Micar Innovation
Drug Discovery Factory

# Micar21 Scientific Approach

In this project we are going to repurpose our CCR5/CCR7 and SLC6A5 proprietary inhibitors and to discover new ACE2 allosteric binders for the treatment of COVID-19.

Micar Innovation (Micar21) addresses the urgent unmet needs of patients with cardiovascular disease. At the moment, one of the most urgent unmet needs is treatment for COVID-19 as it has been demonstrated that patients with cardiovascular diseases are at increased risk of death due to COVID-19. Our innovation will benefit all patients affected by COVID-19 and other related coronaviruses.

Micar Innovation (Micar21) is a **drug discovery "factory"** and in the last years we have discovered and pre-clinically developed inhibitors of specific protein targets for the treatment of different diseases. For instance, we have already identified a nanomolar range inhibitors of the Glycine 2 transporter[1], AKT1 binders[2], PPARγ[3] and others. Moreover, we also developed an **improved in silico virtual screening** platform to identify new hits [4] and also improved one of the most sophisticated computational approaches during the lead optimization phase - FEP+[5]. **In this project, we are going to**:

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**- repurpose our proprietary CCR5/7 and *SLC6A5* molecules;**

**- discover and optimize by the aforementioned approaches new ACE2 allosteric ligands;**

**- develop them as treatment for COVID-19.**

## Aims and Objectives:

Our overall aim is to discover and develop treatment for COVID-19. For this we have identified the following objectives:

1. To test the activity of our proprietary and also newly identified inhibitors against COVID-19 in vitro;
2. To perform the hit-to-lead FEP+ guided optimization and the preclinical development of the potential hit COVID-19 inhibitors via iterative rounds of in vitro, in silico and in vivo testing in alternative animal models in order to select the most promising molecules with which to proceed to in vivo proof-of-concept;
3. To provide in vivo proof-of-concept and validate the in safety and efficacy of 3 lead candidates in vivo;
4. To start the negotiations for an early-stage licencing deal with a reliable partner who can perform the clinical development of the leads;

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5. Should the latter turn impossible, to grow the company into a clinical development company and perform the clinical development of the leads and enter into a licensing deal with a pharma company.
We have the support of Bulgarian Academy of Sciences, University of Medicine in Plovdiv, Bulgaria, The Ministry of Economy in Bulgaria and other major organizations and technology clus (see letters of support) who will support us throughout the development of the inhibitors into a COVID-19 treatment by providing advice, access to infrastructure and resources.

## Addressed challenges:

By this project and aiming at bringing our innovation to the market we will address the multiple layer of challenges presented by the COVID-19 to society – growing number of infected patients (close to 200 00 at the time of submission), high mortality (more than 6% in Italy), lack of treatment, rapid spread (from 1 to close to 200 000 cases worldwide in less than 3 months; the peak in Europe and USA is yet to come), huge impacts on the economy and daily life of everyone worldwide.

## Background of the innovation:

There are seven known types of coronavirus (CoVs), which include 229E and NL63 (Genfa Alphacoronavirus), OC43, HKU1, MERS and SARS (Genus Betacoronavirus). While 229E, NL63, OC43 and HKU1 typically infect the human population, SARS and MERS epidemics in 2002 and 2012 respectively were results from transition of the virus from animals to humans, raising significantly the mortality rates of the diseases. The recent 2019-nCoV (COVID-19 ) pathogen that has emerged in China has been characterized as a new member of the Betacoronavirus genus, closely associated with several types of coronavirus in bats, as well as a severe acute respiratory syndrome coronavirus (SARS-CoV). Compared to SARS-CoV, COVID-19 appears to be more easily transmitted from person to person, and has spread across multiple continents, leading to the WHO's Global Health Emergency Declaration on Novel Coronavirus, followed by a pandemic status, all of which reflecting the impact of the virus as an international problem.

