ORIGINAL RESEARCH ARTICLES

DOCKING STUDY OF NOVEL N-SUBSTITUTED 2,5-BIS[(7-CHLOROQUINOLIN-4-YL)AMINO]PENTANOIC DERIVATIVES AS SELECTIVE HIGH-BINDER WITH ANGIOTENSIN CONVERTING ENZYME 2

Mohammed Oday Ezzat[a], Basma M. Abd Razik[b] and Kutayba F. Dawood[c]

(Received 01 April 2020) (Accepted 15 July 2020)

ABSTRACT

The prevalence of a novel coronavirus (2019-nCoV) in the last few months represents a serious threat as a world health emergency concern. Angiotensin-converting enzyme 2 (ACE2) is the host cellular receptor for the respiratory syndrome of coronavirus epidemic in 2019 (2019-nCoV). In this work, the active site of ACE2 is successfully located by Sitmap prediction tool and validated by different marketed drugs. To design and discover new medical countermeasure drugs, we evaluate a total of 184 molecules of 7-chloro-*N*-methylquinolin-4-amine derivatives for binding affinity inside the crystal structure of ACE2 located active site. A novel series of *N*-substituted 2,5-bis[(7-chloroquinolin-4-yl)amino]pentanoic acid derivatives is generated and evaluated for a prospect as a lead compound for (2019-nCoV) medication with a docking score range of (-10.60 to -8.99) kcal/mol for the highest twenty derivatives. Moreover, the ADME pharmaceutical properties were evaluated for further proposed experimental evaluation *in vitro* or *in vivo*.

Keywords: Molecular docking, Drug Design, Scaffold hopping.

INTRODUCTION

Recently, the prevalence of novel coronavirus 2019-nCoV in China and its spread all across the world has caused several sickness syndromes such as severe respiratory illness with fever and pneumonia leading in many cases to death[1]. According to the reports of the World Health Organization (WHO), there has been more than 40 mn confirmed infected cases globally reported leading to more than 1.1 mn deaths. The pathogen studies already characterize this virus as a new member of betacoronavirus genus close to several bat coronaviruses[2]. The recent scientific reports demonstrated the similarity of new 2019-nCoV S with SARS-CoV S by hosting at the same functional cell receptor and angiotensin-converting enzyme 2 (ACE2)[3]. These findings prompted us to generate leads to find a candidate for this interaction site, especially after the novel reporting of ACE2 bounding to the 2019-nCoV S with about 15 nM affinity which is around ~10- to 20-fold higher affinity more than ACE2 binding to SARS-CoV S and the confirmation of complex formation of ACE2 to the 2019-nCoV S by negative-stain EM and cryo-EM at a high resolution[4]. The high binding affinity of 2019-nCoV S with human ACE2 refers apparently to the ability of 2019-nCoV to spread between humans and the requirement for additional studies to study this possibility for prevention. Depending on the target, there are two main categories to discover effective anti-coronavirus therapies: one is targeting the immune system or cells of humans, while the other is acting on the coronavirus itself. In the targeting of the human immune system, innately its response plays an effective role in administration of the replication process and infection syndromes of coronavirus, and as generally expected the interferon will enhance the immune response action. Moreover, in human cells signal pathways are important for virus replication, and blocking these pathways will show an effective anti-viral action[5]. From previous work, it is already reported that most viruses are bound to the receptor proteins on cell surface to enter human cells. According to this fact, SARS virus also binds to the angiotensin-converting enzyme 2 (ACE2) receptor[6].

[a] Department of Chemistry, College of Education for Women, University of Anbar, 31001, Ramadi, Anbar, Iraq
[b] Department of Pharmaceutical Chemistry, College of Pharmacy, Mustansiriyah University, 10001, Baghdad, Iraq
[c] Department of Chemistry, College of Education for Pure Sciences, University of Anbar, 31001, Ramadi, Anbar, Iraq
* For Correspondence E-mail:edw.mohamed_oday@uoanbar.edu.iq; mohamed_oday@yahoo.com

