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Original article

Multi-targeted molecular docking, pharmacokinetics, and drug-likeness evaluation of coumarin based compounds targeting proteins involved in development of COVID-19



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ABSTRACT

COVID-19 is a progressing pandemic of coronavirus disease-2019, which had drowned the whole world in a deep sorrow sea. Uncountable deaths were extending the list of deaths every single day. The present research was aimed to study the multi-target interaction of coumarins against COVID-19 using molecular docking analysis. The structure of coumarin compounds was checked for ADME and Lipinski rule of five by using SwissADME, an online tool. SARS-CoV-2 proteins such as RdRp, PLpro, Mpro and spike protein were collected from the Protein Data Bank. The molecular docking study was performed in the PyRx tool, and the molecular interactions were visualised by Discovery Studio Visualizer. All the coumarin compounds used in the study were obeyed Lipinski's rule of 5 without any violations. All the three designed derivatives of phenprocoumon, hymecromone, and psoralen were showed high binding affinity and prominent interactions with the drug target. The presence of -OH groups in the compound, His41, a catalytic dyad in Mpro, number of and the distance of hydrogen bond interactions with SARS-CoV-2 targets was accountable for the high binding attractions. The modified drug structures possess better binding efficacy towards at least three targets compared to their parent compounds. Further, molecular dynamic studies can be suggested to find the ligand-protein complex stability. The present study outcome reveals that the designed coumarins can be synthesised and examined as a potent inhibitory drug of SARS-CoV-2. © 2022 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

COVID-19 is a progressing pandemic of coronavirus disease-2019, which had drowned the whole world in a deep sorrow sea. This virus is now known as the novel coronavirus because it is caused by a previously unknown virus of crown shape. Coronaviruses are broad, single-stranded ribonucleic acid (RNA) viruses with a diameter of around 125 nm, which is enclosed by a capsid helical symmetry protein or envelope structured a protein shell

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named as nucleocapsid, and then surrounded by a bulbous bilayer lipid surface which contains various structural proteins (spike, envelope, and membrane) (Prajapat et al., 2020; De Groot et al., 2013; Jahan and Onay, 2020).

After SARS-CoV-2 invasion, there is a hyperinflammatory response by the immune system of the host leading to acute respiratory distress and multiple organ failure. COVID-19 hyperinflammatory response are caused by the elevated levels of proinflammatory factors cytokines and interleukin (Maiti et al., 2020; Tang et al., 2020). It is important to block or reduce the inflammatory action caused by COVID-19 to improve the patient's health and reduce the casualty.

According to a research report in February 2020, there are no specific anti-viral drugs available to treat this SARS-CoV-2. The only choice to treat this disease is by utilising antiviral medications until a specific drug will be discovered. Furthermore, broad-spectrum antiviral drugs like remdesivir have been stated in the report that they are highly effective in managing and controlling

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SARS-CoV-2 infection. Moreover, other clinical candidates like the EIDD-2801 compound, which is still under development (Zhou et al., 2020). We can consider antiviral therapy, which includes broad-spectrum antiviral drugs like lopinavir or ritonavir, neuraminidase inhibitors, modified OC43-HR2P peptide (EK1) RNA synthesis inhibitors till the world come up with a solution to this disease by creating a specific treatment against this virus. Table 1 shows the list of drugs evaluated for their effect on coronavirus infection (Liu et al., 2020).

Besides, research and development on small molecules show that the drug acting on SARS-CoV and MERS-CoV can be considered as a drug of choice in the treatment SARS-CoV-2. Most vitally, these drugs inhibit proteases and RdRp, thereby reducing the virus infectivity (Lu et al., 2020). The approved vaccine like Pfizer is currently based on a genetic molecule called messenger RNA (mRNA) (Polack et al., 2020). Furthermore, blood plasma protein treatment, also known as a convalescent plasma treatment, is available to treat severely infected patients with COVID-19 (Li et al., 2020).

The consequences of the SARS-CoV-2 pandemic are severe on global health as well as on the global economy. There is a need to develop new drugs or drugs to cure the COVID-19 since there are no specific drugs available to treating COVID-19. So, antiviral, anti-malarial, and herbal medicines have been alternative options for the treatment of COVID-19 (Tobaiqy et al., 2020). Coumarin has various pharmacological activities, including antiviral anti-inflammatory and antimicrobial activity. Thus, this research focuses on the interaction mechanism of coumarin derivatives with COVID-19; as more discoveries about coumarin are uncovered, much research is still going on.

Even though few studies (Ait-Ramdane-Terbouche et al., 2020; Chidambaram et al., 2020; Koparir, 2020; Kumar et al., 2020; Lyndem et al., 2020; Maurya and Mishra, 2021; Milenković et al., 2020; Mishra et al., 2020; M Özdemir et al., 2020; Mücahit Özdemir et al., 2020; Yañez et al., 2020) have been reported on coumarin analogues interactions against SARS-CoV2, and reported that the compounds are showing good binding affinity, there are other coumarin derivatives that are not studied and there is no much multitargeted studies on coumarines which can help to find a single compound to interact with the multiple targets which are responsible for the entry into human, replication and virulence of SARS-CoV2. Moreover, the coumarin derivatives are reported for

 Table 1

 Drug candidates that reportedly act on the corresponding targets in similar viruses.

