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Molecular Docking Study of the Binding Interaction of Hydroxychloroquine, Dexamethasone and Other Anti-Inflammatory Drugs with SARS-CoV-2 Protease and SARS-CoV-2 Spikes Glycoprotein

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Abstract

Aims: The outbreak of the novel coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is still accountable for millions of deaths worldwide and declared as a global pandemic by the World Health Organisation. Despite efforts, there is still limited evidence available on a successful potent inhibitor with a low toxicity profile that can aid in the prevention and/or treatment of COVID-19. This study will focus on four main aspects: 1) screening 19 Food Drug and Administration (FDA) approved drugs using computational molecular docking; 2) assessing drug toxicity profiles using biological data; 3) recommending potential therapies against COVID-19 and 4) supplementing currently used therapies. **Methods:** 19 FDA approved drugs were investigated against the crystal structure of SARS-CoV-2 protease (6LU7) and SARS-CoV-2 glycoprotein (6VXX) using a computational molecular docking software, Molecular Operating Environment (MOE). Separately, on MOE, 6LU7 and 6VXX were loaded, prepared, and the binding pockets located. The drug's canonical SMILES were imported, minimised, and docked on the prepared proteins using a search algorithm to establish the highest stability conformation. Drugs were ranked depending on binding properties and biological data to assess safety; steric clashes and voids in the binding site were also analysed. **Results and discussion:** Out of the nineteen (19) FDA approved drugs, 18 inhibited 6LU7 and 13 inhibited 6VXX. High-ranked drugs based on binding properties for 6LU7 were hydroxychloroquine, dexamethasone, naproxen, etoricoxib, and ibuprofen. For 6VXX were hydroxychloroquine, celecoxib, etoricoxib,

meloxicam, and parecoxib. Considering safety profile, the top 3 drugs in descending order for 6LU7 were etoricoxib, naproxen and dexamethasone and for 6VXX were etoricoxib, meloxicam, and parecoxib. Compared to the literature, the results were consistent for dexamethasone which was effective against 6LU7. However, for hydroxychloroquine and ibuprofen, there was conflicting literature regarding safety and efficacy. **Conclusion and future work:** The findings suggest that against COVID-19 etoricoxib might be effective as a therapeutic and prophylactic measure. Naproxen and dexamethasone would be more effective as treatment only while meloxicam and parecoxib as prophylaxis. However, future studies are needed to validate these findings. Compared to previous literature, the findings in this study also support the use of dexamethasone over hydroxychloroquine and ibuprofen for COVID-19 based on the binding and safety properties. Despite this, future research should explore the impressive binding properties displayed by hydroxychloroquine and ibuprofen to aid in developing a new drug against COVID-19.

Keywords

Anti-Inflammatory Drugs, COVID-19, FDA Approved Drugs, MOE Software, SARS-CoV-2, Spike Glycoprotein

1. Introduction

The emergence of the novel coronavirus disease (COVID-19) caused by the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) has been accountable for a staggering number of deaths worldwide and was affirmed as a global pandemic and public health crisis on 11 March 2020 by the World Health Organisation [1]. Although a specific efficacious treatment against COVID-19 is not yet available, the discovery of one is crucially needed. This has led to the investigation of other treatment options particularly repurposing readily available therapies that have already been assessed for safety. In this chapter, we will discuss our current understanding of COVID-19, critique the literature available, and discuss computational approaches in the aid of efficient drug design.

1.1. Epidemiology of SARS-CoV-2

The WHO epidemiological reports [2] have concluded that globally as of 3rd January 2021, the cumulative number of cases was over 83 million and deaths were over 1.8 million since the beginning of the pandemic (**Figure 1**). Last week, the United States of America, the United Kingdom, Russia, and India reported the highest number of cases. Americas accounted for 47% of all new cases and 42% of all new deaths. Europe accounted for 38% of new cases and 43% of new deaths. Western Pacific region had comparable new cases to last week but a rise in deaths (10%). South-East and Eastern Mediterranean regions had a decline in new cases and deaths. The greatest increase in new cases (13%) and deaths (28%)

was in Africa. Furthermore, recent reports have detected a SARS-CoV-2 variant VOC-202012/01 in the United Kingdom and 501Y.V2 in South Africa [2].

1.2. The Structure of SARS-CoV-2

The coronaviruses originate from Coronaviridae and are enveloped, positive-sense single-stranded ribonucleic acid (ssRNA) viruses [3]. At present, seven human coronaviruses are known: hCoV-229E, hCoV-HKU1, hCoV-OC43, hCoV-NL63, SARS-CoV, Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2 [4]. The SARS-CoV, MERS-CoV, and SARS-CoV-2 have similar structural characteristics and are majorly responsible for severe pneumonia [5].

The coronaviruses infect humans and animals; SARS-Cov-2 is a β coronavirus that mainly affects mammalians [6]. The highly pathogenic SARS-CoV-2 targets the respiratory system and is primarily transmitted through airborne respiratory droplets from an infected person [7].

The SARS-CoV-2 ribonucleic acid (RNA) genome compromises of flanked 5'-capped and 3'polyadenylated untranslated regions, composed of multiple opening reading frames to encode for structural proteins. SARS-CoV-2 compromises of five proteins that have different roles (**Figure 2**); this includes the nucleocapsid (N) protein for viral replication, transcription, and host cell hijacking, membrane (M) protein for assembly, organisation, viral fusion, and budding, envelope (E) protein for viral assembly, host permeability, and viral-host interaction [8]. Hemagglutinin-esterase dimer (HE) is associated with the viral entry [9] and encoded by the HE gene [10]. Lastly, the projecting spike (S) glycoprotein found on the envelope of the corona-virion acts as the main point of interaction with the host cell receptor and is composed of 3 replicate chains each having 1273 amino acids [9]. The S glycoprotein consists of two subunits: S1 for viral-host interaction and S2 which medicates membrane fusion [6]. Since the S glycoprotein interacts with the host receptors first, it can be utilised as a drug target to prevent viral entry and spread.

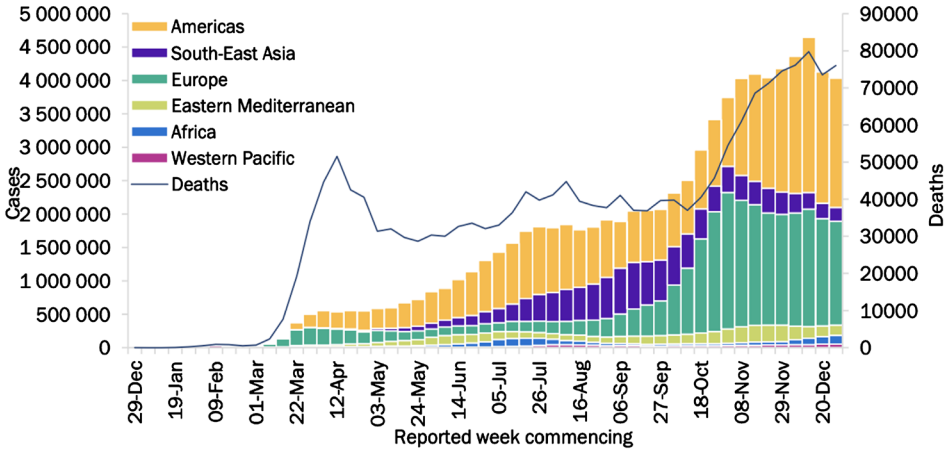


Figure 1. The weekly figures of cases and deaths from COVID-19 by region, from the 3rd January 2021 [2].

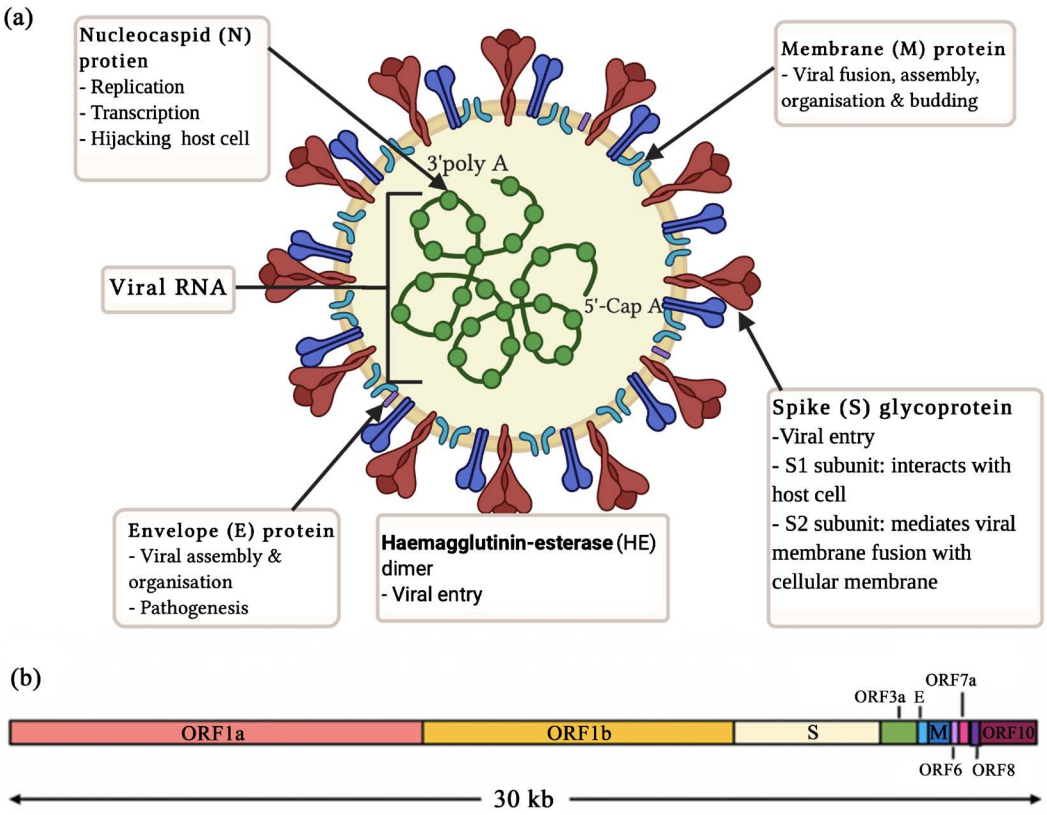


Figure 2. SARS-CoV-2 structure and viral genome organisation. (a) BioRender generated the diagram. (b) The SARS-CoV-2 RNA genome organisation was collected from GenBank (AN: MN908947).

