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# Virtual Screening Investigation for Anti Covid-19 Potential Drugs

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**ABSTRACT**

Coronavirus (COVID-19, SARS-COV-2) has become in short time a global pandemic. It causes after two weeks of exposure severe respiratory symptoms. Herbal and non-herbal drugs were tested for treatment of coronavirus. In this study, virtual screening was applied on a small library of compounds; 7 drugs which were clinically effective; Chloroquine, Hydroxyl chloroquine, and Azithromycin, Zinc, cepharanthine, Mefloquine, Remdesivir. Drug-Drug interactions data was also mentioned and discussed. As aresult, all compounds have bonds to the main core of coronavirus protease. This can interpret the mechanism of action previous researches pointed out to. It also explains the clinical effectiveness of the studied drugs used as monotherapy or combined one and assist in discovering more effective drugs against this unseen assassin.

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## INTRODUCTION

Coronavirus was reported as an unknown factor for severe pneumonia cases first appeared in Wuhan, China in the last month of 2019. It soon becomes a global pandemic; in four months. Media, websites, scientific researchers are all oriented to spotlight on this new version of coronavirus, or as it is known; new coronavirus. (1)

According to World Health Organization (WHO), more than million and a half cases with thousands of deaths has been globally confirmed in 212 countries to date. (1) COVID-19; another synonym for coronavirus, is transferred from bats to human causing severe respiratory symptoms. (2) After two weeks of exposing to the virus, symptoms like influenza appear such as fever, cough, and shortness of breath appear. (2) However, these symptoms soon are developed to severe pneumonia leading to death especially in old patients with coronary diseases and/or diabetic patients and those who have weak immune system.

Coronaviruses naturally exist. They used to be found in animals such bats and pangolins. The type of corona which is found in bats and pangolin as main reservoirs for the virus, transferred to human causing COVID-19 pandemic. (3) However, this virus is different from other members in coronavirus family which ranges from SARS (Severe Acute Respiratory Syndrome), first appeared in 2003 to MERS (Middle East Respiratory Syndrome) first appeared in 2012. However, this virus is more alike to SARS; therefore, it was named after it; SARS-CoV-2. (4) (5)

One of the unique characteristics of this spherical with one RNA strand coronavirus is that it still alive for 3 hours in the air. It also has more resistance so it remains on surfaces made of plastic and stainless steel though its infectious potency decreases on the previous surfaces by time. SARS-CoV-2 half life in aerosol is similar to SARS-CoV-1; 1.1-1.2 hours. (5) However, SARS-CoV-2 is more resistant than SARS-CoV-1 on cardboard.

Healthcare professionals can only reduce the symptoms of the infection as there is no confidential treatment against the virus. (6) In China, herbal and non-herbal treatments were applied for curing of SARS-CoV-2, but according to Dudani T *et al.*, herbal drugs are not sufficient alone. It must be combined with non-herbal medicines and changing of lifestyles. (7)

As corona infection becomes a global issue, many pharma companies and research centers are still trying to figure out a treatment for it, but none came to a confident conclusion yet. Different drugs were clinically and in-silico investigated. (8)

FDA (Food and Drug Administration) has approved 20 drugs to be used as a treatment for coronavirus infection, but none of them is a drug of choice. (9) seventeen of them are safe and have inhibition effects on the virus. However, Chloroquine still has the safest profile among anti-corona drugs. (10) We mention of these drugs hydroxychloroquine (HCQ) and chloroquine phosphate. (9)

However, a number of possible mechanisms were proposed for those drugs, but none is confirmed; "Among the pharmacotherapeutic agents evaluated, some of the highlighted agents, which are being evaluated, are high-dose Vitamin C, Favipiravir, Adalimumab, Dihydro-artemisinin piperaquine, Leflunomide, Dipyridamole, Chloroquine or HCQ, Suramin Sodium, Lopinavir/Ritonavir and Arbidol (Umifenovir) tablets, and IFN-alpha 2b. Other important agents being evaluated are Huo-Shen particles, Xiyanping injection, Shen-Fu injection, etc., many of which are from traditional Chinese medicines background. Use of stem cells is also evaluated frequently". (11)

Shang *et al*., found by virtual screening that Remdesivir bonds to the COVID-19 protein (SARS-CoV-$2^{Mpro}$). (12) It is confirmed that it also has an inhibition efficacy resulted from *in-vitro* investigation which found Remdesivir causes pre-mature termination in the virus RNA as Remdesivir is an adenine analogue. (13)

On the other hand, Zhang *et al*. found that Teicoplanin prevents the virus from entering the cells. (14) Moreover, Wang et al., found that Chloroquine reduces virus replication. (15) Zinc lozenges is known to be used against common cold caused by coronavirus. (16), (17)

In silico study showed that Cepharanthine, a natural compound, can be a potential antiviral for the prevention and treatment of SARS-CoV-2 infection. (18)