The second therapeutic strategy is based on the action of coronavirus itself by blocking the synthesis pathway of viral RNA through genetic material and inhibition of virus proliferation. This step is effected by blocking the virus binding ability to human cell receptors or the inhibition of the self-assembly process of the virus by working on structural proteins[7]. In the process to fight coronavirus, researchers have found three main ways of designing and developing new drugs. The first strategy is based on testing several broad-spectrum available anti-virals such as ribavirin, cyclophilin, and interferons which are already being used to treat and inhibit coronavirus pneumonia[8]. There are several advantages of therapies under this strategy because of the clearly approval of efficacy, metabolism, dosages, and side effects for the treatment of viral infections. But also, there are several disadvantages of this strategy as its therapies cannot kill the viruses in a targeted stage and they are too "broad-spectrum" with side effects. The second strategy is based on screening available molecule databases that may have potential therapeutic activity against the coronavirus. This strategy required a high-throughput screening procedure leading to the discovery of various new drugs, for example, the design and discovery of lopinavir/ritonavir drug as anti-HIV drug. Finally, the third strategy is based on the availability of pathological and genomic information of various coronaviruses to generate novel targeted drugs. Hypothetically, the new drugs found within these therapeutic strategy would exhibit higher anti-coronavirus activity, but the researcher's plan to obtain most of the new drugs might need more than 10 years [9]. QSAR and drug design modelling is the fastest way for the development of new medicines in the plan fo treat coronvirus by discovering possible molecules from the drug database. Once the efficacy is evaluated by bioinformatics analysis, it can further be confirmed by systematic experimental and clinical procedures and methods.

In this work, a novel series of 2,5-bis[(7-chloroquinolin-4-yl)amino]pentanoic acid derivatives are molecularly designed and computationally pharmacological evaluated with for binding at ACE2 active site by docking score. The final result is a promising novel series of high binding and potent active molecules that can be used further as medical drugs against 2019-nCoV ability to bind with ACE2 enzyme better than currently available drugs. Moreover, the ADME pharmaceutical properties of all novel molecules were evaluated by using the QikProp prediction tool for further proposal experimentally evaluation *in vitro* or *in vivo*.

METHODOLOGY COMPUTATIONAL METHOD

Among four recently reported crystal structures, 6M17, 6M18 and 6VW1 of Entity 2 containing Chain, the best resolution structure of 6M1D was selected in this study to propose docking and virtual screening of derivatives. Enzyme preparation process was performed by protein preparation tool wizard (ProPrep) under Maestro v12.2 (Schrödinger LLC, 2017) with preparation prior for docking by energy minimization using Optimized Potential for Liquid Simulations (OPLS_2005) method by adding hydrogen atoms, sequencing of bond order, corrections of charges, removing atomic clashes, statues adjustment of tautomerization and ionization of enzyme crystal. Highest proposed active sites were located by using the Sitemap tool under Schrödinger software at normal mode on the obtained crystal structure. After grid generation, the active site box was saved in maegz file format for future procedures.

For ligands preparation, a total of 184 molecules of 2,5-bis[(7-chloroquinolin-4-yl)amino]pentanoic acid derivatives were collected from previously reported literatures and the 3D structures of all derivatives were obtained by using ChemDraw, 18.0 from ChemOffice software (ChemOffice, 2018). For geometry optimization, the MM+ energy force field optimization was applied individually for each derivative and saved as .mol file format in the Hyperchem program ver. 8.0. After this step, an additional energy optimization by semi-empirical calculation process from RM1 (Recife Model 1) tool was performed by using Spartan 14.0 (Spartan, 2014) with selecting Monte Carlo calculation (100 optimization cycles and 1000 interactions) and the best low 3D structure optimized file of each derivative was saved as mol2 file format. QSAR Molecular design and docking study were evaluated applied by using the Glide tool under Maestro v12.2 (Schrödinger, 2017). Docking study was adjusted with flexibly of ligand option by selecting Glide-extra (XP) simulation precision during docking process and the enzyme active site was kept as rigid and all RMSD data was saved. Finally, scaffold generation and chemical group replacement were applied by generating different derivatives with deferent N- substituted chemical groups by applying the R-Group generation method under Maestro and all was saved for further use. All these computational process (preparation, energy minimization, molecular modelling, and docking study) were performed on Dell Precision PC T-1578 workstation model supported with Intel (R) Core (TM) i7 processor, 3.60GHz, 32 GB RAM, 1 TB of HD under Windows 7 system Service Pack 1.

Results and Discussion

The propagation of a novel coronavirus (2019-nCoV) in the last months has become a worldly pandemic threat a crossing many countries causing. Nowadays, the reporting of the prefusion Cryo-Em conformation structure for (2019-nCoV) spike and the cloning of ACE2-B0AT1 complex crystal structure in open conformation open a promising way for design and modelling of promising hit drugs for prevention or treatment[10]. Moreover, early in March 2020, Renhong Yan & *et al* reported the interaction site position of (2019-nCoV) with ACE2 by locating the receptor-binding domain (RBD) of glycoprotein (S protein) at the extracellular peptidase domain (PD) of ACE2 enzyme[11]. Because it is recently cloned, the obtained ACE2 crystal has been deposited without any inhibitor ligand and could be useful to locate the active site. To solve this problem, the site map tool under Schrödinger software is applied to evaluate the promising active site positions of ACE2 crystal. This tool locates five proposed sites that could be very promising for ligand interaction between drug and enzyme, leading to causing inhibition or prevent the binding ability of virus with the enzyme. One of these five sites is the same which has been already proposed by Renhong and his group (Fig. 1).

