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Docking Study of Novel *Ortho*-Phenylenediamine Derivatives as Spike Glycoprotein Coronavirus 2019-Ncov Inhibitors

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Abstract

A series of new *ortho*-phenylenediamine derivatives were designed. The crystal structure of the post fusion core of 2019-nCoV S2 subunit and prefusion 2019-nCoV spike glycoprotein with a single receptor-binding domain were used as a target protein for molecular docking of *ortho*-phenylenediamine derivatives and a protein-ligand interaction analysis was performed using Auto Dock 4.2 software. Based on the docking score and after additional three-dimensional similarity analysis, NHM7 [(10,10'-((1E,1'E)-(1,2-phenylenebis(azanylylidene))bis(methanylylidene))bis(anthracen-9(8aH)-one)] had the highest binding energy. The calculated binding energy of *ortho*-phenylenediamine indicated effective binding of the proposed inhibitors to fusion core of 2019-nCoV S2 subunit and prefusion 2019-nCoV spike glycoprotein with a single receptor-binding domain.

Keywords: *Severe Acute Respiratory Syndrome; Coronavirus; COVID-19 Spike Glycoprotein Inhibitors; Chloroquine; Hydroxychloroquine; Molecular Docking*

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Abbreviations: EEs-Early Endosomes; ELs-Endolysosomes

Introduction

The eruption of coronavirus disease 2019 (COVID-19) has a serious threat to worldwide public health and economies. Human coronaviruses (CoV) are enveloped positive-stranded RNA viruses belong to the category Nidovirales, and are frequently conscientious for digestive tract and upper respiratory infections [1,2]. Acute respiratory condition coronavirus (SARS-CoV) showed that it can cause severe and occasionally deadly respiratory tract infections in humans [3-5]. The consecutive outbreaks, in addition, emphasize the threat of these viruses and caused a pandemic warning that has been declared a public health crisis of international anxiety [5-7]. Coronavirus entry into host cells is arbitrated by the transmembrane spike (S) glycoprotein that forms homotrimers overhanging from the viral

exterior [8-10]. S consists of two functional subunits in charge for binding to the host cell receptor (S1 subunit) and fusion of the viral and cellular membranes (S2 subunit). For many CoVs, S is cleaved at the boundary between the S1 and S2 subunits, that stay non-covalently bound in the prefusion conformation [11-14]. The distal S1 subunit comprises the receptor-binding domain(s) and contributes to stabilization of the prefusion state of the membrane-anchored S2 subunit that contains the fusion machinery [15-17]. There are many potential targets against COVID-19 and among targets replication-related enzymes, such as spike glycoprotein are extremely conserved [18;19]. It has been reported that drugs that inhibit spike glycoprotein are able preventing proliferation and replication of the virus [18,19].

Chloroquine is extensively used anti-malarial with immunomodulatory effects, Chloroquine and hydroxychloroquine were reported to have anti-SARS-CoV activity in vitro. The molecular mechanism of action of chloroquine and hydroxychloroquine has not been completely understood. Findings from earlier studies have suggested that chloroquine and hydroxychloroquine may inhibit the coronavirus through a series of steps [20-22]. Hydroxychloroquine was found to be more potent than chloroquine to inhibit SARS-CoV-2 *in vitro* [23,24]. Following the spike glycoprotein inhibition approach two supposed standard spike glycoprotein inhibitors were used as lead including chloroquine and hydroxychloroquine inhibitors and their chemical structures were shown in Figure 1.

