Newly developed semi-synthetic chloroquine and hydroxychloroquine-phytochemical conjugates as prospective COVID-19 drug(s)

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ABSTRACT

COVID-19 continues to be belligerent health threats nowadays and the Corona pandemic is the global burden. Herein, two series of chloroquine (CQ) and hydroxychloroquine (HCQ) derivatives were chemically conjugated with established small phenolic phytochemicals namely, thymol, vanillin, guaiacol, eugenol, 4-hydroxycoumarin and vanillin analogues by the principles of Williamson ether reaction. During the reaction, deprotonated pentoxides of phytochemicals were substituted by chlorine atom of CQ and HCQ through SN_2 reactions. The virtually designed molecules were evaluated further through validation by RO5, level of toxicity, drug-likeness parameter and concomitant validation by ADME. Moreover, the molecular docking study of these congeners was also carried out by using COVID-19 protease (PDB ID:6lu7); from which, the analogue 2e had a greater binding affinity. Thus, the HCQ-vanillin conjugation would be coveted as a potential candidate against human COVID-19.

Keywords: Chloroquine, Hydroxychloroquine, Phytochemical, Coronavirus

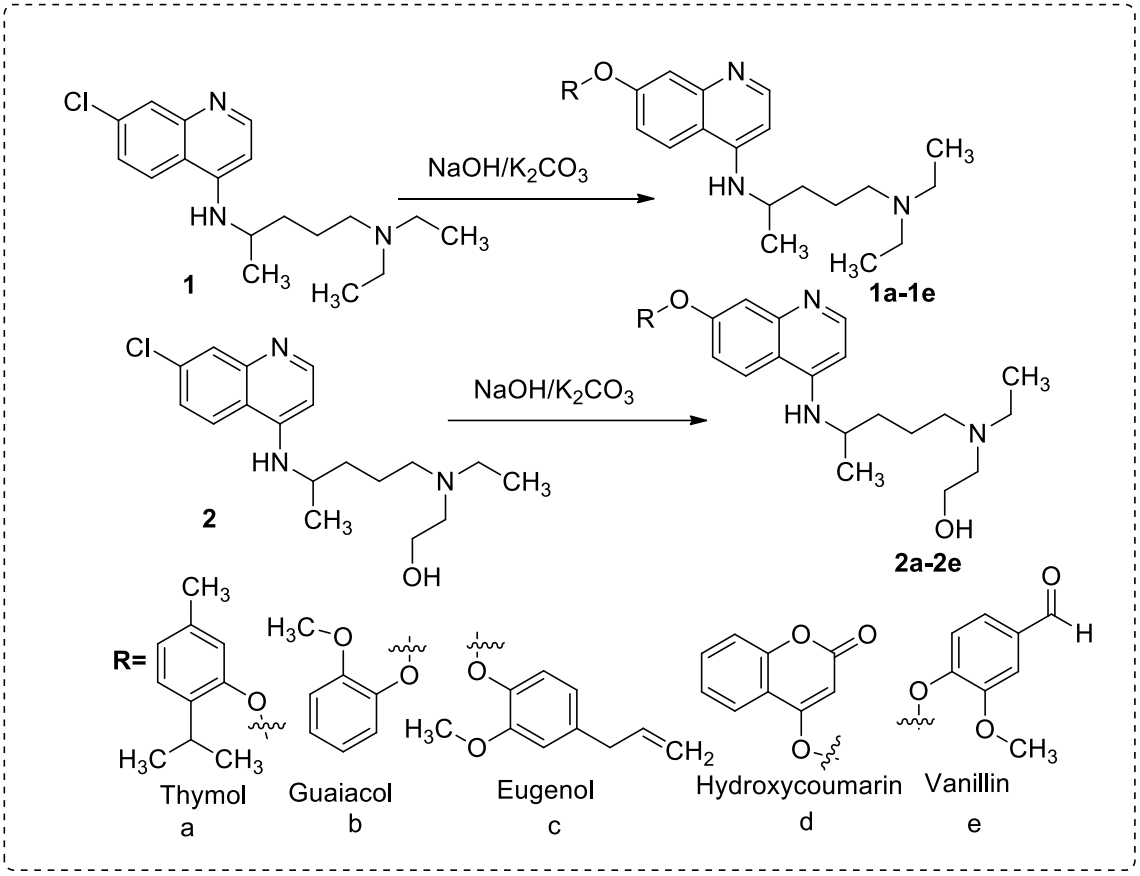
1. INTRODUCTION

Human Coronavirus-19 (COVID-19) has been an emerging infectious virus causing significant staggering respiratory morbidity for 8-10 days with eventual mortality, worldwide (Sun et al. 2020; Kannan et al. 2020); consequently, infection by COVID-19 remains as the serious most global health challenge today, from the account of ever increasing rather gargantuan global death rates. Without purported clinical experiences, currently evaluated for COVID-19 antiviral medicines are azithromycin, chloroquine (CQ**),** hydroxychloroquine(HCQ), corticosteroids**,** lopinavir, ritonavir and a few more (Phadke and Saunik 2020; Dhama et al. 2020). Those are protease inhibitors and certain nucleotide as inhibitors; however, WHO has not recommended particular drugs for this COVID-19. Since the medical fraternity is unable to contain the COVID-19 infection anywhere globally, due to its gigantic size harbouring host enzymes from the previous host before infecting a new, which have rendered COVID-19 as the most developed virus. Obviously, control measures have been taken in war footings, everywhere.