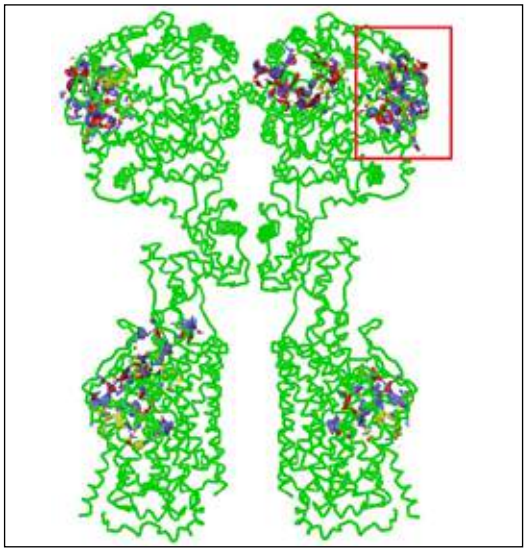


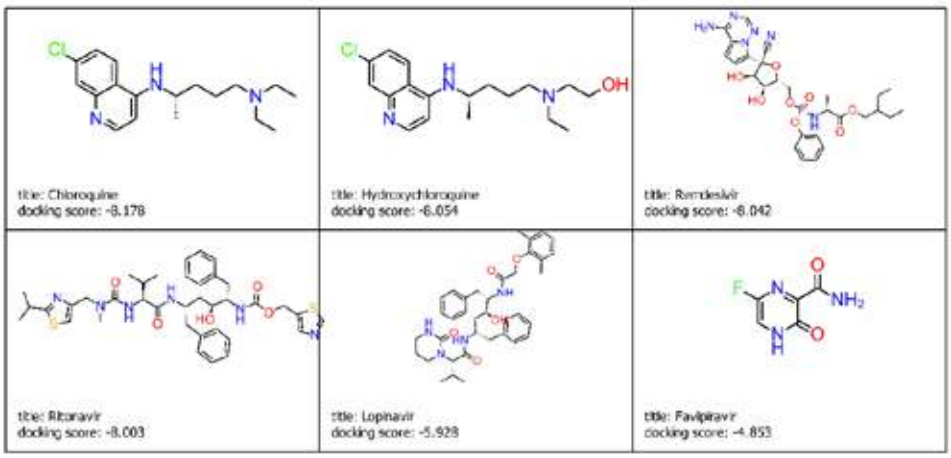
Fig. 1: ACE2 crystal structure (green wire) with predicted active sites (colored contours). The selected active site is in the red box

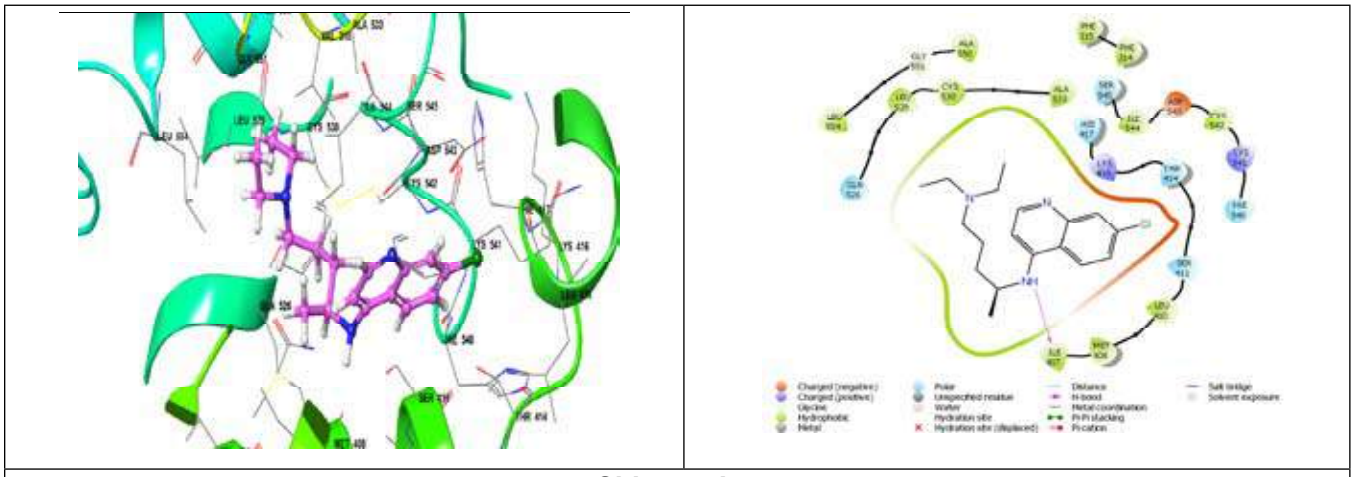
Next, this site was booked as an active site to dock and predict a list of compounds to design a new series of the ligand which could be used as a hit drug for further study. To evaluate this active site and molecular design of new compounds, a docking study was performed for a list of available antiviral drugs, and all binding scores are listed in Table I.

