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Computational Study of Oseltamivir, Chloroquine, Hydroxy Chloroquine, Ribavirin and Kaletra against Lysosomal Protease of COVID19

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Abstract--- *A contagious respiratory disease caused by COVID19 has spread out from China to worldwide, on 30 January 2020; World Health Organization (WHO) declared officially the COVID19 is pandemic disease. Iraqi Health Ministry (IHM) recommended a list involved six drugs (Oseltamivir, Chloroquine, Hydroxy Chloroquine, Ribavirin and Kaletra) as drug of choice to treating COVID19 infected patients. In this study, computational study was performed to evaluate the effectiveness of these drugs against lsysomal protease of COVID19.Chloroquine and Hydroxy chloroquine (D2, D3) respectively showed the highest functional score (-12.3, -10.7 kcal/mol) with appropriate orientation and full fitness (-1244, -1336) inside the active site, and the lowest functional score was Ribavirin (D4) with (8.1 kcal/mol). The other candidate drug Oseltamivir and Kaletra (Lopinavir, Ritonavir) (D1,D5,D6) showed low affinity to bind with the target active site (-3.7 kcal/mol), (5.2 kcal/mol), (5,7kcal/mol) respectively. The computational study showed that Chloroquine and Hydroxy chloroquine have potential inhibitor candidate against lysosomal protease of COVID19, but the other drugs (Oseltamivir and Ribavirin and Lopinavir, Ritonavir) showed low inhibition capacity against same target. To this study we get significant information and we highly recommended utilizing both Chloroquine and Hydroxy chloroquine as dug of choice to treating Iraqi patients infected with COVID19.*

Keywords--- *Chloroquine and Hydroxyl Chloroquine, Lysosomal Protease, Molecular Docking and COVID19.*

I. INTRODUCTION

Last two decade much viral infectious disease emerged, such as Middle East respiratory syndrome-related coronavirus (MERS) and severe acute respiratory syndrome (SARS), still present a big concern to the world health (1). Recently a severe contagious viral infection was reported as it's started in China and transmitted to worldwide. Till 23 march 2020, at least more than 365.000 cases has been reported since first case was hospitalized in Chine on 12 December 2019. The viral infection caused by a newly identified coronavirus, this virus was initially named as the 2019- novel coronavirus (2019-nCoV) on 12 January 2020 by World Health Organization (WHO). WHO officially named the disease as coronavirus disease 2019 (COVID19) and Coronavirus Study Group (CSG) of the International Committee proposed to name the new coronavirus as SARS-CoV-2, both issued on 11 February 2020 (2).

Depend on the phylogeny tree analysis (GISAID accession no. EPI_ISL_402124) (3), COVID 19 belong to

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lineage B of β-coronavirus and share high genetic sequence identitywith that of human severe acute respiratory syndrome coronavirus-related coronavirus (SARS-CoV) and bat SARS-like coronavirus (SL-CoV) (3). WhereCOVID19 was most closely related to the bat coronavirus (SL-CoV) with 82.3% amino acid identity and around 77.2% amino acid identity to SARS-CoV (4).

Genetically, the isolated COVID19 from Wuhan-Hu-1 coronavirus (WHCV) showed positive-sense RNA and complete genome length is 29.9 kb (5), compare with SARS-CoV (27.9 kb) and MERS-CoV (30.1 kb) (6). Also the COVID19 genome showed a variable number (30-33) of open reading frames (ORFs) (7). Where two – thirds of viral RNA, mainly located in the first ORF (ORF1a/b) they are responsible for translates two polypeptides (pp1a, pp1ab), and also encodes 16 non-structural proteins (NSP), while the remaining ORFs encodes other structurally and accessory proteins. The rest parts of viral genome encodes mainly the four major structural proteins involve Nucleocapsid (N), Matrix (M), Envelope (E) and Spike (S) glycoprotein. COVID19 exhibits some genomic and phylogenetic similarity to SARS-CoV, particularly in the S-glycoprotein gene and receptor-binding domain (RBD), as most genomic genes are encoded proteins of COVID19 are similar to SARS-CoVs, with tiny differences (8).