1.3. The Pathophysiology of SARS-CoV-2

SARS-Cov-2 enters by endocytosis or fusing the viral envelope directly with the host membrane [11] (**Figure 3**). Viral S-protein cleaves into S1 and S2 subunits with the assistance of Type II Transmembrane Serine Protease (TMPRSS2) present on the host's type II pneumonocytes [12]. The S1 subunit binds onto the Angiotensin-Converting Enzyme 2 (ACE2) [13] also found on the host type II pneumonocytes; the S2 subunit later cleaved initiates membrane fusion by liberating the fusion peptide. This facilitates S-protein activation and fusion which enables viral entry and spread.

Next, the coronaviruses uncoats the nucleocapsid (N) protein which releases viral ssRNA into the host cytoplasm. This viral RNA uses mRNA to translate replicase polyprotein 1a (pp1a) and 1ab (pp1ab) which undergo autoproteolytic cleavage by viral protease to form numerous non-structural proteins including RNA-dependent RNA polymerase enzyme and helicase which facilitate the production of 6-9 sub-genomic messenger RNA (mRNA) which form accessory and structural proteins (N, M, E, S) [14]. These proteins are then transported to the endoplasmic reticulum-Golgi intermediate compartment (ER-GIC) and the viral RNA in the cytoplasm to produce nucleocapsids which combine within the ER-GIC membrane to self-assemble the new virions, which are released by exocytosis and spread to neighbouring cells [14].

Viral production damages the ER present on the type II pneumocytes and initiates a viral-induced inflammatory cascade to prevent viral dissemination [15]. After viral antigen recognition, the cells release cytokines, including interleukin IL-1, IL-2, IL-6, IL-8, and tumour necrosis factor-alpha (TNF-α) [16]. This stimulates chemotaxis and recruits natural killer cells to the damaged site to releases reactive oxygen species (ROS), cytotoxic T lymphocytes, and nitric oxide [15] to destroy the virus.

This overly exaggerated immune response during the infection can result in

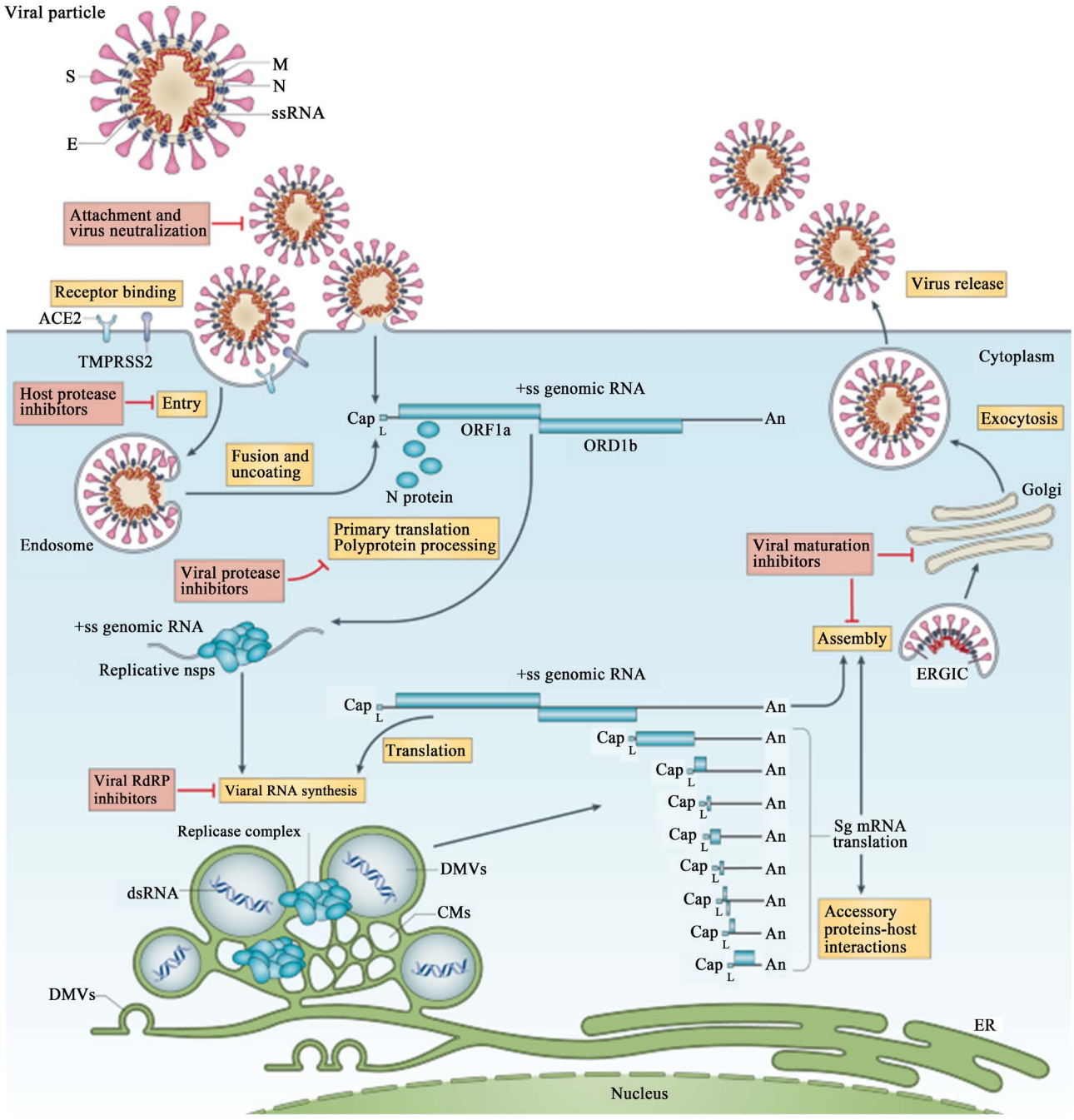


Figure 3. The SARS-CoV-2 Lifecyle [14].

substantial damage to the lung epithelium and endothelium which can lead to alveolar collapse, impaired gas exchange, hypoxemia, tachycardia, and shortness of breath [15]. Serious complications, include acute respiratory distress syndrome (ADRS), septic shock, organ failure, and death [17], which are more common in the elderly with comorbidities and high-risk groups.

1.4. Literature Review of Drug Therapies Currently Available against the SARS-CoV-2

The pathological and clinical features of SARS-CoV-2 resemble the emergence of SARS-CoV in 2002 and MERS-CoV in 2012 [14] as they all utilise a similar mechanism that dysregulates the host immune response [18] through spike (S) protein interaction with the respiratory tract. Indicating that effective drug treatments used to combat the SARS and MERS outbreak (corticosteroids and antimalarials) may potentially be suitable for the SARS-CoV-2 virus [19] [20].

Repurposing existing treatments that were previously effective against similar viruses can be an efficient approach to drug design as the properties and safety profiles are already established. Immunotherapy treatment options for SARS-CoV-2 can be arranged into antivirals that interfere with viral replication or anti-inflammatory drugs which diminish the risk of further injury induced by the exaggerated inflammatory response.

At the start of the pandemic, the FDA approved the emergency use of chloroquine and hydroxychloroquine on March 28, 2020 [21]. Both are indicated for malarial prophylaxis, but hydroxychloroquine is also indicated for systemic autoimmune diseases. Initially, in COVID-19 patients both drugs demonstrated viral load reduction/disappearance [22] perhaps because mechanistically based on the chemical structure, hydroxychloroquine and chloroquine can raise endosomal pH to stop viral fusion on the host cell and prevent viral entry and release [6]. However, the sample size of this study was small and larger controlled trials were necessary to assess the effectiveness. Then, it was discovered at higher doses chloroquine was linked to an increased risk of toxicity and so was avoided in critically ill patients, for this reason concerning safety hydroxychloroquine was preferred over chloroquine against COVID-19 [23]. Further molecular studies have also supported the use of hydroxychloroquine over chloroquine as it has shown to be a more potent inhibitor that is can interrupt S-glycoprotein interaction with the host cell membrane [24] by binding to the protease enzyme and preventing replication [25]. On the 15th October 2020, the largest international randomised trials for COVID-19 (Solidarity Trial) found that hydroxychloroquine had minimal to no effect on overall mortality, initiation of ventilation and duration of hospitalisation [26]. However, these results only apply to hospitalised patients and not the pre- or post-exposure prophylaxis for COVID-19 [26] indicating that hydroxychloroquine may still be effective in prophylaxis, but further studies are required to determine this.

Furthermore, there was still serious safety concerns associated with the use of hydroxychloroquine which include cardiac complications which can unexpec-

tedly develop into respiratory or cardiac arrests [27] and increased fatality risks especially when administered in high doses or with other drugs like azithromycin [28]. Other findings have also recognized that cardiac involvement is a complication linked with the SARS-CoV-2 infection [29].

Similarly, dexamethasone also gained significant interest but also controversy surrounding its use against COVID-19. Corticosteroids such as dexamethasone exhibit rapid anti-inflammatory and immunosuppressive properties by inhibiting pro-inflammatory cytokines including IL-1, IL-2, IL-6, IL-8, and TNF-*α*, [16] which as previously mentioned are involved in SARS-CoV-2 severity. Also, in contrast to other corticosteroids dexamethasone is long-acting and the most potent [30].

On 16th June 2020, the results from the large Recovery trial revealed that patients taking 6 mg dexamethasone daily with a severe SARS-CoV-2 infection had an 8% - 26% lower mortality rate than those given standard care and increased 28-day survival for patients developing ADRS from COVID-19. The Recovery findings also supported the use of dexamethasone only for patients with severe symptoms of COVID-19 but not with mild symptoms or in an outpatient setting as it showed no clinical benefit [31]. On 2 September 2020, WHO published an interim guideline which supported dexamethasone and other corticosteroids in treating COVID-19 [32]. Currently, only corticosteroids have been effective in critically ill COVID-19 patients. WHO recommends a once-daily dose of 7 - 10 days to avoid complications associated with long-term high dose usage which can dampen the immune response, lead to secondary infections, prolong viral shedding, and in critically ill patients irreversible pneumonia from cytokine-related injury [33]. Long-term corticosteroid use can also increase the risk of arrhythmias in high-risk group patients with underlying heart conditions [34]. The lack of longer-term follow-ups in the RECOVERY trial means that the associated risks must be anticipated as they can outweigh the benefits.

