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OVERVIEW OF ANTIVIRAL DRUGS AGAINST SARS-CoV-2 FOR THE TREATMENT OF COVID-19

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The new coronavirus SARS-CoV-2 has quickly spread all over the globe causing a global pandemic. The intrinsic properties of the virus make it potentially fatal, as COVID-19 disease severity varies among individuals with currently unknown background. The world community is thus in a desperate need for novel antiviral drugs against SARS-CoV-2. In this work, we focus on macromolecular targets present on SARS-CoV-2 virus as potential targets for antiviral drugs. In the continuation, we offer a brief presentation of drugs or drug candidates that act directly on virus life cycle and have promising effects in COVID-19 therapy.

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Key words: *antiviral drugs, SARS-CoV-2, COVID-19, coronavirus*

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Introduction

The new coronavirus SARS-CoV-2 is a beta-coronavirus that contains a single RNA strand surrounded by a nucleocapsid and enveloped in a phospholipid membrane envelope (1). The virus obtains the membrane by budding from human cells, so it is very similar in structure to the human cell membrane. Structural glycoproteins are embedded in the membrane, the most well-known of which are S-shaped proteins in the form of spikes (1). Protein S binds to the enzyme angiotensin converting enzyme type 2 (ACE2), which is present on the membrane surface of host lung epithelial cells (2). In addition to this important structural protein S, SARS-CoV-2 also contains the next structural proteins:

- N (nucleocapsid),
- E (envelope) and
- M (membrane).

The SARS-CoV-2 virus also contains sixteen nonstructural proteins, some of which have enzymatic function, such as: RNA-dependent RNA polymerase (RdRp), the major cysteine 3C-Like protease (3CLpro), and the papain-like protease PLpro (2, 3). All of these macromolecular targets provide an excellent basis for drug design with action against the SARS-CoV-2 virus. Research is most intensive in the direction of the development of 3CLpro protease and RdRp RNA polymerase inhibitors, for which the crystal structures that enable structure-based drug design are known (4).

The rapid development of potential antiviral agents is possible due to the high similarity of SARS-CoV-2 with SARS-CoV and MERS-CoV (5). This similarity allows us to test a large number of known compounds with proven activity on SARS-CoV and MERS-CoV in a short time, thus already identifying potential antiviral agents that are in preclinical and clinical stages of development (6). The interaction between protein S and ACE2 is also crucial for the detection of antiviral agents and vaccines, as this is the initial contact that allows the virus to enter the host cell (1). The rapid pace of research and the accelerated evaluation of promising compounds in clinical trials give us hope that a safe and effective drug and/or vaccine against COVID-19 will soon be available. In the following, we will present key compounds with antiviral activity, especially their mechanism of action, while the results of clinical trials of the most promising compounds is out of the scope of this brief review.

Antiviral drugs

Due to the pandemic dimensions of SARS-CoV-2 virus infections, research into the development of antiviral agents for the treatment of COVID-19 is very intensive. As drug development is a very time-consuming process that can take more than ten years, in the current situation it is crucial to

identify and evaluate the active substances or candidates who are or have been in clinical trials. Numerous safety data and a side effect profile are known for these compounds, and safety and efficacy in the treatment of patients with COVID-19 can be evaluated. This process of detecting active substances is called "drug repurposing" or "drug repositioning" (7), which means that the safety of these active substances for an indication other than the one for which it was previously registered is known. Of course, there is a fear that none of the already known active substances will be successful in clinical trials for the treatment of COVID-19, so the discovery and development of new compounds is crucial. To start with a new drug discovery and development, one has to identify macromolecular targets involved in the different phases of the SARS-CoV-2 virus life cycle that can be interfered/inhibited with drug molecules (Figure 1).