Structurally, Coronaviruses family is large, enveloped, single-stranded RNA viruses, where the virus is enclosed by a membrane that carries Spike protein (S) which will mediate the attachment step and entry into the host cell. Matrix (M) which involved in organizes the nucleoprotien inside, and Envelope (E) made up from lipid and protein and it is involved in the viral budding step and may be incorporated into the virion. Finally the Nucleocapsid (N) inside membrane that bounded the genomic RNA as showed in (Fig.1) (9, 10). An envelope-anchored spike protein guides coronavirus entry into host cells (11, 12). It first attaches and bind with host Angiotensin-converting enzyme 2 (ACE2) receptor through viral S1subunit (found in viral surface spikes) and the fused the whole virus body into host membranes by aids of its S2 subunit (7, 14).

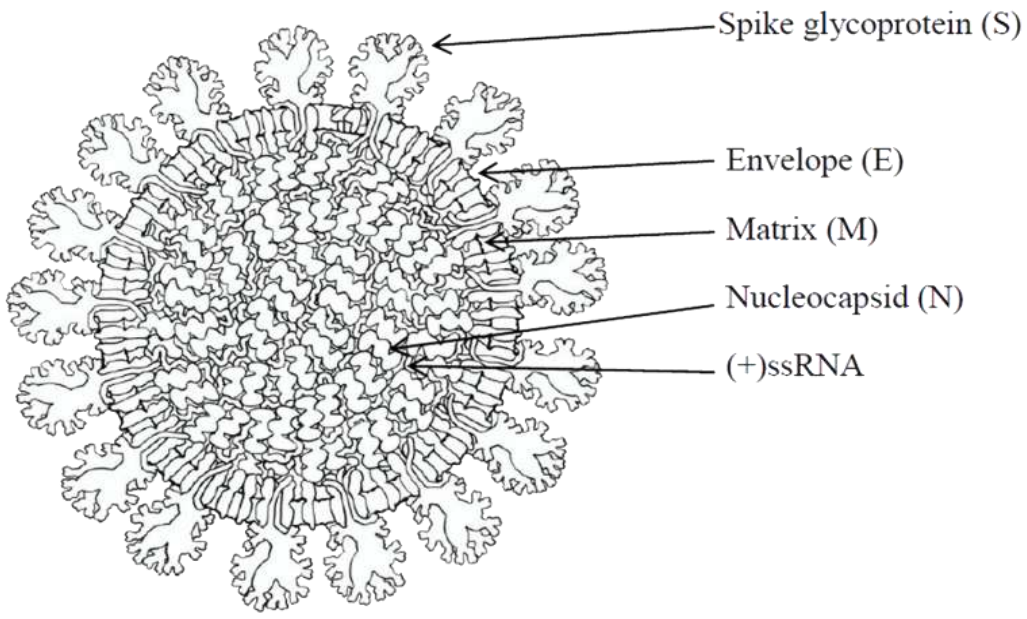

Figure 1: COVID 19 Structure

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One of the most important features of COVID19 is their tropism (14), where the viral entry into the host is one of the important determinants of viral tropism (15). The entry of COVID19 involves two main steps: Angiotensin-converting enzyme 2 (ACE2) receptor binding and membrane fusion into the host cell. Where the COVID 2019 enters endosome, and proceeds to lysosomes, then fused into lysosomal membrane. The lysosomes play critical roles in cell metabolism by breaking down biomolecules and cellular debris and also by providing nutrients for other cellular functions (16). The lysosomal protease activities are central to the functions of lysosomes (17). They are also required to activate the membrane fusion of a variety of viruses, including coronaviruses and filoviruses (18-20). Understanding the correlation between lysosomal protease activities and viral tropism has important implications for investigating viral pathogenesis (21).

Lately, Iraqi Health Ministry recommended to utilize six different drugs as antiviral agentand they are (Oseltamivir, Chloroquine, HydroxyChloroquine, Ribavirin and Kaletra (Lopinavir, Ritonavir)) in their hospitals, as drug of choice for COVID19 infected patients who started to attend in Iraqi hospitals during march 2020. Clearly these different drugs have different targets, as Oseltamivir has potential inhibitor for neuraminidase enzyme of Inflenza A virus (strain A/Chile/1/1983 H1N1) (22), Chloroquineand HydroxyChloroquine both have potential to inhibit the action of heme polymerase in malarial tropozoit and preventing the conversion of heme to hemazoin, also both drugs have ability to diffuse into lysosome and increase the PH (23). Recently Chloroquine and HydroxyChloroquine are used to prevent the glycosylation of ACE2 receptor that consider as a target for CONVID19 (24). Ribavirin also has ability to inhibit the protein synthesis of Influenza A virus (strain A/Beijing/11/1956 H1N1), HBV, HCV, other RNA viruses. The specific target of Ribavirin is RNA-directed RNA polymerase catalytic subunit (25). Kaletra (Lopinavir, Ritonavir) is involved in inhibition of Human immunodeficiency virus type 1 (HIV-1) protease enzyme that normally cleaves the structural and replicative proteins that arise from major HIV genes, such as Gag and Pol proteins (26,27). Gag gene encodes proteins involved in the core and the nucleocapsid, while Pol gene encodes the HIV reverse transcriptase (28). So the present study is computational study of these drugs to investigate their effectiveness against their target in COVID19.