The next category of drugs are non-steroidal anti-inflammatory drugs (NSAIDs) which work by inhibiting cyclooxygenase (COX) enzymes to reduce the production of prostaglandins which are essential mediators in inflammation, pain, and fever; symptoms associated with COVID-19. In March 2020, there were apparent observations and unconfirmed anecdotal reports raised by the French Health Ministry stating that ibuprofen had exacerbated the COVID-19 symptoms in patients [35]. However, observational evidence is difficult to assess due to protopathic bias. There were also concerns on the possibility of ibuprofen prolonging recovery time by dampening the immune response and increasing the likelihood of other opportunistic infections [35] but no evidence exists to reinforce this in COVID-19 patients and as previously demonstrated with dexamethasone, immune suppression can be beneficial. On the 19th April 2020, WHO published a systematic review on NSAIDs and viral respiratory infections to conclude that there was no direct and existing scientific data from infected COVID-19 patients to suggest that NSAIDs cause severe adverse events, long-term

survival, or quality of life [36].

Another review evaluated the clinical trials of naproxen on a similar virus, influenza which is also an RNA virus like the SARS-CoV-2. The studies showed that naproxen inhibited the nucleoprotein and prevented viral replication, but these results may not be generalisable to all RNA viruses and need to be investigated on the SARS-CoV-2. Also, the safety concerns of naproxen on COVID-19 patients still need to be evaluated [37].

The above is a detailed overview of the current literature available which has addressed efficacy and safety concerns of hydroxychloroquine, dexamethasone, and NSAIDs. However, the evidence to support or reject the use of these drugs particularly NSAIDs is insufficient as most of the evidence was based on observational studies rather than controlled clinical trials. There was also limited binding research available to aid in drug design and discovery. Furthermore, although some of these drugs have particular safety concerns associated with their use this does not mean they are not potent inhibitors that can potentially be used in fragment-based drug discovery to treat or prevent COVID-19. This study will focus on contributing to the existing knowledge and limited binding studies available with a particular focus on hydroxychloroquine, dexamethasone, and NSAIDs on the SAR-CoV-2 protease and SARS-CoV-2 glycoprotein using computational studies.

1.5. Computer-Aided Drug Design

The advances of computational approaches have enabled the testing of repurposed therapies and aided the identification of effective inhibitors on specific targets. The molecular docking procedure is a computer-aided prediction that uses search algorithms to estimate the preferred ligand orientation, if it binds, to the binding site on the targeted protein. It also locates the binding site region to increase docking efficacy, ligand interactions, and binding affinity.

The computational analysis of binding properties of the drug structure with the target can then be used to aid in novel drug design. Computational studies also aid in drug filtration so only the most potent inhibitors which are most likely to be effective during *in vivo* studies on the disease patient can be investigated further [9]. This is particularly effective during current periods of urgency as there is a necessity for decreasing the time and cost to approve drugs for use and reduce the risk of failure during drug development.

1.5.1. Selection of the Target Protein

The resolution was an important factor that was considered before selecting the appropriate protein to prepare for modelling. Both SARS-CoV-2 protease (PDB ID: 6LU7, Resolution: 2.16 Å, Method: X-RAY diffraction) [38] and SARS-CoV-2 spikes glycoprotein (PDB ID: 6VXX, Resolution: 2.80 Å, Method: Electron microscopy) consisted of a relatively low-resolution structure compared to the other SARS-CoV-2 protease available. This was beneficial as it meant that the protein was more constricted in available binding regions which reduced the li-

kelihood of false-positive sites thus enhancing docking accuracy. In addition, low-resolution proteins are more likely to withstand structural deformation which further increases docking accuracy [39].

1.5.2. Structure and Function of SARS-CoV-2 M^{pro}

This study will focus on assessing drug leads which target the crystal structure of SARS-CoV-2 main protease (M^{pro}) (**Figure 4**) through computer-aided drug design. SARS-CoV-2 M^{pro} mediates viral replication and transcription making it an appealing drug target [40]. SARS-CoV-2 M^{pro} has one polypeptide and three domains with the CYS-HIS catalytic dyad and is highly conserved between M^{pro} in all coronaviruses indicating that inhibition of this pocket could result in broad-spectrum activity against other coronaviruses [41].

1.5.3. Structure and Function of SARS-CoV-2 Spike Glycoprotein

We will also target the protruding homotrimers, SARS-CoV-2 S glycoprotein [43] (**Figure 5**) present on the surface of the virus which is involved in viral entry. This is a pleasant target for neutralising antibodies during infections which means it could be used in treatment and vaccine design. The S trimer consists of N-linked glycans which have a significant role in folding and regulating access to the host. The SARS-CoV-2 S2 subunit shares 88% of amino acid sequence identity with SARS-CoV-2 and is structurally conserved meaning accessibility to Abs will be similar to the coronaviruses [44].

1.6. Aims and Objectives

Although many drugs have been investigated against SARS-CoV-2, there is limited computational and biological data reported on all anti-inflammatory drugs which have the potential to reduce SARS-CoV-2 severity through immunosuppression. Therefore, this study will aim to screen 19 FDA approved drugs with immunosuppressive activity (**Figure 6a**) against the crystal structure (6LU7 and 6VXX) of COVID-19 using computational molecular docking to recommend drugs with the best binding properties and the potential to be the most effective during

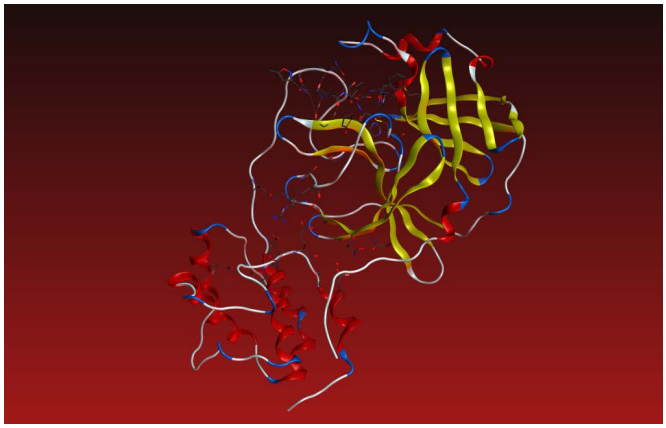


Figure 4. 6LU7: The crystal structure of SARS-CoV-2 main protease complex with an inhibitor N3 [42].

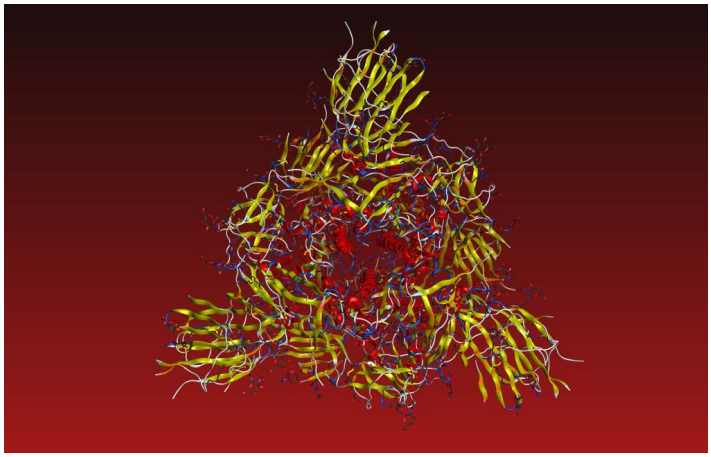


Figure 5. 6VXX: The structure of the SARS-CoV-2 spike glycoprotein (closed state) [45].

novel drug design and *in vivo* studies. Assess biological data from Drug Bank and emc to select drugs with the most tolerable toxicity profile, reduce risk of failure during development, and improve patient outcomes. This study will also supplement the current regimens from the literature.

2. Materials and Methods

2.1. Methodological Approach

The FDA-approved drugs were ranked by computational-aided drug design (CADD) to determine the optimal orientation, binding affinity, and interactions of the ligand-receptor complex. CADD was the preferred method for this study compared to the traditional high-throughput screening and combinational chemistry as it was able to increase the hit rate of the drug compounds by utilising a more target search as well as identifying possible derivatives that can enhance therapeutic activity [46]. CADD is also very efficient for urgent drug design as the docking speed is fast however, it still lacks experimental approaches which is why biological data was also incorporated in the ranking process to improve drug development and discovery success.

2.2. Modelling Platforms

The computational molecular docking analysis of the 19 FDA approved drugs against the crystal structure of SARS-CoV-2 protease and SARS-CoV-2 spikes glycoprotein was performed using the Molecular Operating Environment (MOE) software (version 2019.0102). XQuartz (version 2.7.11) was used as the open-source display server for macOS. ChemDraw (version 17.1.10) was used to produce the two-dimensional structures of the drug molecules.

The three-dimensional crystal structure of SARS-CoV-2 protease (PDB ID: 6LU7) and SARS-CoV-2 spikes glycoprotein (PDB ID: 6VXX) was obtained from RCSB Protein Data Bank (PDB). The canonical simplified molecular-input line-entry system (SMILES) of the FDA approved drugs was obtained from PubChem.

2.3. Preparation of the Drug Database

The canonical SMILES of the selected drug were imported onto the MOE software and converted into a three-dimensional structure. The energy of each structure was minimised using the MOE -minimize feature to ensure the structures were in the most stable conformation with the lowest potential energy and steric hindrance; this was to increase structure optimisation during docking. The minimised structures were saved as database chains in the form of an MDB file and equipped for docking in the upcoming stages.

2.4. SARS-CoV-2 Protease Preparation

The retrieved SARS-CoV-2 protease (PDB ID: 6LU7) file was loaded onto the MOE software and prepared for molecular docking by protonation and allocation of partial charges [47] using the MOE-Quick prep feature. The ligands were then selected and removed from the active site to enable interaction of the chosen drugs with the binding pockets of the protein. The binding pockets are the targets for drug inhibition, which was located using the MOE-Site finder tool; the application of dummies was also required to coordinate the binding pockets. This was saved as a MOE file.