However, learning the main targets in the virus can assists in discovering new drugs to fight the virus. The goals are the spike protein, envelop protein, membrane protein, protease, nucleocapsid protein, hemagglutinin esterase, and helicase, figure 1. (11) (19) (20)

As coronavirus protein might be a main goal for discovering drugs to treat coronavirus, it worth mentioning its composition. In short. Corona protease consists of two chains (A and C). The chain C is a polymer rather than a protein. unlikely, chain A is a protein. the sequence of chain C is bonded to three molecules which are also connected to each other by hydrogen bonds. The molecules IDs are (010, 02J, PJE). The piece (02J) is considered to have a main role in protease activation. Hence, virus replication. (21)

In this study, we have done virtual screening for the most efficient drugs clinically which are somehow chemically similar to the triple (010,02J, PJE) which is located in coronavirus main protease. (8) (22)We aim by that to match between the in-vitro studies done by other colleagues and our in-silico study to propose a treatment for SARS-COV-2 with suggesting mechanisms for them.

## MATERIALS AND METHODS

Chloroquine, Hydroxychloroquine, Mefloquine, Azithromycin, Remdesivir, Cepharanthine, and Zinc chemical structures were downloaded from PubChem database, figure 1. (23) The crystal structure of the coronavirus main protease ($M^{PRO}$) was downloaded from Protein Data Bank. The crystal structure ID is (6LU7), (11) (21). Rigid docking was applied using Autodock 2.4.1, Chimera, Pymol, PLIP, were used for virtual screening and interactions analyzes in default settings. Grid Box was focused on the binding site with dimensions ( X= 50, y=56, y= 28).

Validation of the software was done by re-docking in the rigid mode the isolated (o2J); the original ligand from 6lu7 crystal structure, with the protein after optimization. Then, we have determined the binding residues in the binding site of (02J) with the crystal structure and orientation.

Scoring function used was the binding energy in addition to the evaluation of binding interactions residues and orientation.

Data was also collected about these drugs interactions depending on Drug Bank. Interaction Checker. (24)

## RESULTS AND DISCUSSION

The binding site of the protease is found to be consisted of 8 residues; 3C, 25A, 26A, 143A, 144A, 163A, 164A, 166A, figure 2. This is also where the triple sequence (0.10,02J, PEJ) bonds to the chain C and A, as well. (21)

As figure 3 shows, Root Mean Square Deviation (RMSD) for the superimposing between the original optimized 6LU7 crystal structure and the re-socked 02J with the optimized protein is 0.00. this can be because superimposing RMSD counts compare between the complexes as pairs not orientations. However as can be seen in Figure 4 and 5, the orientation is the same for the original 02J and the re-docked one. They also have bonding in the same binding site with the same residue. The binding energy for the re-docked ligand is -3.43. This interprets that the coming results are reliable and tests can go thoroughly as the method found to be valid.

All the investigated compounds have bonds in the mentioned binding site. The score functions, binding energy for the studied compounds and the binding residues are shown in Table 1.

As figure 6 and 7 show, HCQ has two complexes with the enzyme. It conforms H-bonds with chain C. the distances of these bonds for complex 1 are 2.7$^{o}$A and 2.09 $^{o}$A and 1.84 $^{o}$A for complex2. The binding energies for the complexes represented as score functions are -3.35 and -3.33 respectively.

Azithromycin conforms no H-bonds with the protein. It bonds with chain C by hydrophobic interactions, as figure 8 illustrates. The binding energy is -4.16.

Chloroquine score function is -4.44. it conforms no H-bonds, figure 9. It only has Wan Der Vals Interactions with the residues in the binding site. On the other hand, mefloquine conforms two H-bonds with the binding site residues. The length of them are 2.17 $^{o}$A and 2.7 $^{o}$A. the binding energy for Mefloquine is -4.7.

Zinc has contacts with the binding site with binding energy of -.0.81. Ten out of ten conformations are in the same pocket of the binding site, figure 10.

Cepharanthine conforms two complexes with 6LU7 binding site. Complex 1 has no H-bonds with the residues. Its binding energy is -5.79, figure 11. On the other hand, Complex 2 binding energy is – 5.59. it conforms H-bonds, unlike complex1, in addition to Wan Der Wals interactions, figure 12.

Remdesivir has four complexes out of ten in the binding site (complex 1, complex 2, complex3, complex 7), figures12-15. The binding energies for these complexes are -2.05, -1.91, -1.41, -0.94. they all conforms H-bonds and hydrophobic contacts.