II. METHODS

The three dimensional (3D) crystal structure of the lysosomal protease of COVID 19 were obtained from Protein Data Bank server (6Y84 accession No.), with high resolution1.37 A° created experimentally by X-Diffraction method (35). The lysosomal protease consists of two subunit (A1, A2) (29). Further optimization was done by UCSF Chimera, moreover the determination and visualization of the active site done by Discovery.Studio v2.5 software (Fig. 2). The next step is construction and preparation of six chosen small molecules (Oseltamivir (Tamiflu), Chloroquine, HydroxyChloroquine, Ribavirin and Kaletra (Lopinavir, Ritonavir)) (D1-D6) respectively as ligand, involving manufacturing 2D sketching and transforming into 3D structure (Fig.3), and minimizing the field energy to meet the requirement for molecular docking algorithm. The third step is submitting to the molecular docking algorithm online by use the Swiss Dock Server. The final step is the analysis the computational simulation results docking by Chimera 1.10 software.

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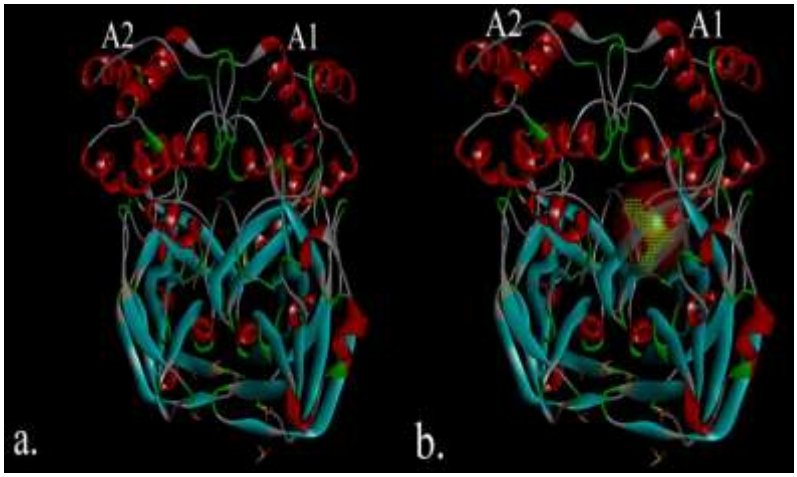

Figure 2: a. 3D crystal structure of Lysosomalprotease of COVID19. b. Visualizing the Active Site of Target A1 Subunit

