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Hydroxychloroquine and curcumin conjugates as multifunctional co drugs for the potential treatment of COVID-19: An *in-silico* based study

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ABSTRACT

The present study has been undertaken to search the novel co drugs for fighting against the COVID-19 disease through an *in- silico* approach. We have designed eight co drugs by merging two drugs namely hydroxychloroquine, used in the treatment of COVID-19 and curcumin an immuno modulator. The two drugs were linked through a bio-cleavable hydrolyzable linker by three-step process. The designed co drugs were subjected for molecular docking studies to know their therapeutic efficacy against the COVID-19 main protease (M^{pro}) and Interleukin-1β. The designed co drugs have a promising binding affinity towards M^{pro} in the narrow range of binding energies between -28.2498 to -35.8648 kcal/mol when compared to the standard Remdesivir which has -21.8600 kcal/mol. Similarly the binding energies of designed co drugs against Interleukin -1β range between -25.8032 to -34.6973 kcal/mol when compared to the standard curcumin which has -17.3274 kcal/mol. Our findings demonstrated that the designed co drugs may be useful for the treatment of viral respiratory infection due to their additive therapeutic actions on the viral protein and modulating the immune response synergistically.

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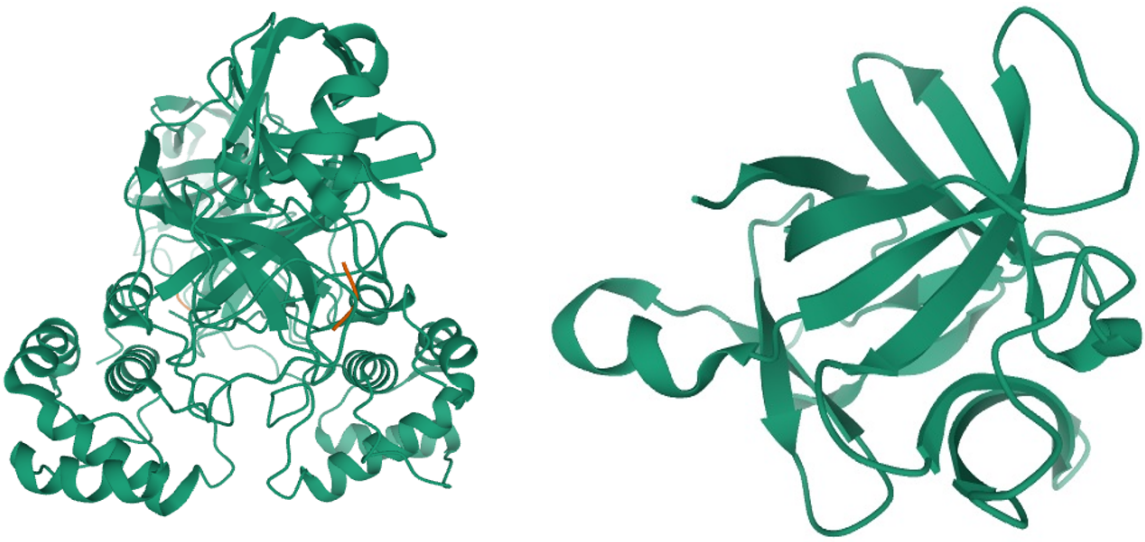
INTRODUCTION

The COVID-19 outbreak originated from Wuhan during December 2019, affects patients in three levels. The first level is patients with asymptomatic, the second level is patients showing the symptoms of fever, cough and the third level is patients suffer very severe conditions like difficulty in breathing and other respiratory illness or cytokine storm, which means the body's immune system cause damage to the host organs (COVID-19, 2020a). To help the global researchers to hunt the COVID-19 infection, on April 9, 2020, China research laboratory issued the three-dimensional structure of coronavirus



a b

Figure 1: (a): The 3D crystal Structure of COVID-19 main Protease (6LU7) (b): The 3D crystal Structure of Interleukin 1 beta (6Y8M)

