Binding ability studies of arginine, citrulline, N-acetyl citrulline and thiocitrulline with SARS Cov-2 main protease using molecular docking studies.

Thimmasandra Narayan Ramesh

Department of Studies and Research in Chemistry, University College of Science, Tumkur University, Tumkur 572 103, India

Corresponding author email: adityaramesh77@yahoo.com

Abstract

In this article, the binding abilities of arginine, citrulline, N-acetyl citrulline and thiocitrulline on the active sites of SARS-COV-2 protease have been investigated using in-silico studies. All the above ligands bind selectively and preferentially to Cys-145 active site and also to other amino acids surrounding to it in the main protease. Of which arginine forms less number of weaker bonds compared to the other ligands, it by itself is a precursor for the formation of citrulline analogues with in the cell. Major advantage of using the above ligands is that in addition to its preferential binding they have the ability to increase the immunity by assisting NO generation. Our results show that N-acetyl citrulline, citrulline, thiocitrulline and arginine may be used as a supplement during the treatment of SARS-COV-2.

Key words: SARS-COV-2 main protease, citrulline analogues, nitric oxide, immunity

Graphical abstract:

COVID-SARS-2 Main protease interaction with N-acetyl citrulline

Introduction
As per the WHO situation status report dated 24th April 2020, 26,26321 have been infected by SARS-COVID-2, of which 1,81,938 are dead (WHO situation reports 95). The spreading of this pandemic SARS-COVID-2 continues and seems to not cease very soon. One of the major hindrances is in its higher rate of spreading and there are no effective drugs for treatment of infected ones (Lai et al., 2020). Scientists and researchers across the world are intensively engaged in the development of vaccine and development of drugs to curb it (Le et al 2020; Eynde et al., 2020). Development of new vaccine and also new drug molecules cannot meet the immediate requirement as they have to go through clinical trials and its efficacy as well the protocol for its administration has to be established. An alternative option is to screen the drugs which have already been approved by FDA and used for the treatment of various types of other viruses and test them on SARS COVID-2 virus (Dai et al., 2020; Ul Wamar et al., 2020; Univ. of British Columbia group, 2020; Kadiuglu et al., 2020;Tu et al., 2020). Computational chemists and biologists by adopting the combinatorial procedures have verified majority of the available drugs/compounds and the best of them have been examined for their binding ability to main protease of SARS COV-2 (Wang et al., 2020;). Recently, hydroxychloroquine in combination with azithromycin was considered to be a potent drug and has been used to treat the infected patients (Rolain et al., 2007; Gautret et al., 2020; Lin et al., 2020). If the patients are already suffering from health issues then this drug may have other secondary effect as well (Moling et al., 2020). Also remdesivir have also been tested for the COVID 19 infected patients and has been found to be not very effective (Wang et al., 2020). Antiviral drug niclosamide has been proposed to be as a potential drug for the treatment of SARS-COVID-2 (Xu et al., 2020).

Majority of patients die due to lack of oxygen supply to the system as spike protein of SARS-COVID-2 binds to ACE2 enzyme thereby affecting alovelar cells of the lungs (Jin et al., 2020). It is also found that SARS-COVID-2 is also affecting the neurons/hemoglobin and other organs as well (Basilio et al., 2020). Recently there was a report on insilico studies on the use of N-acetyl cysteine/Zinc-acetyl cysteine to treat infected lungs (Guthappa 2020). Instead of targeting only lungs, it will be highly recommended, if we could choose the molecules/ligands which has both the binding affinity to m-protease as well boost the overall immune system. Nitric oxide is known to have antibacterial and antiviral property (Jones et al., 2010). NO is produced by the tissues in the body and controls cardiovascular, immune and nervous systems (Akaike et al., 2020). Gaseous nitric oxide is also released in the respiratory tract of humans during the exhaling
process (Lorenzo Berr et al., 2020). Recently there was a report on the use of nitric oxide for the treatment of SARS-COV-2 (Basillo et al., 2020). Major disadvantage is that instead of using directly nitric oxide it will be safer to use the molecules/compounds which could generate nitric oxide in-vivo thereby enhancing its permeability to all the tissues in the body and bind to m-protease. L arginine acts as a precursor to nitric oxide generation in the body (Rajapakse et al., 2009). We could also find citrulline and its analogues, as citrulline is a non-essential amino acid produced from arginine by a peptidyl arginine deaminase enzyme. L-citrulline can also be reversibly converted to L-arginine by argininosuccinate synthase (Guayao et al., 1992). Citrulline residues have been found in myelin basic protein (MBP) and some of the histone proteins (Drug Bank, Canada). Hence, L citrulline has been tested as a nutraceutical and found to have least side effects. NO is used as precursor for several enzymatic reactions. Of which different forms of NOS induce immunity inflammatory/immunological stimuli in certain tissues and lungs (Virarkar et al., 2013). L-citrulline enhances NO generation thereby results in the improvement in obese asthmatics (Fernando et al., 2019). Therefore we have used arginine, citrulline, acetyl citrulline and thiocitrullline as these are produced within the system thereby minimizes secondary complications. In this report, we have explored arginine, citrulline (FDA approved drug), acetyl citrulline and thiocitrullline (experimental stages) for their binding ability with main protease of SARS-COV-2 by using molecular docking studies.

