therapeutics are needed.
  - ✓ Emergence of new sars-cov-2 variants.
  - ✓ Many people are not becoming vaccinated.
  - ✓ Repurposing approach with the small molecules to accelerate the development process.

https://doi.org/10.1155/2021/1828792

SARS-CoV-2 Characteristics

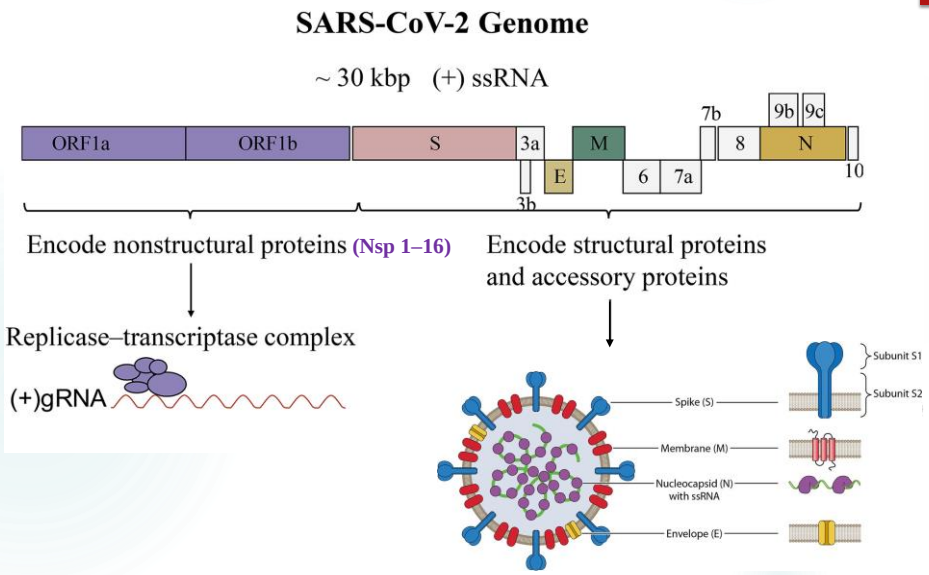
- Genus: *Betacoronavirus.*
- Enveloped, positive-sense single-stranded RNA virus.
- The outer surface peppered with 24–40 spike proteins: attachment/entry.
- The receptor for the S protein is ACE2, expressed on : alveolar cells, esophageal cells, enterocytes, and others.