Among all selected drugs, chloroquine and hydroxychloroquine were the most active with higher binding docking score at (-8.178 & -8.054 k/mol) as already reported to use these drugs (under brand name Plaquenil and Chloroquine FNA) for successful treatment to combat the disease[12]. But even though they are reported as active drugs, many side effects appeared on several patients after long term usage. Moreover, these two compounds surrounded by a list of amino acids were selected to refer as active binding site (Fig. 2).

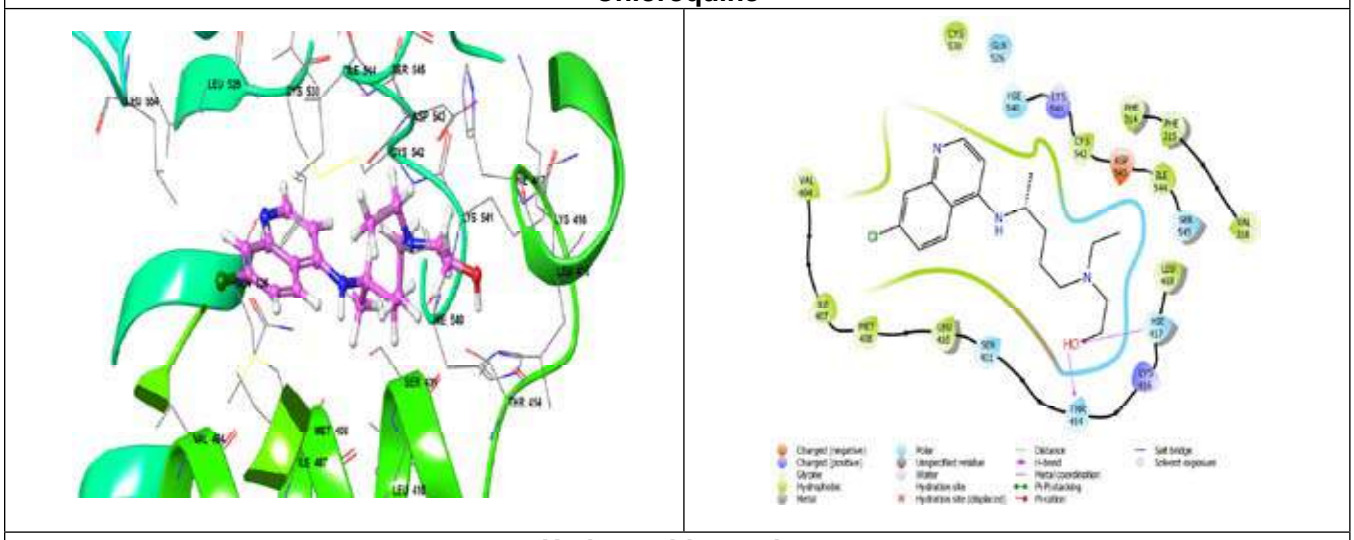
Inside ACE2 pocket, chloroquine bound by H-bond interaction appears between ILE407 and the hydrogen atom of amine group surrounded by several amino acids as following: MET408, LEU410, SER411, THR414, LYS416, HIE417, ALA533, CYS530, LEU529, GLN526, HIE540, LYS541, CYS542, ASP543, ILE544, SER545, PHE314, PHE315, ALA550, GLY551, and LEU554. In same

Table I: List of docking binding score and chemical structure of available antiviral drugs





Chloroquine



Hydroxychloroquine

Fig. 2: Chloroquine and hydroxychloroquine in 3D and 2D structures surrounded by amino acids inside ACE2 active site pocket with interactions

ACE2 pocket, hydroxychloroquine binds by two H-bond interactions: one appears between HIE417 and oxygen and while the second between THR414 and hydrogen atom of hydroxyl group surrounded by several following amino acids: VAL404, ILE407, MET408, LEU410, SER411, THR414, LYS416, HIE417, LEU418, SER545, ILE544, ASP543, CYS542, LYS541, HIE540, GLN526, CYS530, VAL318, PHE315, and PHE314.