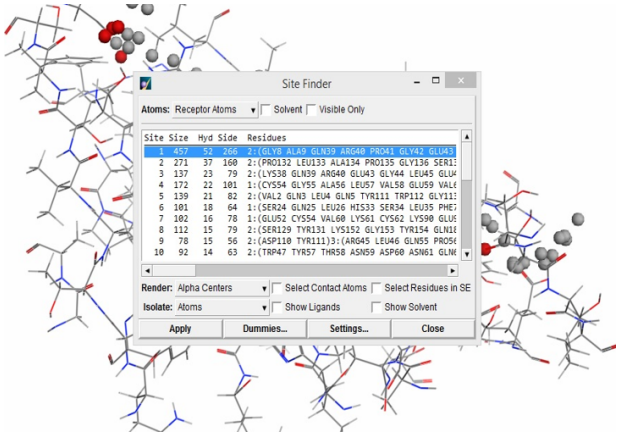


Figure 2: Binding pocket through active site finder module of the MOE

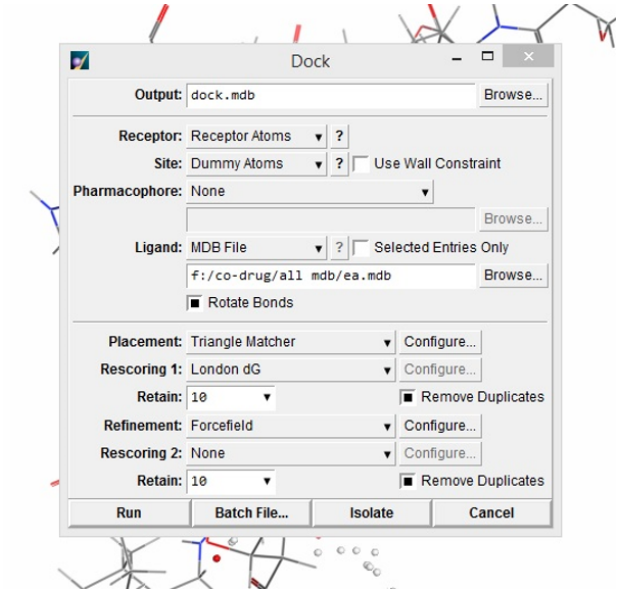


Figure 3: Docking Parameters Set for the simulation

main protease (Jin *et al.*, 2020). This helped the researchers around the world to use different molecular modeling and virtual screening methods to identify the existing drug that could inhibit this enzyme possibly.

There are several drugs have been tested against this COVID-19 infection, includes Japanese influenza drug favipiravir, anti epileptic drug valproic acid, anti-malarial drug-hydroxy chloroquine along with anti-biotic azithromycin (Gautret *et al.*, 2020), anti-HIV drugs lopinavir and ritonavir, and anti-viral drug remdesivir. However, the problems in these drugs have their unique side-effects like heart palpitation, permanent blindness, migraine, and in some cases, even death (Zeitlinger and Koch, 2020).

The proteins/enzymes involved in the budding and exocytosis processes which are essential for viral replication may be considered as the important targets for the development of novel anti-viral drugs. Anti-viral drugs most commonly target the proteins, which is essential for the virus to synthesize the offense protein from human cells. In novel coronavirus, there are four proteins, namely, M (membrane protein), required for virus budding; S (viral spike glycoprotein), that has receptor binding and membrane fusion activities; E (small membrane protein), used for the assembly of coronavirus; N (nucleo capsid phosphoprotein), the viral

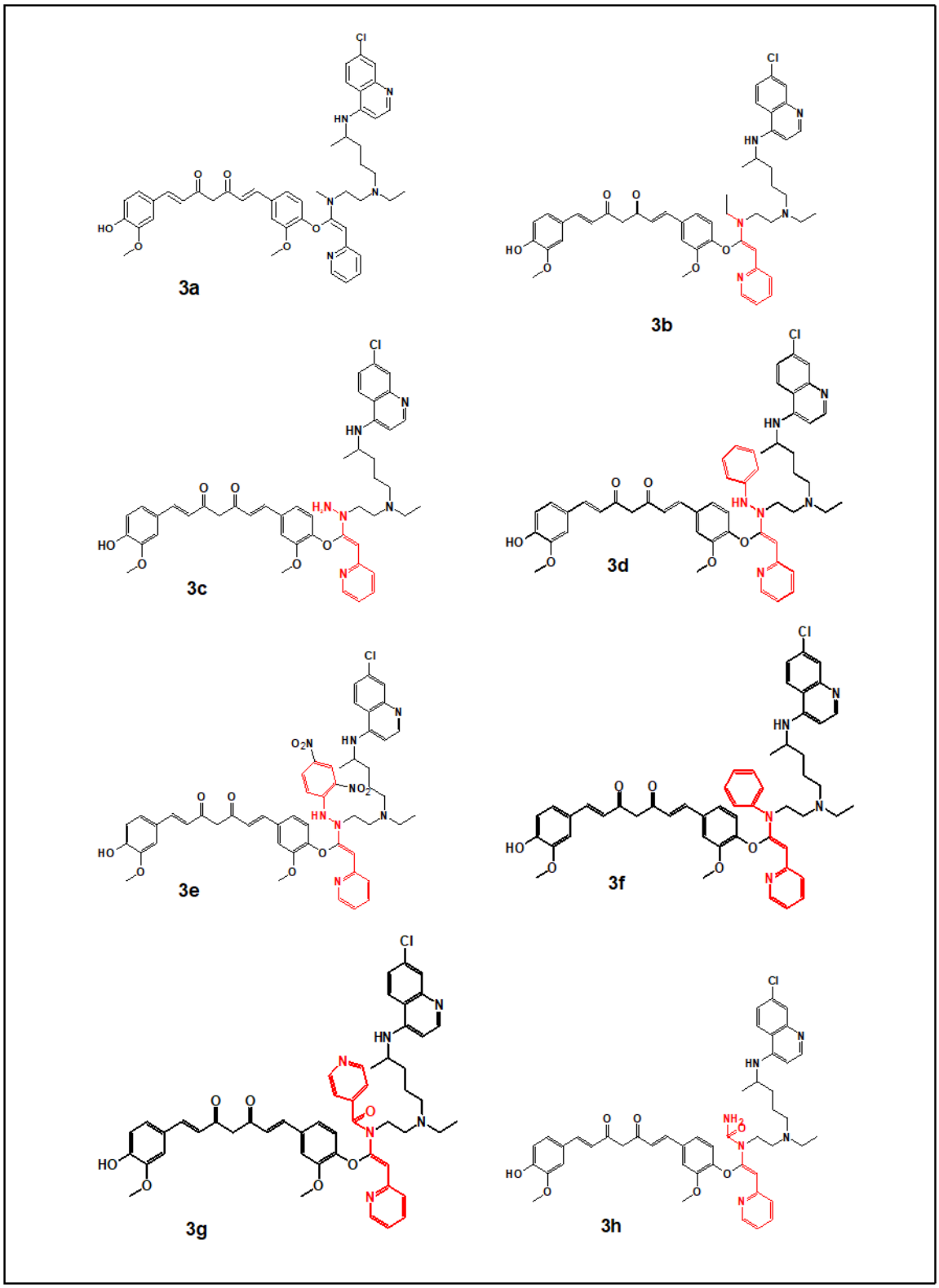
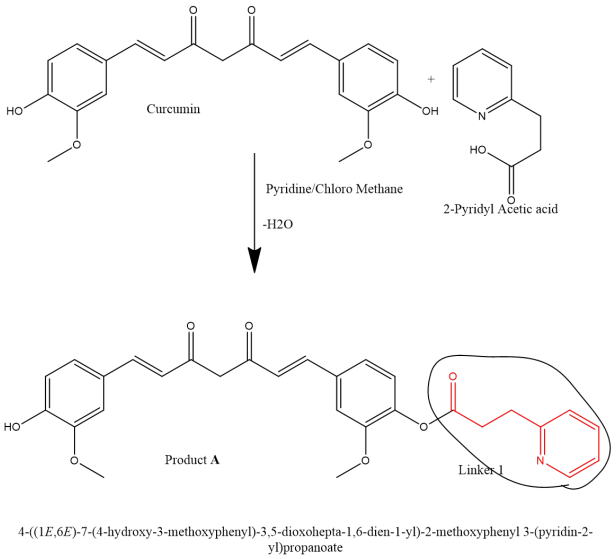
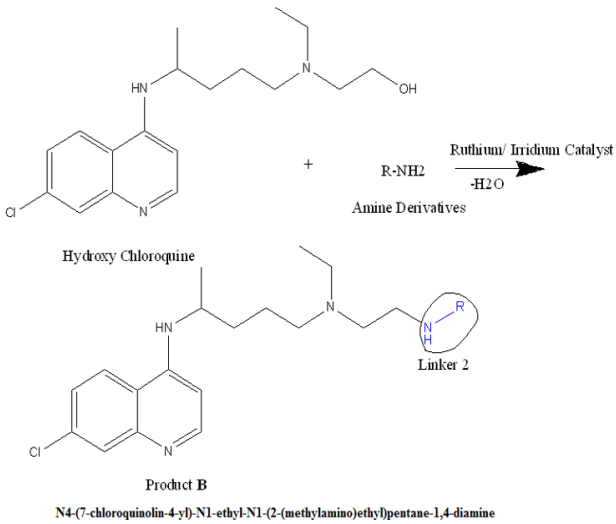