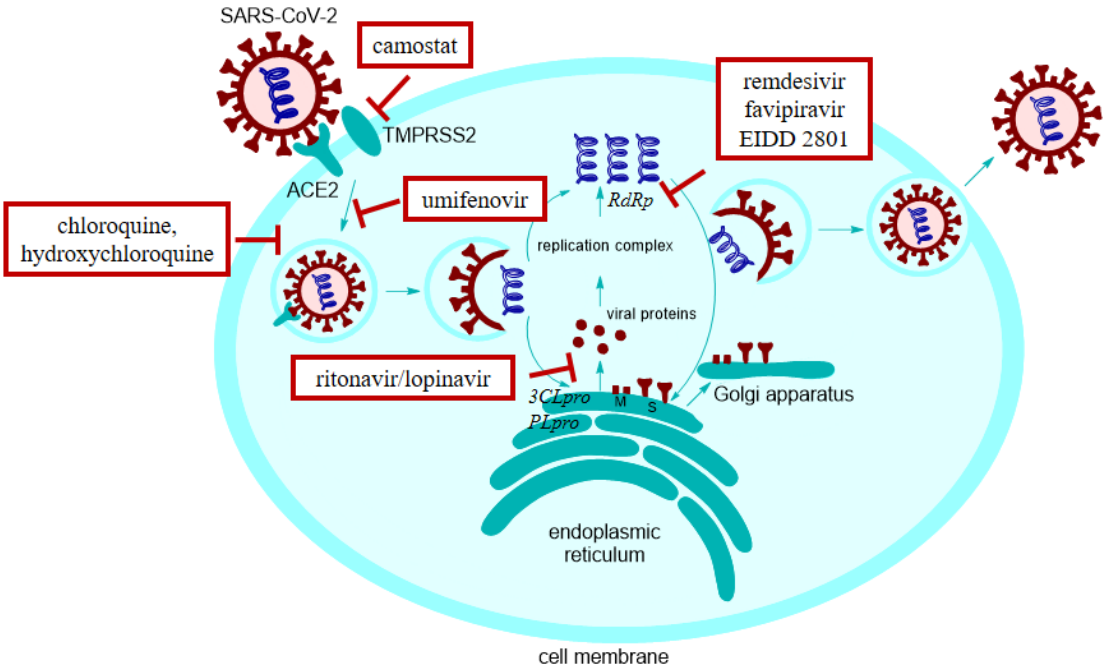

Figure 1. Targets of drugs displaying antiviral activity against SARS-CoV-2

Viral entry inhibitors

In preventing the viral entry into the host cell, one can act on both viral proteins and host cell proteins, or we can inhibit endocytosis by changing the pH in the endosomes.

Camostat

Camostat (Figure 2) acts as a serine protease inhibitor that has been registered in Japan in 2006 for the treatment of chronic pancreatitis. It also acts as a transmembrane serine protease 2 (TMPRSS2) inhibitor, which plays a key role in the entry of SARS-CoV-2 into the host cell. TMPRSS2 is responsible for the cleavage and activation of the SARS-CoV-2 protein S, which binds to the ACE2 receptor of the host cell. A study performed on the Calu-3 cell line displayed that inhibition of TMPRSS2 by camostat can prevent the entry of various coronaviruses such as SARS-CoV, MERS-CoV and SARS-CoV-2, and also reduce cell infection with SARS-CoV-2 (2). Based on this study, camostat was proposed as a potential active ingredient for the treatment of COVID-19, but the results of clinical studies are not yet known.

Umifenovir

Umifenovir (Figure 2), also known as arbidol, is a broad-spectrum antiviral compound used to treat influenza in Russia and China. The mechanism of action of umifenovir is multifaceted, describing both direct virucidal activity and its influence at different stages of the viral cycle, such as virus entry into the host cell and replication. Umifenovir is a hydrophobic and weakly basic compound that incorporates into membranes due to its amphiphilic structure. Direct antiviral activity is thus associated with interaction with aromatic amino acid residues of structural viral glycoproteins and/or with the viral lipid envelope (8). X-ray crystallography has also shown that umifenovir binds to influenza virus haemagglutinin, thus preventing its conformational