The findings interpret the suggested mechanism of action for Chloroquine, HCQ, and Azithromycin, Zinc, Cepharanthine, Mefloquine, Remdesivir that they might be virus transcription preventers. By that, azithromycin has two suggested mechanisms; one resulted from our study as virus transcription preventer and the other mechanism is by binding to SARS-CoV-2 Spike (S)Protein - ACE2 Complex. (25) same goes for HCQ considering the same source of information. (25) despite which of the mechanisms are right, probably both, these findings agree with in-vitro study done by Andreani J *et al*. which confirmed a synergic effect for using hydroxyl chloroquine and azithromycin. (26) another open label study conducted in France augments the same findings, combination of azithromycin and HCQ increase the virus elimination. (27) chloroquine has same effects as well. (28) Chloroquine is effective. In Brazil, a clinical trial investigated using HCQ and azithromycin against the virus. (29)

However, Redmesvir is not as close as the other ones are to the named core. This augments a study found that remdesivir as admin analogue inserts in the virus RNA.

Moreover, the results agree with the clinical studies for Chloroquine and Remdesivir drugs which were done in Wuhan, China by Wang *et al*, 2020. (30) though they have different binding sites.

Drug-drug interactions has a contrast point of view. A total of 348 drugs are known to interact with mefloquine (61 major drug interactions, 278 moderate drug interactions, 9 minor drug interactions). According to drug bank interaction checker, "Using mefloquine together with azithromycin can increase the risk of irregular heart rhythm that may be serious and potentially life-threatening. You may be more susceptible if you have a heart condition called congenital long QT syndrome, other cardiac diseases, conduction abnormalities, or electrolyte disturbances (for example, magnesium or potassium loss due to severe or prolonged diarrhea or vomiting)." (31)

Same goes for HCQ and azithromycin combined. (32) but no interaction is recorded for using zinc lozenges with any of these two drugs.

As a conclusion for the previous discussion, HCQ and azithromycin are about to be recommended as a bi-therapy as they are more effective than using any of them alone as a monotherapy against COVID-19, but the moderate interaction between them is crucial and must be considered. Moreover, drug developers can also derivate from quinine chemical structure to find a drug of choice against COVID-19.

## CONCLUSION

Drug discovery against SAR-CoV-2 is still challenging. So far, no drug has been known to be a drug of choice to treat coronavirus infection. Different drugs were tested but none gave a relief to be a certified drug by WHO or FDA. In this paper, we suggest a new mechanism of actions for drugs known to be clinically effective against the COVID-19 and studied their interactions. We recommend these known drugs to be used as combined therapy but taking drug-drug interactions in consideration till a selective anti-corona virus drug is discovered, so the best choice for now is either Azithromycin plus Zinc lozenges, or HCQ plus Zinc lozenges. Chloroquine is not preferred though it has same properties but it self-causes irregular heart rhythm. Other drugs can be used as monotherapy or in combination with Zinc. However, we leave the judgment for the treatment protocol for physicians and experts in the medical field.



**Declaration of interest:**

All the authors have no conflict of interest to declare.

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**Ethical approval:**

No ethical approval is requested.

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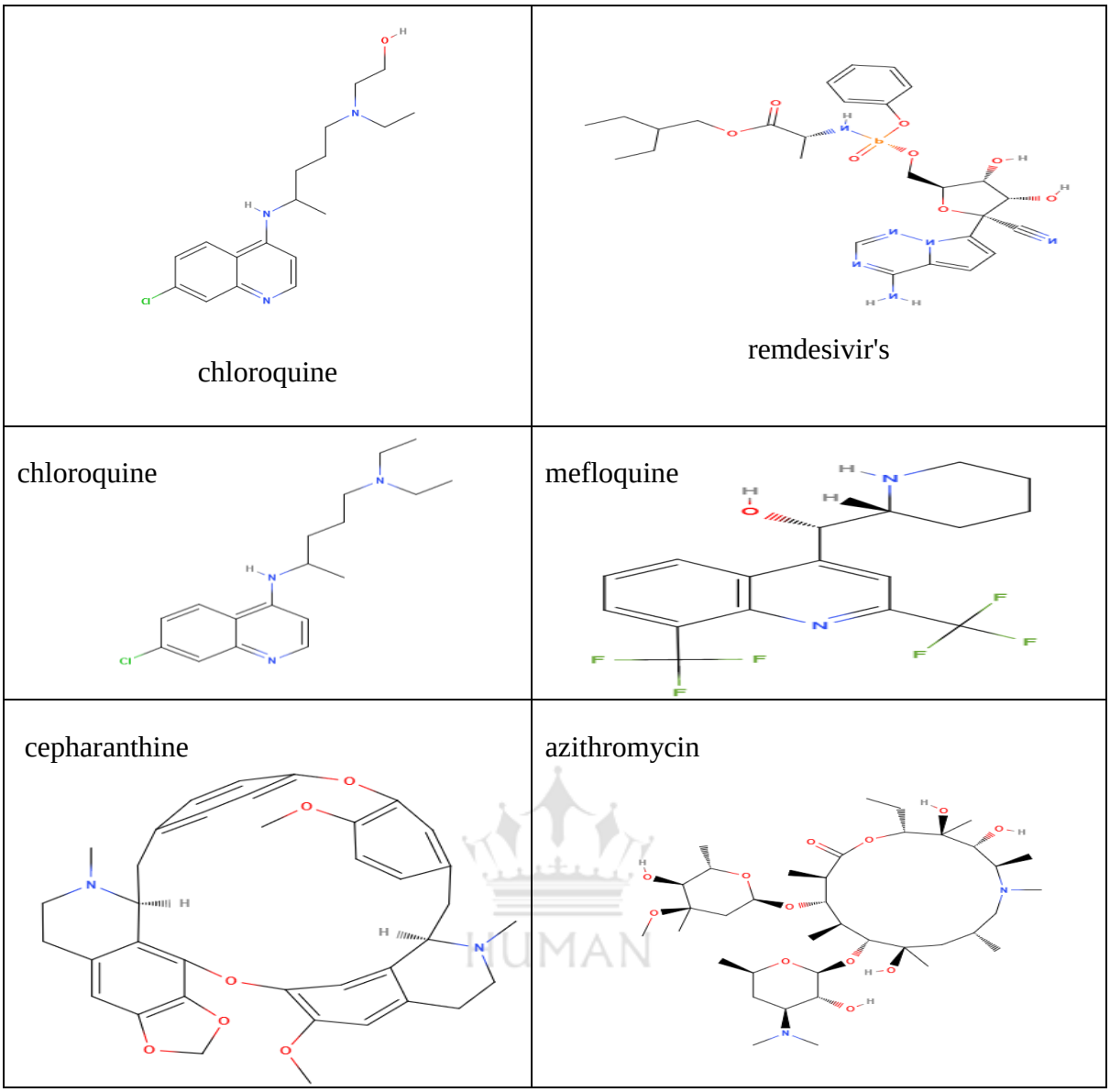