Target candidate	Full name	Role during viral infection	Drug candidate
3CLpro	coronavirus main protease 3CLpro	A protease for the proteolysis of viral polyprotein into functional units	Lopinavir
PLpro	papain-like protease PLpro	A protease for the proteolysis of viral polyprotein into functional units	Lopinavir
RdRp	RNA-dependent RNA polymerase	An RNA-dependent RNA polymerase for replicating viral genome	Remdesivir, ribavirin, favipiravir
S protein	viral spike glycoprotein	a viral surface protein for binding to host cell receptor ACE2	Arbidol
TMPRSS2	transmembrane protease, serine 2	A host cell-produced protease that primes S protein to facilitate its binding to ACE2	Camostat mesylate
ACE2	angiotensin- converting enzyme 2	A viral receptor protein on the host cells which binds to viral S protein	Arbidol
AT2	angiotensin AT2 receptor	An important effector involved in the regulation of blood pressure and volume of the cardiovascular system	L-163491

having good anti-inflammatory activity which can help to improve the inflammation caused by SARS-CoV2. The rationale behind the selection of coumarin derivatives is as said above and the selection of four enzymes RdRp (PDB ID: 7BV2), PLpro (PDB ID: 6W9C), Mpro (PDB ID: 6W63) and spike proteins (SP) (PDB ID: 6M0J) are because of their important role in the entry of virus into human cell, replication and virulance. This insisted us to carry out the present multi-targeted molecular docking study on coumarin drugs, and to design and predict molecular interaction of some unstudied coumarin analogues against COVID-19. The outcome of the present study reveals that the modified coumarin derivatives possess better binding efficacy towards at least three targets out of four targets studied when compared to their parent compounds. ADME studies of all these compounds indicate lipophilic, high gastrointestinal absorbable, and blood-brain barrier permeability of the compounds studied.

2. Materials and methods

A modelling software such as Chem Office-16 (https:// www.cambridgesoft.com/Ensemble_for_Chemistry/details/ Default.aspx?fid=16), Discovery Studio Visualizer 3.0 (https://discover.3ds.com/discovery-studio-visualizer-download), Swiss Protein Data Base Viewer (https://spdbv.vital-it.ch/), Open Babel (https://openbabel.org/wiki/Main_Page), PyRx (https:// pyrx.sourceforge.io/), and AutoDock Vina (https://vina.scripps. edu/) was used in the present study. The online tool Swiss ADME (https://www.swissadme.ch/) was also used. The 3D structures of SARS-CoV-2 proteins such as RdRp (PDB ID: 7BV2), PLpro (PDB ID: 6W9C), Mpro (PDB ID: 6W63) and spike proteins (SP) (PDB ID: 6M0J) were downloaded from Protein Data Bank (Fig. 1).

2.1. Preparation of ligands

Structure of the coumarin compounds [phenprocoumon (PHE), hymecromone (HYM) and psoralen (PSO)] were downloaded from PubChem, and the designed derivatives were drawn by using the Chem Draw tool in Chem Office-16 software. Then, all the structures were checked for ADME and Lipinski rule of five by using online tools such as SwissADME, and the structures that met Lipinski rule of five, Veber's Law and ADME threshold were used for docking. The information obtained from the SwissADME were molecular weight, MlogP value, a number of hydrogen bond donors and acceptor, a number of rotatable bonds, topological surface area and the bioavailability score. The selected structures' energy minimisation was done using the MM2 force field and then saved as either.sdf or.mol file for further use.

2.2. Preparation of proteins

The crystallographic structures of SARS-CoV-2 proteins downloaded from Protein Data Bank (PDB) were checked for broken chain and errors using Swiss Protein Data Base Viewer and corrected. The information about the protein's active site was gathered by using Discovery Studio Visualizer. Furthermore, the water and other heteroatoms were removed, and polar hydrogens were added to the protein structure, and saved as PDB format for docking study (Dallakyan and Olson, 2015).

2.3. Docking studies

The selected chemical compounds and protein structures were uploaded in the Virtual Screening software interface PyRx. Using the conjugate gradient algorithm, the energy minimization was performed with the Universal Force Field (UFF). The total number



Fig. 1. 3D structures of SARS-CoV-2 proteins. A) RdRp (PDB ID: 7BV2), B) PLPro (PDB ID: 6W9C), C) Mpro (PDB ID: 6W63), D) spike proteins (PDB ID: 6M0J).

of steps was set to 200, and the number of steps for the update was 1. In addition, the minimization was set to stop at an energy difference of less than 0.1 kcal/mol. Then, both chemical compounds and protein structures were saved in '.pdbqt' format using the Open Babel tool in PyRx. The active binding site grid box was generated by using the forward option in the PyRx. The grid box's size and coordinate were adjusted by tracking the boundary line of the box or by entering the values in the appropriate box (Dallakyan and Olson, 2015; Veerasamy and Karunakaran, 2022). The coordinate and distance of the \times , y and z axes of the grid boxes of each protein molecule were given in Table 2. The conformational search algorithm used in PyRx is the Lamarckian genetic algorithm. The docking method used in the present work was semi flexible docking.

After docking, the software displayed the binding energy with different conformers, and it was saved in '.csv' format. The results of PyRx were split into individual conformers by using Autodock Vina. Then, the docking output files were analysed for the interactions between the chemical compounds with the amino acid of protein using Discovery Studio Visualizer (Adeniji et al., 2020). All binding conformations of the re-docked ligand within the binding pocket of protein produced by the PyRx tool were similar to the binding mode of the co-crystallised ligand, and the root means square deviation (RMSD) for these conformations were below 2 Å (Vianna and Azevedo, 2012). Each conformer and the protein were loaded on Discovery Studio Visualizer and observed for the interactions. The best conformer was selected based on the docking score and better non-covalent bond interaction. The photographs of the docking pose and interactions were collected and saved (Adeniji et al., 2020).