Corona=crown

https://doi.org/10.1016/j.it.2020.10.004
doi:10.3390/jcm9061885

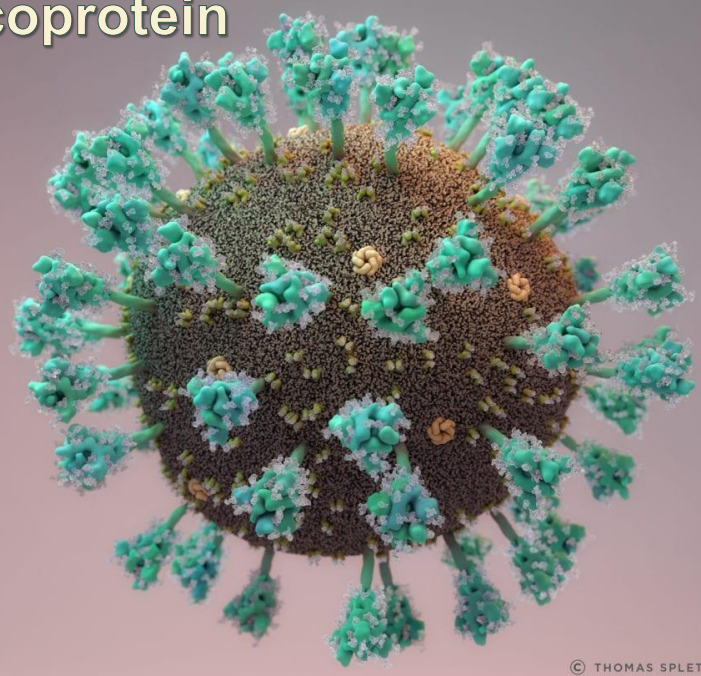
SARS-CoV-2 Characteristics



https://doi.org/10.1016/j.apsb.2021.06.016

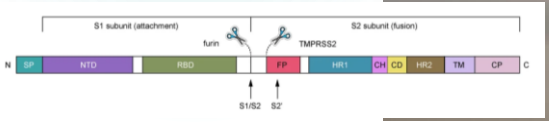
Spike Glycoprotein

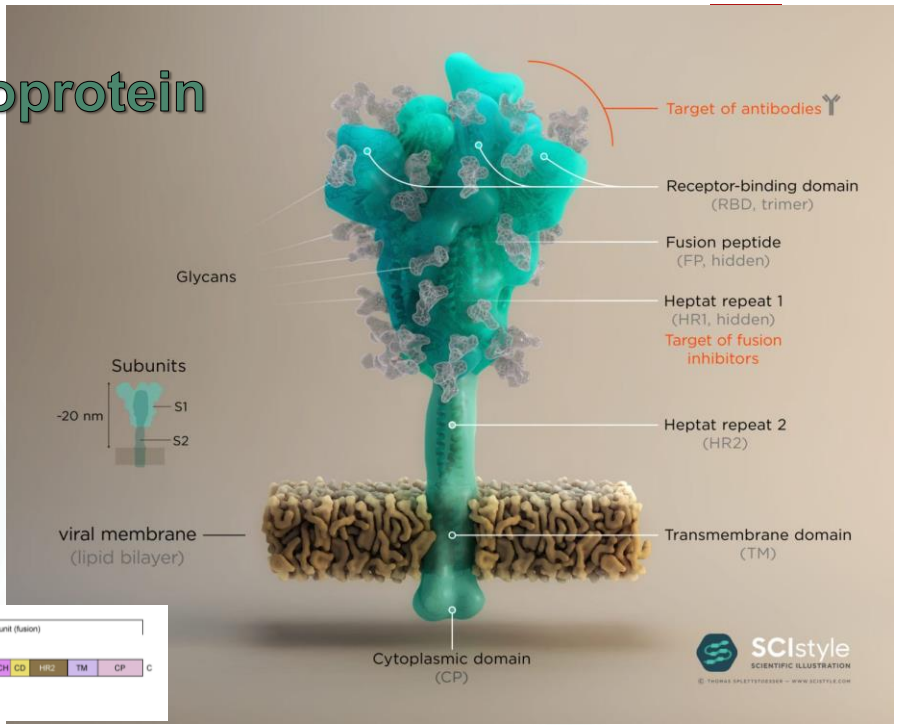
Spike is a homotrimer



Spike Glycoprotein

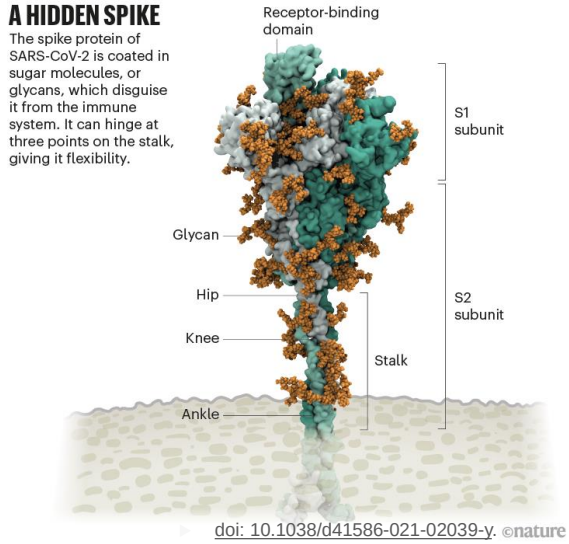
- A RBD: a key region that interacts with hACE2 in a 1:1 ratio.
- S1/S2 polybasic furin cleavage site.