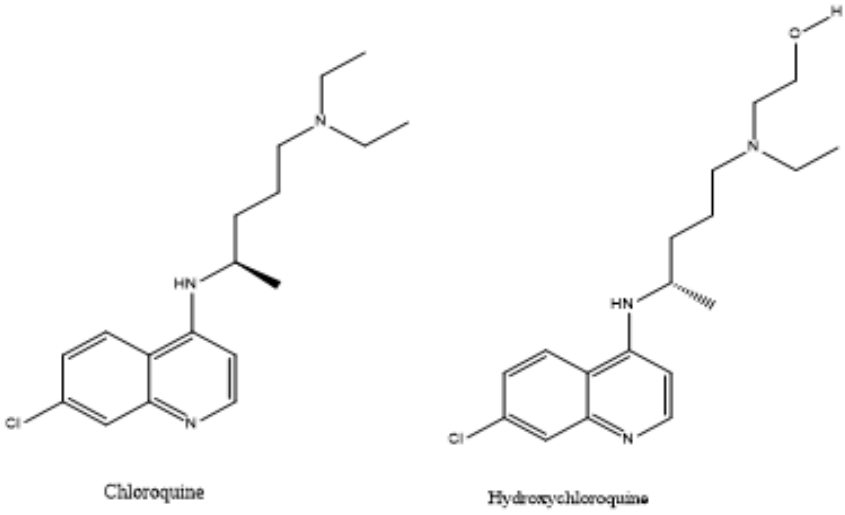


Table 1: *Chemical structure of chloroquine and hydroxychloroquine*

The post fusion core of 2019-nCoV S2 subunit and prefusion 2019-nCoV spike glycoprotein with a single receptor-binding domain may be utilized as a homologous target for screening of *ortho*-phenylenediamine derivatives that could inhibit the proliferation and replication of COVID-19 [25-28]. The *ortho*-phenylenediamine derivatives are Schiff bases recognized for their therapeutic value as they were reported to have anti-inflammatory, analgesic, antiviral, antitumor, antifungal and antibacterial properties [29-34]. Molecular modeling is a recognized computational tool to aid early drug discovery and development. It is used to generate ideas of a compounds or macromolecules 3D conformation, protein–ligand interactions, and allows forecasts about biological activities. The integration of molecular modeling in drug or vaccine design can help in early drug or vaccine discoveries [35-37]. The main aim of this study is to further identify spike glycoprotein as a target, and by computational drug repurposing procedures to allocate appropriate inhibitory agents.

Materials and Methods

Molecular Docking

The starting geometry of the *ortho*-phenylenediamine derivatives was constructed using chem3D Ultra software (version 8.0, Cambridge soft Com., USA). The optimized geometry of *ortho*-phenylenediamine derivatives with the lowest energy was used for molecular dockings. The crystal structure of prefusion 2019-nCoV spike glycoprotein with a single receptor-binding domain up (6VSB)

was downloaded from the Protein Data Bank https://www.rcsb.org/structure/6vsb and post fusion core of 2019-nCoV S2 subunit (6LXT) was downloaded from the Protein Data Bank https://www.rcsb.org/structure/6lxt. Molecular dockings of *ortho*-phenylenediamine derivatives with 6VSB and 6LXT was accomplished by Auto Dock 4.2 software from the Scripps Research Institute (TSRI) (http://autodock.scripps.edu/). Firstly, polar hydrogen atoms were added into protein molecules. Then, partial atomic charges of the protease enzymes and *ortho*-phenylenediamine derivatives molecules were calculated using Kollman methods [38]. In the process of molecular docking, the grid maps of dimensions: (60Å X 60Å X 60Å) and (36.8Å X 64.6Å X 60Å) for 6VSB and 6LXT, respectively, with a grid-point spacing of 0.376Å and the grid boxes is centered. The number of genetic algorithm runs and the number of evaluations were set to 100. All other parameters were default settings. Cluster analysis was performed on the basis of docking results by using a root mean square (RMS) tolerance of 2.0Å, dependent on the binding free energy. Lastly, the dominating configuration of the binding complex of *ortho*-phenylenediamine derivatives and spike glycoprotein fragments with minimum energy of binding were determined which relied strongly on the information of 3D-structures of the spike glycoprotein binding site and ultimately generated a series of spike glycoprotein-binding complexes.

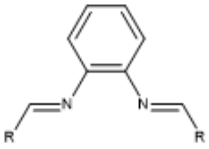
Results and Discussion

Molecular Docking

Table 1 shows the binding energies of chloroquine and hydroxychloroquine, (Figure 1, as standards), *ortho*-phenylenediamine derivatives, and spike glycoprotein (6VSB and 6LXT) obtained by the molecular docking strategy. Molecular dockings of the *ortho*-phenylenediamine derivatives with spike glycoprotein (6VSB and 6LXT) were performed using Auto Dock 4.2 to obtain information about interaction forces between *ortho*-phenylenediamine derivatives and spike glycoprotein (6VSB and 6LXT). *ortho*-phenylenediamine derivatives and spike glycoprotein (6VSB and 6LXT) were kept as flexible molecules and were docked into seven forms of rigid spike glycoprotein (6VSB and 6LXT) to obtain the preferential binding site to ortho-phenylenediamine derivatives on spike glycoprotein (6VSB and 6LXT). The molecular docking results are shown in Table 1. The modeling studies indicate *van der Waals*, hydrogen bonding (Table 1) and electrostatic interactions between *ortho*-phenylenediamine derivatives with spike glycoprotein (6VSB and 6LXT). The contribution of *van der Waals* and hydrogen bonding interaction is much greater than that of the electrostatic interaction because the sum of *van der Waals* energy, hydrogen bonding energy and desolvation free energy is larger than the electrostatic energy, [39,40].