**Figure No. 1: Chemical structure for potential drugs treat COVID-19** *(23)*

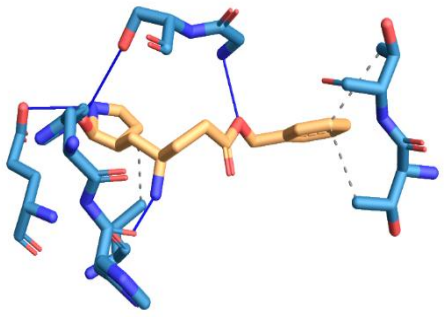

**Figure No. 2: binding site in COVID-19 Protease, (32)**

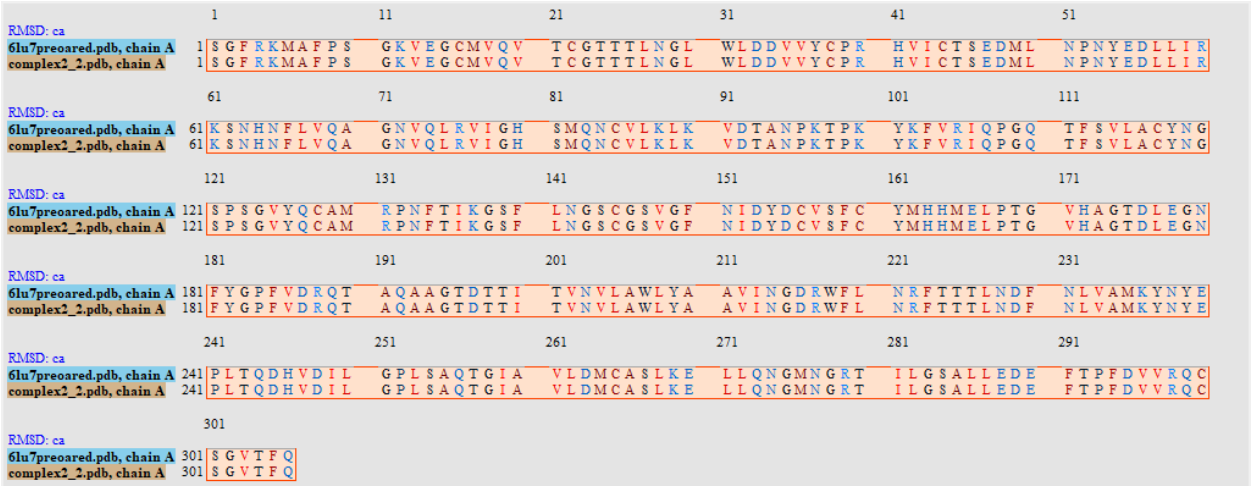


**Figure No. 3: superimposing result between the optimized 6LU7 and the re-docked ligand complex**

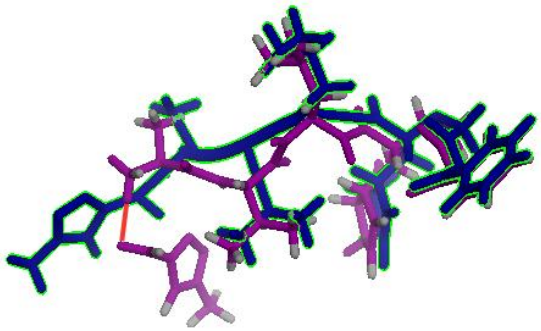

**Figure No. 4: superimposing result for orientation of the ligands, the original structure is in blue, the re-docked compled is in magenta.**