change required for membrane fusion and thus virus entry into the host cell (9). As some active substances for the treatment of influenza were also used in China in the early stages of COVID-19, the antiviral activity of umifenovir, baloxavir, laninamivir, oseltamivir, peramivir and zanamivir against SARS-CoV-2 was investigated *in vitro*. Among these, umifenovir showed the strongest activity as it was found to prevent the binding of SARS-CoV-2 to the host cell and also affect the release of the virus from intracellular vesicles formed after the virus enters the cell (10). The results of a small clinical study involving 86 patients with mild to moderate COVID-19, of whom 34 received lopinavir/ritonavir, 35 umifenovir, and 17 no antiviral therapy, showed that umifenovir monotherapy had no significant effect on course of the disease (11).

Chloroquine and hydroxychloroquine

Chloroquine and hydroxychloroquine (Figure 2) are orally active drugs that have been used for many years in the prophylaxis and treatment of malaria. Hydroxychloroquine also has immunomodulatory activity and has been granted marketing authorization for the treatment of rheumatoid arthritis, porphyria and generalized lupus erythematosus. Both active substances are also known to act against certain RNA viruses, with hydroxychloroquine having stronger antiviral activity against SARS-CoV-2 (12). The mechanism of action of these two drugs is multifaceted. Chloroquine and hydroxychloroquine are known to raise pH in intracellular organelles such as endosomes and lysosomes due to their basic properties. Because acidic pH in endosomes is a prerequisite for fusion of the viral membrane with the lysosome membrane, they prevent SARS-CoV-2 from entering the host cell. Chloroquine can further prevent the virus from entering the cell also through its effect on the posttranslational glycosylation of the ACE2 receptor and SARS-CoV-2 S protein. Chloroquine also acts as an ionophore for zinc ions, which at higher intracellular concentrations reduce the biosynthesis of viral RNA-dependent RNA polymerase. In particular, hydroxychloroquine additionally has an anti-inflammatory effect by reducing the expression of TNFα, interleukin-1β, interleukin-6 and reducing the activation of the MAPK kinase signalling pathway, which could have a beneficial effect on the course of COVID-19 in the second stage before cytokine storm (13).

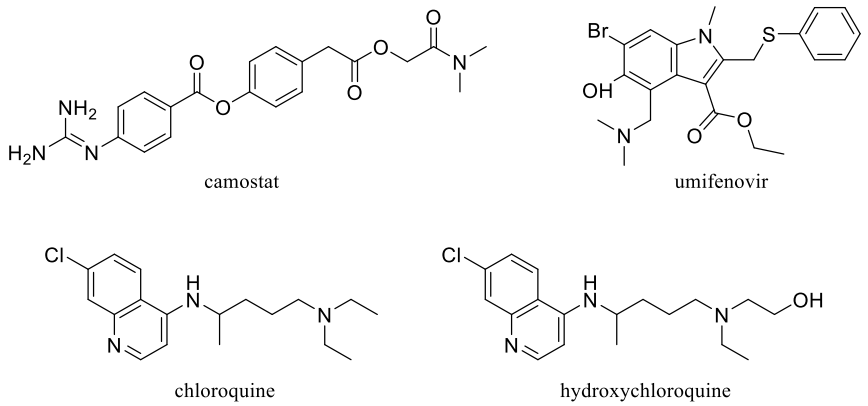

Figure 2. SARS-CoV-2 host cell entry inhibitors

SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) inhibitors

RdRp plays a key role in the replication of SARS-CoV-2 virus and is therefore an important target for the detection of antiviral agents. RdRp from SARS-CoV-2 and SARS-CoV have more than 95% amino acid sequence similarity, which points to a rather high likelihood of high success rate in testing a wide range of compounds that have previously shown activity against SARS-CoV as well as other RNA viruses (14, 15).