The *ortho*-phenylenediamine derivatives, and spike glycoprotein (6LXT and 6VSB) interactions are shown in Figure 2 and 3, respectively. *Ortho*-phenylenediamine derivatives provide higher binding energy (-7.8 to -11.6 kcal/mol) compared to chloroquine and hydroxychloroquine standards spike glycoprotein (6VSB and 6LXT) (-4.7 to -6.0 kcal/mol) Table 1. Figure 2 indicates four hydrogen bonds between NHM7 and 6LXT. In addition, NHM7 showed good docking interaction of -11.6 kcal/mol with the 6LXT binding site (Figure 2). Compound NHM7 has the highest binding energy of the series. This compound has an extra phenyl moiety attached to the naphthyl analogue of the phenylenediamine Schiff`s base derivative with a log P value of 7.49 indicating the importance of the lipophilicity for the interaction with the active site. The interaction of similar Schiff's base *ortho*-phenylenediamine derivatives with the spike glycoprotein binding site is essential for effective inhibition as previously reported [41,42]. Therefore NHM7 (*ortho*-phenylenediamine derivatives) may be considered the most effective spike glycoprotein inhibitors.

The obtained results using computational drug repurposing is an efficient way to find novel applications for already known drugs [43]. Molecular docking and binding free energy calculations for *ortho*-phenylenediamine derivatives can be used to forecast drug-target interactions and binding affinity. The appearance of resistance to existing antiviral drugs or vaccines is a major challenge in antiviral drug development. The drug repurposing technique allows finding novel antiviral agents within a short period in order to overcome the challenges in antiviral therapy. Computational drug repurposing has previously been used to recognize drug candidates for viral infectious diseases like ZIKA, Ebola, influenza and dengue infections. These methods were also utilized to recognize possible drugs against MERS-CoV and SARS-CoV [44,45] and following the COVID-19 outbreak, computational repurposing has been and are used for COVID-19.



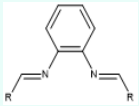
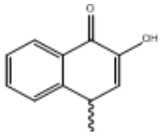
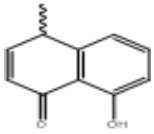
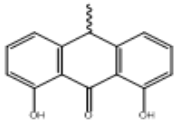
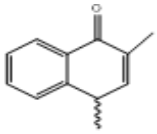
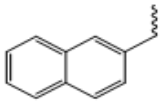
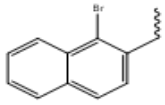
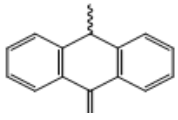
 Substituent (R)	Compounds (M Wt) g/mol	Log P* Calculated	Hydrogen bonds**		Binding energy (ΔG) kcal/mol.	
			Donors	Acceptors	6LXT	6VSB
Chloroquine	319.89g/mol	4.63	1	2	-5.2	-4.7
Hydroxychloroquine	335.87g/mol	3.58	2	3	-6.0	-5.6
	NHM1 420.42 g/mol	4.36	2	6	-9.3	-8.2
	NHM2 420.42 g/mol	5.04	2	6	-10.1	8.2
	NHM3 552.53 g/mol	7.17	2	8	-10.4	-8.7
	NHM4 416.47 g/mol	5.40	0	4	-9.4	-7.8
	NHM5 384.47 g/mol	7.02	0	2	-8.1	-9.3
	NHM6 542.26 g/moL	8.40	0	2	-8.5	-8.7
	NHM7 488.53 g/mol	7.49	0	4	-11.6	-9.0

Table 1: *Various energies in the binding process of ortho-phenylenediamine derivatives, chloroquine and hydroxychloroquine with COVID-19 spike glycoprotein (6VSB and 6LXT) obtained from molecular docking. The unit of all energies (ΔG) is kcal/mol.*