Figure 4: Designed co-drug for the treatment of COVID-19 disease



Scheme 1: Designed synthetic scheme for the synthesis of Product A (4-((1E,6E)-7-(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dien-1-yl)-2-methoxyphenyl3-(pyridin-2-yl) propanoate)



Scheme 2: Designed synthetic scheme for the synthesis of Product B (N4-(7-chloroquinolin-4-yl)-N1-ethyl-N1-(2-(methylamino)ethyl)pentane-1,4-diamine)

RNA has been connected with this protein inside the virion (Holmes, 2003).

The WHO declared that the outbreak of COVID-19 has been considered as a pandemic, and on the 13th of May, it has officially declared the statistics on outbreak has reached to 4139794 confirmed cases, 285328 confirmed deaths, and 215 countries are affected with this virus (WHO, 2019). Whereas, in India, the statistics on COVID-19 Dashboard as on: 13th of May 2020, 08:00 GMT+5:30 articulate that, there were 47480 Active Cases, 24385 Cured/Discharged cases, and 2415 deaths (COVID-19, 2020b). This pandemic is still enduring and hence there is an emerging call to find out the novel preventive measures to combat this novel pandemic situation. However, until now, there is no specific treatment was found to cure this disease. With response to this statement by WHO, there is an emerging need to find curative measures against this virus through diverse techniques like adjusting the immune system through various immunomodulators, by the development of diagnostic, vaccines, re-purposing of drugs, etc. Furthermore, in agreement with the WHO report, the development of anti-coronavirus vaccines or anti-coronavirus drugs might take another 18 months and this COVID-19 is considered a seasonal disease shortly.

We have chosen the co drug or mutual pro drug approach, which means conjugating two or more therapeutically active compounds through covalent chemical linkage or a cleavable spacer. The co drug consists of two different scaffolds have different therapeutic actions molded together in a single entity through a specified bio cleavable linker to release the parent drugs at their site of action (Das *et al.*, 2010). Co drugs are made through the combination of two scaffolds/drugs with similar or different pharmacological activities into a single hybrid form to complement each other to elicit the therapeutic effect. Co drug strategy is employed to ameliorate physicochemical, biopharmaceutical, and drug delivery/targeting of the therapeutic actives (Das *et al.*, 2010; Aljuffali *et al.*, 2016) Co drug-based therapy is reported to treat various diseases such as neurodegenerative disorders, cardiovascular problems, tumors, glaucoma, retinal neovascularization, alcohol addiction, virus infection, and bacterial infection (Das *et al.*, 2010).