**Methods**

*Molecular docking studies*

3CLpro/Mpro structure (6LU7) of COVID-SARS-2 was obtained from protein databank (PDB format (https://www.rcsb.org/). While the structure of ligands (arginine, citrulline, N-acetyl citrulline, thiocitrulline) in PDB format was collected from the database of Drug Bank, Canada (https://www.drugbank.ca/).

6LU7 was prepared, its active sites (His-41, Cys-145 and Gln-189) were chosen based on the reported data and confined in a grid box (Zhang et al., 2020) to compute the binding ability with ligands. Prior to it, 6LU7 and ligands was optimized, genetic algorithm and Lamarckian algorithm was used in Auto dock tools 1.5.6. The graphical presentations and results were analyzed using UCSF Chimera.
Results and Discussion

Major part of research focus on targeting SARS-COV-2 main protease (Mpro) to prevent its replication. As an alternative to the development of new drugs, we have screened and chosen the potential ligands which have been used earlier for their beneficial effects in enhancement of immune system. Table 1 shows the properties of various ligands which fulfil Lipinski’s five rule.

Table 1: Properties of drug molecules/ligands (Ref: Drug Bank, Canada: https://www.drugbank.ca/)

| Ligands       | Citrulline | N-acetyl citrulline | Thiocitrulline | Arginine |
|---------------|------------|---------------------|----------------|----------|
| Molecular formula | C₆H₁₃N₃O₃ | C₈H₁₅N₃O₄ | C₆H₁₃N₃O₂S | C₆H₁₄N₄O₂ |
| Molecular mass (g mol⁻¹) | 175.18 | 217.10 | 191.25 | 174.11 |
| LogP          | -3.19      | -2       | -2.6   | -3.5    |
| H-bond acceptor count | 4 | 4 | 3 | 6 |
| H-bond donor count   | 4 | 4 | 4 | 5 |
| Rotatable bond count | 5 | 6 | 5 | 5 |

In-silico studies have been carried out to evaluate the potential binding abilities of these ligands to main protease (Mpro). Arginine, citrulline, N-acetyl citrulline and thiocitrulline were allowed to bind to the active sites of SARS COV-2 (3CLpro) separately using Auto dock 1.5.6. In Table 2 is shown the structure of different ligands, lowest binding energies with best conformations by which they bind to the active sites of Mpro.

In Table 3 is given the binding energies of different ligands to active sites of Mpro and their preference are arranged as follows: N-acetyl citrulline > arginine > thiocitrulline > citrulline. Table 4 summarizes the binding energies of different ligands in several conformations with Mpro.
Table 2: Interaction of SARS COV-2 protease with ligands