Spike Glycoprotein

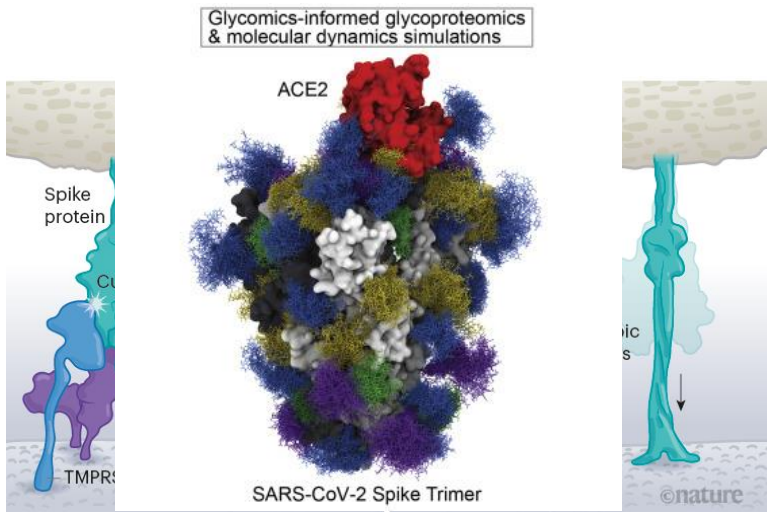
- Wildly flexible and hinge at three points.
- The S-glycoprotein has 22N-linked glycosylation sites that are spread throughout the NTD and RBD.
- ACE2 N-glycans interacts with SARS-CoV-2 spikes.



doi: 10.1038/d41586-021-02039-y. ©nature

SARS-CoV-2 Entry Mechanism

- Activation by host proteases are required.
- SARS-CoV-2 binds to ACE2 on the target cell
- TMPRSS2 cuts a site on S2 subunit.
- That cut exposes hydrophobic amino acids that rapidly buries itself in the host cell membrane.

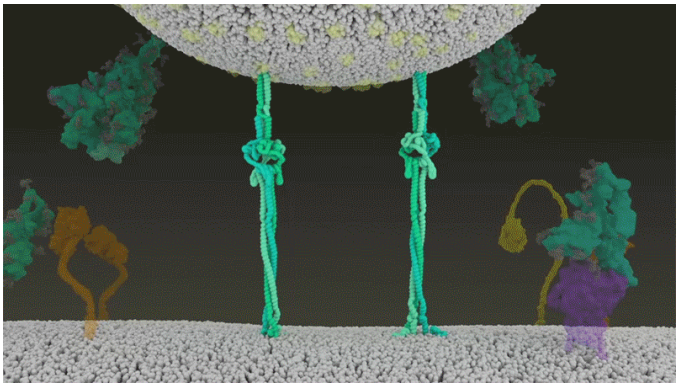


https://doi.org/10.1146/annurev-pharmtox-061220- 093932

doi: 10.1038/d41586-021-02039-y.

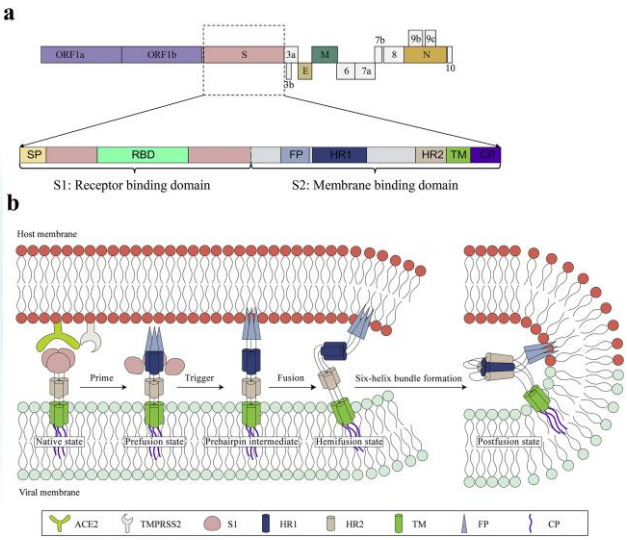
SARS-CoV-2 Entry Mechanism

- The extended spike folds back onto itself, like a zipper, forcing the viral and cell membranes to fuse.
- The virus then ejects its genome into the cell.



doi: 10.1038/d41586-021-02039-y.