Chloroquine and hydroxychloroquine have been found to be efficient on SARS-CoV-2 and reported to be efficient in Chinese COVID-19 patients. Hydroxychloroquine is widely used worldwide for the prophylaxis is derived from available evidence of benefit as treatment and supported by preclinical data. On the other hand, the immune system is a very important and dynamic one to regulate the body's inadequate immune response which could cause many diseases, while excessive and unregulated immune responses produce autoimmunity. Interleukin plays a vital role in maintaining this immune in a balanced condition (Waters *et al.*, 2018).

Cytokines play a vital role in host cell normal immune responses. When a severe immune reac-

Table 1: Docking results for designed co-drug molecules with proteins viz. PDB Id: 6LU7 and 6Y8M

Ligands	Targets	Molecular Docking parameters (kcal/mol)					
		S	rmsd_refir	E_conf	E_place	E_score1	E_refine
3a	6LU7	-29.1905	3.1332	114.8776	-35.8992	-8.9094	-29.1905
	6Y8M	-30.1818	1.9245	131.7791	7.4570	-7.2985	-30.1818
3b	6LU7	-32.3370	2.8464	119.8081	-60.6725	-10.3698	-32.3370
	6Y8M	-29.3281	2.2041	110.3413	-51.5425	-9.7877	-29.3281
3c	6LU7	-30.9454	2.8067	156.8525	-105.1475	-10.9808	-30.9454
	6Y8M	-29.2795	3.8143	157.7194	-41.7619	-8.2759	-29.2795
3d	6LU7	-35.0135	2.0840	153.5767	-68.8002	-10.9257	-35.0135
	6Y8M	-25.8032	2.8920	149.0033	-9.9911	-8.0958	-25.8032
3e	6LU7	-32.5917	4.6357	207.1254	-76.1159	-11.3205	-32.5917
	6Y8M	-34.6973	4.3967	200.1437	-10.5559	-8.8317	-34.6973
3f	6LU7	-28.2498	3.0425	136.5072	-44.2248	-8.4560	-28.2498
	6Y8M	-25.9017	2.8366	129.9287	-16.5249	-6.6655	-25.9017
3g	6LU7	-32.2662	5.2570	113.9838	-69.1081	-9.6591	-32.2662
	6Y8M	-28.8448	3.3834	119.5122	7.1257	-6.8749	-28.8448
3h	6LU7	-35.8648	2.2907	39.3722	-67.9918	-10.6689	-35.8648
	6Y8M	-27.4442	3.3478	41.1756	-17.9602	-7.9996	-27.4442
RDV	6LU7	-21.8600	3.3018	-95.7516	-37.4017	-9.1901	-21.8600
CU	6Y8M	-17.3274	1.2218	32.6083	-47.7222	-10.8938	-17.3274

rmsd_refine- Root mean square deviation; *E_conf* - Conform erenergy; *E_place* - Placement stage, *E_score1* - Rescoring stage; *E_refine*- Refinement stage; *RDV* -Remdesivir; CU-Curcumin

tion occurs, the body could synthesize and release too many cytokines into the bloodstream quickly. However, having overproduction of this immune cell (cytokines) in the bloodstream could be very harmful and as a result, a cytokines storm may occur, which leads to infection, organ failure, high fever, an autoimmune condition, and other associated disease (NIH, 2020). These conditions may occur during the flu infection, lung inflammation, and fluid buildup, causing pneumonia and might cause mortality in patients (The Scripps Research Institute , 2014).

However, not only the allopathic treatment has been considered for treating the COVID-19. China has been using traditional medicine along with allopathic drugs for prophylactic and treatment of this COVID-19. Besides, our Indian traditional system does not just boost immunity but also have antiviral properties (Kumar *et al.*, 2012). Curcuminoids, a mixture of three different curcumins are isolated from the spice herb of *Curcuma longa*, Indian saffron, showed the effectiveness in several preclinical and clinical study data and used in the prophylaxis and treatment of diverse diseases such as cancer, cardiovascular disorders, inflammatory responses, metabolic and neurological disorders, and skin problems (Kunnumakkara *et al.*, 2017). The immunomodulatory effect of this active ingredient might be due to the interaction with assorted immunomodulators like cellular components, macrophages, lymphocytes, cytokines, and other transcription factors (Momtazi-Borojeni *et al.*, 2018).

Keeping all the above facts in our minds, we have designed eight co-drugs by combining the Curcumin, a well-known plant-based immuno booster along with hydroxychloroquine markedly available prophylactic treatment drug for COVID-19. In this present scenario with this life-threatening virus, failure to find the targeted therapy and treatment options to this situation is inadequate, unfortunately. However, to accelerate the discovery of the novel leads, we move forward to think of an alternate remedy for prophylaxis and treating this situation. In order to achieve this strategy, we have chosen the *in-silico* molecular modeling approach to design the novel co drugs to combat this COVID-19.