The Dearth of drug to control Corona bewilders clinicians and new drug(s) with novel molecular targets are desperately needed. There is no FDA approved antiviral to treat these infections. A few classes of antiviral compounds are being used, which are limited, and that might resist, soon. Recently, hydroxychloroquine being used as COVID-19 (Guastalegname and Vallone 2020). Though, it is not clinically proved for the viral agent.

Natural phytochemicals with structural phenolic system are readily help forming phenoxides by treating with potassium carbonate or sodium hydroxide. These phytochemical- phenoxides were reacted with repurposed antimalarial drug such as, chloroquine (CQ) and hydroxychloroquine (HCQ) for obtaining the desired congeners. During the reaction, the nucleophile phenoxide of phytochemical was readily substituted the chlorine atom of either CQ or HQC through .biomolecular nucleophilic substitution' reaction.

We purpose herewith, semi-synthesis approaches of chloroquine-phytochemical (CQP) hydroxychloroquine-phytochemical (HCQP) conjugates **(Scheme 1)** for developing active molecules against COVID-19. Individually, HCQ has hepatotoxic and its conjugate-derivative with phytochemical might reduce toxicity with increases of control-effectively.



Scheme 1. Synthetic route of CQP and HCQP derivatives

2. MATERIALS AND METHODS

2.1. Preparation of CQP and HCQP

Two series of CQC and HCQC derivatives were structurally drawn by ChemDraw Ultra12v., and ACD-Chemsketch and verified neatly with the principle of stereo-chemistry. Subsequently, 2D-structures were reinstated as 3D-structures saved as mol2 format. Furthermore, those formats were converted to PDB lay-out by PyMol-program (Baral et al. 2019).

2.2. Physicochemical parameter and ADMET

Lipinski rule of five (RO5) and Toxicity level with LD_{50} (lethal doses 50) were predicted through the previously described software. Consequently, ADME (absorption, distribution, metabolism, and excretion) prediction were performed by pre-ADME tool (Chita Ranjan Sahoo et al. 2019).

2.3. Molecular-docking

Optimized prepared ligands were taken further molecular docking studies. Simultaneously, the crystal structure of COVID-19 protein protease (PDB ID:6lu7) was retrieved from protein data bank and removal of heteroatoms and previously attached ligand. The docking study was carried out by AutoDock suite-4.0 with the addition of the grid parameter, Lamarckian genetic algorithm followed by energy minimization thoroughly monitored. After completion of docking, the post-docking analysis was carried out by Discovery studio v17 and LigPlot+ V.2.2 for protein-ligand-complex structure observation (Dehury, Behera, and Mahapatra 2017; Jin et al. 2020).