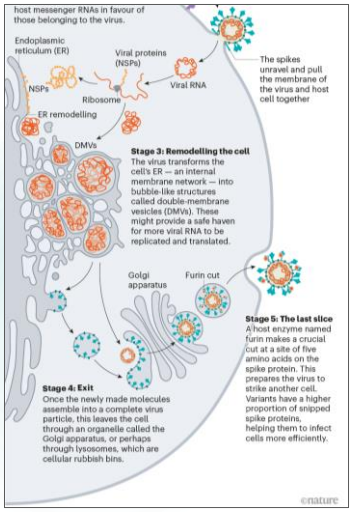
SARS-CoV-2 Entry Mechanism



- During fusion with the host, the S1 and S2 subunits separate. The S2 subunit forms a 6-HB, with a HR1 and HR2 region that fuses with the host cell membrane.

https://doi.org/10.1146/annurev-pharmtox-061220- 093932
https://doi.org/10.1016/j.apsb.2021.06.016

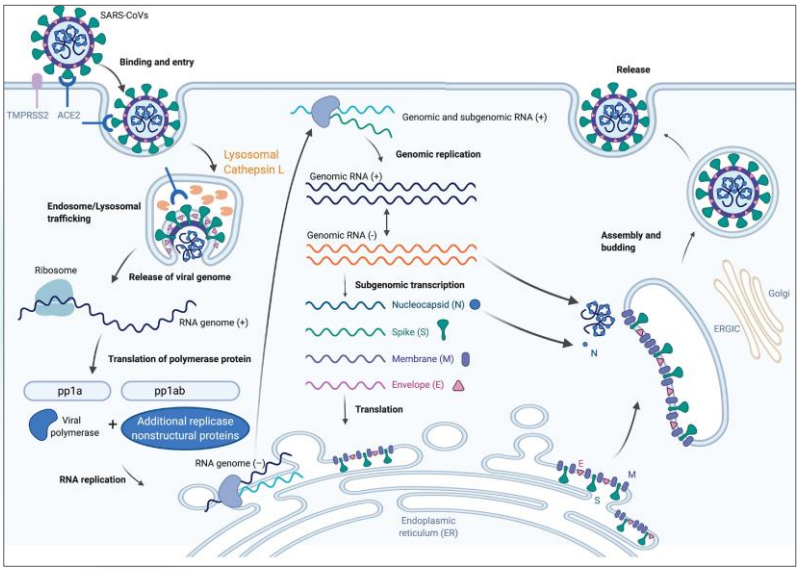
SARS-CoV-2 Translation



- ✓ On the inside of the cell, SARS-CoV-2 transforms the ER into DMVs providing a safe place for replication and translation.
- ✓ Proteins involved in making DMVs could be good drug targets.

doi: 10.1038/d41586-021-02039-y.

SARS-CoV-2 Translation



- ► Ribosomes translate viral RNA.
- ▪ Papain-like protease (PLpro)
- ▪ 3C-like protease (3CLpro, Mpro)
- ▪ RNA- dependent RNA polymerase (RdRp)

https://doi.org/10.1016/j.it.2020.10.004
doi: 10.1038/d41586-021-02039-y.
https://doi.org/10.1038/s41392-021-00733-x