METHODS

Computational workup

Computer-assisted simulated docking experiments were carried out under an MMFF94X force field on COVID-19 main protease (PDB Id-6LU7, Figure 1 a)

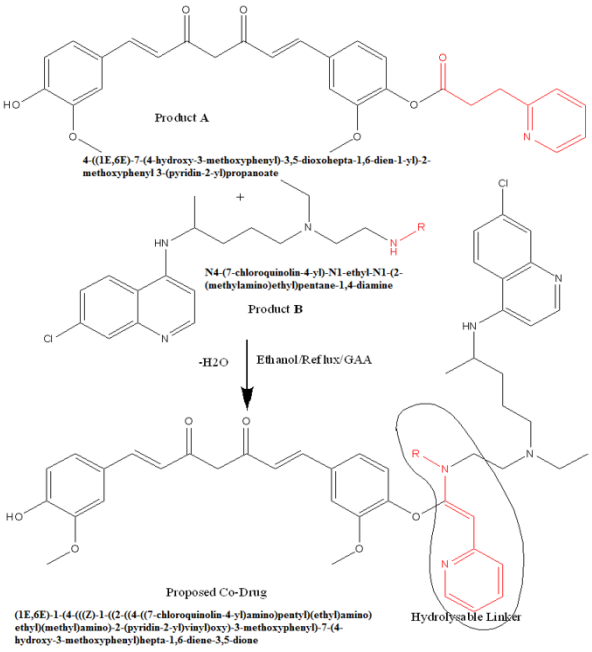
Table 2: Ligand Protein interaction details at the active site of the enzymes viz. COVID-19 main protease and Cytokines (Interleukin-1β)

Ligand molecules	Ligand – Protein interaction	
	PDB Id: 6LU7	PDB Id: 6Y8M
3a	Side Chain Donor (-OH)- Thr 25; Side Chain Acceptor (C=O) Asn 142; Polar centre- Thr 24; Thr 190; Lys 44; Gln 158; Greasy centre- Met 43; Acidic centre- Gln 156; Receptor Contact – All the above-mentioned amino acids.	Side Chain Acceptor (O-CH3)- Lys 77; Arene-Cation interaction (Phenyl ring)- Lys 71; Polar centre- Gln 81; Glu 28; Ser 21; Lys 74; Asn 128; Asp 78; Thr 72; Lys 77; Greasy centre- Leu 52; Pro 131; Met 133; Leu 134; Receptor Contact – All the above-mentioned amino acids.
3b	Side Chain Acceptor (C=O)- His 163; Polar center- Thr 24; Thr 25; Ser 144; Ser 46; Gln 153; Asn 142; Arg 155; Greasy center- Met 165; Phe 140; Leu 141; Pro 165; Acidic center- Gln 155; Receptor Contact – All the above mentioned amino acids.	Arene-Arene interaction (Quinoline Phenyl ring)- Tyr 24; Arene-Cation interaction (Curcumin Phenyl ring)- Lys 77; Polar center- Gln 81; Glu 28; Ser 54; Lys 74; Asp 78; Thr 34; Lys 77; Greasy center- Leu 52; Leu 82; Pro 131; Acidic center- Gln 25; Asp 75; Receptor Contact – All the above mentioned amino acids.
3c	Back bone Donor (-OH)- Gln 164; Polar center- Cys 145; Gln 145; Thr 24; Thr 25; Thr 180; Gln 180; Grease center- Asn 181; Leu 58; Met 40; Receptor contact- All the above mentioned amino acids.	Side Chain Acceptor (C=O)- Lys 77; Thr 78; Side Chain Donor (-OH)- Ser 123; Arene-Arene interaction (Curcumin Phenyl ring)- Phe 133; Polar center- Gln 23; Glu 28; Ser 21; Lys 74; Ser 123; Asp 142; Thr 24; Lys 77; Greasy center- Pro 131; Met 133; Leu 134; Acidic center- Asp 73; Asp 142; Receptor Contact – All the above mentioned amino acids.
3d	Side Chain Donor (-OH)- Thr 25; Thr 24; Polar center- Thr 24; Thr 25; Thr 48; Ser 46; Thr 92; Gln 153; Gln 183; Asn 142; Greasy center- Ala 191; Leu 141; Receptor Contact – All the above mentioned amino acids.	Side Chain Acceptor (C=O)- Lys 77; Arene-Arene interaction (Quinoline Phenyl ring)- Tyr 24; Polar center- Gln 41; Thr 137; Ser 21; Lys 74; Ser 44; Asp 142; Thr 24; Lys 77; Asp 77; Greasy center- Phe 138; Leu 82; Acidic center- Asp 75; Asp 142; Receptor Contact – All the above mentioned amino acids.
3e	Side Chain Donor (-OH)- Thr 24; Polar center- Asn 142; Gly 143; Thr 25; Ser 44; Thr 42; Thr 183; Greasy center- Pro 144; Ala 191; Met 48; Leu 141; Receptor Contact – All the above mentioned amino acids.	Side Chain Acceptor (C=O)- Lys 77; Side Chain Donor (-OH)- Asp 142; Arene-Arene interaction (Curcumin Phenyl ring)- Phe 133; Polar center- Gln 81; Gln 33; Ser 21; Lys 74; Ser 133; Thr 36; Lys 71; Asp 142; Thr 24; Lys 77; Lys 72; Greasy center- Phe 133; Met 130; Leu 80; Acidic center- Asp 75; Asp 142; Receptor Contact – All the above mentioned amino acids.