2.5. SARS-CoV-2 Spikes Glycoprotein Preparation

The obtained SARS-CoV-2 spikes glycoprotein (PDB ID: 6VXX) file was loaded onto the MOE software. Once loaded the repeated chains and "NAG" chains were deleted manually using the MOE-SEQ tool resulting in the final protein consisting of only the first single chain (6VXX.A). This was performed as 6VXX is a tetramer with multiple domains, binding sites, and ligands originating from the duplicate chains so eliminating these chains reduced the number of available atoms thus the duration required to prepare the protein for molecular docking. The resulting protein was then prepared using the MOE-QuickPrep feature and bindings pockets located with the MOE-Site finder tool and defined by the application of dummy atoms. This was saved as an MOE file.

2.6. Molecular Docking Protocol

The molecular docking procedure is an automated process that docks the drug molecule into the binding pockets [48] of the protein using a search algorithm that evaluates the ligand conformation repeatedly until minimum energy is attained [49]. The molecular docking algorithm also calculates quantitively predictions of binding affinity and the number as well as the type of interactions with the binding site surfaces and ligands [48], which are later ranked to determine the ligands which are best suited against the SARS-CoV-2 protease and SARS-CoV-2 spikes glycoprotein.

For this process the MOE-dock tool was used, the site was amended from ligand atoms to the selected protein (6LU7/6VXX) at the time. The selected ligand was converted from a MOE to an MDB file with the chosen drug database pre-

viously saved from step 2.2. This procedure was left to run until the binding affinity and ligand-receptor interaction results had accumulated. The best conformations with the greatest number of complementary ligand interactions and the greatest binding affinity were selected for analysis in the next stage.

2.7. Molecular Analysis of Ligand-Protein Interaction

In this stage, only highly ranked drugs with the most number ligand interactions and the largest binding affinity were selected for analysis. This involved loading the previously prepared protein (6LU7 or 6VXX) with the docked drug on to the MOE software; to which the ligand was allocated using the MOE-ligand tool. Then the MOE-interaction (VDW) feature was applied to observe steric clashes and voids in the binding site for ligand expansion. To visualise the atoms protruding onto the molecular surface or to estimate the favourable locations of the ligand atoms the MOE-Surface, and Maps feature was selected, and the surface was amended from interaction (VDW) to molecular surface. Lastly, the MOE-Render-Atoms feature was used to select the icon of choice and personalise the colours of atoms to enhance clarity and visualisation. This procedure was repeated with the selected drugs and the gap regions between the ligand and binding site were compared and analysed in the discussion section.

2.8. Assessing the Drug Toxicities from Biological Databases

Drug properties including the mechanism of action, contraindications, adverse drug reactions, and toxicity were obtained from biological database sources including DrugBank and electronic medicines compendium (emc) which acquires the content pharmaceutical companies and medicine regulators (Medicine and Healthcare products Regulatory Agency or European Medicines Agency); this makes the content more reliable. Biological data was incorporated into the ranking procedure to reduce the possibility of failure during development and improve patient outcomes by predicting adverse effects and the patients that are most at risk of developing these reactions.

3. Results

This study screened 19 approved drugs using the binding properties from computational molecular docking and assessed the safety profile from the biological data. From the literature, the evidence was conflicting for certain treatments particularly NSAIDs and there was limited binding research available to aid in drug design and discovery for potential inhibitors against COVID-19. To supplement the available evidence this study utilised computational drug repurposing techniques for expeditious treatment recognition and the discovery of novel therapeutic options.

Summary of Key Findings

Out of the 19 approved drugs in descending order, hydroxychloroquine, etori-

coxib, dexamethasone, naproxen, and ibuprofen ranked highly (Table 1), in terms of binding properties against the SARS-CoV-2 protease (6LU7) (Figure 6b). Compared to the SARS-CoV-2 glycoprotein (6VXX) naproxen and ibuprofen did not interact with the binding site instead high ranked drugs in descending order were hydroxychloroquine, etoricoxib, celecoxib, and parecoxib (Table 2) (Figure 6c). From the high ranked drugs, only hydroxychloroquine and etoricoxib were effective against both the SARS-CoV-2 protease and SARS-CoV-2 glycoprotein. However, in terms of safety from the toxicity profile (Table 3) etoricoxib, naproxen and dexamethasone were safer than hydroxychloroquine and ibuprofen. Whereas for the SARS-CoV-2 glycoprotein etoricoxib, meloxicam and parecoxib ranked higher in safety than hydroxychloroquine and celecoxib (Table 4). Furthermore, referring to Figures 7-15 all the selected drugs apart from celecoxib had occupied most of the space in the binding site.

Despite these promising results there is still an associated toxicity profile displayed in Table 3 and Table 4 which can have severe implications, particularly on high-risk group patients who are more likely to experience severe COVID-19 symptoms; therefore the risks and benefits of the use of these drugs still need to be critically reviewed before implementing as a treatment suggestion and this will be discussed below.

Table 1. The binding properties and ranking of the repurposed drug against the 6LU7 protein.

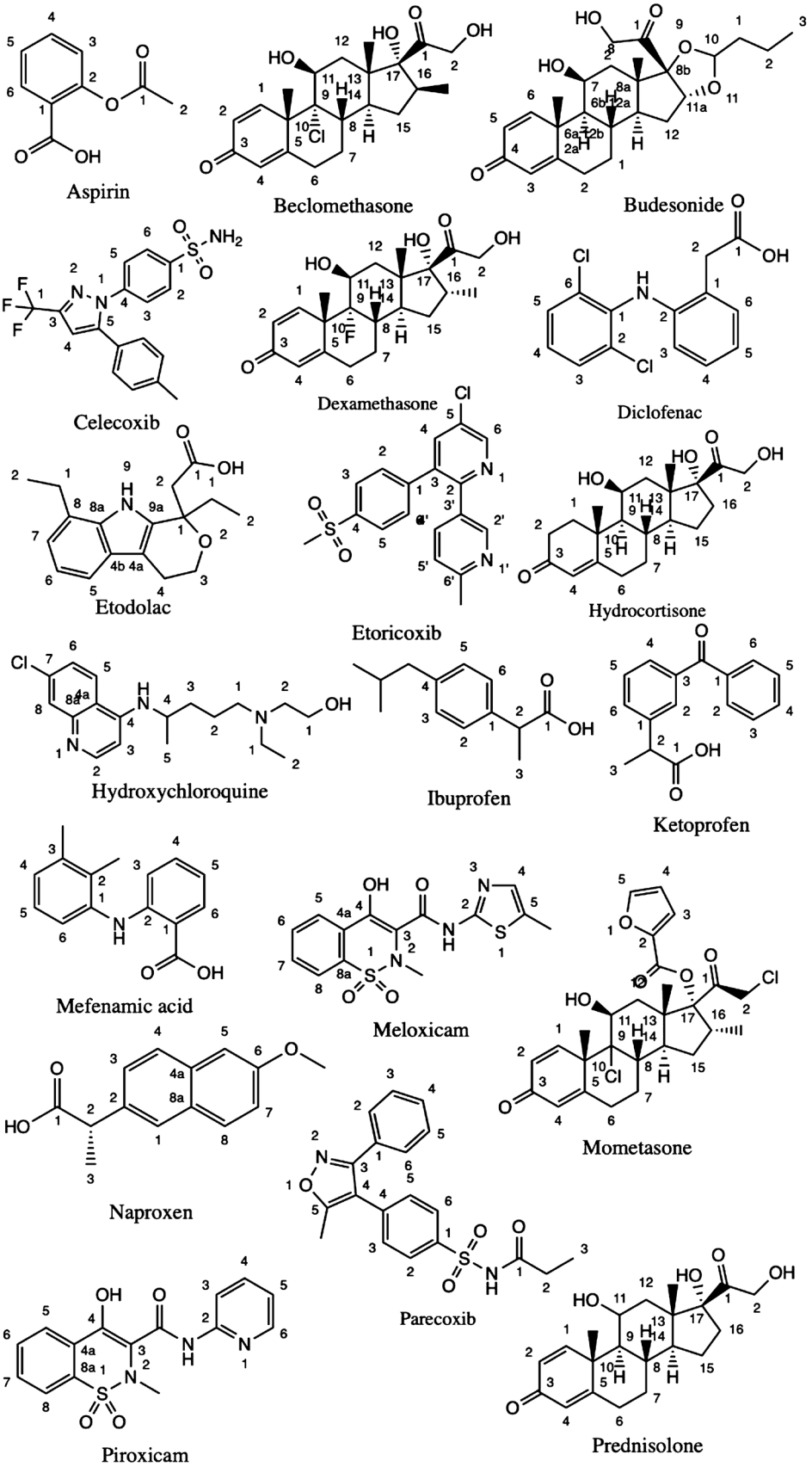
Drug	Number of interactions	Interaction types	Binding affinity (kcal/mol)	Ranking (1 - 20, 1 = best, 20 = worst)
Hydroxychloroquine	4	MET 165 (A) H-donor HIS 41 (A) H-pi HIS 41 (A) H-pi GLY 143 (A) pi-H	−6.3615	1
Dexamethasone	4	GLU (A) H-donor LYS 5 (A) H-acceptor LYS 137 (A) H-acceptor HOH 454 (A) H-acceptor	−5.1879	3
Naproxen	4	LYS 5 (A) H-acceptor LYS 137 (A) pi-cation LYS 137 (A) pi-cation HOH 454 (A) pi-H	−4.6286	4
Celecoxib	2	GLU 270 (A) H-donor GLU 270 (A) H-donor	−5.2343	12
Mefenamic acid	2	MET 49 (A) H-donor SER 46 (A) H-acceptor	−4.4983	17