By selecting 7-chloro-*N*-methylquinolin-4-amine as hit scaffold for drug design, a list of chloro-*N*-methylquinoline derivates were collected (total of 184) and docked inside active site and listed in Table II (the most active 20 compounds). This step located compound 2,5-bis[(7-chloroquinolin-4-yl)amino]pentanoic acid as the highest active compound with docking score at 9.838 k/mol.

To define the preferable replacement chemical group,

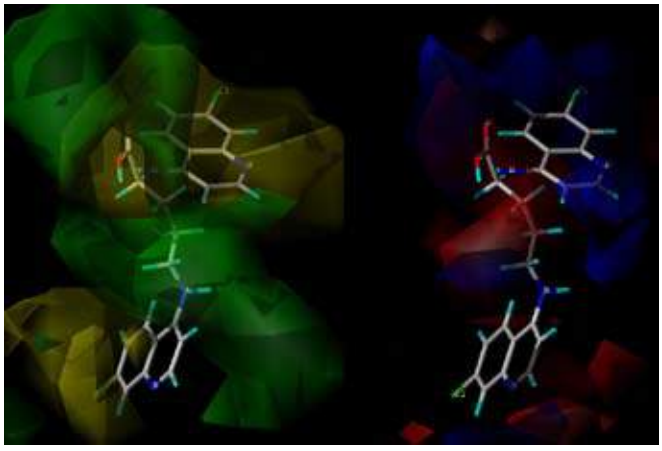
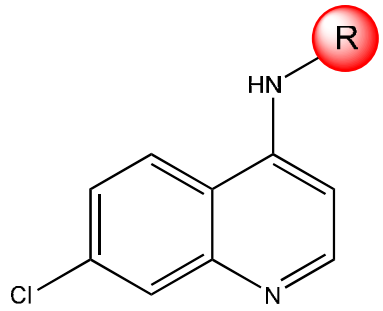
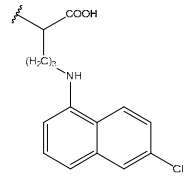
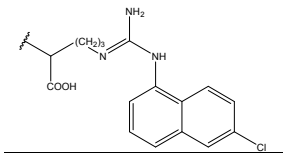
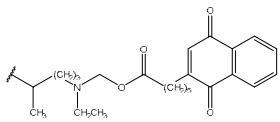
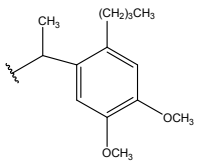
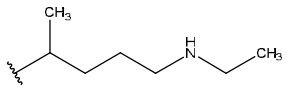
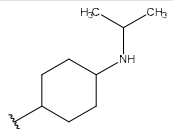
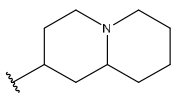
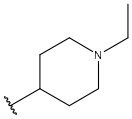
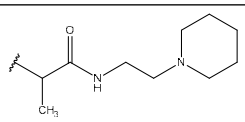
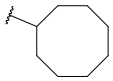
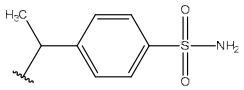
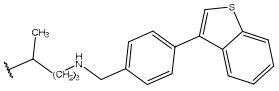
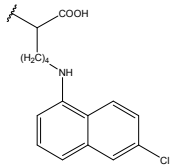
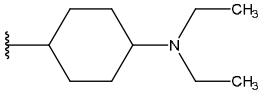
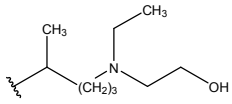
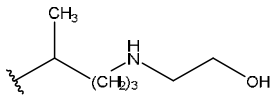
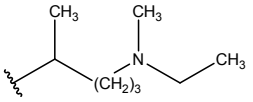
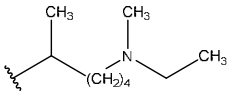
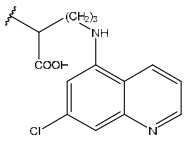
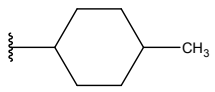


Fig 3. The highest lead active compound 2,5-bis [(7-chloroquinolin-4-yl)amino]pentanoic acid surrounded by contours as desirable and undesirable regions after molecular modelling process

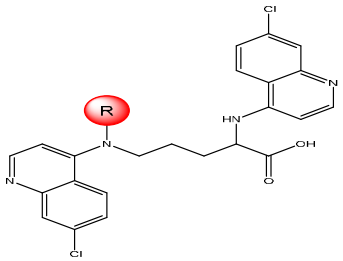
Table II: The structure and docking score of the most active 20 chloro-N-methylquinolin derivates