Continued on next page

Table 2 continued

3f	Side Chain Acceptor (O-CH3, -OH)- Thr24; Thr 43; Side Chain Donor (-OH) – Thr 24; Polar centre- Asn 142; Gly 168; Thr 25; Ser 48; Gln 183; Greasy centre- Phe 140; Pro 163; Acidic Center- Gln 168; Receptor Contact – All the above-mentioned amino acids.	Side Chain Acceptor (C=O)- Lys 77; Ser135; Arene-Arene interaction (Curcumin Phenyl ring)- Tyr 24; Arene-Arene interaction (Quinoline Phenyl ring)- Phe 139; Polar center- Gln 81; Gln 25; Ser 44; Lys 74; Asp 75; Ser 13; Lys 77; Asp 142; Thr 24; Ser 135; Greasy center- Phe 133; Met 130; Leu 83; Acidic center- Asp 75; Glu 85; Asp 142; Receptor Contact – All the above mentioned amino acids.
3g	Back bone Donor (-NH)- Gln 168; Polar center- Asn 142; Gln 168; Gln 153; Thr 25; Thr 183; Gly 142; Ser 48; Thr 34; Asn 119; Thr 28; Thr 23; Thr 118; Grease center- Met 43; Met 143; Receptor contact- All the above mentioned amino acids.	Arene-Cation interaction (Quinoline Phenyl ring)- Lys 77; Backbone Donor- Ser21; Polar center- Gly 123; Gly 128; Ser 21; Tyr 24; Asp 72; Gly 22; Lys 77; Greasy center- Thr 123; Leu 52; Pro 23; Leu 134; Acidic center- Asp 75; Gln 25; Receptor Contact – All the above mentioned amino acids.
3h	Back bone Donor (-OH)- Thr 142; Polar center- Asn 119; thr 28; Gly 143; his 41; Asn 142; Thr 24; Ser 40; His 134; Glu 155; Gln 150; Grease center- Pro 141; Met 141; Receptor contact- All the above mentioned amino acids.	Side Chain Acceptor (C=O)- Lys 77; Arene-Arene interaction (Quinoline Phenyl ring)- Tyr 24; Polar center- Gln 41; Thr 24; Ser 134; Ser 123; Asp 142; Thr 79; Lys 77; Asp 142; Glu 23; Greasy center- Phe 133; Leu 82; Met 130; Acidic center- Gly 23; Asp 142; Receptor Contact – All the above mentioned amino acids.
Remdesivir	Side Chain Acceptor- Asn 151, Lys 102, Gln 110; Side Chain Donor- Asp 153; Arene-Arene Interaction centre- Phe 294; Grease centre- Val 104, Phe 294, Ile 106; Polar centre- Thr 111, Gln 110, Asn 151; Acidic centre- Asp 153; Basic center- Lys 102, Arg 105; Receptor contact- All the above-mentioned amino acids.	-
Curcumin	-	Side Chain Acceptor- Lys 77; Backbone Donor- Ser 21; Polar center- Gly 123; Gly 128; Ser 21; Tyr 24; Asp 72; Gly 22; Lys 77; Greasy center- Thr 123; Leu 52; Pro 23; Leu 134; Acidic center- Asp 75; Gln 25; Receptor Contact – All the above mentioned amino acids.



Scheme 3: Designed synthetic scheme for the synthesis of co drug

and IL-1β (PDB Id- 6Y8M, Figure 1 b) using Chemical Computing Group's Molecular Operating Environment (moe-dock 2005) software, Montre´al, Canada.

Design of co drugs

The proposed scheme consists of three steps reaction as follows; In step 1, an equimolar mixture of curcumin is treated with 2-pyridyl acetic acid in the presence of pyridine, chloromethane to give the Product **A**. In step 2, animation process, an equimolar concentration of hydroxychloroquine is treated with amine derivatives (R) in the presence of specialized catalysts like ruthenium or iridium type of catalyst to get the Product **B**. In step 3 process, by merging step 1 and step 2 products together with the diverse linkers such as, primary amines, hydrazine, and carbazide compounds in the presence of the suitable solvents like ethanol and glacial acetic acid to obtain the proposed co-drugs **3a-3h** (Schemes 1, 2 and 3).

Preparation of ligand files

The ligand files of eight proposed co drugs were constructed using the builder module of MOE-DOCK and the energies were minimized. The molecular geometries were drawn; correct 3D structures were ensured and followed by energy optimization at a standard MMFF94 force field level, with a 0.0001 kcal/mol energy gradient convergence criterion. The molecule builder of the (MOE, 2009). 10 program was used for this purpose and after building the molecule, the energy was minimized, potential and partial energy were corrected and then saved as a molecular database (.mdb) file in a local directory for the further process.

Selection of targets

The prime target, coronavirus main protease (Mpro), which is a chief culprit enzyme, guides in mediating the transcription and replication process of the virus (Anand, 2002; Yang *et al.*, 2003) has been assigned for our study. There are 30,000 nucleotides have compressed together in the virus genome of novel COVID-19, its gene may perhaps encode with two polyproteins namely, pp1a and pp1ab, which are desirable for the viral replication and transcription (Zhou *et al.*, 2020; Wu *et al.*, 2020). The main proteolytic enzyme, Mpro, through the proteolytic process, could release the polypeptides from these polyproteins. Besides, this Mpro enzyme is the main protein in the viral cell life cycle together with the absence of closely related homologs of human protein, recognizes the Mpro as a promising target for the anti-viral drug discovery (Pillaiyar *et al.*, 2016). Therefore, the inhibition of Mpro may perhaps block the biosynthesis viral polyproteins, resulting in viral death. Interleukin-1β (IL-1β), signaling protein, a type of cytokines regulates and promotes the development of white blood cells mostly lymphocytes, which is responsible for immunity. IL-1 regulates the body's natural response towards any microbial and viral pathogenic infection (Smith and Humphries, 2009). When a coronavirus enters into the body system, the immune capacity of the COVID-19 infected patients is suppressed that is characterized by a decrease in the T cell counts and the increase in immunosuppressive cytokines. However, in COVID-19 disease, the overproduction of IL-1 (immune-stimulatory cytokines) is thought to regulated by this co drug approach and thereby it could decrease the immune response towards this COVID-19 infection (Verdecchia *et al.*, 2020; Pedersen *et al.*, 2014; Guimond *et al.*, 2009; Gao *et al.*, 2015).

