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Evaluation Study Of Some Benzo (B) Thiophene Derivatives Against The Lysosomal Protease Of SAR-Cov-2

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Abstract

A contagious respiratory disease caused by COVID19 has spread out from China to worldwide, on30 January 2020; World Health Organization (WHO) declared officially the COVID19 is pandemic disease.In this study, computational studywas performed to evaluate the effectiveness of chemical compounds (M1 & M2) against lsysomal protease of SAR-CoV-2. The molecular docking results showed that the two molecules (M1& M2) have pretty good potential affinity to bind with preferred active site of A1 subunit of lysosomal protease of SAR-CoV-2, where the compounds (M1, M2) showed highest functional score (-12.5, -21.6 Kcal/mol) with appropriate orientation and full fitness (-1271, -1308) inside of the active site compared with Chloroquine and Hydroxychloroquine (-12.3, -10.5 Kcal/mol) respectively.The results of ADME toxicity profile of compounds (M1, M2) were computed and compared with Chloroquine and Hydroxy chroloquine. Table (1) showed the two molecules (M1, M2) meet the drug likeness parameters Both compounds have high Pharmacokinetics with ability to inhibit CYP1A2, CYP2C19 and CYP2C9 with high ability to absorption in gastrointestinal (GIA), effluated in central nerve system (CNS) and brain-blood barrier permeability (BBB). Based on the computational study results, the molecules (M1 & M2) have pretty potential inhibitor candidate for Lysosomal protease of SAR-CoV-2.

Two benzo (b) thiophene containing triazole moity especially 3-(3—chloro-1-benzothien-2-yl)-4H-1,2,4-triazol-3-N-piperidine (M1) and 3-(chloro-1-benzothien-2-yl)4H-1,2,4-triazole-3-N-pyrole (M2) were synthesized and succefully characterized by FT-IR spectrum.…

Key Words SAR-CoV-2, Lysosomal protease, molecular docking, compounds (M1 &M2).

1 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 (Wolfeet al., 2007). Recently a severe contagious viral infection was reported as it's started in China and transmitted to worldwide. Till 7September 2021, at least 225.326.000 cases have 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 (Yan-Rong et al., 2019).

Depend on the phylogeny tree analysis (GISAID accession no. EPI_ISL_402124) (3), COVID 19 belong to lineage B of β-coronavirus and share high genetic sequence identity with that of human severe acute respiratory syndrome coronavirus-related coronavirus (SARS-CoV) and bat SARS-like coronavirus (SL-CoV) (Chan et al., 2020). Where COVID19 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 (Zhou et al., 2020).

Genetically, the isolated COVID19 from Wuhan-Hu-1 coronavirus (WHCV) showed positive-sense RNA and complete genome length is 29.9 kb (Fan Wu et al., 2020), compare with SARS-CoV (27.9 kb) and MERS-CoV (30.1 kb) (De Wit et al., 2016). Also the COVID19 genome showed a variable number (30-33) of open reading frames (ORFs) (Belouzard et al., 2009). 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-CoV, with tiny differences (Yan-Rong et al., 2019).

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 nuceloprotien 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 (Enjuanes et al., 2006, Perlman and Netland, 2009). An envelope-anchored spike protein guides coronavirus entry into host cells (Li, 2015,Li, 2016). 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 (Belouzard et al., 2009, Nomaguchi et al., 2012).

One of the most important features of COVID19 is their tropism (Nomaguchi et al., 2012), where the viral entry into the host is one of the important determinants of viral tropism (Li et al., 2006). 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, and 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 (Lim and Zoncu., 2016). The lysosomal protease activities are central to the functions of lysosomes (Turk et al., 2012). They are also required to activate the membrane fusion of a variety of viruses, including coronaviruses and filoviruses (Millet and Whittaker, 2015, Simmons et al., 2017). Understanding the correlation between lysosomal protease activities and viral tropism has important implications for investigating viral pathogenesis (Simmons et al., 2018).

Chloroquine and Hydroxychloroquine were prescribedextensively for the prevention and treatment of Malaria, rheumatoid arthritis and systemic lupus erythematosus.The main mechanismof action of these drugs against Plasmodium parasites isbelieved to be partly related to its interaction with DNA andthrough inhibition of the polymerization of heme (Tönnesmannet al., 2013).