Favipiravir

Favipiravir (T-705, Figure 3) is an antiviral drug discovered by screening chemical libraries. It works by selective inhibition of RdRp. A more precise mechanism of action reveals that it is a prodrug, for favipiravir is only phosphoribosylated to the active form, favipiravir ribofuranosyl-5'-triphosphate, by the cell enzyme within the cell, which is then recognized as a RdRp substrate (false purine nucleotide), leading to RNA-polymerase inhibition (16, 17). The incorporation of false nucleotides into viral RNA results in its high rate of mutations creating an inanimate viral phenotype (18). Because the catalytic domain of RdRp is preserved among many types of RNA viruses, favipiravir has broad-spectrum antiviral activity (16). It is effective not only against many types and subtypes of influenza virus, including the bird flu subtype, but also other RNA viruses such as arenaviruses, hantaviruses, bunyaviruses, noroviruses, and filoviruses that cause hemorrhagic fever

(16, 18, 19). Due to its unique mechanism of action, favipiravir is also a promising active substance in the fight against other RNA viruses, such as SARS-CoV-2.

Favipiravir has been registered in Japan since 2014 for the treatment of influenza, but only for individual virus subtypes and in specific disease circumstances, and has also been studied in the treatment of Ebola (19). Favipiravir is teratogenic requiring extra caution in use. However, in clinical trials to date it has shown a favourable side effect profile with mild gastrointestinal problems and asymptomatic uric acid elevations, and the potential for oral treatment and the relatively low risk of drug interactions represent additional benefits of the drug (20). Several clinical studies are currently underway to investigate the efficacy of favipiravir as a stand-alone or combination treatment for COVID-19.

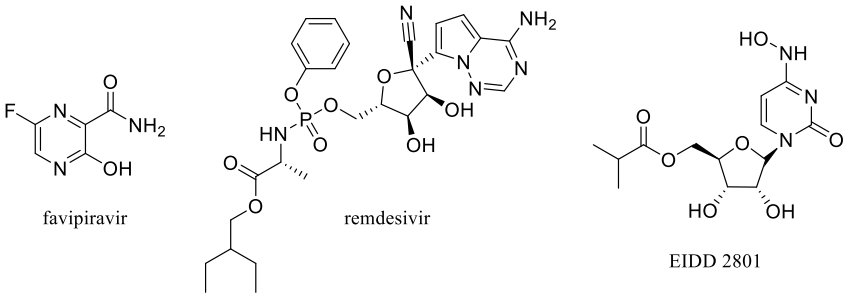

Figure 3. RNA-dependent RNA polymerase inhibitors with antiviral activity against SARS-CoV-2