**As a vaccine is not available and the number of patients worldwide is growing by hours, a treatment is urgently needed.**
The complete structure of COVID-19 was obtained literally a couple of weeks ago by cryogenic electron microscopy and published on March 4th, 2020. This provides many new opportunities for scientists to create vaccines and therapeutics. Based on this structure and many other research it is clear now that the virus entrance into the cell is guided by the interactions of the virus S1-spike protein receptor binding domain (RBD) and the Angiotensin II receptor (ACE2);

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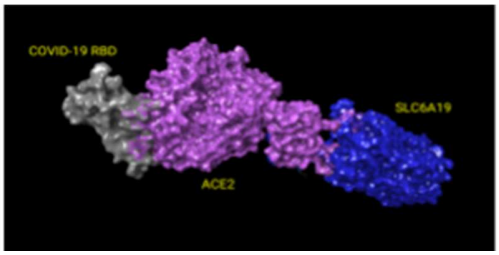
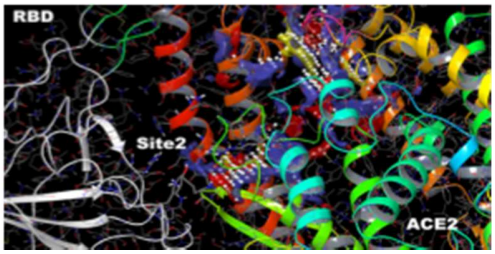
A)




B)




**Figures 1A and 1B. Several cleavage proteins, such as for example TMPRSS2, are also involved in this system as it has been shown some days ago[6]. However, the inhibition of TMPRSS2 is not the best drug design approach because the ACE2 cleavage can be done also by Furin and 6 other proteins. On the other hand, the B0AT1 neutral transporter (SCL6A19) plays also a key role restricting the cleavage process thus is an attractive indirect drug target.** It is also clear that the change in ACE2 enzymatic activity cannot restrict the S1-RBD-ACE2 interactions and it cannot be inhibited even with a strong (0.55nM) inhibitor. **Thus, only a small molecule that binds to the ACE2 pocked located close to the S1-RBD can effectively inhibit the RBD-ACE2 interactions and is the best approach to stop the virus entrance**. ***Currently, despite the urgent need there are no known binders for this pocket.*** Indeed, the immune response is also very important and as it has been shown that B and T cell epitopes for SARS-CoV-2 are present too[7]. As for these cells the CCR5 and CCR7 receptors are crucial factors for their migration and have a role in inflammatory responses to infection, **CCR5 and CCR7 receptors are also an attractive target in the treatment of COVID-19 infected patients**.

*In summary, we suggest a 3-way approach to stop the COVID-19 cell entrance and response via inhibition of:*
*- CCR5/7;*
*- SLC5A19;*
*- most importantly the ACE2 allosteric site, which is close to the virus S1 spike receptor binding domain.*

**Our proprietary molecules and rationale for their re-purposing into COVID-19 treatment**

***Discovery of the first class small molecule S1-RBD-ACE2 inhibitors***
CardioMol Ltd. is part of Micar Innovation (Micar21). Micar Innovation (Micar21) has a strong track record in the development of drug molecules that bind to the so-called chemokine receptors, in particular CCR2, CCR5, CCR6, CCR7, and CCR9 receptors. Based on the aforementioned and previous scientific reports for the COVID-19 immune response, Micar Innovation scientists have redirected their efforts from developing a potent CCR7 antagonist to one that has dual action, including inhibition of the CCR5 receptor; e.g dual CCR5/7 antagonist. **Micar Innovation (Micar21) has discovered and will obtain next week patent for a drug candidate that is a good CCR5/7 antagonist**. The compound showed an antagonist activity with IC50 values of 980 nM and 1.1 uM for CCR7

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and CCR5, respectively **(Figure 2)**. In this project, we are going to test our proprietary series based on the scaffold of the CCR5/7 antagonist against the coronavirus in vitro and if we see inhibition of the virus in vitro we will start a pre-clinical development program based on this scaffold with our improved pre-clinical drug discovery platform. A FEP+ guided hit-to-lead optimization will be also performed increasing both the activity and selectivity and pharmacology profile as well. This is our first approach to indirectly influence the virus action and improve the immune response.