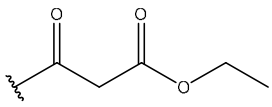
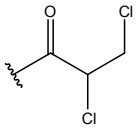
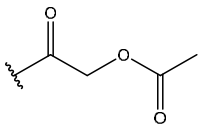
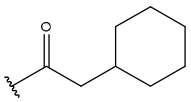
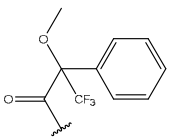
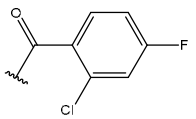
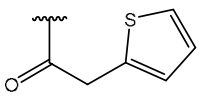
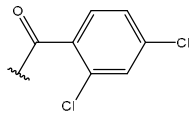
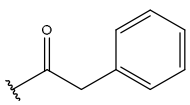
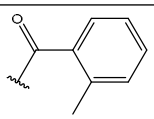
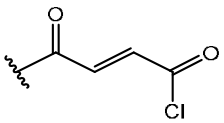
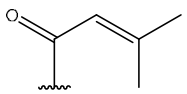
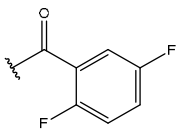
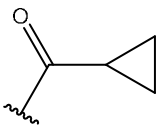
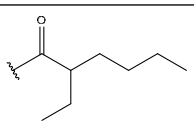
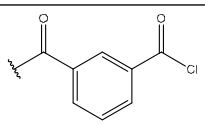
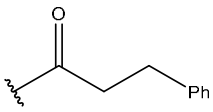
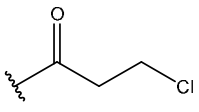
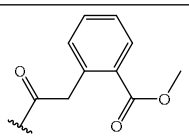
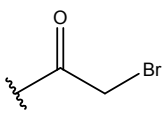


Compound	R	Docking Score in kcal/mol
1		-9.838
2		-9.710
3		-9.414
4		-9.275
5		-9.174
6		-9.166
7		-8.980
8		-8.771
9		-8.594
10		-8.519
11		-8.485
12		-8.388
13		-8.241
14		-8.182
15		-8.154
16		-8.057
17		-8.048
18		-7.981
19		-7.865
20		-7.819

molecular modelling with application of QSAR evaluation study is performed and an active pharmacophore is generated with a highly preferred area (in green) for promising replacement position to generate higher active compound (Fig. 3). Here, the heigher selected lead compound is surrounded by desirable and undesirable contours sequentially coloured in green and yellow. The colouring concept of contours reigns is based on the yellow contours region for steric bulk undesirable, green contours region for steric bulk desirable positions, blue contours region for positive charge positions, finally red contours region for a negative charge.

Table III: The structure of novel N-substituted 2,5-bis[(7-chloroquinolin-4-yl)amino]pentanoic acid derivatives with docking score



Compound	R	Docking Score in kcal/mol	Compound	R	Docking Score in kcal/mol
N1		-10.603	N11		-9.158
N2		-10.537	N12		-9.129
N3		-10.126	N13		-9.128
N4		-9.908	N14		-9.124
N5		-9.861	N15		-9.042
N6		-9.650	N16		-9.016
N7		-9.485	N17		-9.014
N8		-9.453	N18		-8.998
N9		-9.388	N19		-8.995
N10		-9.382	N20		-8.990

QSAR model study helped to locate and select the effective position for chemical group replacement based on the replacement of chemical group under green regions to increase the pharmacological potency by increasing binding docking score leading to increased compound activity. By applying replacement at selected nitrogen atom (N-substitution) with a list of chemical groups of fragments, a new series of compounds are listed with high binding affinity and docking score listed in (Table III)

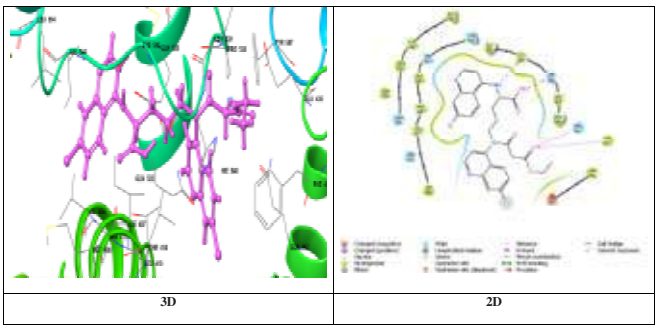


Fig 4. Compound N1 in 3D & 2D structure surrounded by active site amino acids inside ACE2 crystal pocket interactions