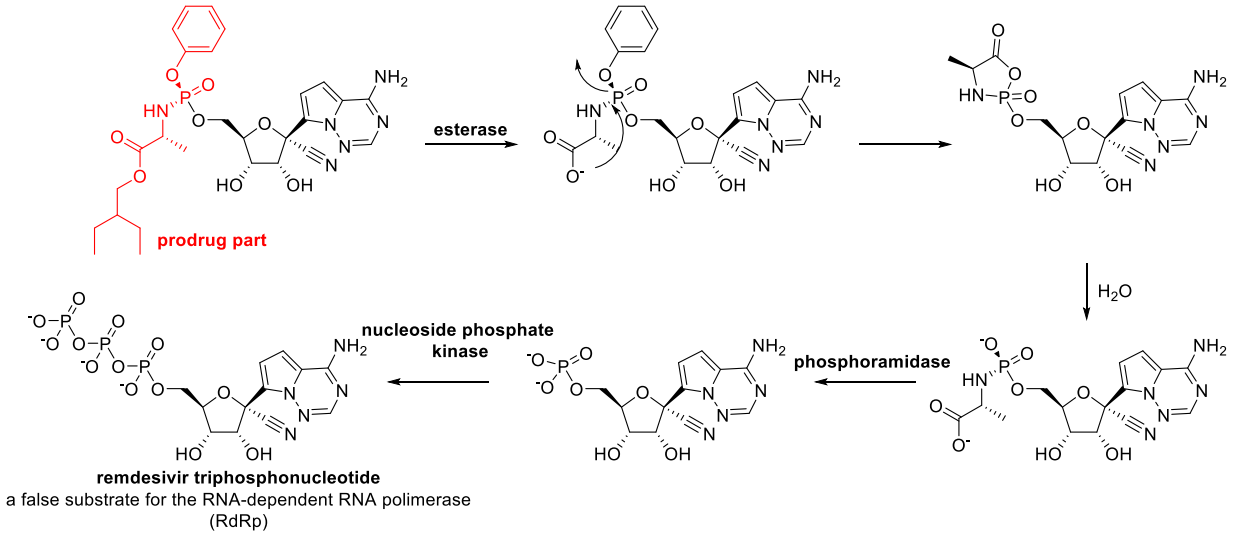

Figure 4. Bioactivation of remdesivir

Remdesivir

Remdesivir (GS-5734, Figure 4) has been developed for the treatment of Ebola virus infections but its use is not yet authorized (21, 22). It is a phosphoramidate prodrug of an adenosine C-nucleoside, which is converted into active species by a mechanism similar to that of sofosbuvir: liver esterases and tissue phosphoramidases convert the corresponding phosphoramidate prodrug to 5'-monophosphate. The latter is converted to triphosphate in cells (Figure 4), which in turn inhibits the activity of viral RdRp. The active species competes with ATP for incorporation into RNA and interferes with control reading by introducing mutations into the viral genome (23, 24). It thus slows or inhibits viral RNA synthesis. This mechanism suggests the possibility of action against a wide range of viruses with RNA as hereditary material. In *in vitro* human cell studies, remdesivir has been shown to be effective against a variety of RNA viruses, including SARS-CoV, MERS-CoV, and SARS-CoV-2. In experimental

animal studies, remdesivir inhibited the replication of Ebola virus in rhesus monkeys and SARS-CoV virus in mice with early initiation of treatment. Remdesivir is one of the most promising compounds for the treatment of COVID-19 according to the results of clinical trials and has first been available for compassionate use in patients who are not eligible for inclusion in clinical trials. Very recently, remdesivir was approved by the American Food and Drug Administration for COVID-19 treatment based on the results from three clinical trials (25).

EIDD 2801

EIDD 2801 (Figure 3) is an orally administered prodrug with favourable pharmacokinetic properties that is in the preclinical stage of development and has a broad spectrum of action against viruses with the RNA genome (26). Isopropyl ester EIDD 2801 is hydrolyzed to β-D-N4-hydroxycytidine after ingestion, which is then converted to the corresponding N-hydroxycytidine triphosphate. The latter inhibits coronaviruses' RdRp including that of SARS-CoV-2, and thus interferes with the "normal" viral RNA replication. Once incorporated into RNA, it does not act as a chain breaker, but triggers the formation of a very large number of viral RNA mutations, which in translation leads to defective, non-functional viral proteins, thus achieving antiviral activity. The potential for the development of resistance to EIDD 2801 is reported to be very low (27). One of the important features of EIDD 2801 is that in the case of MERS-CoV it acts on remdesivir-resistant MERS-CoV. However, because mutated amino acids (F480L and/or V557L) are retained in the RdRp of most coronaviruses, they also represent the potential for the development of remdesivir resistance in SARS-CoV-2 (27). Nevertheless EIDD 2801 is a promising compound for further development and possibility of combination therapy with remdesivir in the event of a clinically relevant development of SARS-CoV-2 resistance to remdesivir. The promising antiviral activity of EIDD 2801 *in vitro* and *in vivo* indicates the great potential of this compound for the treatment of COVID-19, and we await the results of planned clinical studies to demonstrate its safety and efficacy.

Coronavirus 3C-Like protease inhibitors

Lopinavir/ritonavir

The active substances lopinavir and ritonavir (Figure 5) are inhibitors of HIV-1 aspartate protease and are used in the treatment of HIV infections. Lopinavir was developed as an optimized ritonavir analogue with action on ritonavir-resistant HIV-1. The active substances are used in combination, as ritonavir acts as a cytochrome P450 inhibitor, resulting in higher plasma concentrations of lopinavir (28). In SARS-CoV-2, the active substances are thought to inhibit the coronavirus cysteine 3C-Like protease (3CLpro), thereby preventing the cleavage of viral polyprotein into functional proteins and achieving antiviral activity.