Later, a few coumarin analogues named phenprocoumon-1 (PHE1), hymecromone-1 (HYM1) and psoralen-1 (PSO1) were designed based on the docking results, then the molecular interaction of designed analogues with SAR-CoV-2 was studied by molecular docking study. Their ADME properties and synthetic accessibility was also calculated using SWISSADME web tool.

Table 2

Coordinate of the $\times,$ y and z centers of grid boxes of SARS-CoV-2 proteins with dimensions.

Protein molecule	x- centers (Dimension)	y-centers (Dimension)	z-center (Dimension)
RdRp	91.52	92.38	103.73
Mpro	-35.19	13.22	25.43
PLpro	-20.46	18.11	-26.91
SP	-25.43	11.59	4.57

3. Results

3.1. ADME properties of known drugs

In the present research work, the molecular interactions of coumarin compounds and the selected SARS-CoV-2 viral proteins were studied using molecular docking. RdRp, PLpro, Mpro and spike protein are known as a promising targets for SARS-CoV-2 drug development. In the present study, structures of the coumarin drugs were also modified and tested against the target to look for better binding efficiency and interactions.

In this study, three coumarin derivatives PHE, HYM and PSO, were tested. Phenprocoumon is a long-acting anticoagulant, it works by inhibiting the vitamin K epoxide reductase enzyme and hence prevents the formation of the reduced, active form of vitamin KH2, and this prevents the activation of vitamin K-dependent coagulation factors. Some studies supporting that the direct Xa inhibitors may hinder the entry of SARS-CoV-2 by preventing the splitting of the spike protein (Al Horani, 2020). PHE has not been tested against any COVID-19 target until now.

The SwissADME web tool was used to compute the absorption, distribution, metabolism, and excretion (ADME) parameters of compounds. From this computation, the drug-likeness of coumarin derivatives used in the docking analysis was predicted. Lipinski's rule of five defines numbers for some parameters of compounds which are molecular weight lesser than \leq 10 hydrogen bond acceptors, \leq 5 hydrogen bond donors, logP value of \leq 5. The compounds which has no more than one violation then they are considered as the drug-likeness. The ADME properties of coumarin drugs are depicted in Table 3. The ADME properties of known drugs are calculated for only comparison with the designed drugs properties, which is discussed in latter part of the article.

3.2. Docking results of known drugs

A known drugs PHE, HYM and PSO, were considered for studying their docking behaviour with SARS-CoV-2 protein 7BV2, 6W9C, 6W63 and 6M0J. The docking results of PHE, HYM and PSO showed the least binding energy. Table 4 shows the binding affinity of drugs with the target and their predicted pKi (Shityakov and Förster, 2014), while Table 5 shows the amino acids involved in interaction with coumarin derivatives.

3.2.1. Binding to RdRp (7BV2)

RNA-dependent RNA polymerase (RdRp) or Non-structural protein (nsp)12 is a vital enzyme of coronavirus replication or transcription complex (Kirchdoerfer and Ward, 2019). In the study of SARS-CoV-2 inhibitors, RdRp has been used as a significant drug target. In the current study, coumarin derivatives PHE, HYM and

Table 3

ADME properties of coumarin drugs and derivatives.

No.	Drug	Pubchem ID	Molecular Weight (g/mol)	Mlog P	Hydrogen bond acceptor	Hydrogen bond donor	Rotatable bond	Topological Polar Surface Area (Å)	Bioavailabilityscore	Synthetic Accessibility*
1	Phenprocoumon	54,680,692	280.32	3.20	3	1	3	50.44	0.55	-
2	Hymecromone	5,280,567	176.17	1.34	3	1	0	50.44	0.55	-
3	Psoralen	6199	186.16	1.48	3	0	0	43.35	0.55	-
4	Phenprocoumon- 1	-	296.32	2.62	4	2	3	70.62	0.55	4.11
5 6	Hymecromone-1 Psoralen-1	-	178.14 202.16	0.45 0.89	4 4	2 1	0 0	70.67 63.58	0.55 0.55	2.64 2.62

* Synthetic accessibility was calculated using SWISSADME tool. The synthetic accessibility is accessed as 1 is very easy to 10 is very difficult.

Table 4

Binding affinity (kcal/mol) betwee	n coumarin drugs and derivatives	against targets of SARS CoV-2 د
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No.	Coumarin derivatives and its synthesised compounds	RdRp		PLpro		Mpro		SP	
		kcal/mol	pKi ^a						
1	Phenprocoumon	-5.2	3.83	-5.7	4.19	-6.8	5.00	-6.4	4.71
2	Hymecromone	-5.5	4.05	-5.8	4.27	-5.9	4.34	-5.3	3.90
3	Psoralen	-6.3	4.63	-5.5	4.05	-6.5	4.78	-6.0	4.41
4	Phenprocoumon-1	-6.4	4.71	-6.0	4.41	-7.2	5.30	-6.1	4.49
5	Hymecromone-1	-5.8	4.27	-5.4	3.97	-6.0	4.41	-5.5	4.05
6	Psoralen-1	-6.5	4.78	-6.2	4.56	-6.6	4.86	-6.4	4.71
7	Remdesivir	-6.1	-	-7.4	-	-7.8	-	-6.9	-

^a Negative decimal logarithm of predicted inhibition constant Ki (Ki predicted = exp($\Delta G/RT$), where R = 1.98 × 10⁻³ Kcal/mol*K, T = 298.15 K (Shityakov and Förster, 2014).

PSO, showed the binding affinity of -5.2, -5.5 and -6.3 kcal/mol, respectively, with RdRp (7BV2).