SARS-CoV-2 Translation

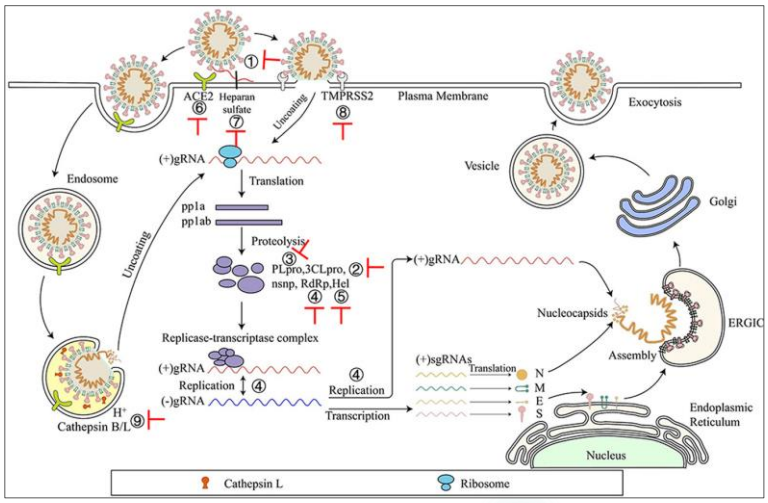
- Three mechanisms by which SARS-CoV-2 suppresses the translation of host mRNA in favour of its own:

1. 1. Nsp1 recruits host proteins to chop up all cellular mRNAs that don't have a viral tag.
2. 2. Reduces overall protein translation in the cell by 70%. Nsp1 physically block the entry channel of ribosomes so mRNA can't get inside.
3. 3. The virus shuts down the cell's alarm by preventing cellular mRNA from getting out of the nucleus.

doi: 10.1038/d41586-021-02039-y.

Therapeutic Targets

- **S-protein** and **Nsps**.
- Protease inhibitors: prevent a virus from using **TMPRSS2**, **cathepsin L** or other **proteases** to enter host cells.
- Absence of a human homolog make **Mpro** an attractive target.



https://doi.org/10.1016/j.apsb.2021.06.016
https://doi.org/10.1146/annurev-pharmtox-061220- 093932

Targeting Viral Entry via the Endosomal Pathway

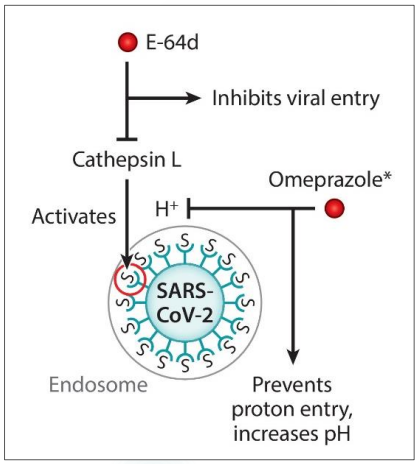
Drugs targeting endosome acidification:

- **HCQ, CQ, and ammonium chloride**: inhibit viral replication by increasing lysosome pH.

  Questionable benefit.

- **Omeprazole**: interferes with lysosomal activity and inhibits double-stranded RNA formation.

  Combinations.

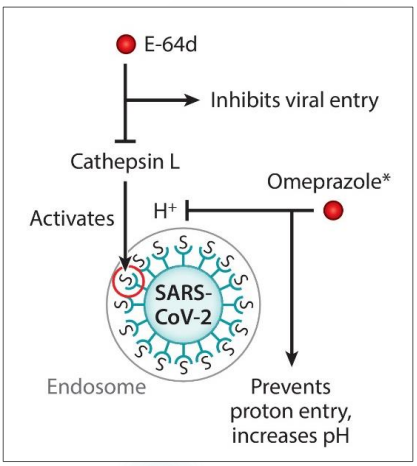


https://doi.org/10.1146/annurev-pharmtox-061220- 093932

Targeting Viral Entry via the Endosomal Pathway

Drugs targeting cathepsin:

- **E64d:** a broad-spectrum inhibitor, targets lysosomal cathepsin.
- In combination with camostat mesylate could fully block viral entry.