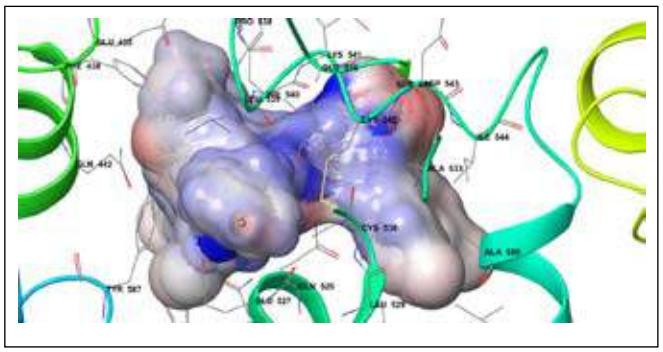


Fig 5. The potential electrostatic contour of all derivatives aligned in the same orientation inside the ACE2 active site

(The most active 20 compounds). Moreover, the highest generated compound is surrounded by a list of active site amino acids referring to the superimposition of this compound inside active site (Fig. 4).

Inside ACE2 active site, compound N1 bound by four interactions gives the high docking affinity score at -10.60 kcal/mol. Two interactions occur between hydrogens atoms of hydroxyl and amine groups with HIE540 and CYS540, while tho other two occur between the oxygen of ketone group with GLN442 and TYR587. The rest of active site amino acids are surrounding compound N1 as follows: VAL404, ILE407, MET408, LEU410, SER411, THR414, ILE544, CYS542, HIE540, LEU539, PRO538, GLN442, TYR587, PHE438, GLU435, GLN526, LEU529, CYS530, ALA533, and LEU554.

The prediction application of ADME (absorption, distribution, metabolism, and excretion) pharmacological properties is highly desired to clarify the procedure of lead drug optimization and development to improve the final discovered drug properties. In order to predict the ADME values, the QikProp tool under Schrödinger software is

Table III: Recommended values of ADMET prediction parameters

Property or Descriptor	Description	Range or recomm ended values
CNS	Predicted central nervous system activity.	–2 (inactive), +2 (active)
QPlogPw	Predicted water/gas partition coefficient.	4.0 to 45.0
QPlogPo/w	Predicted octanol/ water partition coefficient.	–2.0 to 6.5
QPlogS	Predicted aqueous solubility, log S. S in mol dm^{-3}.	–6.5 to 0.5
ClQPlogS	Conformation-independent predicted aqueous solubility.	–6.5 to 0.5
QPlogHERG	The predicted IC_{50} value for the blockage of HERG K^+ channels.	concern below –5
QPPCaco	Predicted apparent Caco-2 cell permeability in nm/sec.	<25 poor, >500 great
QPlogBB	Predicted brain/blood partition coefficient.	–3.0 to 1.2
QPlogKhsa	Prediction of binding to human serum albumin.	–1.5 to 1.5
Human Oral Absorption	Predicted qualitative human oral absorption.	1, 2, or 3 for low, medium, or high.
Percent Human Oral Absorption	Predicted human oral absorption on 0 to 100% scale.	>80% is high <25% is poor
Rule of Five	A number of violations of Lipinski's rule of five.	maximum is 4

Table IV: ADME predicted values of the highest active twenty novels *N*-substituted 2,5-bis [(7-chloroquinolin-4-yl)amino]pentanoic acid derivatives

Entry ID	CNS	QP log Pw	QP log Po/w	QP logS	CIQP logS	QP log HERG	QP Pcaco	QP log BB	QP log Khsa	Human Oral Absorption	Percent of Human Oral Absorption	Rule Of Five
N 1	-2	15.986	5.192	-7.65	-7.924	-5.349	21.583	-2.341	0.403	1	55.31	2
N 2	-2	16.293	4.674	-7.167	-7.641	-5.19	14.231	-2.42	0.286	1	61.996	1
N 3	-2	14.274	7.685	-9.01	-11.182	-5.496	104.897	-1.195	1.125	1	82.194	2
N 4	-2	14.628	6.449	-7.994	-9.234	-5.681	84.903	-1.426	0.768	1	73.312	2
N 5	-2	14.615	6.368	-7.773	-9.171	-5.537	77.333	-1.457	0.764	1	72.115	2
N 6	-2	16.206	5.147	-7.629	-7.972	-5.44	17.635	-2.259	0.283	1	53.474	2
N 7	-2	14.854	6.881	-8.726	-9.62	-5.821	105.322	-1.116	0.846	1	77.52	2
N 8	-2	13.347	6.978	-8.605	-8.976	-5.36	67.639	-1.83	0.965	1	74.641	2
N 9	-2	14.578	6.864	-8.158	-9.458	-5.735	87.001	-1.488	0.906	1	75.935	2
N 10	-2	17.065	6.007	-8.156	-9.256	-5.882	32.874	-2.003	0.669	1	63.349	2
N 11	-2	14.319	6.739	-8.872	-8.951	-5.508	92.216	-1.163	0.809	1	75.652	2
N 12	-2	14.034	6.929	-9.119	-8.906	-5.455	70.972	-1.646	1.061	1	74.73	2
N 13	-2	14.584	6.881	-8.701	-9.957	-5.441	80.886	-1.165	0.876	1	75.464	2
N 14	-2	14.775	7.128	-9.053	-10.294	-5.661	84.247	-1.158	0.95	1	77.23	2
N 15	-2	14.742	6.541	-8.15	-9.171	-5.532	87.281	-1.35	0.868	1	74.068	2
N 16	-2	14.095	6.142	-8.09	-8.079	-5.243	83.879	-1.418	0.747	1	71.423	2
N 17	-2	14.276	5.843	-7.98	-7.601	-5.509	75.936	-1.517	0.618	1	68.897	2
N 18	-2	17.162	5.963	-8.696	-9.346	-6.088	20.839	-2.204	0.603	1	59.548	2
N 19	-2	13.891	5.847	-7.493	-8.25	-4.884	84.017	-1.225	0.563	1	69.709	2
N 20	-2	13.923	5.526	-7.214	-8.869	-4.87	68.614	-1.304	0.444	1	66.255	2