Continued

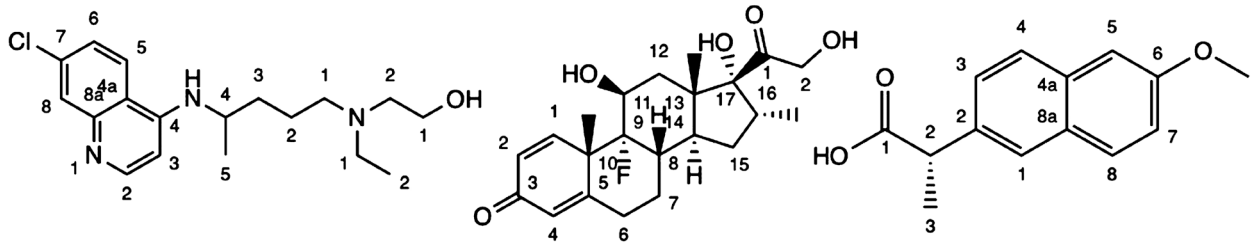
Etoricoxib	6	MET 6 (A) H-donor MET 6 (A) H-donor ARG 298 (A) H-acceptor ARG 298 (A) H-acceptor TYR 154 (A) H-pi TYR 154 (A) pi-H	−4.7708	2
Aspirin	2	GLN 110 (A) H-donor HIS 246 (A) H-acceptor	−4.4247	18
Diclofenac	2	THR 25 (A) pi-H THR 26 (A) pi-H	−4.7717	16
Meloxicam	2	ASP 153 (A) H-donor TYR 154 (A) H-pi	−4.9258	15
Ibuprofen	5	CYS 145 (A) H-donor HIS 163 (A) H-acceptor HIS 41 (A) H-pi MET 165 (A) pi-H GLU 166 (A) pi-H	−5.6501	5
Ketoprofen	2	HIS 41 (A) H-acceptor HIS 41 (A) H-pi	−5.7020	11
Parecoxib	3	GLU 288 (A) H-donor LYS 137 (A) H-acceptor LYS 137 (A) pi-H	−5.2472	7
Etodolac	3	GLN 74 (A) H-donor LEU 67 (A) pi-H LEU 67 (A) pi-H	−4.8638	8
Piroxicam	3	ASP 48 (A) H-donor ASP 48 (A) H-acceptor ASN 53 (A) pi-H	−4.9016	9
Prednisolone	2	GLN 107 (A) H-donor HIS 246 (A) H-acceptor	−5.0142	14
Hydrocortisone	2	ASP 153 (A) H-donor HOH 440 (A) H-donor	−5.0211	13
Budesonide	4	GLN 107 (A) H-donor ASP 245 (A) H-donor HIS 246 (A) H-acceptor HOH 483 (A) H-acceptor	−4.8355	6
Beclomethasone	3	HOH 434 (A) H-donor LYS 5 (A) H-acceptor LYS 5 (A) H-acceptor	−4.8445	10
Mometasone	0	-	0	20

Table 2. The binding properties and ranking of the repurposed drugs against the 6VXX glycoprotein.

Drug	Number of interactions	Interaction types	Binding affinity (kcal/mol)	Rank (1 = best 15 = worst)
Hydroxychloroquine	4	ASN 165 (A) H-acceptor LYS 129 (A) H-acceptor GLU 132 (A) pi-H GLU 132 (A) pi-H	−4.6855	1
Dexamethasone	2	SER 297 (A) H-donor LYS 300 (A) H-acceptor	−4.4664	8
Naproxen	0	-	0	15
Celecoxib	3	LYS 964 (A) H-acceptor SER 50 (A) H-acceptor VAL 47 (A) pi-H	−5.4664	3
Mefenamic acid	0	-	0	15
Etoricoxib	2	GLN 321 (A) H-acceptor LYS 537 (A) pi-H	−4.8107	2
Aspirin	0	-	0	15
Diclofenac	0	-	0	15
Meloxicam	3	ASN 343 (A) H-donor ASN 343 (A) H-donor TRP 436 (A) pi-pi	−5.0303	5
Ibuprofen	0	-	0	15
Ketoprofen	0	-	0	15
Parecoxib	3	LYS 964 (A) H-acceptor VAL 47 (A) pi-H LYS 964 (A) pi-H	−5.3639	4
Etodolac	2	THR 912 (A) H-donor THR 912 (A) pi-H	−4.8000	7
Piroxicam	2	PHE 374 (A) H-donor ALA 372 (A) pi-H	−4.3556	9
Prednisolone	3	ASP 364 (A) H-donor THR 385 (A) H-donor ASN 370 (A) H-acceptor	−3.7528	10
Hydrocortisone	1	ARG 34 (A) H-acceptor	−4.6782	12
Budesonide	2	ASN 856 (A) H-acceptor ASN 856 (A) H-acceptor	−5.5163	6
Beclomethasone	1	LYS 129 (A) H-acceptor	−4.4156	14
Mometasone	1	VAL 608 (A) H-acceptor	−4.9213	11


Aspirin
Beclomethasone
Budesonide
Celecoxib
Dexamethasone
Diclofenac
Etodolac
Etoricoxib
Hydrocortisone
Hydroxychloroquine
Ibuprofen
Ketoprofen
Mefenamic acid
Meloxicam
Mometasone
Naproxen
Parecoxib
Piroxicam
Prednisolone

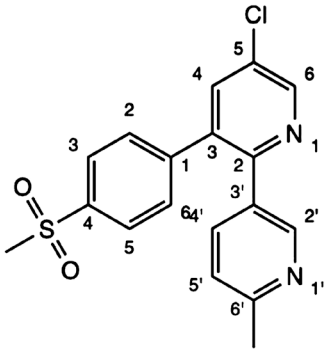
(a)



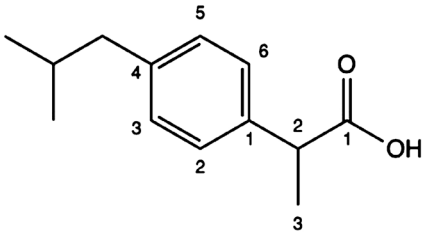
Hydroxychloroquine

Dexamethasone

Naproxen

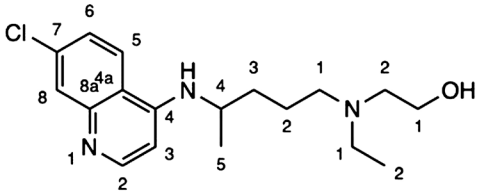


Etoricoxib

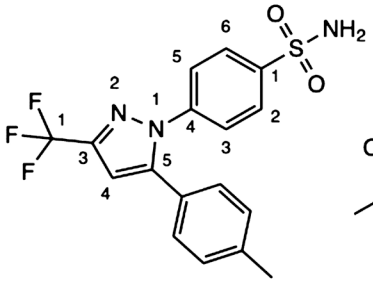


Ibuprofen

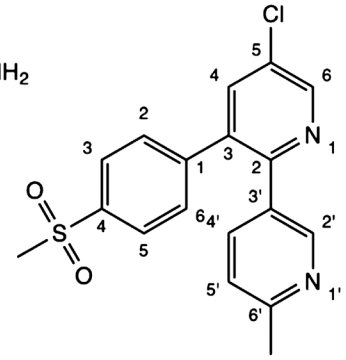
(b)



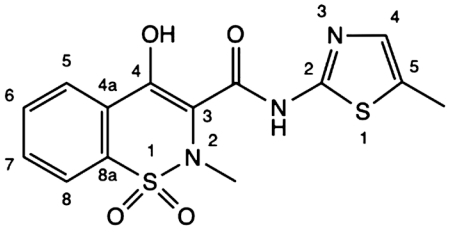
Hydroxychloroquine



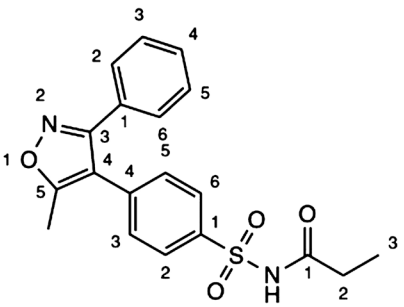
Celecoxib



Etoricoxib



Meloxicam



Parecoxib

(c)

Figure 6. (a) The structures of the 19-FDA approved drugs built on ChemDraw; (b) The structures of the five highest ranked drugs against the 6LU7 protein built on ChemDraw; (c) The structures of the five highest ranked drugs against the 6LU7 protein built on ChemDraw.

Table 3. The properties of the repurposed drugs against 6LU7 [50] [51].

Drug name	Main indication	Mechanism of action	Drug toxicity	Contraindications
Hydroxychloroquine	Malaria prophylaxis, systemic lupus.	Raises lysosomal pH in antigen-presenting cells to prevents viral fusion and entry to host cell.	QTc prolongation, hypokalaemia, torsades de pointes, ventricular tachycardia, and fibrillation, sudden cardiac or respiratory arrest.	Hypersensitivity to 4-aminoquinolone, pre-existing maculopathy of eye, pregnancy.
Dexamethasone	Suppression of inflammatory and allergic disorder, inflammation, mild croup, macular oedema.	Inhibition of pro-inflammatory cytokine production.	Increased susceptibility of infection, impaired healing, osteoporosis, hyperlipidaemia, hyperglycaemia, metabolic acidosis.	Systemic infection and immunocompromised patients.
Naproxen	Rheumatoid arthritis, osteoarthritis, acute gout, dysmenorrhoea.	Inhibits COX-1 and COX-2 enzymes to reduce prostaglandin synthesis.	Nausea, vomiting, epigastric pain, lethargy, and drowsiness.	Hypersensitivity to NSAIDS, pregnancy, severe hepatic, heart, renal failure.
Etoricoxib	Rheumatoid arthritis, acute gout, acute pain.	Inhibits COX-2, prevents prostaglandin production.	Gastrointestinal and cardiorenal events.	Hypersensitivity to NSAIDS, ischaemic heart disease, cerebrovascular disease, pregnancy and lactation, severe hepatic impairment.
Ibuprofen	Mild to moderate pain from dysmenorrhea, migraine, rheumatoid arthritis, pyrexia.	Blocks COX-1 and COX-2, decreases prostaglandin synthesis involved in inflammation, pain, fever and swelling.	CNS depression; rarely metabolic acidosis, acute renal failure, liver failure, hyperkalaemia, respiratory depression, and cyanosis.	Hypersensitivity to ibuprofen, severe hepatic, renal or heart failure, asthmatic patients, heart diseases, gastrointestinal bleeding.

Table 4. The properties of the repurposed drugs against 6VXX [50] [51].