3. RESULTS AND DISCUSSION

3.1. Toxicity and drug-likeness

Natural phytochemical is being less toxic than synthetic drugs due to biosynthetically preparatory methods (C.R. Sahoo, Paidesetty, and Padhy 2019; Chita R. Sahoo, Paidesetty, and Padhy 2019). The phenolic or polyphenolic compounds are more potent anti-infective agent(s) and free radical scavengers due to liberation of H-bond with weak ionisation, which is forming as phenoxide by treated with strong bases. Thus, the phytochemical containing phenolic hydroxy moiety would produce phenoxide which is acting as nucleophile and further react with CQ and HQC. During the reaction, simple nucleophile phenoxide of phytochemical substituted chlorine atom of CQ and HQC by the principle of well-known Williamson ether via. SN2 reaction.

Both CQC HCQC derivatives were remarked as low toxicity at level-IV. The physicochemical derivative parameters are presented (**Table 1**). Furthermore, it was, violations of RO5 at cLogP value (<5) of the compounds **1a-1c** & **2a**, **2c** were observed. Moreover, compounds **1d-1e** have a topological polar surface area below 70Å. Compound **2e** has a significant drug-likeness score among all the derivatives. Indeed, compounds **2d-2e** were taken for further ADME assessments.

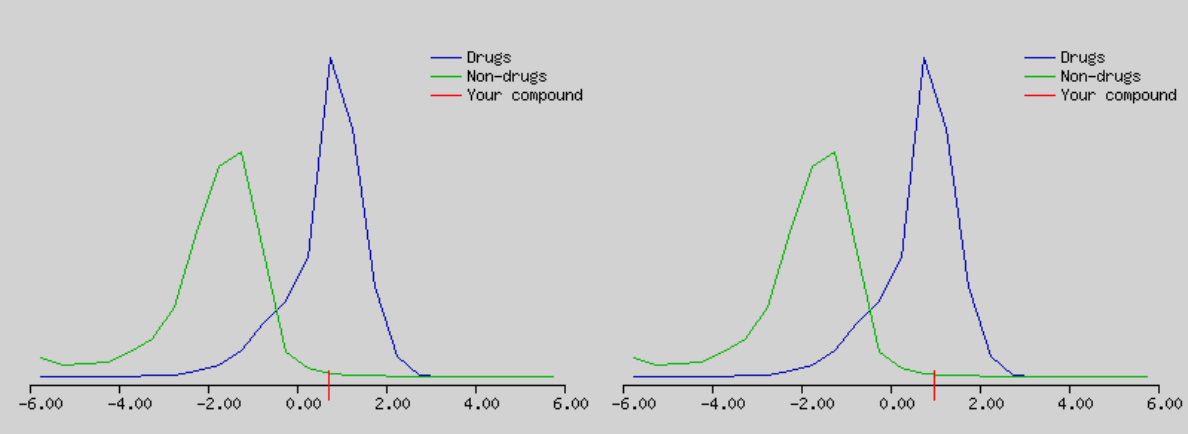


Fig 1. Graphically **v**isualization of drug-likeness scores of the compound 2d-left and 2e-right**.**

Table 1. Physicochemical properties of CQP and HCQP derivatives.

Name of Compound	Conjugation details	Lipinski-rule of five (RO5)					DL score	Toxicity	
		MW	H-ba	H-bd	cLogP	tPSA		LD_{50}	Level
1a	Chloroquine+ Thymol	433	3	1	7.19	37.39	1.16	557	IV
1b	Chloroquine+ Guaiacol	407	4	1	5.36	46.62	1.06	695	IV
1c	Chloroquine+ Eugenol	447	4	1	6.15	46.62	1.20	557	IV
1d	Chloroquine+ Coumarin	445	5	1	4.77	67.60	0.79	1000	IV
1e	Chloroquin + Vanillin	435	5	1	4.91	63.69	1.02	416	IV
2a	Hydroxychloroquine + Thymol	449	4	2	6.32	57.62	1.11	557	IV
2b	Hydroxychloroquine + Guaiacol	423	5	2	4.49	66.85	1.02	1040	IV
2c	Hydroxychloroquine + Eugenol	463	5	2	5.28	66.85	1.16	416	IV
2d	Hydroxychloroquine + Coumarin	461	6	2	**3.90**	87.83	0.73	1000	IV
2e	Hydroxychloroquine + Vanillin	451	6	2	**4.04**	83.92	0.98	416	IV

*MW-molecular weight, H-ba-hydrogen bond acceptor H-bd-hydrogen bond donor, cLogP-calculated logP, tPSA-total polar surface area, DL score- drug-likeness score, LD50- lethal doses (mg/kg).