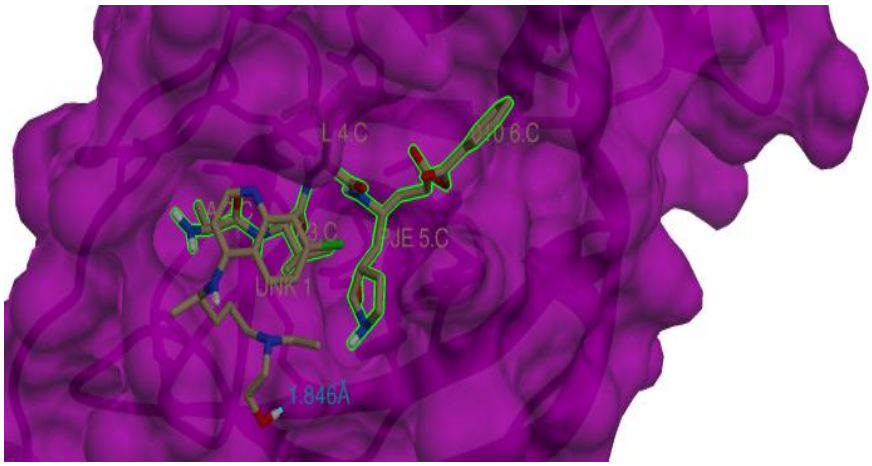


**Figure No. 5: hydroxychloroquine complex 1 interactions with 6LU7**

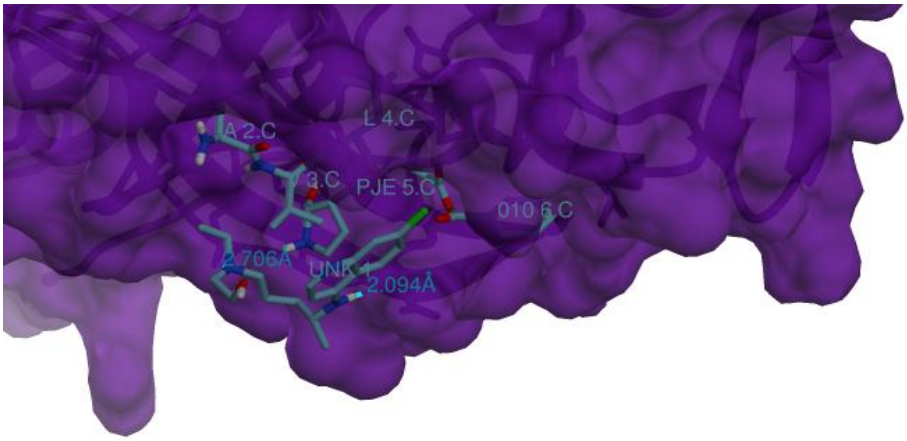


**Figure No. 6: Hychloroquin complex 2 interactions with 6LU7**

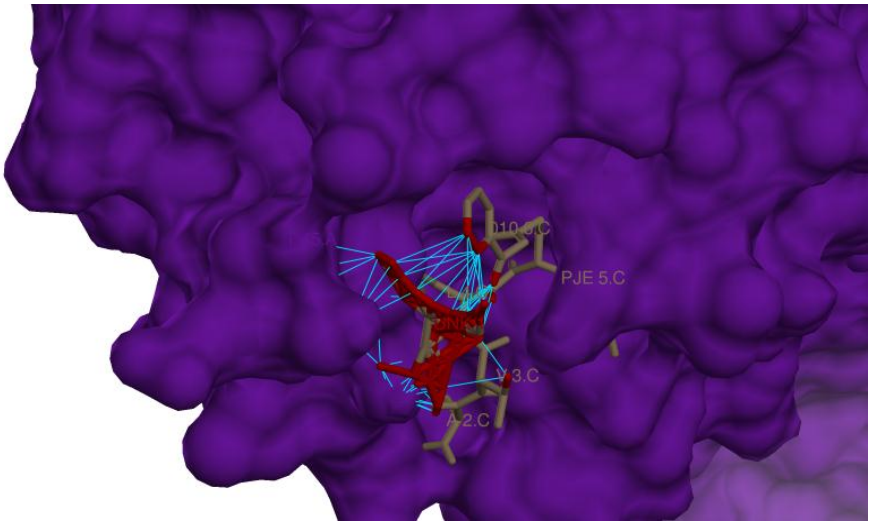


**Figure No. 7: AZITHROMYCIN complex1 interactions with 6LU7**

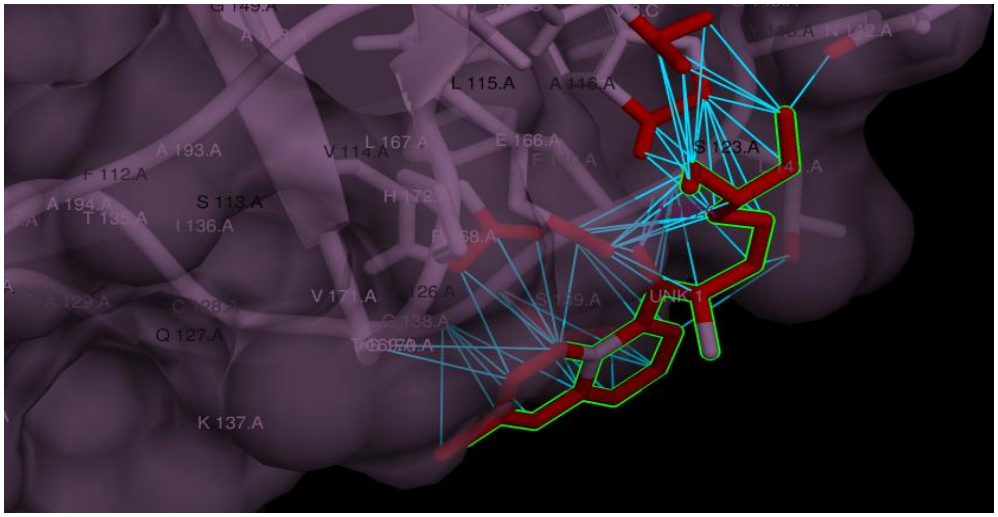