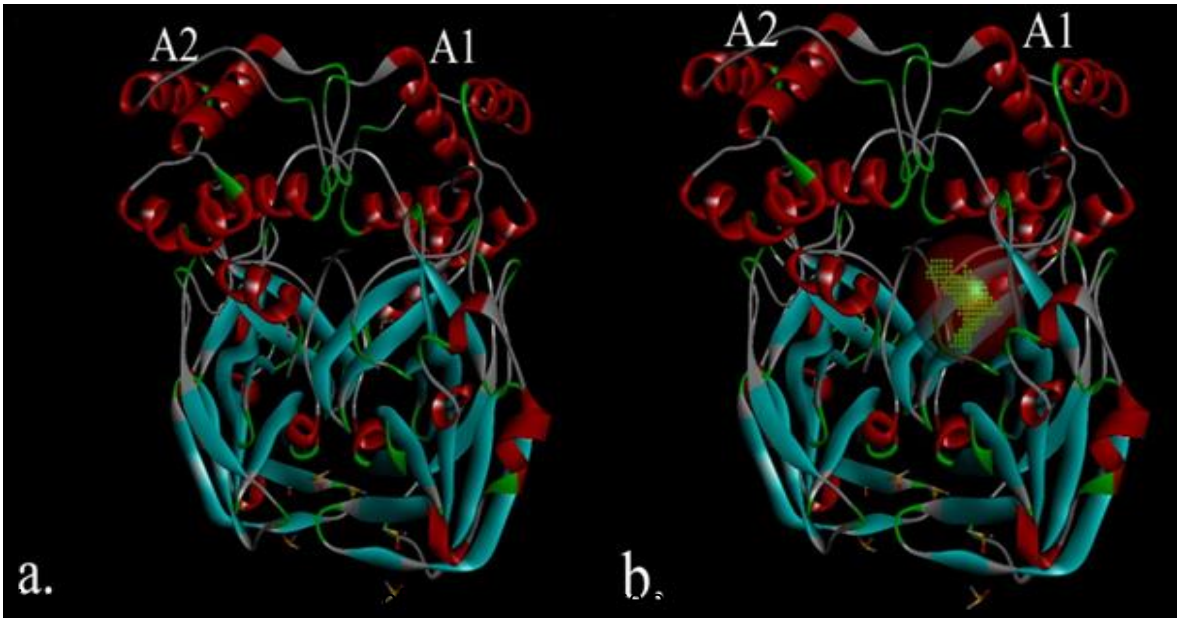
Lately, Chloroquine and Hydroxychloroquine have been labeled against SARS-CoV-2 , As well as Chloroquine might prevent the SARS-COV-2 from attach and binding to ACE-2 receptor by inhibition the terminal glycosylation. New study showed that Hydrochloroquine may additionally prevent SARS-CoV-2 from binding with gangliosides leads to inhibit the virions to touch the ACE-2 receptor (Fantini et al., 2020).Both Chloroquine and Hydroxychloroquine are able to enter into the lysosomes andendosomes led to increased pH of intracellular components.These organelles normally require an acidic environment forhomeostasis. Ultimately, this increase in pH results in theirdysfunction, leading to defective protein degradation, endocytosis,and exocytosis needed for viral infection, replication, andpropagation (Al-Bari, 2015). The Entry intothe endolysosome might be essential for the viral genome to bereleased into the cytoplasm of infected host cells (Mingo et al., 2015).

2- 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, The lysosomal protease consists of two subunit (A1, A2) (Abdalkader et al., 2020). Further optimization was done by UCSF Chimera, moreover the determination and visualization of the active site done by Discovery.Studio v2.5 software (Fig. 1). The next step is construction and preparation of three small molecules which entitled (M1,M2) respectively as ligand, involving manufacturing 2D sketching and transforming into 3D structure (Fig.2), 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 thecomputational simulation results docking by Chimera 1.10 software.

Computer-based methods have been employed to predict of ADMEtoxicity profile properties of designed molecules. The molecules (M1, M2) were sketched in 3D structures and energy minimizationand subjected to SWISSADME Server for assessment of the ADMET profile.These rules are distributed among molecular weights ofdesigned molecules and assessment the physicochemical properties (Hussein et al., 2005).



b. Visualizing the active site of target A1 subunit.