used by structural evaluation of the highest generated derivatives, and various recommended parameter values are listed in Table III. As a new series of promising drugs, the newly generated series is evaluated for toxicology prediction study. The final result is a list of values with safely recommended ranges and this could light the way for referring to a perfect agreement of pharmaceutically properties and the synthesis of new series of (2019-nCoV) drugs which can be further clinically evaluated.

Moreover, the alignment of the highest active novel derivatives inside ACE2 enzyme active site is in the same position and orientation and orderliness by same N-substituted chemical groups which approve the favorability of this substitution at selected N-amine to the highest active compound in traying data set as shown in Fig. 5 with potential electrostatic contour.

CONCLUSION

Scaffold hopping application by selected chemical group replacement is one of the best applicable approaches used in molecular drug design and discovery to improve medication potency and binding affinity inside active site. In this work, the active site of the ACE2 enzyme was successfully located and approved by the docking affinity of several anti-viral drugs. The obtained active site was applied for virtual screening of 2,5-bis[(7-chloroquinolin-4-yl)amino]pentanoic acid derivatives and generated QSAR pharmacophore to approve the ability of N position substitution to increase binding affinity. A series of novel *N*-substituted 2,5-bis[(7-chloroquinolin-4-yl)amino]pentanoic acid derivatives were generated and theoretically evaluated to predict the potential and pharmacological activity and ADME properties. The final findings confirm the ability of the novel series for

prospect and application as lead compounds for Covid-19 medication.

REFERENCES

1. Hui DS, I Azhar E, Madani TA, Ntoumi F, Kock R, Dar O, et al. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health — The latest 2019 novel coronavirus outbreak in Wuhan, **China. Int J Infect Dis.** 2020, 91, 264–6.
2. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. **New Engl. J. Med.** 2020, 382(8), 727–33.
3. Hoffmann M, Kleine-Weber H, Krüger N, Müller M, Drosten C, Pöhlmann S. The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. **bioRxiv.** 2020, 929042.
4. Coutard B, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. **Antiviral Res.** 2020, 176, 104742.
5. Omrani AS, Saad MM, Baig K, Bahloul A, Abdul-Matin M, Alaidaroos AY, et al. Ribavirin and interferon alfa-2a for severe Middle East respiratory syndrome coronavirus infection: A retrospective cohort study. **Lancet Infect Dis** [Internet]. 2014, 14(11), 1090–1095.
6. Ge XY, Li JL, Yang X Lou, Chmura AA, Zhu G, Epstein JH, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. **Nature**. 2013, 503(7477), 535–538.
7. Jin YH, Cai L, Cheng ZS, Cheng H, Deng T, Fan YP, et al. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia. **Mil Med Res.** 2020, 7(1), 1–23.
8. Ung COL. Community pharmacist in public health emergencies: Quick to action against the coronavirus 2019-nCoV outbreak. **Res. Social and Admin. Pharma.** Elsevier Inc.; 2020, 16, 583–586.
9. Robson B. Computers and viral diseases. Preliminary bioinformatics studies on the design of a synthetic vaccine and a preventative peptidomimetic antagonist against the SARS-CoV-2 (2019-nCoV, COVID-19) coronavirus. **Comput Biol Med.** 2020, 119, 103670.
10. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh C-L, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. **Science**. 2020, 367(6483), 1260–1263.
11. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of the SARS-CoV-2 by full-length human ACE2. **Science.** 367(6485), 1444-1448.
12. Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. **Cell Discov.** 2020, 6(1), 1–4.