Fig. 3A to 3B shows the interactions between PHE, HYM and PSO with 7BV2. Benzene nucleus of PHE formed pi-anion interaction with Asp760 at 3.48 Å, while the pyrone group formed pi-cation interaction with Arg555 at 3.73 Å. PHE also formed hydrogen bond interactions with 7BV2 at Asp760 (2.12 Å) and Arg555 (3.26 Å), shown in Fig. 3Ba. HYM formed pi-alkyl interactions with residues Arg555 (3.94 Å, 4.52 Å) and Ala547 (4.25 Å), and one pi-donor hydrogen interaction of pyrone with Ser549 at 3.83 Å. HYM showed less prominent hydrogen bond interaction with different amino acids of 7BV2 compared to PHE, at Ala547 (2.65 Å) and Arg553 (3.13 Å), as shown in Fig. 3Bb. Psoralen shows the highest binding affinity among three drugs, with a -6.3 kcal/mol binding score. The pyrone group formed carbon-hydrogen interaction with Ala554 at 3.60 Å and two pi-donor hydrogen interactions with Ala554 at 3.59 Å and Tyr455 at 3.64 Å. The benzene and pyrone nucleus formed 3 pi-alkyl interactions with Ala554 (4.64 Å), Arg553 (5.24 Å), and Ala448 (5.24 Å). Psoralen also formed hydrogen bond interaction with 7BV2 at Ala554 (2.96 Å) and Asn552 (3.07 Å, 3.08 Å), as shown in Fig. 3Bc. Based on the binding results, it is clearly shown that all the coumarin-based drugs can bind with RdRp with different efficacies. Both HYM and PSO exhibited high binding affinity against RdRp compared to PHE.

3.2.2. Binding to PLpro (6W9C)

Drugs that binding to the PLpro catalytic triad can destroy the role of PLpro in the host immune response evasion to reduce the inflammation of host cells (Shin et al., 2020).

PHE showed moderate binding potential towards 6W9C, with –5.7 kcal/mol binding energy as shown in Fig. 4Aa and 4Ba. The hydroxyl group of PHE formed hydrogen bonds with 6W9C at Met206 and Glu203 at distances of 2.46 Å and 2.51 Å, respectively. One amide-pi stacked interaction formed with Tyr207 at 4.93 Å, and van der Waals interaction with adjacent amino acids. The coumarin benzene of PHE formed two pi-alkyl interactions with Leu199 at 5.07 Å and Leu185 at 5.41 Å, while alkyl interaction was observed for the alkyl group with Leu199 at 4.43 Å. On the

other hand, HYM shows a slightly stronger binding affinity of -5.8 kcal/mol for 6W9C. Fig. 4Ab and 4Bb shows the interaction between HYM and 6W9C. HYM formed hydrogen bond interactions with 6W9C at Tyr305, Glu214, Lys217 and Tyr213 with distances of 2.04 Å, 2.14 Å, 2.22 Å, 2.31 Å, respectively. One pisigma interaction was observed between pyrone and Glu214 at 3.55 Å.

Among the three drugs tested, PSO showed a slightly lesser binding affinity of -5.5 kcal/mol with PLpro, as shown in Fig. 4Ac and 4Bc. PSO formed hydrogen bonds with Met208 of 6W9C at distances of 2.14 Å and Arg166 at distances of 2.70 Å and 5.42 Å. The furan ring of PSO formed a carbon-hydrogen bond and one pi-sigma bond interaction with Tyr207 at 3.41 Å and3.41 Å, respectively. The pyrone ring of PSO formed a pi-sulfur bond interaction with Met206 at 4.25 Å, while the same amino acid formed an interaction with the benzene ring at the distance of 5.05 Å.

From the results obtained, HYM possesses the highest binding affinity among the three drugs tested towards PLpro, with the binding affinity of -5.8 kcal/mol. It showed a more prominent hydrogen bond interaction with 6W9C.

3.2.3. Binding to Mpro (6W63)

Mpro is a dimer with cysteine and histidine in the active site, forming a catalytic dyad, conserved among coronaviruses, making it an ideal therapeutic target (Anand et al., 2003).

PHE showed the highest binding affinity towards 6W63 at -6.8 kcal/mol, as shown in Fig. 5Aa and 5Ba. PHE formed hydrogen bonds with Gln189 and Arg188 at 2.39 Å and 2.44 Å, respectively, and three pi-alkyl interactions were observed towards Pro168, Met49 and His41 at 4.80 Å, 5.26 Å and 5.40 Å, respectively. Moreover, an alkyl bond was observed with Met165 at a distance of 4.72 Å. Pi-pi *T*-shaped bonding was formed at the benzene ring with His41 at a distance of 5.41 Å, one pi-sulfur bond interaction and a pi-donor hydrogen bond were formed by the interaction of the pyrone ring of PHE with Met165 and Glu189 at 5.90 Å and 3.20 Å, respectively (Fig. 5Ba).

Table 5

SARS CoV-2 protein amino acids involved in interactions with the coumarin derivatives.