Drug name	Main indication	Mechanism of action	Drug toxicity	Contraindications
Hydroxychloroquine	Malaria prophylaxis, systemic lupus.	Raises lysosomal pH to prevents viral fusion and entry to host cell.	QTc prolongation, hypokalaemia, torsades de pointes, ventricular tachycardia, and fibrillation, sudden cardiac or respiratory arrest.	Hypersensitivity to 4-aminoquinolone, pre-existing maculopathy of eye, pregnancy.
Celecoxib	Osteoarthritis, rheumatoid arthritis, ankylosing spondylitis.	Selective COX-2 enzyme inhibitor, reduces prostaglandin, prostaglandin F2, thromboxane, prostaglandin D2, reduces pain and inflammation.	Difficulty breathing, gastrointestinal disorders, high blood pressure, myocardial infarction, kidney failure, and cerebral infarction.	Hypersensitivity to sulphonamides, active peptic ulceration, pregnancy and breastfeeding, severe hepatic impairment, congestive heart failure, creatinine clearance < 30 ml/min.
Meloxicam	Relief of pain and inflammation in musculoskeletal disorders.	Preferentially blocks COXI-2 which inhibits prostaglandin synthetase decreases prostaglandin, reduces symptoms mediated from inflammation.	Gastrointestinal disorders, shallow breathing, oliguria.	Hypersensitivity to NSAIDS, third trimester pregnancy, person under 16 years old, hypersensitivity to NSAIDS, inflammatory disease, severe impaired liver function, severe renal and heart failure.
Etoricoxib	Rheumatoid arthritis, chronic and acute pain, gout, ankylosing spondylitis.	Inhibits COX-1 and COX-2, prevents prostaglandin production, reduces inflammation.	Gastrointestinal and cardiorenal events.	Hypersensitivity to NSAIDS, ischaemic heart disease, cerebrovascular disease, pregnancy and lactation, severe hepatic impairment.
Parecoxib	Short-term perioperative pain control.	Inhibits COX-2, reduces prostaglandin-mediators of pain and inflammation.	Gastrointestinal complications, hypertension, hypotension oedema, renal impairment.	Hypersensitivity to NSAIDS and sulphonamide. Third trimester pregnancy and breastfeeding. Severe hepatic impairment. Congestive heart failure.

4. Discussion

4.1. Investigation of the Selected Drugs

4.1.1. Hydroxychloroquine

Hydroxychloroquine is classified as an anti-malarial drug that has anti-inflammatory and immunomodulatory functions [27]. The main approved indications which are generally safe for use are for malarial prophylaxis and autoimmune diseases such as systemic lupus but with regards to treatment against the SARS-CoV-2 there are various concerns surrounding efficacy and safety which have been stated in the literature.

From the molecular docking results, hydroxychloroquine displayed superior interactions against the SARS-CoV-2 protease and glycoprotein when compared to the other drugs in **Table 1** and **Table 2**, for this, reason it was ranked first in both instances. Hydroxychloroquine displayed a high binding affinity with the 6LU7 protein, and the benzene functional group formed three hydrophobic pi-H interactions with the HIS-41 and GLY-143 amino acids on the binding site. Hydroxychloroquine also interacted through hydrophilic hydrogen bonds with MET-165. Whereas on the 6VXX glycoprotein the binding affinity of hydroxychloroquine was considerably less but it still managed to display strong hydrophobic interactions with GLU-132 and hydrophilic interactions with ASN-165 and LYS-129 on the binding site. In both cases, with 6LU7 and 6VXX, the combination of hydrophobic and hydrophilic interactions with hydroxychloroquine will further lock the ligand tightly into the binding site and compensate for voids shown in **Figure 7** and **Figure 8**, which can imply that hydroxychloroquine will behave as a potent inhibitor against the SARS-CoV-2 protease and SARS-CoV-2 glycoprotein.

Despite the promising results which suggest that hydroxychloroquine may act as an effective treatment and prophylactic measure; there is conflicting published data regarding efficacy in the literature which in recent findings has concluded that hydroxychloroquine was ineffective in treating hospitalised patients [26]. However, since these results do not apply to non-hospitalised patients or show that hydroxychloroquine was ineffective as prophylaxis it can suggest that

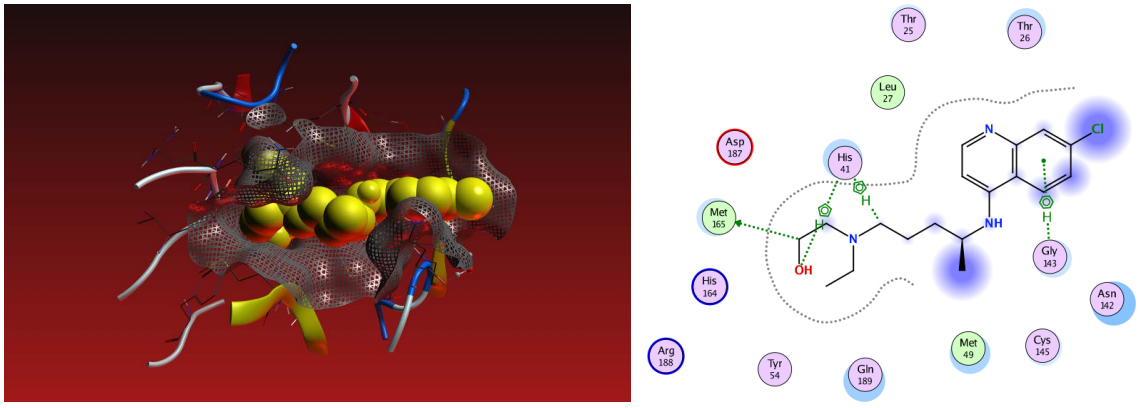


Figure 7. The binding of hydroxychloroquine with the 6LU7 binding pocket.

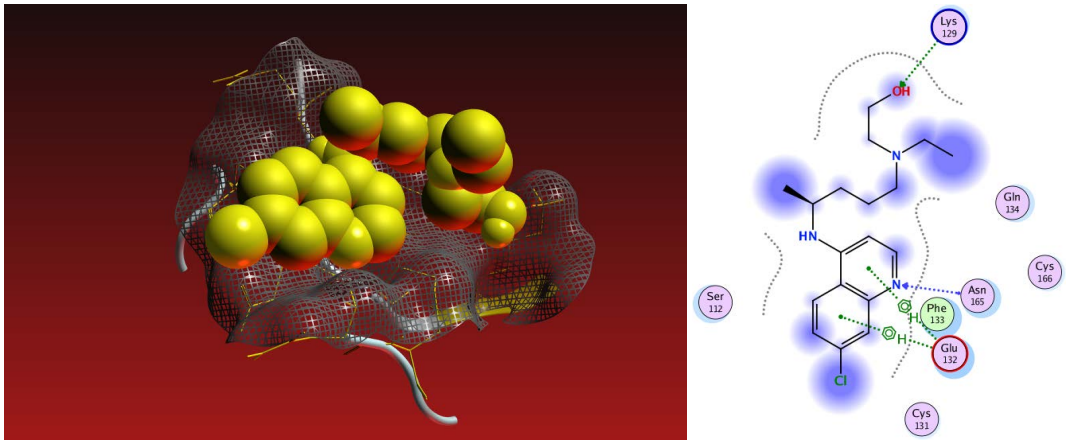


Figure 8. The binding of hydroxychloroquine with the 6VXX binding pocket.

hydroxychloroquine may still be effective as a prophylaxis and treatment for non-hospitalised patients, but further studies will need to validate this.

Regardless, the safety concerns associated with hydroxychloroquine still need to be weighed with the benefits, especially at high doses and unmonitored usage which can lead to overdose and cause serious drug toxicity including QTc prolongation, hypokalaemia, torsades de pointes, ventricular tachycardia, and fibrillation (**Table 3** and **Table 4**). Also, since the minimal fatal dose of hydroxychloroquine is still not well-defined serious poisoning can be common particularly with improper self-medicating which has been reported before [52] [53]. Other linked concerns include the secondary effect of hydroxychloroquine shortage caused by the rapid rise in demand which could mean patients suffering from an indicated autoimmune disease such as lupus will be unable to receive their prescribed medications and treat their condition [54].

4.1.2. Dexamethasone

Dexamethasone is a systemic corticosteroid indicated for immune-related inflammatory disorders; dexamethasone functions through suppression of the immune response by preventing naïve T cell proliferation and differentiation [55] which prevents pro-inflammatory cytokine production. This reduction in systemic inflammation diminishes ARDs progression, exudative fluid in the lung tissue, inflammasome production which prevents additional alveolar harm, respiratory insufficiency, and hypoxaemia [56].

The molecular docking results for dexamethasone against the 6LU7 protein have demonstrated a considerably high binding affinity compared to the other compounds in **Table 1**; ranking dexamethasone as the third top drug against 6LU7. This aligns with the RECOVERY trial from the literature which has shown that dexamethasone was effective against COVID-19; the high binding affinity displayed by dexamethasone may also explain how a low dose of only 6mg was effective in reducing mortality for those with a severe SARS-CoV-2 infection [31].

However, unlike hydroxychloroquine and etoricoxib, dexamethasone only displayed hydrophilic hydrogen interactions with GLU, LYS-5, LYS-137, and

HOH-454 on the 6LU7 binding site and SER-297, LYS-300 on the 6VXX binding site. The lack of hydrophobic interactions may indicate the insufficiency for dexamethasone to pass through the cell membrane and exert maximum pharmacological efficacy. However, from the literature dexamethasone was effective in COVID-19 patients perhaps suggesting that the presence of fluorine on dexamethasone, which is indirectly bonded to the 6LU7 binding site (Figure 9), could have reduced cytochrome P540 metabolic lability and increased drug availability, permeability, and molecular potency [57].

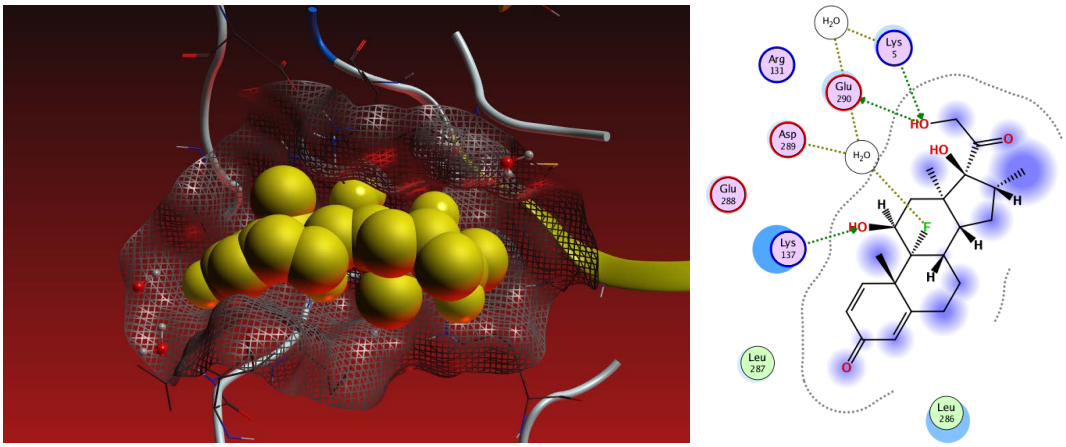


Figure 9. The binding of dexamethasone with the 6LU7 binding pocket.