**Figure No. 8: Chloroquine Wander Vals interactions with 6LU7**

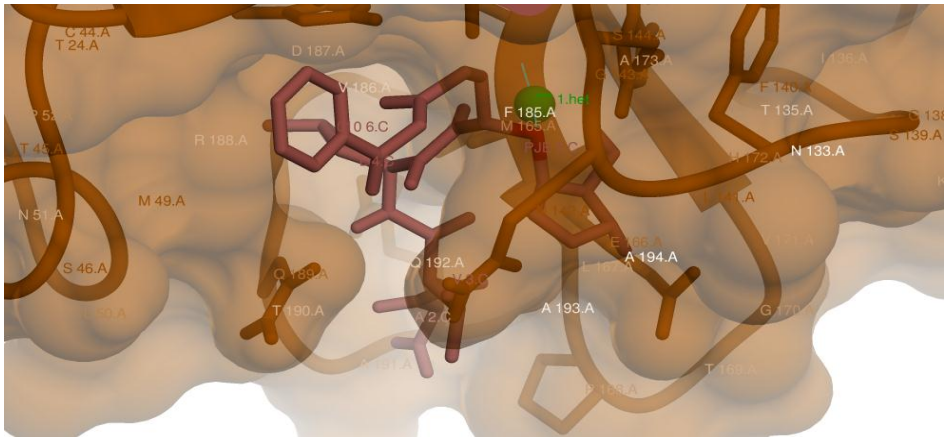


**Figure No. 9: zinc claches with 6LU7**

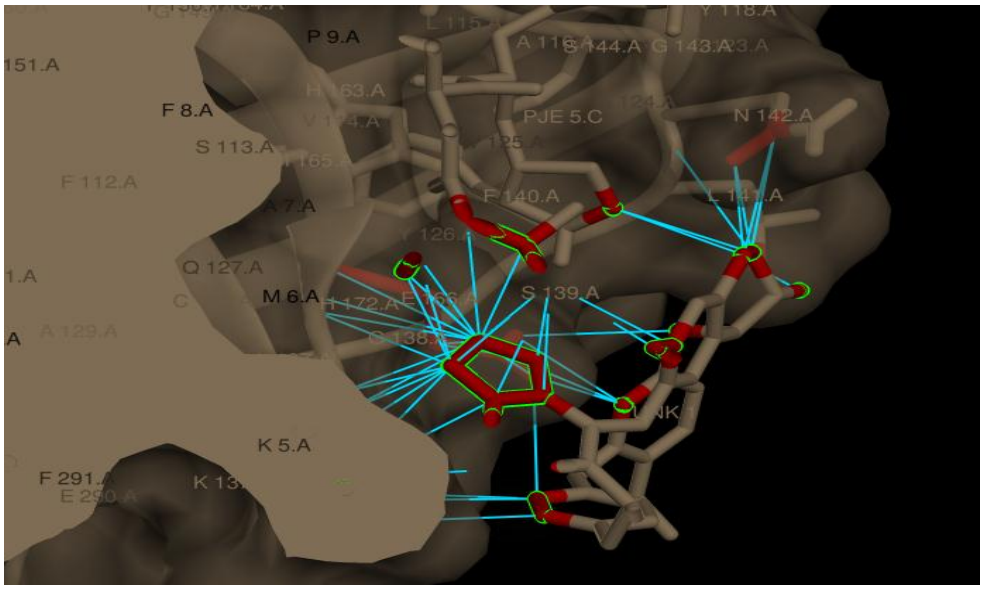


**Figure No. 10: Cephranthine complex1 interactions with 6LU7 binding site**

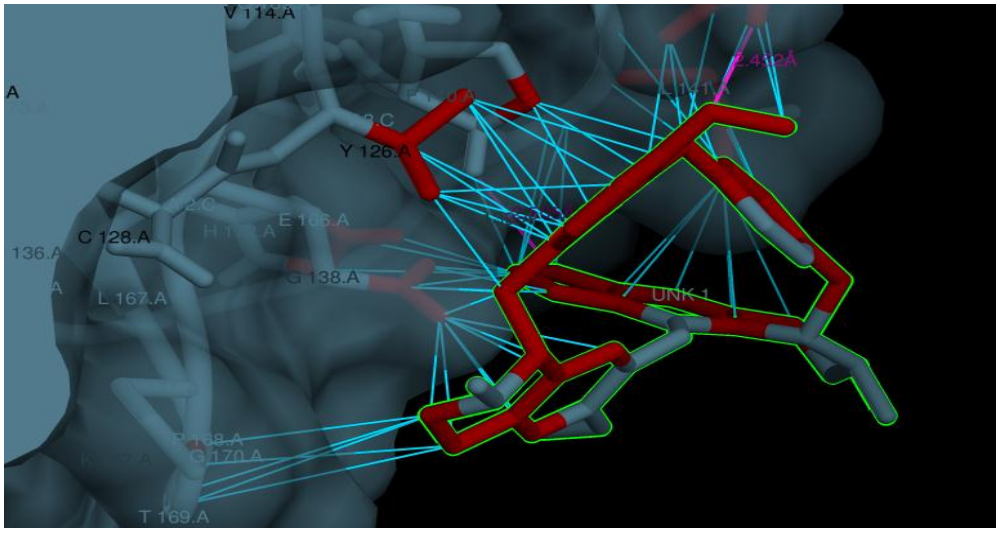


**Figure No. 11: Cephranthine complex2 interactions with 6LU7 binding site**

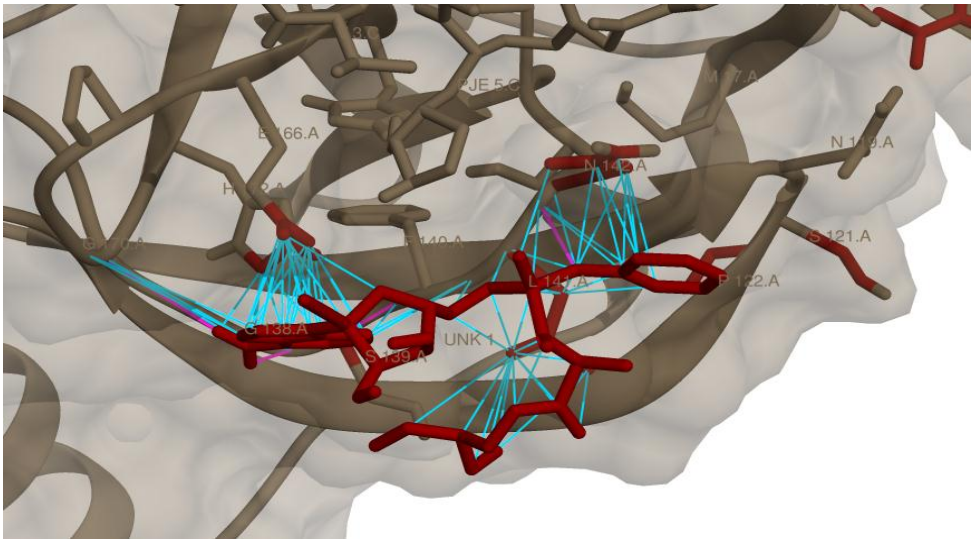


