# Molecular docking studies of N-acetyl cysteine, zinc acetyl cysteine and niclosamide on SARS Cov 2 protease and its comparison with hydroxychloroquine

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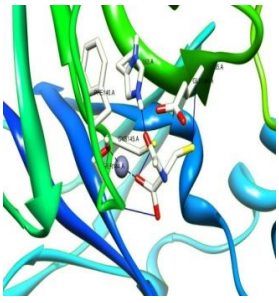
**Abstract**

In this article, we have evaluated the binding abilities of N-acetyl cysteine, zinc acetyl cysteine and niclosamide (antiviral drug) with SARS-COV-2 protease. All the four compounds investigated are effective and selectively bind to active sites of main protease. N-acetyl cysteine being a derivative of cysteine interacts with Cys-145, His-163, Gly-143 of COV-2 protease, zinc acetyl cysteine binds to Gly-143, Ser-144, Cys-145, Glu-166 of COV-2 protease and niclosamide bind to Glu-166, Cys-145, His 41 of main protease. The data has been compared with hydroxychloroquine which effectively binds to Cys-145, Glu-166, Arg-188. The binding affinities of N-acetyl cysteine, zinc-acetyl cysteine and niclosamide are -4.24, -4.29 and -7.5 kcal $mol^{-1}$ while for hydroxychloroquine it is -6.66 kcal $mol^{-1}$. Niclosamide with its lowest binding energy interacts with His-41 and Cys-145 which may be the first molecule to show such binding interaction. The results indicate that N-acetyl cysteine, zinc-acetyl cysteine and niclosamide can also be explored for the treatment for SARS COV-2 as an alternative for hydroxychloroquine.

**Key words**: SARS-COV-2 main protease, n-acetyl cysteine, Zn-acetyl cysteine, niclosamide, hydroxychloqine, drug

**Graphical abstract**:



Main protease-Zn-N-acetyl cysteine interaction

## Introduction

SARS-CoV-2 also known as Covid 19 a new strain of coronavirus took its birth in China with its first report in December 2019 (Rodríguez-Morales et al., 2020). From China this pandemic found its way to other parts of the world with 2,399,849 persons infected of which 164,939 deaths and

615,676 recovered as of 19$^{th}$ April 2020 (WHO situation reports 90). Most of the countries in the world are aggressively engaged in combating with the SARS COV-2 virus infection, while scientists and researchers are engaged in the development of vaccine/drugs to fight the infection from spreading/cure (Frank and Grady, 2020). Thousands of compounds/molecules have been screened for its efficacy towards SARS COV-2 but till date we are still unable to get the effective solution. Promising drugs are hydroxychloroquine and remdesivir/azithromycin has been administered to the patients affected with SARS-COV 2 (Devaux et al., 2020; Xu et al., 2020; Wang et al., 2020). Mpro is targeted in COV-2 virus to inhibit its replication (Jin et al., 2020). N-acetyl cysteine has been used for the treatment of lung congestion associated with mucus/bronchitis (Sanguinetti., 2015). Since SARS-COV-2 also infects the lungs, the question arises whether NAC can also be used for its treatment. Recently there was a report on the use of n-acetyl cysteine for the treatment of SARS COV-2, and its utility has to be still explored (Heck et al., 2020). Zinc ion has the tendency to effectively bind to cysteine and histidine (Lin et al., 2020). SARS-COV-2 spike protein attacks the ACE2 enzyme (zinc metalloprotease) in the lungs and binds to the zinc thereby affecting the Alveolar epithelial type II cells. Since NAC is an approved compound for the treatment of respiratory disorders and acts as an anti oxidant and exhibits antiviral property (for HIV). N-acetyl cysteine zinc (NAC-Zn) is an analog of N-acetyl cysteine (NAC) and has also been used as antioxidant and inhibits antiviral replication. One of the major advantages of using NAC and Zn-NAC is that they can be directly administered without minimum clinical trials. Also the use of broad spectrum antiviral drug niclosamide for the treatment of SARS-COV-2 has been proposed recently (Zu et al., 2020). Therefore, computational studies of protein-ligand (NAC/Zn-NAC/niclosamide/hydroxychloroquine) interactions can provide insight into binding ability of the proposed drugs/molecules. In this article, we have reported the molecular docking studies for SARS COV-2 main protease with N-acetyl cysteine, N-acetyl cysteine zinc, niclosamide and compared the results with hydroxychloroquine.

## Methods

*Molecular docking studies*

Proteins/Macromolecules COVID-19 3CLpro/Mpro structures were obtained in PDB format (https://www.rcsb.org/). The native ligand for 3CLpro/Mpro structures.