Preparation of macromolecules

The coordinates of the crystal structure of COVID-19 main protease in complex with an inhibitor N3 (PDB Id: 6LU7; Figure 1 a) (Jin *et al.*, 2020) and Interleukin-1 beta bound with bikinin (PDB Id: 6Y8M; Figure 1 b) (Nicola and Nichols, 2020), were retrieved from RCSB Protein Data Bank. These PDB files were imported into the MOE suite in which the receptor preparation module was performed to prepare the proteins. All the bound water molecules and hetero atoms were removed from the complex by using the sequence editor (SEQ) window, which is a default program MOE. Both the polar and non-

polar hydrogens added and 3D structures were corrected. The binding pocket was identified via using MOE-Alpha Site Finder. In order to visualize the binding pocket, the alpha spheres were created followed by the generation of dummy atoms on the centers of these spheres (Figure 2). The pockets were found to be deep small canyons lined with the key residues, including both hydrophobic and hydrophilic amino acids.

Docking methodology

With an objective to explore the potential binding affinities of the proposed co drugs towards the validated COVID-19 Mpro enzyme and IL-1 beta, docking was performed. (Jin *et al.*, 2020; Nicola and Nichols, 2020). For docking simulations, the placement was set as a triangular matcher, rescoring was set as London dG, the number of retaining was set as 10, and the refinement was set as a force field to generate the 10 poses of each target ligand conformations (Figure 3). As a result of the docking run, the mdb.output files were created with scoring and multiple conformations of each compound. All the docked conformations were analyzed and the best-scored pose for each compound had been selected for further ligand interaction studies. Besides, the ligand-receptor interaction, followed by the surface analysis of the selected best pose ligand molecule, was generated and viewed for an interpretation.

RESULTS AND DISCUSSION

The new series of proposed co-drugs are depicted in Figure 4. By combining hydroxychloroquine and curcumin scaffolds into one chemical signature through the hydrolysable linker, it is assumed that both drugs may act together synergistically for the treatment of the COVID-19 situation. When the pathogen enters the host cell, the inflammatory response mediates the host immune cell system and thereby eliminates the pathogen which causes the tissue repair. On the other hand, the highly pathogenic virus-like CoVs induces the prolonged cytokines response which could cause morbidity and mortality in CoVs affected patients. Owing to this excessive cytokines responses in CoVs infection guides to the viral sepsis, where the viral cell replicates further and experience the excessive uncontrolled systemic inflammation which could cause pneumonia, acute respiratory symptoms, respiratory and organ failure, shock, and eventually death (Aljuffali *et al.*, 2016; Mesaik *et al.*, 2012).

It is assumed and reported that coronavirus enters into the host cell through interaction with angiotensin-converting enzyme-2 (ACE-2) (Magrone *et al.*, 2020). Many researchers showed that the high expression of ACE-2 may help to bind the cell membrane of the coronavirus to use its spike (S) glycoprotein to enter, replicate, and to infect the human cells, Many human cell enzymes like furin (Braun and Sauter, 2019), which is present in tissues, lungs, and small intestines, could help to synthesize this coronavirus S protein. During the infection, this S protein has cleaved into its subunit like S2 and S2 proteins (Kirchdoerfer *et al.*, 2016). This S2 protein has the receptor-binding domain, whereas; the S2 protein plays a role in membrane fusion. This S1 protein helps the coronavirus to directly bind to the peptidase domain of the ACE-2 through the help of a host cell enzyme. Further, this enzyme helps to allow the virus-cell into the host and thereby it uses the host cell machinery for its replication. The ACE-2 is not only the receptor to accept the S protein of the coronavirus and also involved in post-infection regulation such as immune response (Verdecchia *et al.*, 2020), B cell-mediated immunity regulation, and the regulation of cytokines secretion like IL-1, IL-10, IL-6, and IL-8 (Li *et al.*, 2020).

At present, there is restricted information is available on host immune response towards CoVs. Zhoe *et al.,* in 2020 reported that, in the investigation of 99 CoVs infected patients in Wuhan city, it was found that there is a considerable increase in total neutrophils, decrease in lymphocytes, and increase on serum IL-6 levels which in turn modulates the innate immune system. This report prompted us to investigate how the host immune system responds or recognize the invaded virus.