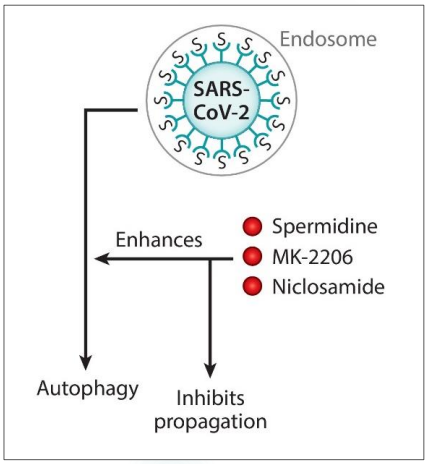
https://doi.org/10.1146/annurev-pharmtox-061220- 093932

Targeting Viral Entry via the Endosomal Pathway

Drugs targeting the autophagy pathway:

- **Spermidine**: induces autophagy.

  Questionable
- **MK-2206**: Inhibit of AKT1→upregulates Beclin-1 and promotes autophagy. MK-2206 reduced SARS-CoV-2 propagation by 88%.
- **Niclosamide**: an orally bioavailable, chlorinated salicylanilide. It inhibits SKP2, stabilizes Beclin-1, and enhances autophagy. It also blocks endosomal acidification.

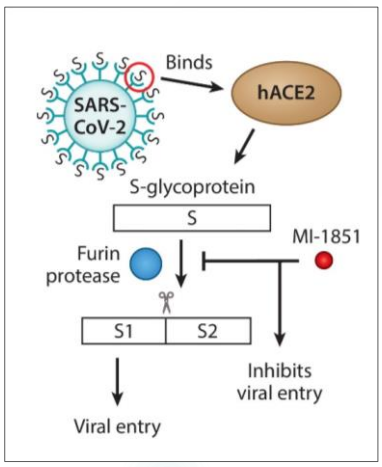


https://doi.org/10.1146/annurev-pharmtox-061220- 093932

Targeting Viral Entry Through the Plasma Membrane Fusion Pathway

Drugs targeting furin and TMPRSS2 proteases:

- **MI-1851**: a synthetic furin inhibitor potently inhibits S-glycoprotein cleavage.
- Reduced viral titers by 30- to 75-fold.

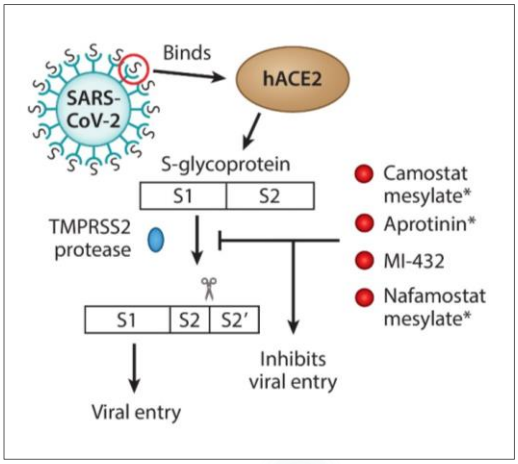


https://doi.org/10.1146/annurev-pharmtox-061220- 093932

Targeting Viral Entry Through the Plasma Membrane Fusion Pathway

Drugs targeting furin and TMPRSS2 proteases:

- **Camostat mesylate/Nafamostat mesylate** : approved TMPRSS2 inhibitors.
- **Aprotinin**: shows a high antiviral effect. Also interferes with formation of dsRNA.
- **MI-432**: a synthetic peptide mimetic inhibitor of TMPRSS2
- Combination of MI-432 and MI-1851 has an increased anti-SARS-CoV-2 activity.