3.2. Assessment of ADME

In ADME, the compounds **2d-2e** were validated by the previously described server (Sahoo et al. 2019). The compound **2d** has a 0.55 p-glycoprotein substrate, whereas **2e** have 0.89 **(Table 2)**. In this evidence compound, **2e** was taken further molecular docking. Coronavirus

has a single-stranded RNA, and structure covered by enveloped glycoprotein, which has played a key role in drug development cascaded (Prajapat et al. 2020). Moreover, pharmacokinetics would bring new opportunities to understanding the drug-membrane complexes with the active participation of both physical and chemical properties (Zheng et al. 2011).

Table 2. Predicted ADME parameters of potent CQP and HCQP derivatives.

Absorption	2d		2e	
Blood-brain-barrier	*BBB-*	0.85	*BBB-*	0.79
Human-intestinal absorption	*HIA+*	0.95	*HIA+*	0.95
p-Glycoprotein-substrate	*Substrate*	0.55	*Substrate*	0.89
Caco-2 permeability	*Caco2-*	0.63	*Caco2-*	0.55
p-Glycoprotein inhibitor	*Non-Inhibitor*	0.86	*Inhibitor*	0.89
Renal-organic cation-transporter	*Non-Inhibitor*	0.74	*Non-Inhibitor*	0.71
Distribution				
Subcellular-organization	*Lysosome*	0.59	*Mitochondria*	0.56
Metabolism				
CYP450 2C9-substrate	*Non-Substrate*	0.84	*Non-Substrate*	0.80
CYP450 2D6-substrate	*Non-Substrate*	0.83	*Non-Substrate*	0.73
CYP450 3A4-substrate	*Non-Substrate*	0.54	*Substrate*	0.65
Excretion & toxicity				
Human ether-related-gene	*Weak-Inhibitor*	0.78	*Weak-Inhibitor*	0.85
Carcinogenicity	*Non*	0.89	*Non*	0.90

3.3. Molecular docking

In these molecular docking studies, compound **2e** with protease protein was docked **(Fig 2)** and the binding interactions at amino acid residues at conventional H-bonding interactions Lys5, Leu287, and Leu271; hydrophobic interactions Glu288, Tyr239, Leu272, Tyr237, Asp289, Lys137, Glu290 **(Fig 3)**. It has been reported that protease could cleave the viral spike-glycoprotein and activate membrane-fusion (Qian, Dominguez, and Holmes 2013).