Herbal plants have rich in flavonoids, carotenoids, and Vitamin C, which could help to increase the host immunity and also promotes the activity of lymphocytes, increase the phagocytosis and induce the interferon production (Khodadadi, 2015). By understanding the pathophysiology of CoVs, i.e. an RNA virus, one might come to know that, the recognition of the virus by endosomal RNA leads to the activation of nuclear translocation. At this stage, the nuclei by the help of transcription factors induce the pro-inflammatory cytokines and this response is considered as the first level of defence mechanism to the entry of the virus into the host cell (de Wit *et al.*, 2016). In CoVs infection, interestingly the transmission of the virus could occur even the infected individual does not show any such type of symptoms. This may be considered as the delayed early response of the immune system. Thus, the innate immune system plays a crucial role in protective or destructive responses. Based on the diverse studies provide evidence that this inflammatory cytokine plays an important role in the pathogenic

condition. Consequently, the therapeutic intervention to target such type of pro-inflammatory cytokines could prove the undesirable inflammatory response in CoVs infection (Prompetchara *et al.*, 2020). Interleukin-1 is a signaling protein necessary to activate the T cells by promoting the production of IL-2 (Berkenbosch *et al.*, 1993). However, the Interleukin-1 (IL-1), which is a drug target for various immunological responses, plays a vital role in the activation and maintenance of an immune response and lymphocyte development (Sobia *et al.*, 2018).

Whereas, ACE-2 has been related to the CoVs infection, and mediates the cytokines and as well related to innate and adaptive immune responses, β-cell regulation, and enhanced the inflammatory responses by IL-1, IL-10, IL-6 and IL-8 (Huang *et al.*, 2020). Hence, the viral infection causes tissue injury, and death is related to the pro-inflammatory process and this might be treated by the use of IL-1 β blockers, which may have some benefit in COVID-19 patients.

The co drug strategy is also beneficial to promote the targeting of drugs to specific proteins by the co drug approach. This strategy can be achieved by modifying the chemical structure and developing new carriers for co-drug delivery. With this aim, we have designed eight co drugs by combining the naturally available plant secondary metabolite curcumin along with presently used prophylactic drug for treating COVID-19 infection, i.e. hydroxychloroquine through the hydrolysable linker. All the docked conformations of the designed eight co drugs were found with the favourable docking poses. This fact was evidenced by the maximum number of amino acid interactions and ranked by the highest score based on the least binding energy (Table 1). The most favourable docking poses of the ten docked conformations for each molecule were analyzed for the further investigation of the ligand interactions within the protein active sites. All the co drug ligands showed a similar binding pattern and anchored tightly inside the active site canyon (Site I) of each protein. The 2D ligand-protein interactions have visualized for all the analogues. The identified compounds had a promising binding affinity with the Mpro receptors, in the narrow range of binding energy for the protein PDB Id: 6LU7 and the score range was between -28.2498 to -35.8648 kcal/mol: London dG was -8.4560 to -11.3205 kcal/mol when compared to the standard drug Remdesivir which had the binding score of -21.8600 kcal/mol. Similarly, all the designed co drugs showed the binding affinity with interleukin -1β in the narrow range of binding energy for the protein PDB Id: 6Y8M and the score range was 25.8032 to -34.6973 kcal/mol: London dG was -6.8749 to -9.7877 kcal/mol when compared to the standard co-crystal which showed the binding score of -17.3274 kcal/mol.

Further, the top docked confirmation of these designed co-drugs depicted a greater alignment with the native ligand pose. The information detailed in the Table 2 showed clearly that, how well the all active ligand molecules were bound and interacted with the amino acid residues through various pharmacophoric features such as side chain acceptor, side-chain donor, backbone acceptor, backbone donor, arene-cation, and arene-arene interaction, etc. with the receptor site (Table 2). The number of conformations generated by these entire molecules is in the range between 6 to 10, which indicates that the flexibility is an important parameter for the ligands to dock deeply within the binding pocket of COVID-19 and as well Interleukin-1β receptor site. Further, a careful calculation of surface analysis of the binding pocket of these molecules indicated that the entire compounds had adopted the position in a hydrophobic cage surrounded by the diverse amino acid residue and these have approached closely to the ligand molecule for the stronger interactions. Among the eight co drugs designed, the co drugs 3d and 3e were identified as topmost HITS. The two co drugs have shown a significant binding affinity towards both proteins when compared to the standards. Among the diverse hydrolysable linkers used in our study, viz. various primary amines, hydrazines, and carbazides; the phenylhydrazine and 2,4-dinitro phenyl hydrazine linkers have shown the increased binding affinity proving these linkers can serve as a building block in the preparation of further compounds.

CONCLUSIONS

The drug design by this co drug approach is one of the versatile and potent strategies that can be applied in a wide range of administration routes. Through this co drug approach, quite a number of issues are resolved like solubility, stability, drug resistance, and biomembrane permeability, etc. we hypothesized that there is a significant correlation between host immune system and the viral load. Base on the hypothesis, eight co-drugs were designed by merging the two pharmacophores, namely hydroxychloroquine (antiviral) and curcumin (immunomodulator) with a high probability of directly inhibiting the novel COVID-19 targets. To support our hypothesis, an *in -silico* docking study was performed. The docking results revealed that the eight designed co drug molecules

(**3a-3h**) have shown significant docking scores with Mpro and as IL-1β protein when compared to the standard drugs. These designed co drugs could serve as useful probes for COVID-19 drug discovery. Of course, further *in-vitro* studies may require to prove the therapeutic action of these designed co-drugs on COVID-19 disease.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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