		-			
No.	Coumarin derivatives	RdRp	PLpro	Mpro	SP
1	Phenprocoumon	Arg555, Asp760	Leu185, Leu199, Glu203, Met206, Tyr207	His41, Met49, Met165, Pro168, Arg188, Gln189	Thr324, Gln325, Gly326, Gly354
2	Hymecromone	Ala547, Ser549, Arg553, Arg555	Tyr213, Glu214, Lys217, Tyr305	His41, Cys44, Met49, Met165, Glu166	Thr324, Gln325, Gly326, Gly354, Asp355
3	Psoralen	Ala448, Tyr455, Asn552, Arg553, Ala554	Arg166, Met206, Tyr207, Met208	Val104, Gln110, Thr111	Gln325, Asn330, Asp355
4	Phenprocoumon1	Agr555, Thr680, Asp760	Leu199, Val202, Met206, Lys232	Met49, Cys145, His163, Met165, Clu166	Met474, Lys475, Arg482, Glu489, Ala614
5	Hymecromone1	Arg555, Ser682, Thr687, Ala688, Asn691, Ser759	Tyr213, Glu214, Tyr305	His41, Cys44, Met49, Tyr54, His164, Met165, Asp187	Lys475, Glu489, Glu495
6	Psoralen1	Asp164, Val166, Pro620, Ser795, Lys798	Tyr213, Glu214, Lys217, Tyr305	His41, Cys44, Met49, His164, Met165, Glu166, Asp187	Arg482, Glu489, His493, Tyr613
7	Remdesivir	Tyr619, Asp760, Trp617, Lys798, Asp760, Pro620	Asp164, Arg166, Glu167, Pro248, Gly266, Asn267, Tyr264, Tyr268	Gly143, Cys145, Glu166, Asn142 Met165, His41, Gln189, Glu166	Glu37, Lys353, Gly354, Phe356, Pro321, Asp355, Ala386

Next, Mpro interaction with HYM is lower than PHE, with the binding affinity of -5.9 kcal/mol towards 6W63. Fig. 5Ab and 5Bb shows the interaction between HYM and 6W63. HYM formed two hydrogen bonds at Glu166 (2.51 Å) and His41 (2.76 Å), two pisulfur bonds formed between pyrone ring and Met49 at 4.41 Å, and Cys44 at 5.58 Å. Moreover, a Pi-alkyl bond was also formed between the phenol ring and Met165 at 4.92 Å. On the other hand, PSO showed stronger binding potential towards 6W63 compared to HYM, with the binding affinity of -6.5 kcal/mol as shown in Fig. 5Ac and 5Bc. One pi-alkyl interaction formed at furan ring with Val104 (4.82 Å), and two hydrogen bond interactions between 6W63 and Gln110 (2.54 Å), and Thr111 (2.85 Å).

3.2.4. Binding to spike glycoprotein (6M0J)

The ability of the corona virus to attach to the host cell can be inhibited if spike glycoprotein is inhibited.

In the current research study, PHE shows the highest binding affinity towards spike glycoprotein compared to HYM and PSO, with the binding affinity of -6.4 kcal/mol. Fig. 6Aa and 6Ba shows

the interaction between PHE and 6MOJ. PHE formed a hydrogen bond with 6MOJ at Gln325 with 2.14 Å and Gly326 with 2.23 Å. One pi-sigma interaction formed at Thr324 with 3.47 Å. The benzene ring of PHE also formed an amide-pi stacked interaction with Gly354 at 4.00 Å and van der Waals interaction with surrounding residues.

Next, HYM possesses a lower binding potential compared to PHE, which is -5.3 kcal/mol, as shown in Fig. 6Ab and 6Bb. The carbonyl group of HYM formed hydrogen bonds with Gly326 at 2.10 Å and with Gln325 at 3.73 Å, while the same carbonyl group formed a carbon-hydrogen bond interaction with Thr324 at the distance of 3.42 Å. One pi-anion interaction formed at Asp355 with 4.97 Å and two amide-pi stacked interactions were observed at phenol and pyrone ring of HYM with Gly354 (4.21 Å and 4.55 Å). PSO possesses slightly higher binding potential, compared to HYM, with -6.0 kcal/mole. Fig. 6Ac and 6Bc shows the interaction between PSO and 6M0J. PSO formed two hydrogen bonds with 6M0J at Gln325 with 2.44 Å and at Asn330 with 2.53 Å, also formed a pi-anion interaction of furan ring with Asp355 at 4.88 Å.



Fig. 2. Structure of coumarin drugs and derivatives. A) phenprocoumon, B) hymecromone, C) psoralen, D) phenprocoumon1, E) hymecromone1, F) psoralen1.

3.3. Docking results of chemically modified coumarin derivatives

After obtaining the docking result of marketed drugs with SARS-CoV-2 targets, the drug compounds were subjected to structural modification (Fig. 2D to 2F) and observed for the binding affinity and interactions against SARS-CoV-2 targets as shown in Figs. 7 to 10. ADME report of the designed coumarin derivatives was given in Table 3. All ligands were found to have suitable ADME values and fulfilled the drug-likeness properties. The docking results of the chemically designed coumarin molecules were obtained, as shown in Tables 4 and 5.

3.3.1. Binding to 7BV2

The interaction between 7BV2 and PHE1 is shown in Fig. 7Aa and 7Ba. PHE1 showed binding towards 7BV2 with the binding energy of -6.4 kcal/mol and formed hydrogen bonds with amino acids Thr680 at 2.30 Å and Arg55 at 3.15 Å. Even though, one pianion was observed towards Asp760 with a distance of 3.71 Å, a pi-cation interaction was observed with Arg555 at 3.42 Å. Next, in HYM1, a total of four hydrogen bond interactions were observed at amino acid Ser759 (2.21 Å), Ser682 (2.24 Å), Asn691 (3.12 Å) and Arg555 (3.15 Å), but with a slightly lower binding affinity of -5.8 kcal/mol compared to PHE1. On the other hand, one pi-





Fig. 3. A) Docking pose of hydrogen binding interaction of 7BV2 with a) phenprocoumon, b) hymecromone, c) psoralen, B) 2D interaction diagram of 7BV2 with a) phenprocoumon, b) hymecromone, c) psoralen.

sigma and a pi-alkyl interaction at the phenol group of HYM1 with Thr687 at a distance of 3.71 Å and Ala688 at a distance of 5.41 Å, as shown in Fig. 7Ab and 7Bb.