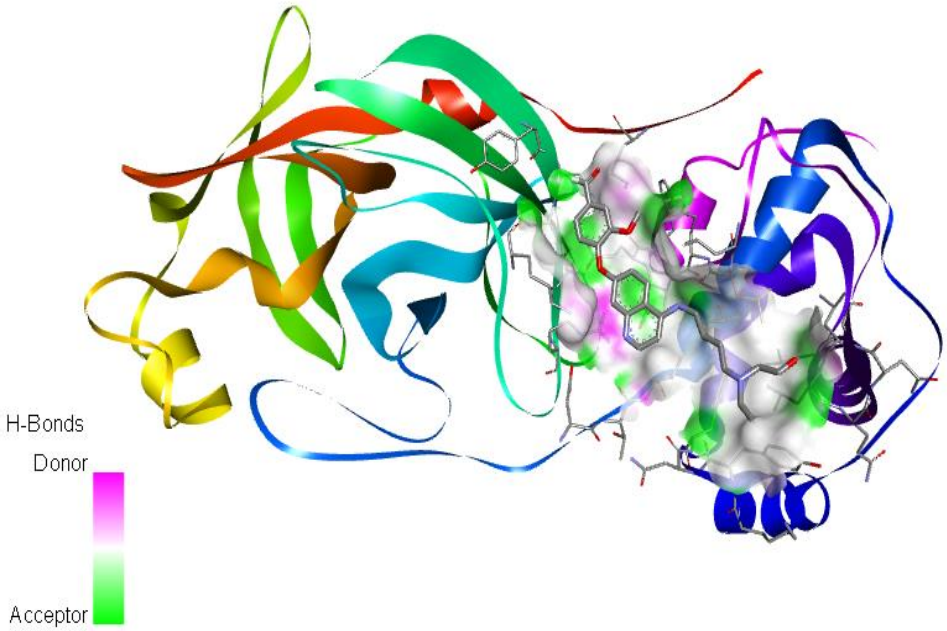


Fig 2. Human COVID-19 protease receptor PDB ID:6LU7 with compound 2e.

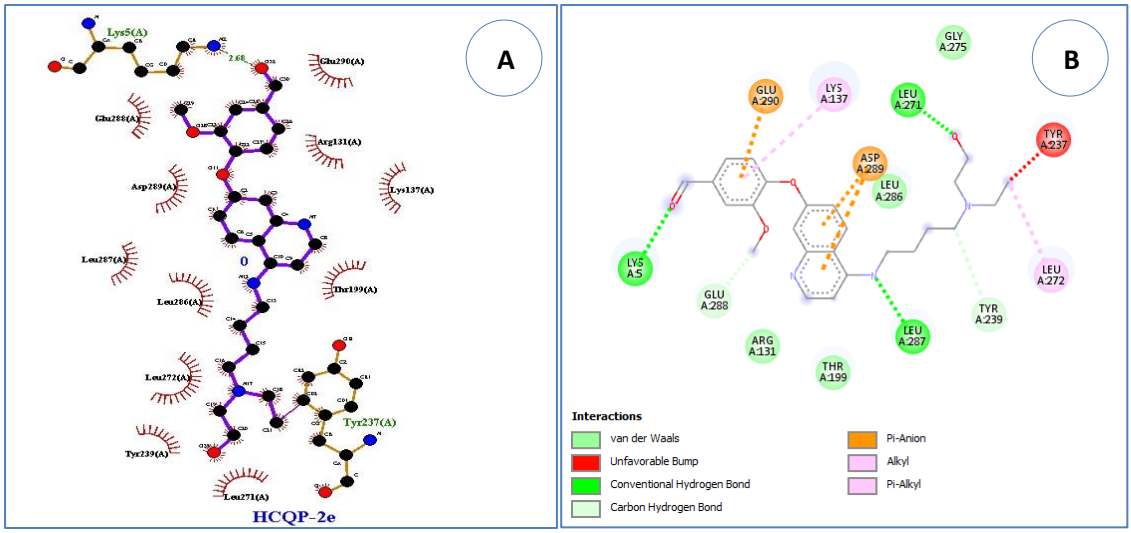


Fig 3. Binding interactions of HCQP-2e with protease receptor.

4. CONCLUSION

In this study, hydroxychloroquine with vanillin analogue would be obliged for the development of COVID-19 potential drug candidates. The HCQC derivatives of compound **2e** was used in this study, and this compound has passed RO5 and pharmacokinetic parameters, concomitant less toxicity. Moreover, molecular docking studies demonstrate flexible complex structure protease-hydroxychloroquine with vanillin conjugant. Thus, it would fasten up to develop novel therapeutic agent(s) through mainstream medicinal chemistry in this contemporary COVID pandemic condition.

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CONFLICT OF INTERESTS

Authors declare no conflict of interest

REFERENCES

Baral, N., S. Mohapatra, B.P. Raiguru, N.P. Mishra, P. Panda, S. Nayak, S.K. Pandey, P.S. Kumar, and C.R. Sahoo. 2019. "Microwave-Assisted Rapid and Efficient Synthesis of New Series of Chromene-Based 1,2,4-Oxadiazole Derivatives and Evaluation of Antibacterial Activity with Molecular Docking Investigation." *Journal of Heterocyclic Chemistry* 56 (2). https://doi.org/10.1002/jhet.3430.