Although the molecular docking results support the use of dexamethasone, there is a harm-benefit ratio to balance before administration which may explain why dexamethasone is not usually recommended in mildly ill COVID-19 patients since the risks can outweigh the benefits. These risks are mainly affiliated with long-term high dose usages (longer than 10 days) shown in Table 3, examples include hyperlipidaemia, hyperglycaemia, osteoporosis, metabolic acidosis as well as increased susceptibility to infections; consistent with the literature. Also, since dexamethasone can only be given for less than 10 days it may not be safe as a prophylaxis which requires long-term exposure; the results from Table 2 also suggest that dexamethasone might not be very effective in preventing viral entry 2 due to the weak interactions with the SARS-CoV-2 glycoprotein.

4.1.3. Ibuprofen

Ibuprofen is categorised as a non-steroidal anti-inflammatory drug (NSAID) which is widely used to relieve mild to moderate pain, decrease inflammation, and pyrexia (Table 3). The commonly available over-the-counter NSAID, ibuprofen, displayed exceptional molecular docking results against the 6LU7 protein in Table 1; this was demonstrated from the high binding affinity and five interactions which consisted of hydrophilic hydrogen interactions with CYS-145, HIS-163, and strong hydrophobic pi-H interactions with HIS-41, MET-165 and GLU-166 on the 6LU7 binding site. The combination of hydrophilic and hydrophobic interactions enhances ligand attachment to the binding site as well as

enabling adequate passage through the cell membrane and cytoplasm to exert its pharmacological effect in treating COVID-19 patients. This compact binding was displayed in Figure 10 as ibuprofen occupied the majority of the 6LU7 binding site. These strong interactions, however, were not replicated on the 6VXX glycoprotein (Table 2) as ibuprofen had no interactions; this can indicate the lack of efficacy ibuprofen may have in preventing viral entry.

Although the molecular docking results suggest that ibuprofen has a strong potential to be useful as a treatment measure against COVID-19 the use is still controversial due to conflicting safety concerns mentioned in the literature and the drug toxicities mentioned in Table 3 which occur from unmonitored dosage and serious poisoning this can include central nervous system (CNS) depression, metabolic acidosis, hyperkalaemia, acute renal and liver toxicity. Although these adverse effects only occur with serious poisoning it is still a risk factor to consider since ibuprofen is easily accessible over-the-counter which provides an opportunity for unmonitored usage, improper self-medicating, drug stockpiling, and shortages. Overall, continuous safety reviews and epidemiological studies on ibuprofen are still required to ensure adequate evidence is available for its use against COVID-19 [58].

4.1.4. Other Anti-Inflammatory Drugs

Etoricoxib

Etoricoxib is a selective COX-2 inhibitor that inhibits prostaglandin production to reduce the inflammatory response and is licensed for acute pain, gout, and rheumatoid arthritis (Table 3). Compared to the other compounds in Table 1 etoricoxib displayed the greatest number of interactions which included four hydrophilic hydrogen bonds with MET-6 and ARG-298 as well as two hydrophobic pi-H interactions with TYR-154. This combination of hydrophilic and hydrophobic interaction was demonstrated by the compact binding displayed in Figure 11 and Figure 12. Furthermore, compared to the 6LU7 protein the number of interactions of etoricoxib on the 6VXX glycoprotein was considerably less and only constituted of one hydrophilic hydrogen bond with GLN-321 and one hydrophobic pi-H interaction with LYS-537. Despite this, the binding affinity

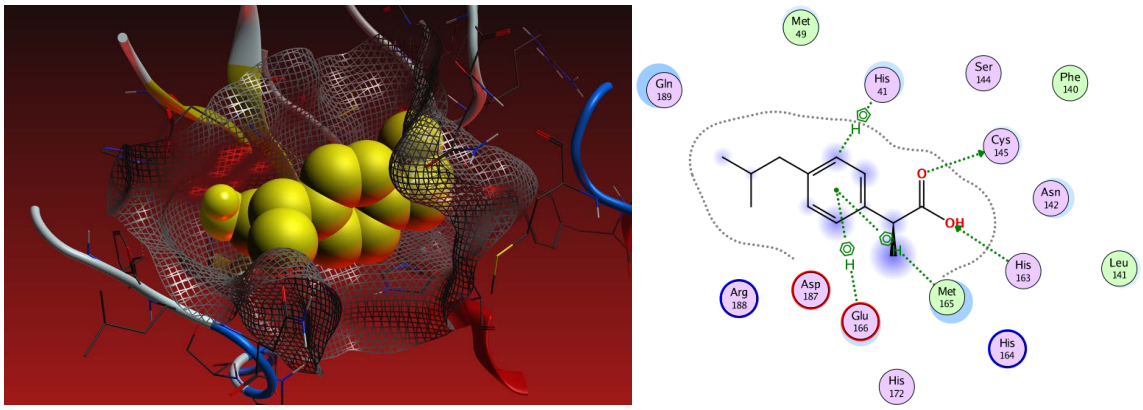


Figure 10. The binding of ibuprofen with the 6LU7 binding pocket.

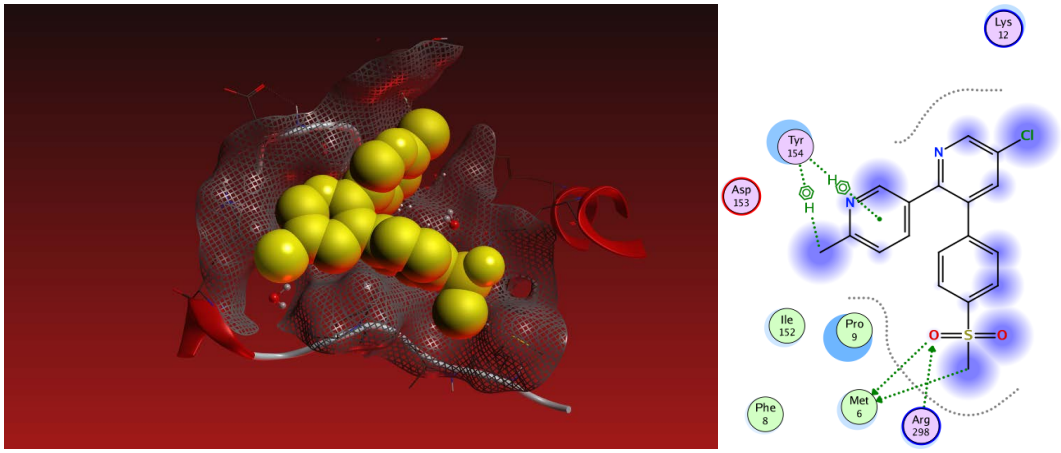


Figure 11. The binding of etoricoxib with the 6LU7 binding pocket.

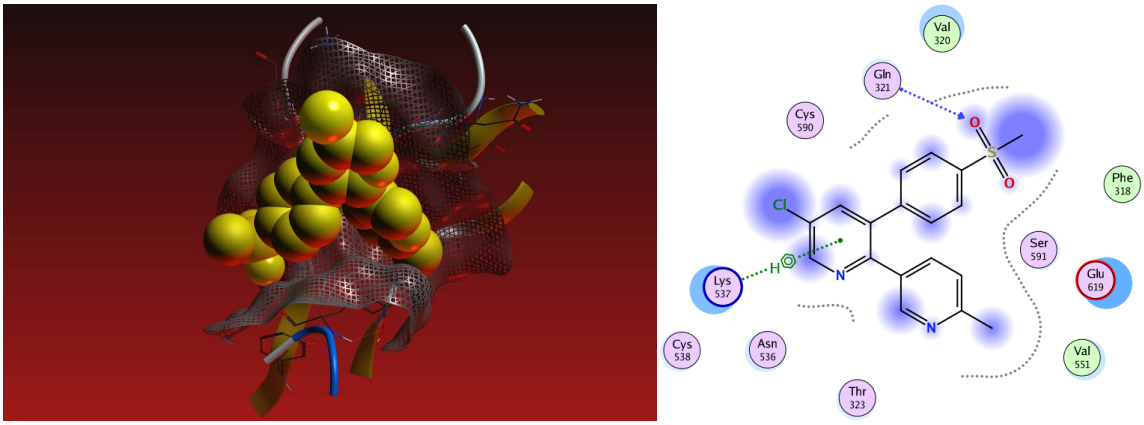


Figure 12. The binding of etoricoxib with the 6VXX binding pocket.

was still considerably high for both 6LU7 and 6VXX which can indicate the effectiveness of etoricoxib to be used as a COVID-19 treatment and prophylaxis.

Regarding the safety profile, etoricoxib adverse effects were not reported in the majority of cases, however, when they were disclosed, they were commonly related to gastrointestinal and cardiorenal events (**Table 4**) with the risk of serious upper gastrointestinal events lowered due to selective COX-2 inhibition from etoricoxib [59].

Naproxen

Naproxen reduces prostaglandin synthesis by blocking COX-1 and COX-2 enzymes and is indicated for pain relief and inflammation in various conditions including osteoarthritis, acute gout, and dysmenorrhoea (**Table 3**). The molecular docking results demonstrated that naproxen had a moderate binding affinity with the 6LU7 protein (**Table 1**) and relatively strong hydrogen hydrophilic interaction with GLU, two hydrophobic pi-cation interactions with LYS-137, and one hydrophobic pi-H interaction with HOH-454. Whereas with the 6VXX glycoprotein (**Table 2**) naproxen shared no interactions with the binding site implying that naproxen may only be effective in the treatment of COVID-19 rather than in the prevention. This is comparable with the literature as naproxen previously

has shown to be effective against viral replication in similar viruses [37] thus signifying the potential to be effective against COVID-19 but more studies are required to confirm this. Figure 13 also shows that naproxen managed to occupy the 6LU7 binding site.

In terms of safety, naproxen is associated with good efficacy and a low incidence of side effects; overdose with naproxen is mild and adverse effects are limited to nausea, vomiting, epigastric pain, lethargy, and drowsiness (Table 3).