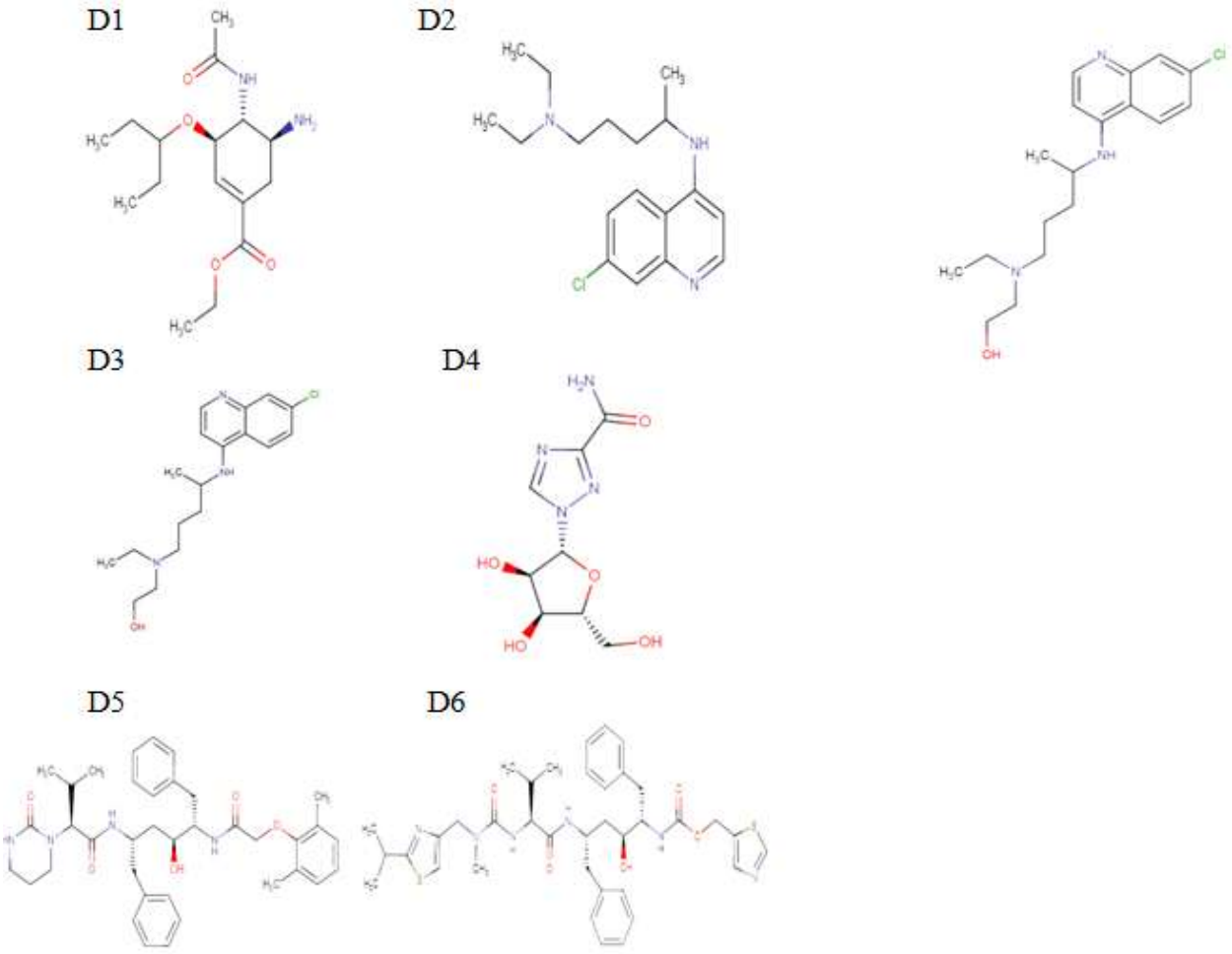

Figure 3: 3D Structure of Six Chosen Molecules (D1: Oseltamivir, D2: Chloroquine, D3: Hydroxy Chloroquine, D4: Ribavirin, D5: Lopinavir, D6: Ritonavir

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III. RESULTS AND DISCUSSION

Computational study (Molecular Docking) was performed to evaluate the effectiveness of six recommended drugs (D1-D6) against lysosomal protease (6Y84 accession No), the binding free energy, full fitness and Gibbs energy (ΔG) are main functional score that reflects the affinity energy to bind process between the ligand- protein to form a complex, and which one that has the lowest affinity energy to bind means has high functional score and become more stable interaction and binding (30). The molecular docking results of six drugs with spike glycoprotein and lysosomal protease showed in (Fig.4.) respectively.