**Figure No. 12: Remdesivir's complex 1 interactions in the binding site of 6LU7**

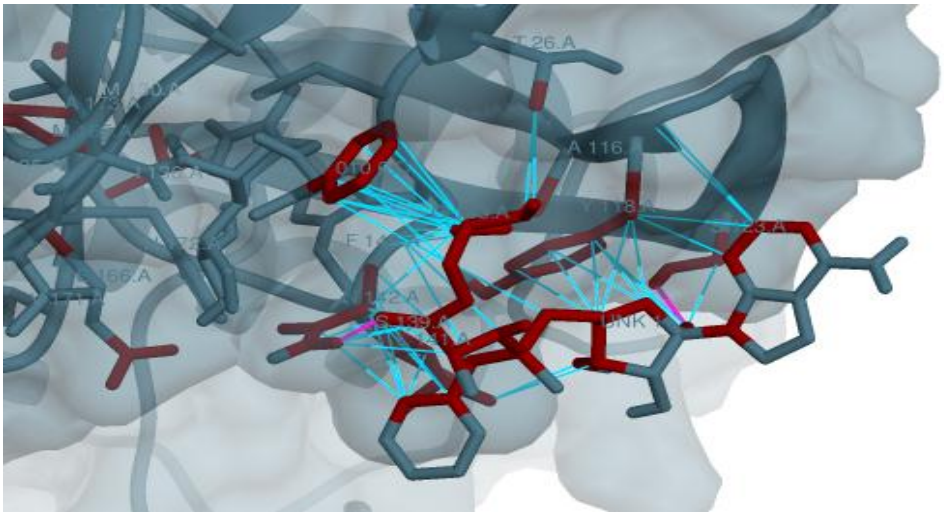


**Figure No. 13: Remdesivir's complex 2 interactions in the binding site of 6LU7**

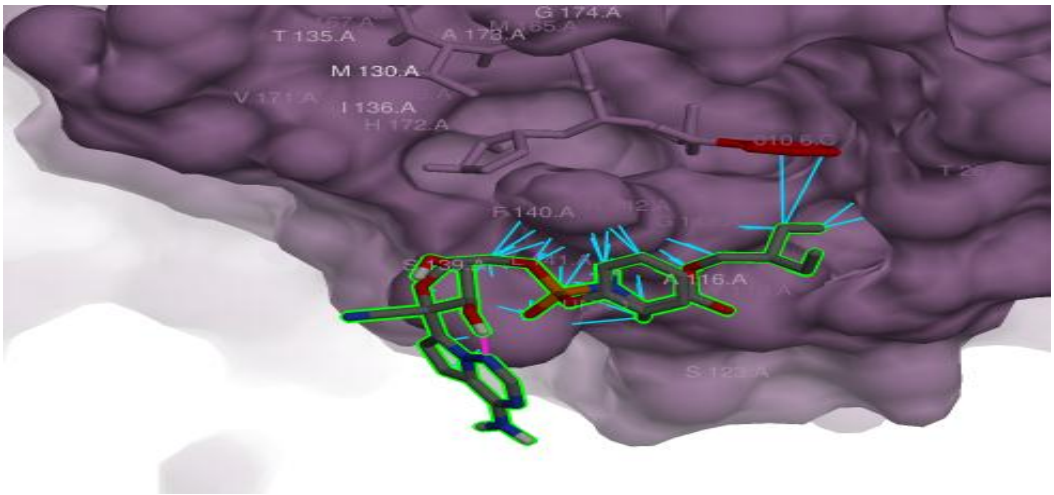


**Figure No. 14: Remdesivir's complex 3 interactions in the binding site of 6LU7**

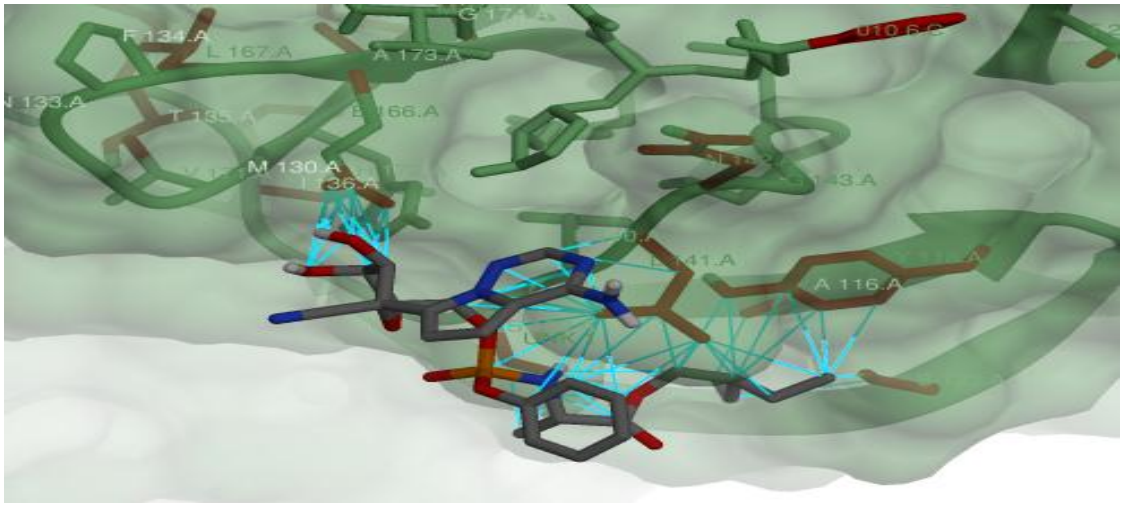


**Figure No. 15: Remdesivir's complex 7 interactions in the binding site of 6LU7**

**Table 1: compound's scoring function**

| compound | cepharanthine | Molfequine | Chloroquine | hydroxychloroquine | Azithromycin | Remdesivir's | zinc |
|---|---|---|---|---|---|---|---|
| Scoring function for complex-1 of each compound | -5.79 | -4.7 | -4.44 | -3.53 | -4.16 | -4.31 | -0.81 |