Meloxicam

Meloxicam is a preferential COX-2 inhibitor licensed for pain relief and inflammation related to musculoskeletal disorders (Table 4). From the molecular docking results, meloxicam had moderate interactions with the 6LU7 protein (Table 1) but exhibited stronger binding with the 6VXX glycoprotein compared to the other compounds which can indicate its function to prevent viral entry. The interactions in Table 2 consisted of two hydrophilic hydrogen bonding with two ASN-343 and one strong hydrophobic π-π stacking interaction with TRP-436 amino acid. The π-π stacking interaction can significantly increase ligand-protein stability [60] which would mean the structural and functional properties of meloxicam would remain unaltered during drug delivery thus increasing therapeutic efficacy [61]. The interactions of meloxicam were weaker with the 6LU7 protein which consisted of one hydrogen hydrophilic interaction with ASP-153 and one hydrophobic pi-H interaction with TYR-154 amino acid. In addition, although meloxicam was capable of fitting within the binding site of the 6VXX glycoprotein (Figure 14) there was more unoccupied space compared to the other drugs which can provide an opportunity for ligand expansion to enhance drug activity in novel drug design.

Furthermore, based on the biological data (Table 4), meloxicam is reasonably safe and the serious adverse events associated with overdose include shallow breathing and decreased urine output.

Parecoxib

Parecoxib inhibits the COX-2 enzyme to decrease prostaglandin-mediators

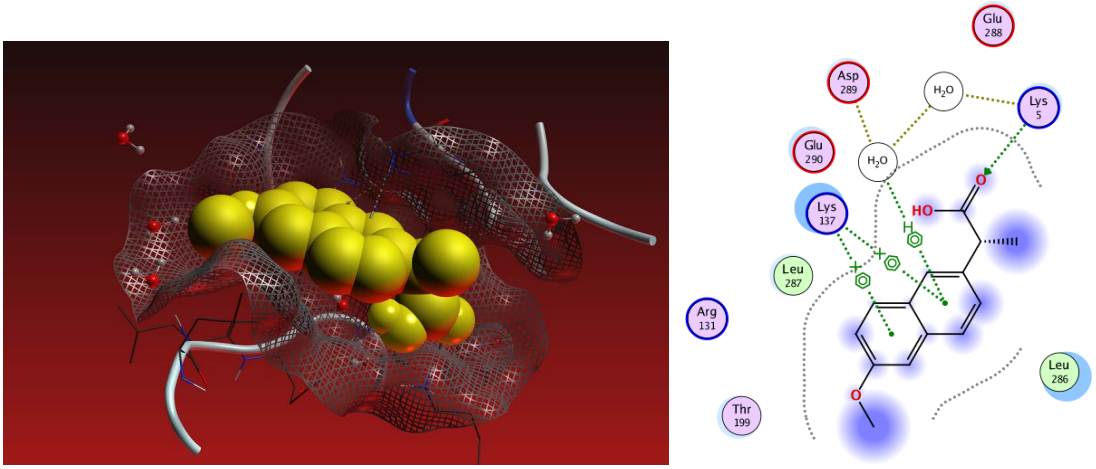


Figure 13. The binding of naproxen with the 6LU7 binding pocket.

related to pain and inflammation; parecoxib is licensed for use in short-term perioperative pain and control (Table 4). Referring to the results in Table 1 and Table 2, parecoxib had a relatively high binding affinity with 6LU7 and 6VXX. Parecoxib displayed hydrophilic hydrogen interactions with GLU-288 and LYS-137 on the 6LU7 binding site and LYS-964 on 6VXX. Parecoxib also interacted through hydrophobic pi-H interactions with LYS-137 on 6LU7 and VAL-47 and LYS-964 on the 6VXX binding site. Referring to Figure 15 parecoxib did occupy the biding site but there were a few voids, also compared to the other drugs in Table 1, parecoxib did not rank as highly due to displaying fewer interactions but against the 6VXX glycoprotein in Table 2, parecoxib ranked highly as many drugs such as naproxen and ibuprofen displayed no interactions. This can indicate that parecoxib may be effective in the treatment and also prevention of COVID-19.

Referring to Table 4, the severe drug toxicities associated with parecoxib were hypertension, hypotension, oedema, and renal impairment. Furthermore, unlike the other drugs parecoxib can only be administered as an injection which means the likelihood of adverse reactions may be increased. Also, parecoxib will be more expensive as it requires sterile conditions and specialist training to administer the drug which means it may not be easily accessible for all patients.

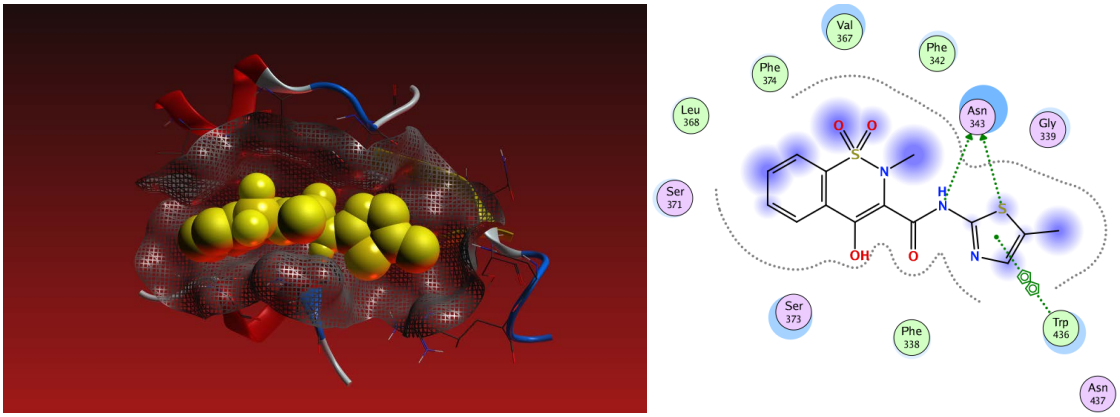


Figure 14. The binding of meloxicam with the 6VXX binding pocket.

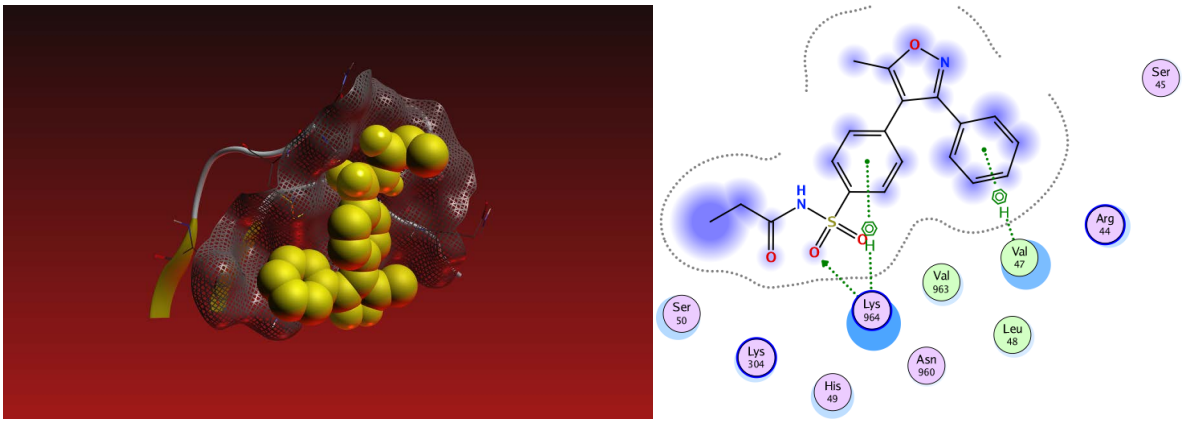


Figure 15. The binding of parecoxib with the 6VXX binding pocket.

4.2. Comparison of the Properties of the Selected Drugs

By referring to Table 3 and Table 4, dexamethasone is mainly contraindicated in systemic infections and immunocompromised patients, however, anti-infective therapy can be employed to prevent opportunistic infections. Whereas, hydroxychloroquine, ibuprofen, and the other NSAIDs, although rare, are associated with known hypersensitivity reactions which can further exacerbate the immune response and worsen infection severity; therefore should be administered with caution in particular groups. Moreover, although hydroxychloroquine displayed superior interactions against 6LU7 and 6VXX, the severe drug toxicity profile will mean that other drugs including etoricoxib, naproxen, dexamethasone, meloxicam, and parecoxib will be more favourable. Also, celecoxib, hydroxychloroquine, and meloxicam were associated with respiratory complications in an overdose which means they should be used with caution for respiratory illnesses like COVID-19.

4.3. Important Amino Acids to Consider for Future Drug Developments against COVID-19

Referring to Table 1, the drugs which exhibited the highest binding affinities with 6LU7 were hydroxychloroquine, ibuprofen, and ketoprofen. All of which interacted with the histidine residue on the binding site, HIS-41, through hydrophobic H-pi interactions. Histidine is known to be the most active amino acid and versatile in terms of protein interactions this derives from its unique molecular structure [62]. Ibuprofen was also shown to covalently bond to the cysteine residue of SARS-CoV-2 M^{pro}. Furthermore, as mentioned in chapter 1, drugs that bind to the CYS-HIS (CYS-145 and HIS-41) catalytic dyad found in the SARS-CoV-2 M^{pro} cleft are more likely to exhibit a broad-spectrum of inhibition with other SARS-CoV-2 proteases [41] like the 3-chymotrypsin-like protease (3CLpro) present on the 3' end which initiates coronavirus replication [63]. Interaction studies have also mentioned other important residues that are significant for the activity of 3CLpro which were GLU-166 and GLY-143 [64] which hydroxychloroquine and ibuprofen interacted with. Further studies need to be conducted on these amino acids and other common amino acids shared with the highest-ranked drugs which may be useful includes MET-165, LYS-137, LYS-5, and HOH-545 for 6LU7 and LYS-964 and VAL-47 for 6VXX.

5. Summary of Conclusion

This study aimed to investigate FDA approved drugs on the SARS-CoV-2 protease and glycoprotein. Overall, from the computational molecular docking results and biological data, the data strongly supports the use of hydroxychloroquine and etoricoxib which might be effective in the treatment and prevention of the COVID-19 virus, with etoricoxib displaying a more favourable safety profile. But further studies are required to validate this. Other useful drugs with tolerable toxicity profile that should be explored further include naproxen against the

SARS-CoV-2 protease and meloxicam against the SARS-CoV-2 glycoprotein. Furthermore, the data also supports the use of dexamethasone over hydroxychloroquine and ibuprofen as therapy for patients infected with the SARS-CoV-2 virus based on the binding properties, and biological data.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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