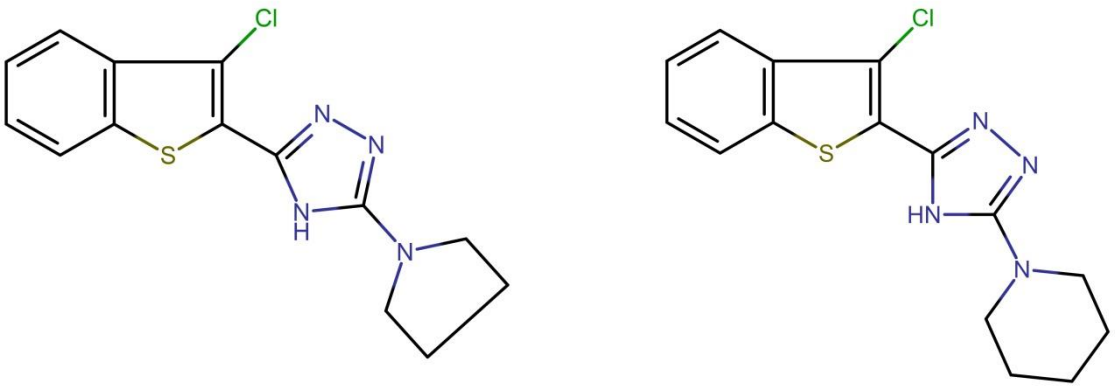


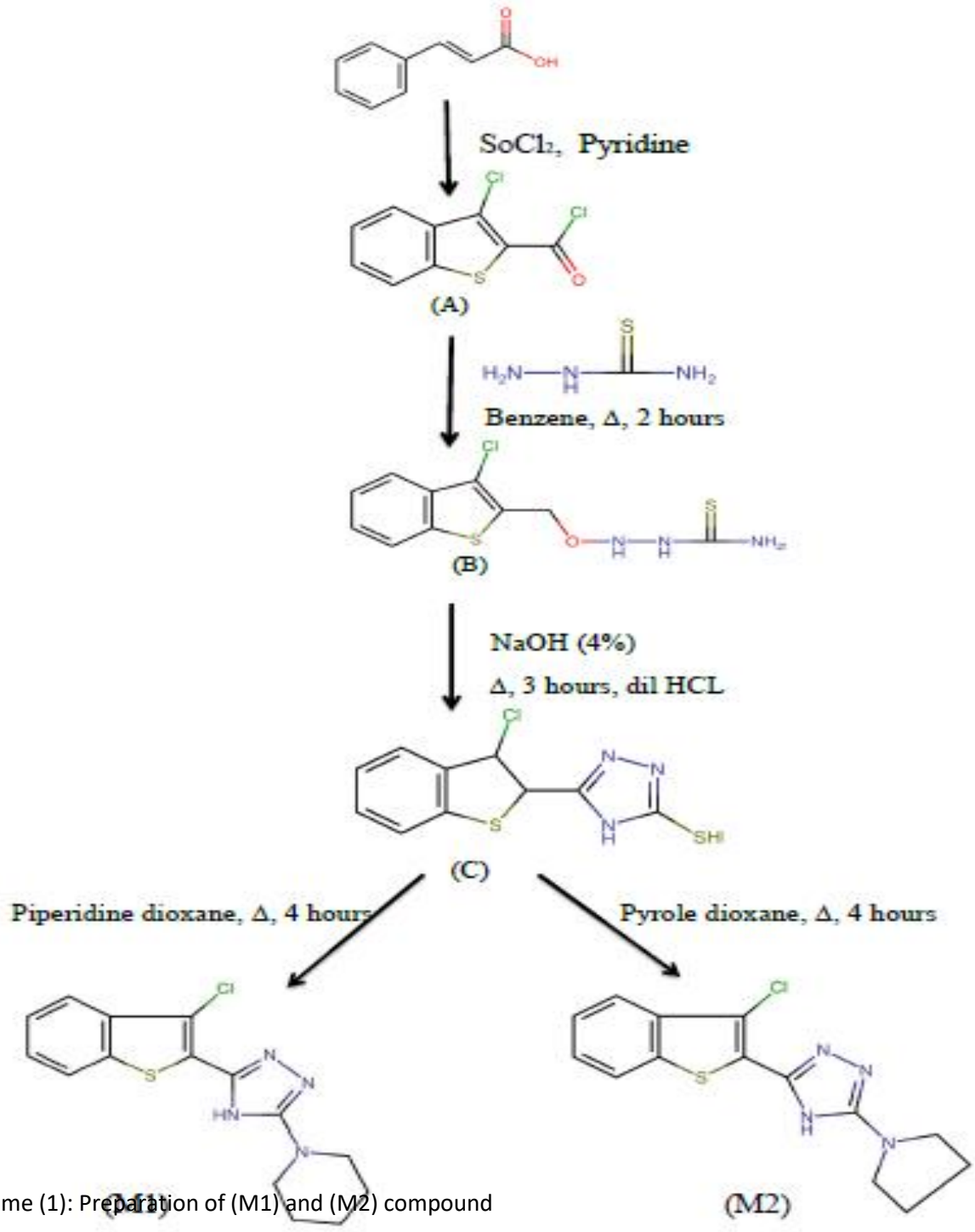
Figure 2: 3D structure of Two chosen molecules (M1, M2).