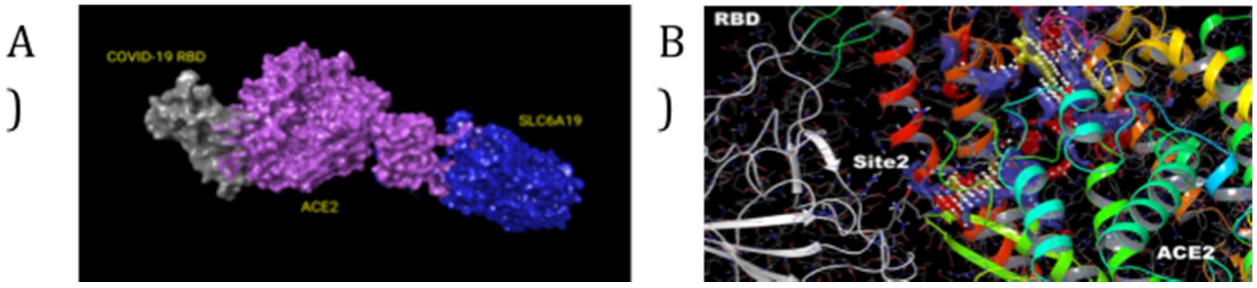


**Figure 1.** ***(A)*** **Structure of Covid-19 S1-RDB, ACE2 and SLC6A19 complex.** ***(B)*** **The interaction surface between S1-RDB (withe color) and ACE2. The allosteric site, Site2, is shown. White dots, blue, red and yellow areas are occupance, donor, acceptor and hydrophobic parts as they were identified by Micar21 scientists.**

Furthermore, besides chemokine receptors immune response, it was demonstrated that COVID-19 uses another membrane protein for cellular entry, namely the angiotensin-converting enzyme 2 (ACE2). There are numerous approved drugs that inhibit homologous ACE protein. They are intended primarily to treat cardiovascular disease and high blood pressure and are not selective for ACE2, therefore cannot be used to treat viral infections. Moreover, a couple of days ago, there was an urgent report stating that people taking ACE inhibitors should immediately cease reception, because inhibiting ACE leads to an increase in ACE2 protein levels 3 to 5-fold, thus increasing virus entry into cells, which leads to increased mortality rates among these patients. **Therefore, based on the structure of the virus already obtained, a specific inhibitor should be developed that only interferes with/inhibits the interaction of COVID-19 and ACE2 and does not affect other physiological processes and does not increase the susceptibility of cardiovascular disease patients to COVID-19.** The scientific team of Micar Innovation (Micar21) is committed to find a suitable and specific inhibitor to prevent ACE2 from binding to COVID-19 within 30-45 days, which would be a complete solution for other similar viral infections (such as SARS) as well. We are targeting now the specific allosteric site close to the S1-RBD and ACE2 surface (**see Figure 1B**) and have already identified promising hits by screening database containing over 4 million drug-like chemical compounds. Biological

evaluations are underway in our labs now. These hits will be further modified and optimized using the Micar Innovation (Micar21) Drug Discovery Platform up to the point to be used as a primary drug ingredient.