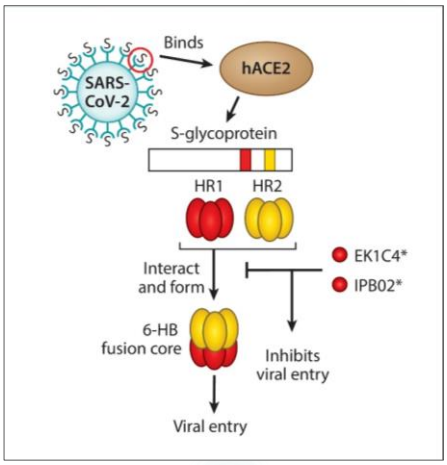


https://doi.org/10.1146/annurev-pharmtox-061220- 093932

Targeting Viral Entry via the Endosomal Pathway

Drugs targeting HR1 of S2 subunit of S-glycoprotein:

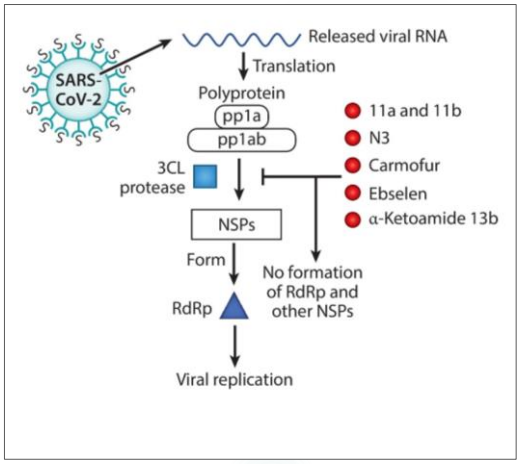
- **EK1C4**: disrupt formation of the 6-HB fusion core by binding to the HR1 domain.
- Modification of EK1 with a cholesterol moiety, EK1C4 forms a more stable complex with HR1, which enhances its antiviral activity (149-fold).
- **IPB02**: lipopeptide fusion inhibitor that targets the HR1 region.



https://doi.org/10.1146/annurev-pharmtox-061220- 093932

Targeting the Main Protease (Mpro)

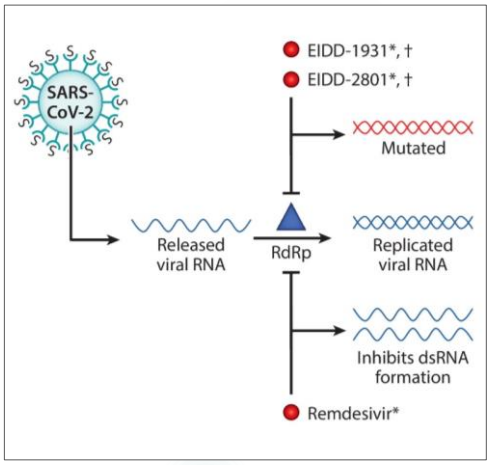
- **11a and 11b:** covalently binds to the catalytic site Cys145 and blocks enzymatic activity.
- **N3:** binds irreversibly in the Mpro substrate recognition pocket.
- **Ebselen and carmofur:** Covalently bind to the catalytic Cys145.
- **α-Ketoamide 13b:** block Mpro by 2 hydrogen bonding interactions.



https://doi.org/10.1146/annurev-pharmtox-061220- 093932

Targeting Viral Replication

- **Remdesivir**: potently stops viral replication activity via RNA chain termination.
- **NHC (EIDD-1931):** potent antiviral activities against RNA virus replication: Ebola virus, SARS-CoV, and MERS-CoV.
- NHC exhibits only a low level of resistance with multiple viruses.
- **EIDD-2801**: an isopropyl ester prodrug of NHC with improved oral bioavailability and pharmacokinetics in vivo.



https://doi.org/10.1146/annurev-pharmtox-061220- 093932

Conclusions

- Despite global efforts, COVID-19 remains a serious concern. Although many clinical trials of the repurposed drugs, immune-based therapies and investigational antivirals have been conducted, there is still no highly effective therapeutic available. The mutation of SARS-CoV-2 make vaccine and drug discovery more uncertain. Accordingly, developing specific or broad-spectrum inhibitors is urgently needed.
- The outbreak of COVID-19 has highlighted the importance for the development of broad-spectrum antiviral agents to combat future coronaviruses.

Thank You and Questions