Dehury, Budheswar, Santosh Kumar Behera, and Namita Mahapatra. 2017. "Structural Dynamics of Casein Kinase I (CKI) from Malarial Parasite Plasmodium Falciparum (Isolate 3D7): Insights from Theoretical Modelling and Molecular Simulations." *Journal of Molecular Graphics and Modelling* 71 (January): 154–66. https://doi.org/10.1016/J.JMGM.2016.11.012.

Dhama, Kuldeep, Khan Sharun, Ruchi Tiwari, Maryam Dadar, Yashpal Singh Malik, Karam Pal Singh, and Wanpen Chaicumpa. 2020. "COVID-19, an Emerging Coronavirus Infection: Advances and Prospects in Designing and Developing Vaccines, Immunotherapeutics, and Therapeutics." *Human Vaccines and Immunotherapeutics*. https://doi.org/10.1080/21645515.2020.1735227.

Guastalegname, Maurizio, and Alfredo Vallone. 2020. "Could Chloroquine /Hydroxychloroquine Be Harmful in Coronavirus Disease 2019 (COVID-19) Treatment?" *Clinical Infectious Diseases*. https://doi.org/10.1093/cid/ciaa321.

Jin, Zhenming, Xiaoyu Du, Yechun Xu, Yongqiang Deng, Meiqin Liu, Yao Zhao, Bing Zhang, et al. 2020. "Structure of Mpro from COVID-19 Virus and Discovery of Its Inhibitors." *BioRxiv*. https://doi.org/10.1101/2020.02.26.964882.

Kannan, S., P. Shaik Syed Ali, A. Sheeza, and K. Hemalatha. 2020. "COVID-19 (Novel

Coronavirus 2019) - Recent Trends." *European Review for Medical and Pharmacological Sciences*. https://doi.org/10.26355/eurrev_202002_20378.

Phadke, Mrudula, and Sujata Saunik. 2020. "COVID-19 Treatment by Repurposing Drugs until the Vaccine Is in Sight." *Drug Development Research*. https://doi.org/10.1002/ddr.21666.

Prajapat, Manisha, Phulen Sarma, Nishant Shekhar, Pramod Avti, Shweta Sinha, Hardeep Kaur, Subodh Kumar, et al. 2020. "Drug Targets for Corona Virus: A Systematic Review." *Indian Journal of Pharmacology*. https://doi.org/10.4103/ijp.IJP_115_20.

Qian, Zhaohui, Samuel R. Dominguez, and Kathryn V. Holmes. 2013. "Role of the Spike Glycoprotein of Human Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in Virus Entry and Syncytia Formation." *PLoS ONE*. https://doi.org/10.1371/journal.pone.0076469.

Sahoo, C.R., S.K. Paidesetty, and R.N. Padhy. 2019. "Norharmane as a Potential Chemical Entity for Development of Anticancer Drugs." *European Journal of Medicinal Chemistry*. https://doi.org/10.1016/j.ejmech.2018.11.024.

Sahoo, Chita R., Sudhir K. Paidesetty, and Rabindra N. Padhy. 2019. "Nornostocine Congeners as Potential Anticancer Drugs: An Overview." *Drug Development Research*. https://doi.org/10.1002/ddr.21577.

Sahoo, Chita Ranjan, Sudhir Kumar Paidesetty, Budheswar Dehury, and Rabindra Nath Padhy. 2019. "Molecular Dynamics and Computational Study of Mannich-Based Coumarin Derivatives: Potent Tyrosine Kinase Inhibitor." *Journal of Biomolecular Structure and Dynamics* 0 (0): 1–10. https://doi.org/10.1080/07391102.2019.1701554.

Sun, Pengfei, Xiaosheng Lu, Chao Xu, Wenjuan Sun, and Bo Pan. 2020. "Understanding of COVID-19 Based on Current Evidence." *Journal of Medical Virology*. https://doi.org/10.1002/jmv.25722.

Zheng, Nan, Hobart Ng Tsai, Xinyuan Zhang, and Gus R. Rosania. 2011. "The Subcellular Distribution of Small Molecules: From Pharmacokinetics to Synthetic Biology." *Molecular Pharmaceutics*. https://doi.org/10.1021/mp200092v.