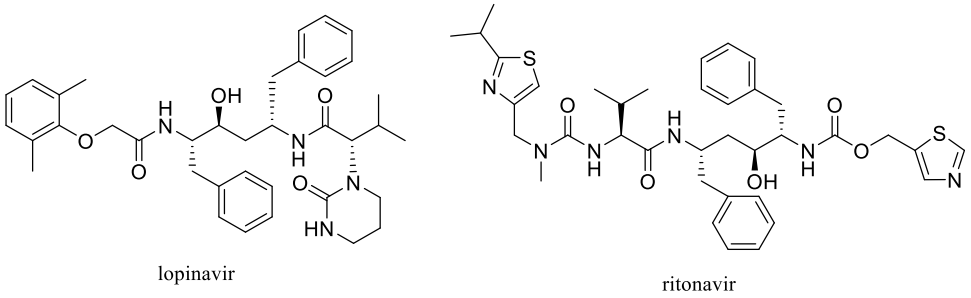

Figure 5. SARS-CoV-2 cysteine protease 3CLpro inhibitors

Interferons

Interferons are a group of cytokines secreted by many types of host cells in response to the invasion of pathogens, including the SARS-CoV-2 virus, and thus regulate the immune system response. The released interferons protect the cells from which they are released as well as the cells in their immediate vicinity from the attack of viruses. Interferons α and β, which are classified as type I interferons, play an important role in viral infections. They act by inducing the production of protein kinase R, which non-specifically inhibits protein synthesis, and RNase L, which degrades cellular and viral RNA. This results in a reduction in the production of new copies of the virus. Interferons also stimulate the expression of the major histocompatibility complex type I, thereby stimulating the action of cytotoxic T88 + lymphocytes, activating natural killer cells, and inducing dendritic cell maturation. Because coronaviruses including SARS-CoV-2 carry the genetic record for proteins that indirectly inhibit the production of type I interferons and thus have an immunosuppressive effect, type I interferons represent promising candidates for the treatment of COVID-19 (29, 30).

Conclusion

The detection of potential candidates for the treatment of COVID-19 is very intensive in both preclinical and clinical stages of development, targeting different stages of the life cycle of the SARS-CoV-2 virus. The true potential of the presented drug compounds for the treatment of COVID-19 will be known only after the results of randomized clinical trials such as SOLIDARITY and DisCoVeRy are revealed, which are currently underway in a large number of patients. The rapid progress in the discovery of treatment for COVID-19 resulted in the approval of remdesivir for the treatment of a subgroup of COVID-19 patients. Last but not least, the preclinical development of new structural types of compounds and compounds with new mechanisms of action for the successful fight against coronaviruses, not only SARS-CoV-2, is also very important for the future antiviral therapy.

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PREGLED ANTIVIRUSNIH LEKOVA PROTIV SARS-CoV-2 VIRUSA ZA LEČENJE COVID-19 BOLESTI

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Novi koronavirus SARS-CoV-2 brzo se proširio svetom, uzrokujući globalnu pandemiju. Karakteristična svojstva virusa čine ga potencijalno fatalnim, jer težina bolesti COVID-19 varira među osobama, sa trenutno nepoznatom pozadinom. Svetska zajednica stoga očajnički traga za novim antivirusnim lekovima protiv SARS-CoV-2 virusa. U ovom radu, fokusiramo se na makromolekularne ciljeve prisutne na virusu SARS-CoV-2, kao i na potencijalne ciljeve za antivirusne lekove. U nastavku nudimo kratku prezentaciju lekova ili kandidata za lekove, koji deluju direktno na životni ciklus virusa i imaju obećavajuće efekte u terapiji COVID-19 bolesti.

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Ključne reči: *antivirusni lekovi, SARS-CoV-2, COVID-19, virus korona*