Fig. 7Ac and 7Bc shows the interaction between PSO1 and 7BV2. PSO1showed the highest binding towards 7BV2 with the binding energy of -6.5 kcal/mol, and hydrogen bond interactions were observed between 7BV2 at Ser795 (3.11 Å, 3.06 Å) and Asp164 (2.56 Å) and the hydroxyl group of PSO1. The pyrone formed pication interaction with Lys795 at a distance of 2.56 Å, and three pi-alkyl interactions were found at Pro620 (3.91 Å, 4.76 Å) and Val166 (4.54 Å).

3.3.2. Binding to 6W9C

Fig. 8Aa and 8Ba shows the interaction between phenprocoumon1 and 6W9C with a binding affinity of -6.0 kcal/mol. The hydroxyl group in the pyrone and benzene ring of PHE1 formed hydrogen bond interactions with Met206 at 2.26 Å and Lys232 at 2.79 Å, respectively. Moreover, one pi-sigma and alkyl interactions at Leu199 (3.84 Å and 5.10 Å) and 2 pi-alkyl interactions were observed at Met206 (3.97 Å) and Val202 (4.55 Å), respectively, as shown in Fig. 8Ba. On the other hand, HYM1 had a binding affinity with the binding energy of -5.4 kcal/mol. Two hydrogen bond interactions formed at the carbonyl group attached to the pyrone with Tyr305 at 2.02 Å and Tyr213 at 2.31 Å, while another hydrogen bond formed at the pyrone ring with Glu214 (2.10 Å). One pisigma bond and one pi-anion bond interaction were observed with the same amino acid at Glu214 (3.73 Å and 4.93 Å), as shown in Fig. 8Ab and 8Bb. Furthermore, PSO1 showed the highest binding energy towards 6W9C among the chemically modified compounds with -6.2 kcal/mol. Four prominent hydrogen bond interactions were found at Glu214 (2.06 Å), Tyr213 (2.11 Å), Tyr305 (2.15 Å), and Lys217 (2.33 Å). Two pi-anion interactions were observed for the phenol and furan ring of PSO1 with the same amino acid Glu214 at 4.54 Å and 4.79 Å, respectively. Fig. 8Ac to 8Bc shows the interaction between PSO1 and 6W9C.

3.3.3. Binding to 6W63

PHE1 had the highest binding score towards 6W63 among the chemically modified compounds with -7.2 kcal/mol. The hydroxyl group attached to the pyrone ring formed two hydrogen bonds with the same amino acid Glu66 at 1.86 Å and 2.21 Å, while the









Fig. 4. A) Docking pose of hydrogen binding interaction of 6W9C with a) phenprocoumon, b) hymecromone, c) psoralen, B) 2D interaction diagram of 6W9C with a) phenprocoumon, b) hymecromone, c) psoralen.

hydroxyl group attached to the benzene ring formed another hydrogen bond with His163 at 2.77 Å. Pi-alkyl interactions were observed for PHE1with Met49 and Met65 at 5.36 Å and 5.38 Å, respectively. Moreover. one pi-sulfur interaction was observed between the benzene ring and Cys145 at 3.86 Å. Fig. 9Aa and 9Ba shows the interaction between PHE1 and 6W63. On the other hand, HYM1 possessed a lower binding affinity compared to PHE1, with a binding score of -6.0 kcal/mol. HYM1 formed hydrogen bond interactions with His164 (2.35 Å), His41 (2.74 Å), Asp187 (2.78 Å), Met49 (2.95 Å), and Tyr54 (3.00 Å). HYM1 also formed a pi-pi stacked interaction with the amino acid His41 at 3.95 Å and 4.18 Å, respectively. The phenol group of HYM1 formed a pisulfur interaction with Met165 (5.92 Å), while pi-alkyl interaction formed with Cys44 (5.04 Å) and Met49 at the distance of 5.37 Å and 5.41 Å. Fig. 9Ab and 9Bb shows the interaction between hymecromone1 and 6W63.

The binding energy of PSO1 towards 6W63 was slightly higher compared to HYM1 with the binding affinity of -6.6 kcal/mol.

PSO1 formed two hydrogen bond interactions at the target with Glu166 (2.28 Å) and His164 (2.52 Å). Next, the furan ring of PSO1 formed a carbon-hydrogen bond with Asp187. Looking into the pi-alkyl interactions, PSO1 formed interaction with Met49 (5.11 Å), Met165 (5.19 Å, 5.22 Å) and Cys44 (5.49 Å), respectively. The furan and phenol rings of PSO1 also formed Pi-Pi T shaped interactions with His41 at a distance of 5.07 Å and 5.70 Å, respectively. Fig. 9Ac and 9Bc shows the interaction between PSO1 and 6W63.

3.3.4. Binding to 6M0I

The interaction between PHE1 and 6M0J were shown in Fig. 10Aa and 10Ba. PHE1 showed binding affinity towards spike glycoprotein with the binding energy of -6.1 kcal/mol. Two hydrogen bond interactions were observed at the hydroxyl group of pyrone and phenol with Glu489 at 2.16 Å and Met474 at 2.45 Å, respectively. Next, PHE1 formed a pi-cation interaction with Arg482 at 5.28 Å, while forming three pi-alkyl interactions with



a







Fig. 5. A) Docking pose of hydrogen binding interaction of 6W63 with a) phenprocoumon, b) hymecromone, c) psoralen, B) 2D interaction diagram of 6W63 with a) phenprocoumon, b) hymecromone, c) psoralen.