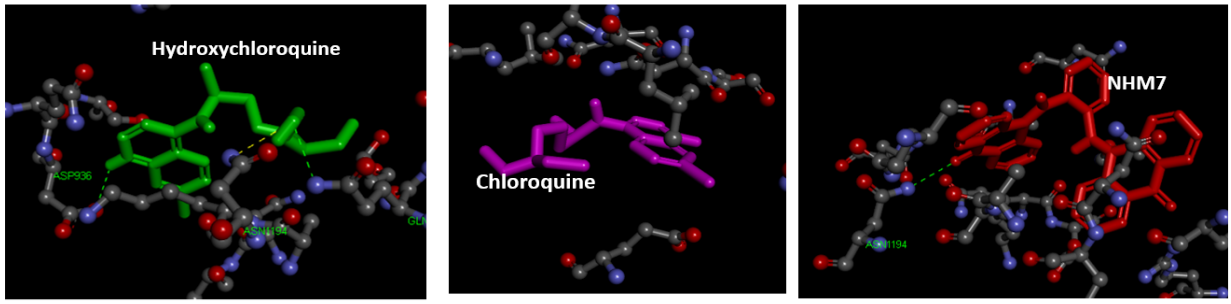


Figure 2: *Interaction model between hydroxychloroquine (green), chloroquine (pink) and NHM7 (red) with 2019-nCoV Spike glycoprotein (6LXT) active site. . Hydrogen bonds green broken line.*

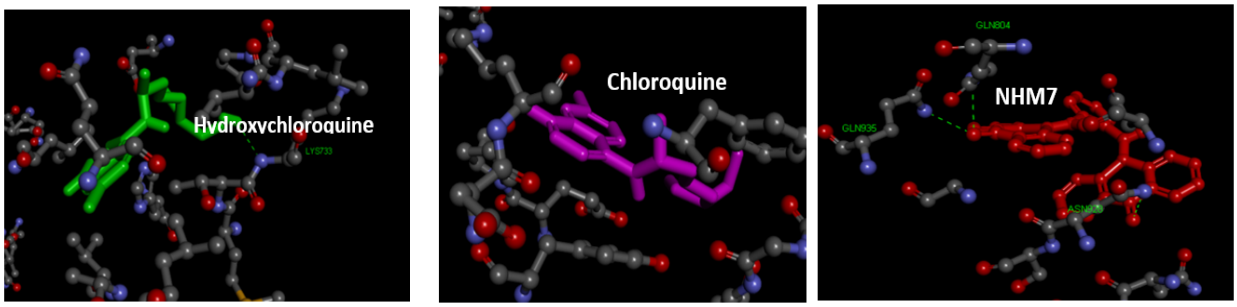


Figure 3: *Interaction model between hydroxychloroquine (green), chloroquine (pink) and NHM7 (red) with 2019-nCoV Spike glycoprotein (6VSB) active site. . Hydrogen bonds green broken line.*

It has been reported that chloroquine and hydroxychloroquine are weak bases that are recognized to raise the pH of acidic intracellular organelles, such as endosomes/lysosomes, which are necessary for membrane fusion [46]. Additionally, chloroquine could inhibit SARS-CoV access throughout altering the glycosylation of angiotensin converting enzyme 2 receptor and spike protein [47,48]. It has been also reported that hydroxychloroquine efficiently inhibited the entry step, as well as the post-entry stages of SARS-CoV-2 that was also established upon chloroquine treatment. Mingo R.M. *et al*. suggested that both chloroquine and hydroxychloroquine blocked the transport of SARS-CoV-2 from early endosomes (EEs) or endolysosomes (ELs) that are needed to release the viral genome of SARS-CoV [49].

Conclusions

Owing to the lack of experimental and clinical data in Libya, as well as the importance to recognize the infectivity of the deadly coronaviruses, we have been mainly relying on computational analyses to study the 2019-nCoV virus in terms of protein structures, phylogeny, functions, and interactions small chemical molecules. In spite of the economic and societal shock of COVID-19 infections and the probability of future outbreaks of even more stern pathogenic COVID-19 in humans, there is still a lack of efficient antiviral strategies to treat COVID-19 and only few options are available to prevent COVID-19 infections. Rapid development and use of a broad-spectrum spike glycoprotein inhibitor alone or in combination with other potent inhibitors of spike glycoprotein might fill the therapeutic gap spanning quarantine and hospital setting.

Further elaborative work is necessary for better understanding the mechanisms of spike glycoprotein inhibition. According to modeling studies *ortho*-phenylenediamine derivatives may have the ability to inhibit COVID-19 spike glycoprotein making them reasonable candidates for consideration of clinical trials and warrant further examination. Results presented in this study shall motivate future efforts in finding potent *ortho*-phenylenediamine derivatives that can be used for COVID-19 protease inhibition *in vivo*. *Ortho*-phenylenediamine derivatives could in the future have possible functional consequences of this cleavage site in the viral cycle, pathogenicity and its possible proposition in the development of antiviral drugs.

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