Covid-19 3CLpro/Mpro with PDB ID 6LU7 a main protease of SARS COV-2 structure in PDB format was obtained from https://www.rcsb.org. Structures of selected drug molecules (NAC/NAC-Zn/niclosamide/hydroxychloroquine were treated as ligands) in PDB/SAF formats were collected from drug bank database, Canada.

6LU7 and ligands were separately prepared and the target amino acid sites (His-41, Cys-145 and Gln-189) were chosen from the reported data and confined in the grid box (Zhang et al., 2020). Binding energies were optimized using search genetic algorithm and Lamarckian genetic algorithm using Auto dock tools 1.5.6. Results were analyzed using Autodock and UCSF Chimera.

## Results and Discussion

In SARS-COV-2, its main protease (Mpro) plays a key role in replication; hence most of the studies have targeted it using various drugs. To explore the interaction of main protease (Mpro) with drug molecules it will be helpful to use the knowledge of computational biology and chemistry and predict the probability of their binding abilities. The N-acetyl cysteine, N-acetyl cysteine Zinc, niclosamide and hydroxychloroquine were docked to main protease (3CLpro) using Auto dock. The properties of potential ligands are listed in Table 1 and were selected based on Lipinski rule. As per Lipinski's rule and further modification on the selection of drug molecules to be effective indicates that the molecular mass should be low and not more than three hydrogen bond acceptors/donors/rotatable bonds. The values of lowest binding energies for NAC/NAC-Zn/Niclosamide/hydroxychloroquine ligands are given in Table 2. Table 3 shows the structure of ligand (drug molecules) and the active amino acids interaction in the pockets of Mpro. Auto docking results of the above drugs with main protease active sites shows that

hydroxychloroquine, N-acetyl cysteine, N-acetyl cysteine zinc and niclosamide were bound to Cys-145 active site of main protease thus inhibiting the main protease activity and thereby affecting the replication of viral RNA inside the host. Niclosamide can form bonds with both active sites Cys-145 and His-41. To the best of our knowledge, till date there are no molecules reported which could selectively target Cys-145 and His-41.

**Table 1: Properties of potential ligands**

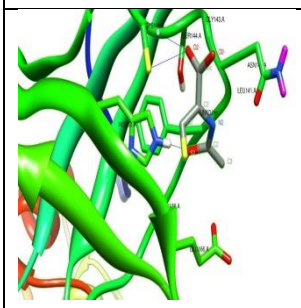
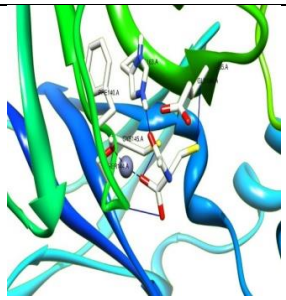
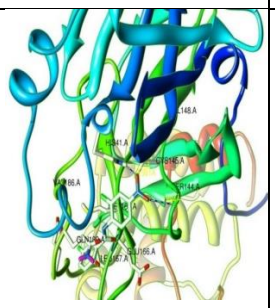
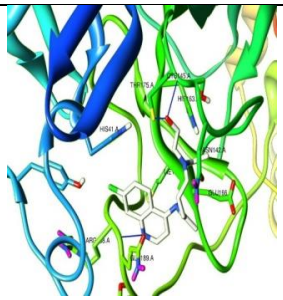
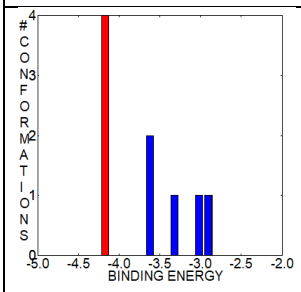
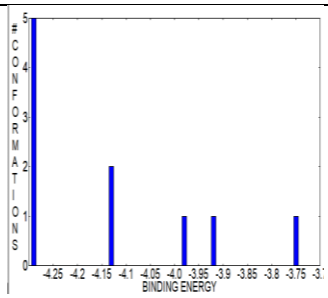
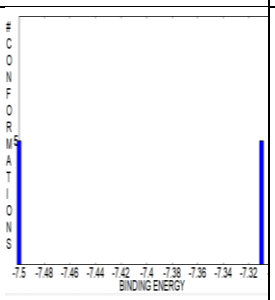
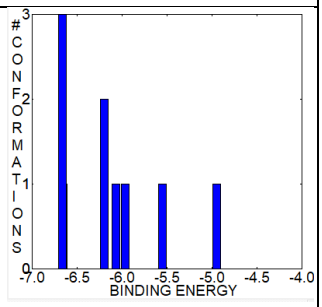
| | **Ligands** | | | |
|---|---|---|---|---|
| | **NAC** | **NAC-Zn** | **Niclosamide** | **Hydroxychloroquine** |
| **Molecular formula** | $C_5H_9NO_3S$ | $C_{10}H_{16}N_2O_6S_2Zn$ | $C_{15}H_8Cl_2N_2O_4$ | $C_{18}H_{26}ClN_3O$ |
| **Molecular mass (g mol$^{-1}$)** | 163.2 | 389.8 | 327.12 | 335.9 |
| **H-bond acceptor count** | 4 | 8 | 4 | 4 |
| **H-bond donor count** | 3 | 4 | 2 | 2 |
| **Rotatable bond count** | 3 | | 2 | 9 |