Lys475 (4.4 Å and 5.3 Å) and Ala614 (5.28 Å). The interaction between HYM1 and 6M0J were shown in Fig. 10Ab and 10Bb. HYM1 showed lower binding potential towards spike glycoprotein with the binding energy of -5.5 kcal/mol. Two hydrogen bond interactions were observed between the hydroxyl groups of HYM1 with Glu489 at 1.96 Å and Glu495 at 2.42 Å. Moreover, HYM1 formed pi-alkyl interactions with 6M0J in the same amino acid Lys475 at 3.82 Å and 4.7 Å, respectively.

Next, the interaction between PSO1 and 6M0J were shown in Fig. 10Ac and 10Bc. PSO1 showed the highest binding potential among the chemically modified drugs towards spike glycoprotein with the binding energy of -6.4 kcal/mol. Two hydrogen bond interactions were observed between the oxygen atom attached to the benzene ring of PSO1 with Tyr613 at 2.11 Å and Arg482 at 2.96 Å of 6M0J. In contrast, another hydrogen bond interaction was found between the carbonyl group of PSO1 with His493 at

2.16 Å. However, pi-cation interactions formed for PSO1 with the same amino acid Arg482 at 3.93 Å and 3.70 Å, while the pi-anion interactions formed with Glu489 at 3.47 Å and 4.07 Å.

4. Discussion

The essential protease (Mpro) and the RNA-dependent RNA polymerase (RdRP), which are responsible for the viral polyprotein proteolytic process as well as viral genome replication and transcription, are two promising drug targets for SARS-CoV-2 related diseases (Gao et al. 2020) and the main protease (Mpro) responsible for virus maturation in addition to crucial roles in mediating viral replication and transcription (Jin et al. 2020). COVID-19 spike glycoprotein that initiates virus internalization by fusing virus membrane to host cell membrane. The papain-like protease PLpro

a



b c A



Fig. 6. A) Docking pose of hydrogen binding interaction of 6M0J with a) phenprocoumon, b) hymecromone, c) psoralen, B) 2D interaction diagram of 6M0J with a) phenprocoumon, b) hymecromone, c) psoralen.

is an essential coronavirus enzyme that is required for processing viral polyproteins to generate a functional replicase complex and enable viral spread. PLpro is also implicated in cleaving proteinaceous post-translational modifications on host proteins as an evasion mechanism against host antiviral immune responses (Frieman et al., 2009). Based on their crucial role in the life cycle of SARS-CoV-2, these four target sites have been extensively docked to design or distinguish structure-based effective drugs for COVID-19 (Dai et al., 2020).

The logic behind the designing of the analogues from the selected coumarin compounds is to find good molecular interactive coumarin with COVID-19 targets than the reported coumarin compounds. We have incorporated hydroxyl group based on the report by Jahan and Onay (2020) suggested that the number of –OH groups of coumarin derivatives can form hydrogen ion bonds with

positively charged amino groups of proteins, which inhibit the activity of a viral protein. The other reason is, it has been already reported that coumarin is a potential nucleus for developing anti-inflammatory drugs, and its hydroxy aromatic derivatives show even more potent anti-inflammatory activity (El-Haggar and Al-Wabli, 2015). So, the coumarins could be concurrently useful to treat inflammation caused by the SARS-CoV2 along with anti-COVID-19 activity.

In this study, PHE showed a great binding affinity towards Mpro with a binding energy of -6.8 kcal/mol, which is the highest binding affinity compared to binding of PHE with RdRp (-5.2 kcal/mol), PLpro (-5.7 kcal/mol) and spike glycoprotein (-6.4 kcal/mol). Looking into the interaction, the research study by Lyndem et al. (2020) revealed that His41 is the catalytic dyad in Mpro. In our present study, PHE interacts with the other residues displayed the best





Fig. 7. A) Docking pose of hydrogen binding interaction of 7BV2 with a) phenprocoumon1, b) hymecromone1, c) psoralen1, B) 2D interaction diagram of 7BV2 with a) phenprocoumon1, b) hymecromone1, c) psoralen1.

binding efficiency. The chemically modified PHE (PHE1) showed the highest binding affinity with Mpro among other COVID-19 targets. Both PHE and PHE1 interacted with Mpro binding site residues of Met49 and Met165. PHE1 formed a more robust interaction with Glu166 (2.21 Å and 1.66 Å) at –OH groups than PHE with Gln189 (2.39 Å) of Mpro. This docking result indicates that PHE could act as the potential inhibitor of SARS- CoV-2 Mpro due to high binding affinity and prominent interactions with the drug target.

HYM is a hydroxycoumarin that has a role as an antineoplastic agent and a hyaluronic acid synthesis inhibitor which could mimic the mechanism that researchers believe protects women from more severe COVID-19 infections. It has been already reported that coumarin is a potential nucleus for developing anti-inflammatory drugs, and its hydroxy aromatic derivatives show even more potent anti-inflammatory activity (El-Haggar and Al-Wabli, 2015).

HYM has not been tested against any COVID-19 targets until now. In this study, the designed HYM1 demonstrated a slightly higher binding affinity towards all the targets than their parent compound, respectively, except PLpro. HYM1 formed more hydrogen bond interactions than their parent compounds with all the targets except for PLpro; this may be why the modified drug has higher binding affinity for all tested targets except for PLpro. This is supported by Milenković et al. (2020) reports that the hydrogen bonds are essential for the overall stability of protein–ligand complexes. Among all the targets, the designed HYM1 formed the highest binding affinity with Mpro. As mentioned previously, the hydrogen bond interaction at His41, known as the catalytic dyad in Mpro, gives the best binding efficiency.