3- Results:

3.1. Chemistry

The new 3-(3-chloro-1-benzothien-2-yl)-4H-1,2,4,-Triazole-3-N-piperdine (M1) and 3-(3-chloro-1-benzothien-2-yl)-4H-1,2,4,-Triazole-3-N-Pyrrolen (M2) were synthesized as following reaction sequences depicted in the scheme (1). The starting material for the synthesis of target compounds (M1 & M2) is 3-chloro-2-chlorocarbonyl (benzo (b) thiophene (A) which was synthesized by the reaction of Cinnamic acid with thionyl chloride in Dimethylformamide (DMF) and dry pyridine according to the report method (29). Reaction between (A) and thiosemicarbazide in dry benzene afforded the 3-chlorobenzo (b) thiophene-2-carboxyylic thiosemicarbazide (B) which upon ring closure with 4% NaOH gave 5-(3-chloro-1-benzothien-2-yl))-4H-1,2,4,-Triazole-3-thiol (C) which is converted into derivatives (M1 & M2) on treatment with piperidine and pyrole respectively (Zainab et al., 2008).

The synthesized compounds were characterized by using some spectroscopic methods such as FT-IR spectra (KBR disc) were recorded with a Pye-Unicam SP-300 spectrometer and ^{1}HNMR on a Hitachi-Perkin and Tetra methyl silane, an internal standard Elemental analysis were done on a 1106 carlo-Erba instrument.



Scheme (1): Preparation of (M1) and (M2) compound

3.2. Insilico study:

Molecular Docking study (Insilico) was conducted to evaluate the effectiveness of two synthesized molecules (M1, M2) against the target protein (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 (Abdalkader et al., 2020).The computational study results of (M1, M2) with lysosomal protease compared with Chloroquine and Hydroxychloroquine as showed in (Fig.3.) respectively.

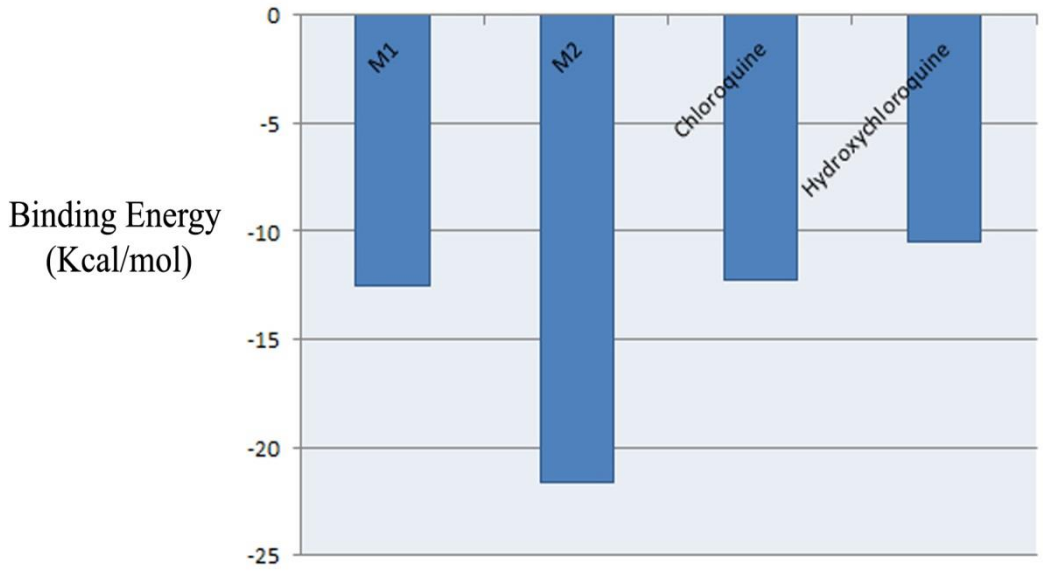


Figure (3): Molecular docking results of (M1, M2, Crolorquine and Hydroxychloroquine) against A1 subunit of lysosomal protease.