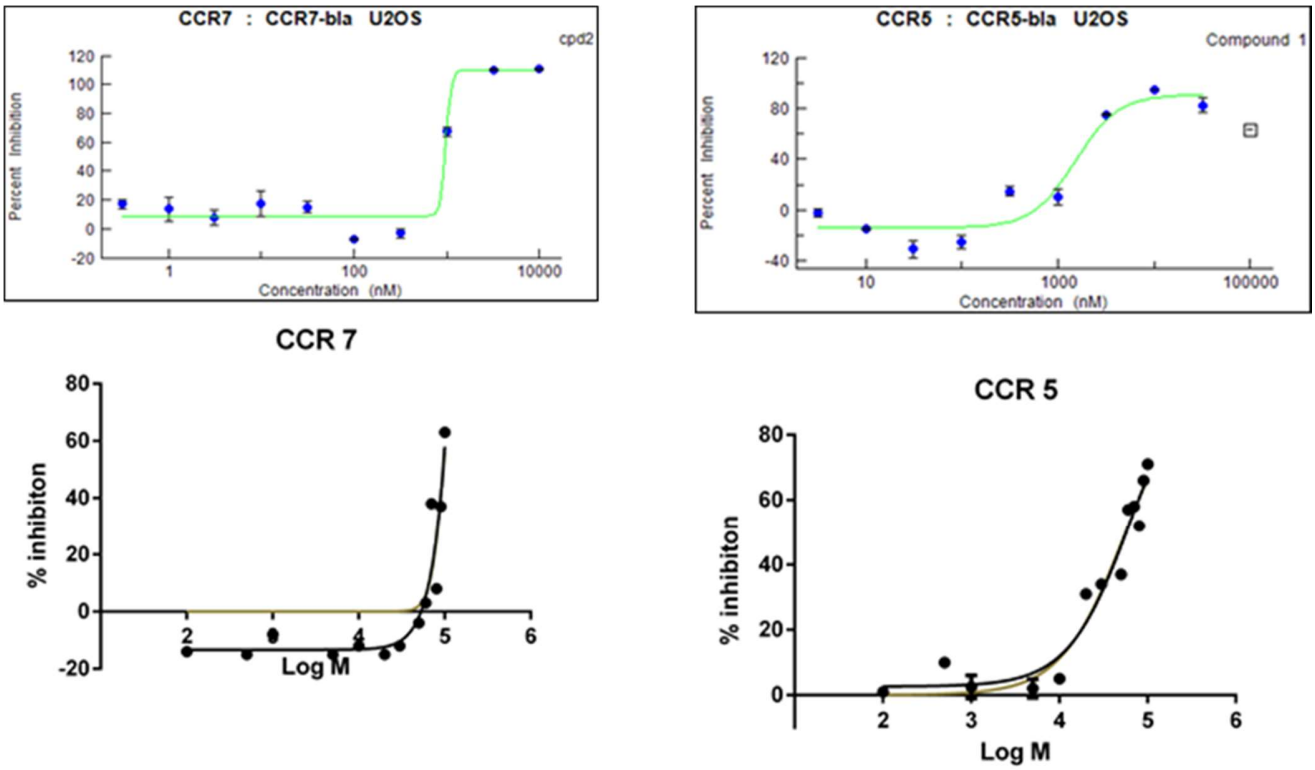


**Figure 2. CCR5 and CCR7 b-arrestin and Ca+ mobilization assays of our hit compound. The assays were performed in Thermofisher and Eurofines companies.**

Finally, as it was already mentioned above the recent research has demonstrated that ACE2 acts in cooperation with the B0AT1 protein, also known as SLC6A19. Currently, ***Micar Innovation (Micar21) has already developed a molecule that binds and inhibits the structural homolog SLC6A14 as well as SLC6A5 (IC50=520nM)*** (ACS Med Chem Lett. 2019 May 22; 10 (6): 904-910). This molecule may also inhibit SLC6A19, if not it can easily be optimized for that purpose. This is our 3th suggestion and an indirect approach in the fight with Covid-19.

## Innovativeness:

### *Competitive advantages*

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Currently there is no treatment for COVID-19 which results in the high mortality rates observed and the rapid spread of the disease worldwide. Joint effort from the EU, WHO, charities such as the Bill Gates foundation resulted in urgent funding calls to address the problem Nevertheless, the expression of interest was not high considering the scope and societal impact of the problem mainly because the world was not expecting or prepared for this challenge. Only 91 proposals were submitted to the urgent EU call in February from which only 17 were funded. Given the unexpected and very recent development of the COVID-19 pandemia, there is scarce information on other innovative solutions or proposals (e.g. the recently approved 17 proposals to the EU urgent call in early 2020. . Treatment for COVID-19 is urgently needed and innovative solutions with potentially positive impact seek funding at any stage of development due the urgency of the problem and any molecule already synthesized and optimized for its ADMET properties (such as the proprietary Micar Innovation (Micar21) molecules) needs to be tested for its activity against COVID-21. This project envisages to explore a number of approaches to identify small molecule hits for antiviral therapy and perform the pre-clinical hit-to-lead development and proof-of-concept in vivo that these inhibitors can treat COVID-19, the disease caused by the most recent coronavirus, by inhibiting specific targets.

## *Other treatments in development*

**Antibodies** – researchers from the Netherlands have discovered a human monoclonal 1 antibody blocking SARS-CoV-2 infection with cross-reactivity to other SARS viruses.[8] They have reported a human monoclonal antibody that neutralizes SARS-CoV-2 (and SARS-CoV). This cross-neutralizing antibody targets a communal epitope on these viruses and offers potential for prevention and treatment of COVID-19 . In fact, in most cases the antibody approaches also target the S1-RBD and ACE2 binding interface.