On the other hand, PSO is a furocoumarin that inhibits DNA synthesis and cell division of viruses. It is used in photochemotherapy with high-intensity long-wavelength UVA irradiation. PSO have a strong tendency to intercalate with DNA base pairs. It has been tested against COVID-19 in the earlier phase of the pandemic as a natural coumarin analogue. Chidambaram et al. (2020) reported the binding affinities of PSO with Mpro (PDB ID: 5 N50) was -5.6 kcal/mol, while in the present study, the binding affinity of Mpro (PDB ID: 6W63) and PSO was -6.5 kcal/mol and has the

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Fig. 8. A) Docking pose of hydrogen binding interaction of 6W9C with a) phenprocoumon1, b) hymecromone1, c) psoralen1, B) 2D interaction diagram of 6W9C with a) phenprocoumon1, b) hymecromone1, c) psoralen1.

highest binding affinity compared than binding towards RdRp (-6.3 kcal/mol), PLpro (-5.5 kcal/mol) and spike glycoprotein (-6.0 kcal/mol). Looking into the interaction of Mpro with PSO by Chidambaram et al. (2020), there was no hydrogen bond interaction found. In contrast, in the current study, PSO showed hydrogen bond interactions with Val104, Gln110 and Thr111.

The chemically modified PSO1 has a higher binding affinity towards all the targets compared to their parent compound. Among the targets, PSO1 has the highest binding affinity towards Mpro (-6.6 kcal/mol) compared to binding with RdRp (-6.5 kcal/mol), PLpro (-6.2 kcal/mol) and spike glycoprotein (-6.4 kcal/mol). Among the chemically modified compound that showed the highest binding affinity with target Mpro, PSO1 formed the shortest distance of hydrogen bond (2.78 Å) than the parent compound (2.85 Å), although with the same number of hydrogen bond interactions shown it better binding efficiency. Docking results indicate that PSO could act as the potential inhibitor of SARS- CoV-2 Mpro due to its high binding affinity and prominent interactions with the drug target.

The research question of the present was whether the selected coumarin drug and the designed coumarin derivatives could prevent SARS-CoV-2 entry and replication. Therefore, the study aimed to explore the coumarin for their potential to combat SARS-COV-2 entry, replication by inhibition of the spike protein, Mpro. PLpro and RdRp of SARS-CoV-2. All the coumarin derivatives showed low binding affinity than remdesivir (Table 4), even though, phenprocoumon-1 and psoralen-1 showed better binding affinity than remdesivir.

The cumarin drugs and its designed derivatives were bind to the active residues Cys145 and His41 of Mpro, indicating potential virus replication inhibition. Though literature reveals that a Cystine-Histidine dyad is essential for the protease activity of Mpro, alanine, glycine, glutamate, serine, and leucine residue also play important roles in the cleavage catalysis process (Gupta et al., 2020). Glu166 residue is a key amino acid involve in the dimerization of Mpro and creation of substrate binding pocket which is required for catalytic regulation. Current study, the cou-





Fig. 9. A) Docking pose of hydrogen binding interaction of 6W63 with a) phenprocoumon1, b) hymecromone1, c) psoralen1, B) 2D interaction diagram of 6W63 with a) phenprocoumon1, b) hymecromone1, c) psoralen1.

marin derivatives bind to Glu166 of Mpro as standard drug, which indicates the catalytic regulation of Mpro by coumarins.

The binding efficacy of coumarins below -6.5 kcal/mole also showed hydrophobic and hydrogen bond interaction with the key amino acids involved in Spike-protein RBD domain and ACE-2 protein-protein interaction (Asp, Ser, and Gln). The results indicate that coumarin derivatives have potential to disrupt spike glycoprotein-ACE-2 protein-protein interaction and thus inhibit viral entry into the cell. The coumarin derivatives has also been shown that RdRp inhibition such as remdesivir, which indicates the potential blocking of the RNA synthesis and thereby delay the chain termination process in SARS-CoV2 RNA synthesis, which generate polyproteins that are responsible for virulence of SARS-CoV2.

Finally, the synthetic accessibility of the designed compounds was predicted by using SWISSADME tool, and the accessibility is 58.9 %, 73.6 %, and 73.8 % for the designed compounds, phenprocoumon-1, hymecromone-1, psoralen-1, respectively. The synthetic accessibility of the designed compounds indicates that proposed coumarin compounds are easily synthesisable, even

though phenprocoumon-1 is least synthesizable than other two designed coumarin derivatives.

5. Conclusion

In conclusion, the interaction of coumarin derivatives towards RdRp, PLPro, Mpro and spike glycoprotein of SARS-CoV-2 was investigated by molecular docking studies. All the coumarin compounds were acquired with the least docking energy against the target proteins. Based on the result obtained in the current study, it can be concluded that all tested compounds were drug-like, passing Lipinski's rule of 5 with 0 violations with molecular weight \leq 500, hydrogen bond acceptors \leq 10, hydrogen bond donor's \leq 5 and Log P value \leq 5. The present study indicates the importance of –OH groups in the compound, His41 catalytic dyad in Mpro, a number and its distance of hydrogen bond interactions with the target for the high binding affinities. The current study results showed that phenprocoumon and its chemically modified structure had the highest binding potential towards the protein





Fig. 10. A) Docking pose of hydrogen binding interaction of 6M0J with a) phenprocoumon1, b) hymecromone1, c) psoralen1, B) 2D interaction diagram of 6M0J with a) phenprocoumon1, b) hymecromone1, c) psoralen1.

targets of SARS-CoV-2 Mpro due to high binding affinity and prominent interactions with the drug target. But the synthetic accessibility of the psoralen-1 is better than the other two designed coumarin derivatives. However, an advanced investigation is necessary to analyse the medicinal substance's likely applications and further research of the compounds. The current ongoing research should lead to more comprehensive drug discovery, leading to better efficacy in combating COVID-19.

Ethical approval

This chapter does not contain any studies with human participants or animals performed by the authors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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