The molecular docking results showed that the two molecules (M1& M2) have pretty good potential affinity to bind with preferred active site of A1 subunit of lysosomal protease of SAR-CoV-2, 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 (Abdalkader et al., 2020). As the two molecules (M1, M2) showed highest functional score (-12.5, -21.6 Kcal/mol) with appropriate orientation and full fitness (-1271, -1308) inside of the active site compared with Chloroquine and Hydroxychloroquine (-12.3, -10.5 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. 4).

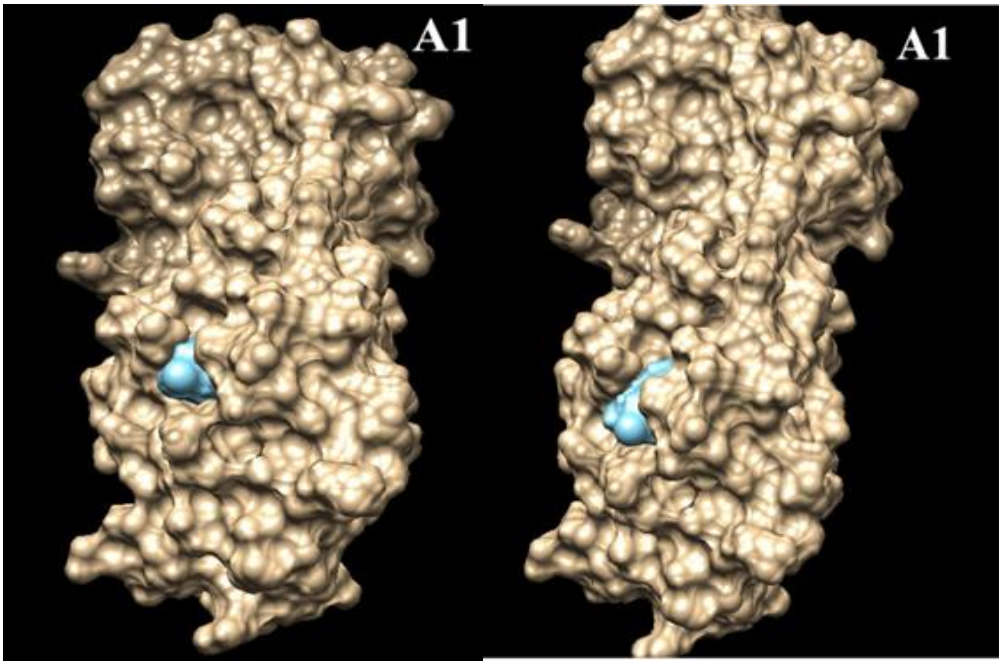


Figure 4: a. Molecule (M1) docked into active site of A1 subunit.

b. Molecule (M2) docked into active of A1subunit.

The results of ADME toxicity profile of designed molecules (M1, M2)were computed and compared with Chloroquine and Hydroxy chroloquine. Table (1) showed the two molecules (M1, M2) meet the drug likeness parameters where molecular weight (MW) ranged (304.8 – 318.8 g/mol), and water Solubility LogSwat were ranged ((-4.4) – (-4.7)), meanwhile the lipophilicity LogP was (2.5 - 2.6) respectively. Both molecules (M1, M2) have Hydrogen bond acceptor and donor (HBA/HBD) (2/1) that enable these molecules to create a covalent bonding withamino acid in active site of target protein. Both molecules have high Pharmacokinetics with ability to inhibit CYP1A2, CYP2C19 and CYP2C9 with high ability to absorption in gastrointestinal (GIA), effluated in central nerve system (CNS) and brain-blood barrier

permeanbilty (BBB) (Abdalkader et al., 2020). Based on the computational study results, the molecules (M1 & M2) have pretty potential inhibitor candidate for Lysosomal protease of SAR-CoV-2.

Table (1): Results of ADME Toxicity profile of molecules (M1&M2) (descriptors) compared with Chloroquine and Hydroxychloroquine.

No.	MW	LogS	LogP	HBA/HBD	GIA	CNS effluated	BBB Permeability	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor
M1	304.8	-4.4	2.5	2/1	High	yes	yes	yes	yes	yes
M2	218.8	-4.7	2.6	2/1	High	yes	yes	yes	yes	yes
Chloroquine	319.8	-4.5	3.9	2/1	High	no	yes	yes	no	no
Hydrochloroquine	335.9	-3.9	3.5	3/2	High	no	yes	yes	no	no

Based on the computational study results, the molecules (M1 & M2) have pretty potential inhibitor candidate for Lysosomal protease of SAR-CoV-2.

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