## *Why now?*

Our innovative approach is technologically possible because the structural basis of the virus recognition by human receptors was revealed literally two weeks ago[9], [10] which allows us to design and perform in silico screenings and identify hits which can be further developed as COVID-19 treatment. COVID-19 treatment is urgently needed as there are currently more than 190 000 cases worldwide and more than 5000 deaths. The number of the infected people is growing rapidly, and the peak of the infection outside of China, including Europe and USA, is yet to come. The mortality rate per country varies, going beyond 7% in Italy[11]. Clearly there is an urgent unmet need for a COVID-19 treatment.

## *Stage of development*

Currently our innovation is at a very early stage of development. We have demonstrated the inhibition of targets related to COVID-19 relevant targets in vitro and have optimized the ADMET properties of our proprietary small molecules. Nevertheless, due to the

urgency of finding a therapy for COVID-19 we seek grant and equity funding to speed up the hit-to-lead development, lead identification, pre-clinical and clinical lead development. Throughout the process, we will seek opportunities for partnership with established pharmaceutical companies and CROs with whom to enter into a licensing agreement and (co-) develop the treatment.

## *Feasibility*

We have performed a preliminary feasibility assessment and we believe that our innovation is feasible because:

- We have access to a unique technology for rapid hit-identification;

- We have all the expertise for rapid hit-to-lead optimization via iterative rounds of in silico, in vitro and in vivo testing, in silico design and organic synthesis;

- We have a committed highly skilled team of professionals with substantial track record and a relevant network of research institutes and laboratories;

- There is an ever pressing urgent demand by the public and governments for devising COVID-19 treatment. In Bulgaria, Micar Innovation (Micar21) achievements and the present project have obtained broad support by major organizations (see Strategic partners below) committed to further enhance and facilitate project progress.

Nevertheless, we have identified potential risk and thought of possible mitigation measures.

**Table 1. Preliminary feasibility assessment**

| Type | Short description | Risk and mitigation plans |
|---|---|---|
| Technological | We have already discovered inhibitors of targets related to targets relevant for treatment of pulmonary infections such as COVID-19 and we have demonstrated the inhibition of these targets by our proprietary molecules in vitro. | Possible risks are that the discovered inhibitors are not active to COVID-19 and or are toxic in vivo. The probability for this is low, as we have used a novel structure-based and computer-aided drug discovery approach which filters out scaffolds which are likely to show poor ADMET properties. We have also performed preliminary ligand-binding mode prediction experiments and we expect our proprietary inhibitors to inhibit also the targets directly relevant to COVID-19. |
| Practical | The company disposes of a small, dedicated team of professionals with a proven track record in | The risk of team members leaving the team is low and we furthermore, mitigate this risk by incentivizing the team members. The risks of |

| | | |
|---|---|---|
| | drug discovery and development and innovation | the team members not being able to meet the requirements of the fast-paced growing of the company we anticipate and the changing needs in the different phases of the drug discovery process, are addressed by clear plans on how to acquire the missing expertise via collaboration with strategic partners nationally and internationally and recruitment of trained staff members (see Implementation and Annex 3. Support letters). |
| Economic feasibility | Drug discovery and development is a costly and lengthy process. COVID-19 pandemic demands for having this process substantially accelerated, by public funding inclusive. | To address the risk of delayed R&D activities due to insufficient funding we have elaborated a strategy for ensuring blended finance, including grant applications, funding from business angels and investors. Furthermore, we will seek support from the Government of Bulgaria, as this is not only an urgent public health issue, but also an opportunity to boost the position of the country as a technological leader in drug discovery and an opportunity to bring the country one level up in the Innovation Scoreboard categorisation - currently Bulgaria belongs to the group of 'modest innovators' and in case of successful R&D and innovation results of the present project this would contribute to shifting to the group of 'moderate innovators'. |
| Regulatory | Drug development is usually a lengthy process also due to the regulatory approvals needed. | The risk of delayed market introduction due to regulatory approvals is addressed by working together with regulatory experts and the Bulgarian government to ensure speedy clearance of experiments, ethical and regulatory approvals, etc. |

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