| Ligand                  | Structure | Binding Energy | Conformations |
|-------------------------|-----------|----------------|---------------|
| Arginine                | ![Arginine Structure](image) | ![Arginine Binding Energy](image) | ![Arginine Conformations](image) |
| Citrulline              | ![Citrulline Structure](image) | ![Citrulline Binding Energy](image) | ![Citrulline Conformations](image) |
| N-acetyl citrulline     | ![N-acetyl Citrulline Structure](image) | ![N-acetyl Citrulline Binding Energy](image) | ![N-acetyl Citrulline Conformations](image) |
| Thiocitrulline          | ![Thiocitrulline Structure](image) | ![Thiocitrulline Binding Energy](image) | ![Thiocitrulline Conformations](image) |
### Table 3: Binding energies on Interaction of ligands with SARS COV-2 protease

| Ligand            | binding energy (kcal/mol) |
|-------------------|---------------------------|
| Citrulline        | -3.9                      |
| n-acetyl citrulline | -5.11                    |
| thiocitrulline    | -3.91                     |
| Arginine          | -4.1                      |

### Table 4. Interactions of main protease with ligand atoms in best conformation

| Ligand            | Arginine | Citrulline | N-acetyl citrulline | Thiocitrulline |
|-------------------|----------|------------|---------------------|---------------|
| Binding energy (kcal mol\(^{-1}\)) | -4.1     | -3.9       | -5.11               | -3.91         |
| Interactions of main protease with ligand atoms | LEU-141 AO:HN; bond distance:2.906Å | LEU 141AO:HN bond distance:2.674Å | LEU-141A O:O; bond distance:3.397Å | PHE-140-AO: H; bond distance:2.080 Å |
|                   | SER-144A OG:HN; bond distance:2.987Å | GLY-143A-HN:O; bond distance:2.04 Å | PHE-140-AO: H; bond distance:2.80 Å | LEU-141-AO: H; bond distance:1.758 Å |
|                   | HIS-163A HE2:O; bond distance:2.026 Å | CYS-145A HN: O; bond distance:2.116 Å | CYS-145A O:O; bond distance:3.426 Å | CYS-145-AHN:HO; bond distance:2.28 Å |
|                   | GLU-166 AOE2:HN; bond distance:2.915Å | GLU-166-AOE2:HN bond distance:2.795Å | HIS-163A HE2:O; bond distance:1.905Å | - |
|                   | GLU-166 OE1:HN; bond distance:2.85Å | HIS-164A O:HO; bond distance:3.225Å | - | - |

All the ligands in one or the other conformation have the ability to bind to CYS-145 i.e one of the active site of main protease thereby exhibiting its ability to inhibit the replication of viral RNA. Of which citrulline also binds to second preferential amino acid His-41. In addition the preferential active site binding, Even though these n-acetyl citrulline, thiocitrulline and arginine could preferentially bind to the other two active sites i.e His-41 and Gln-189, these ligands also bind to other amino acids as well. Citrulline, N-acetyl citrulline and arginine have the tendency to have more number of weaker hydrogen bonds with Mpro. The absence of aromatic groups and long chain of these ligands is an added advantage as they can effectively penetrate and also attach to the other amino acid sites as well. There are reports that COVID-2 can affect other organs in addition to lungs. Therefore it will be difficult to treat in such conditions, enhancing the immunity also should be considered. In addition to the binding affinity, these molecules also
have the ability to enhance the immunity of the cells by the generation of nitric oxide in presence of enzymes thereby protecting them. The results show that n-acetyl citrulline and citrulline even though are produced by the conversion of arginine, the preferential ability of the former indicates that they can be used as potential supplements during the course of SARS-COVID-2 treatment.

**Conclusion**

Even though insilico studies demonstrate the preferential binding abilities of FDA approved hydroxychloroquine/redmesveir/niclosamide/azithromycin drugs, in-vivo there are more secondary complications arises due to which their utility has been severely restricted/limited. As an alternative, we have screened and examined arginine, citrulline, n-acetyl citrulline and thiocitrulline. Arginine and citrulline has been generated within the system itself thus may have benefits compared to other drugs. Advantage of using the above ligands is in addition to the preferential binding ability to one of the active site of main protease these ligands enhance the immunity. Our results show that arginine, acetyl citrulline and thiocitrulline can also be explored as a supplement in supporting the current treatment procedures adopted for the treatment of SARS-COV-2.

**Conflict of interest**

Author declares no conflict of interest.

**Compliance with Ethical Standards**

This article does not contain any studies involving animals or human participants.

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