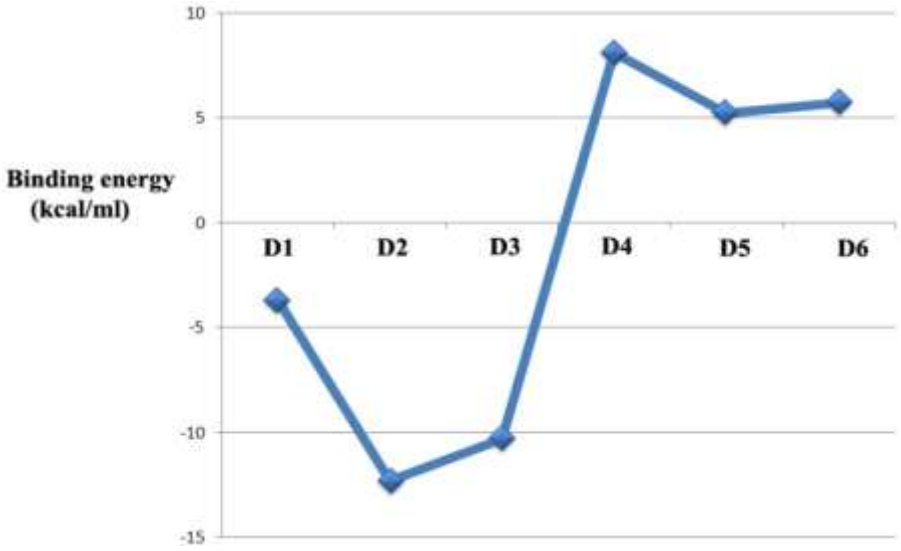

Figure 4: Molecular Docking Results of Drugs against A1 of Lysosomal Protease

The molecular docking results showed that two of six drugs are Chloroquine and Hydroxychloroquine (D2-D3) have pretty good potential affinity to bind with preferred active site of A1 subunit of lysosomal protease of COVID19, where this binding is occupied the allosteric conformation of active site and led to block it and prevent the active site from bind with other substrate, all these events lead to loss the function of the enzyme and disruption the main process of the target protein (30). The Chloroquine and Hydroxychloroquine (D2, D3) respectively showed the highest functional score (-12.3, -10.7 kcal/mol) with appropriate orientation and full fitness (-1244, -1336) inside the active site, and the lowest functional score was Ribavirin (D4) with (8.1 kcal/mol).the other candidate drug Oseltamivir and Kaletra (Lopinavir, Ritonavir) (D1,D5,D6) showed low affinity to bind with the target active site (-3.7 kcal/mol), (5.2 kcal/mol), (5,7 kcal/mol) respectively.

The computational study showed a good indication can be seen by comparing the values of the binding free energy, full fitness, the Gibbs energy (ΔG), molecular weight and the amount of hydrogen interactions as standard inhibitor depending on Lipinski's rule of five. A bond forming can create a strong complex that characterized by a low binding energy, ΔG value, full fitness and the number hydrogen interactions with side chain of amino acid residues of active site of A1 subunit as showed in (Fig. 5).

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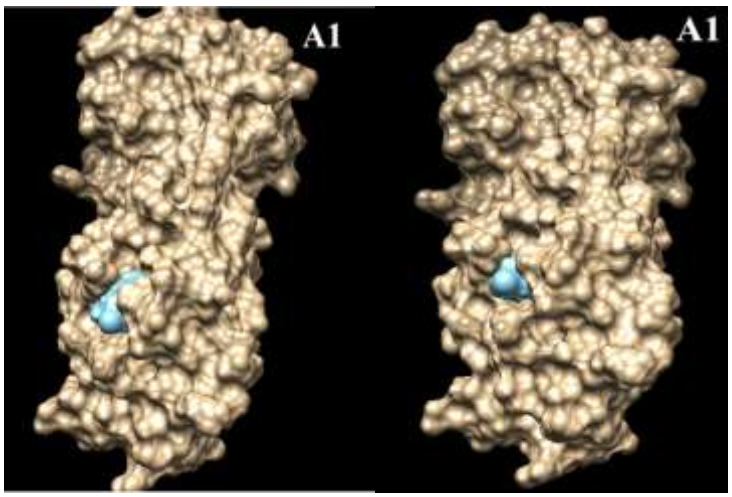

a. b.

Figure 5: a. Chloroquine (D1) Docked in Active Site of A1 Subunit. b. Hydroxy Chloroquine (D3) Docked in Active of A1 Subunit

Based on the computational study results, the chloroquine and hydroxyl chloroquin (D2, D3) have pretty potential inhibitor candidate for Lysosomal protease of COVID19 and eliminating the other drugs (Oseltamivir and Kaletra (Lopinavir, Ritonavir) from drug of choice list in treatment of COVID19 patients.

IV. CONCLUSION

The computational study showed that Chloroquine and Hydroxy chloroquine have potential lysosomal protease inhibitor candidate against COVID19, and other drugs (Oseltamivir and Ribavirin and Lopinavir, Ritonavir) showed low inhibition against same target. Where the molecular docking simulations (insilico) reflecting the initial step in the development of the discovery of new drug candidates. To this study we get significant information and we highly recommended to utilize chloroquine and Hydroxy chloroquine as dug of choice to treat Iraqi patients with COVID19.

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