**Table 2: Binding energies on Interaction of ligands with SARS COV-2 protease**

| **Ligand** | **binding energy (kcal/mol)** |
|---|---|
| N-acetyl cysteine | -4.24 |
| Zinc n-acetyl cysteine | -4.29 |
| Niclosamide | -7.5 |
| Hydroxychloroquine | -6.66 |

Table 3 shows the results of interaction of main protease with NAC/NAC-Zn/Niclosamide/hydroxychloroquine ligands and binding energies vs. conformation graphs. Auto docking tool shows the interaction of NAC, NAC-Zn, Niclosamide and hydroxychloroquine inhibitors with Mpro/3CLpro protein for different conformations based on the score and the conformation with negative binding energies will have maximum stability.

**Table 3: Interaction of ligands with SARS COV-2 protease**

| **SARS-COV 2 Mpro with ligands** | | | |
|---|---|---|---|
| **NAC** | **NAC-Zn** | **Niclosamide** | **Hydroxychloroqine** |
|  |  |  |  |
|  |  |  |  |
| Cys-145<br>NH—O 1.999 Å | Cys-145<br>SG—O 3.537 Å | Glu-166<br>HN—O 1.894 Å | Cys-145<br>HN—O 2.056 Å |
| His-163<br>HE2—O 1.785 Å | Gly-143<br>HN—O 2.039 Å | Glu-166<br>O—O 3.355 Å | Cys-145<br>SG—O 3.17 Å |
| Cys-145<br>SG—O 3.382 Å | Glu-166<br>HN—O2.695 Å | Gly-143<br>HN—O 1.894 Å | Glu-166<br>HN—O 2.053 Å |
| Gly-143<br>HN—O 1.951A | His- 163<br>HE2—O 2.009 Å | Cys-145<br>SG—O 2.98 Å | Arg-188<br>O—N 2.72 Å |
| - | Ser-144<br>HN—O 3.02 Å<br>Ser-144<br>O—Zn 2.014 Å<br>(Angle RMSD=6.8)(Tetrahedral) | His-41<br>HE2—O 2.113 Å | - |

Niclosamide with lowest binding energy forms a strong hydrogen bond with Glu-166 via two hydrogen bonds. Results of docking provide evidence for weak hydrogen bonds between niclosamide with Cys-145 and His-41 (the active sites in SARS COVID-2 main protease). Hyroxychloroquine and N-acetyl cysteine forms stable hydrogen bonds with Cys-145 via sulphur and nitrogen attached hydrogens. N-acetyl cysteine zinc also fits into same pocket where NAC fits. NAC-Zn forms bond with Ser-144, a stable bond via oxygen and zinc (Zn forms form bonds indicating its tetrahedral geometry with angle RMSD=6.8) and hydrogen bond via nitrogen and oxygen. It also binds with Cys-145, Gly-143, Glu-166 and His-163 which provide additional support in bonding with main protease.

Our results show that niclosamide can be an effective drug in the treatment of SARS-COVID-2 but has severe side effects. Hydroxychloroquine also has secondary complications, as an alternative to the above two drugs, we can use Zn-NAC which forms multiple bond with active amino acids. Even though NAC and Zn-NAC have comparable binding energies, Zn-NAC is preferred due to its donor ability of $Zn^{2+}$ to the main protease thus protecting the ACE-2.

**Conclusion**

At present, hydroxychloroquine is the only FDA approved drug to treat SARS-COV-2. We have explored the use of NAC, NAC-Zn, niclosamide, hydroxychloroquine (as control) and targeted main protease (Mpro) to inhibit its replication pathway. Niclosamide has high binding affinity with lowest binding energy compared to hydroxychloroquine. NAC and specifically Zn-NAC have comparable binding energies to that of hydroxychloroquine. Hydroxychloroquine is not a safe drug, hence as an alternative, niclosamide with lowest binding energy can be considered. $Zn^{2+}$ ions in Zn-NAC have the potential ability to target the main protease inhibiting the virus replication via protecting ACE-2. Further in-vitro and in-vivo studies have to be carried out to explore the feasibility of the above compounds and its potentiality.

**Conflict of interest**

Author declares no conflict of interest Compliance with Ethical Standards.

**Compliance with Ethical Standards**

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

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