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Research Article

Molecular docking and dynamic simulations study for repurposing of multitarget coumarins against SARS-CoV-2 main protease, papain-like protease and RNA-dependent RNA polymerase

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Abstract

Proteases and RNA-Dependent RNA polymerase, major enzymes which are essential targets involved in the life and replication of SARS-CoV-2. This study aims at *in silico* examination of the potential ability of coumarins and their derivatives to inhibit the replication of SARS-Cov-2 through multiple targets, including the main protease, papain-like protease and RNA-Dependent RNA polymerase. Several coumarins as biologically active compounds were studied, including coumarin antibiotics and some naturally reported antiviral coumarins. Aminocoumarin antibiotics, especially coumermycin, showed a high potential to bind to the enzymes' active site, causing possible inhibition and termination of viral life. They demonstrate the ability to bind to residues essential for triggering the crucial cascades within the viral cell. Molecular dynamics simulations for 50 ns supported these data pointing out the formation of rigid, stable Coumermycin/enzyme complexes. These findings strongly suggest the possible use of Coumermycin, Clorobiocin or Novobiocin in the fight against COVID-19, but biological evidence is still required to support such suggestions.

Keywords

coumarin, SARS-CoV-2 main protease, papain-like protease, RNA-Dependent RNA polymerase molecular docking

Introduction

COVID-19, a disease caused by the newly emerged virus SARS-CoV-2 of the coronavirus family, was declared a pandemic worldwide on 11th March 2020. Infecting more than 74 million cases and causing more than 1.5 million deaths worldwide in 12 months since its discovery in China in December 2019, it became the fatal outbreak in recent years. Despite the announcement of the ability of dexamethasone to decrease the mortality rate among severely ill and hospitalised patients (Johnson and Vinetz 2020), and the debates discussed on the benefits of using chloroquine in treatment regimens for COVID-19 patients (Mahevas et al. 2020; Principi and Esposito 2020), there is no current treatment strategy for the disease and no drug described to *in vivo* tackle viral growth and replication, and due to the fast growth of the disease and the slow pace of the usual processes of drug discovery and development, most of the current trials to establish anti-COVID-19 drugs are based on drug repurposing (Bleyzac et al. 2020; Cai et al. 2020; El-Din Abuo-Rahma 2020; Guy et al. 2020; Huang et al. 2020; Mohapatra et al. 2020). The well-established safety and pharmacokinetic profiles of old drugs plus the reduced development cost and time made repurposing is a very desirable strategy for targeting new diseases (Pushpakom et al. 2019), as in the case of COVID-19. Recently scientists highlighted repurposing clinically approved drugs to find potential anti-SARS-CoV-2 treatment options focusing on molecules that show inhibition of the critical enzymes of SARS-CoV-2, including protease, papain-like protease, helicase, or RNA-Dependent RNA polymerase. From all the essential enzymes, we selected the main protease of the virus (Mpro), papain-like cysteine protease (PLpro), and RNA-dependent RNA polymerase (RdRp). SARS-CoV-2 PP1ab is a polyprotein encoded by the SARS-CoV-2 replicase gene, which is essential for the virus's replication, transcription, and protein translation (Jin et al. 2020; Wu et al. 2020b; Zhou et al. 2020). Mpro is responsible for the release and maturation of the functional non-structural proteins (Nsps) by cleaving PP1ab at 11 conserved sites (Hegyi and Ziebuhr 2002; Yang et al. 2005). The released Nsps are vital for the virus's life cycle and have a significant role in correcting viral replication. The importance of Mpro in viral replication and the lack of any close homologs in human cells suggest Mpro as an interesting antiviral target (Zhang et al. 2020). PLpro also mediates the maturation of the SARS-CoV-2 PP1ab polyproteins by cleaving them at three sites (Harcourt et al. 2004). Moreover, PLpro antagonises the innate immunity of the host (Yuan et al. 2015; Li et al. 2016). RdRp is one of the Nsps and the crucial enzyme for the replication and transcription of SARS-CoV-2 (Subissi et al. 2014). The three enzymes are the most attractive antiviral targets because of their well-known functions. The main protease (M^{pro}, also called $3CL^{pro}$) enzyme, the well-characterised and most used target, is among those enzymes being critical for viral growth and replication. The heart-shaped enzyme is responsible for proteolytic processes for polyproteins translated from the viral RNA into essential viral enzymes. A cysteine residue, CYS 145, and a neighbouring histidine HIS41 in the active site of a dimeric protein are responsible for the proteolytic reaction (Zhang et al. 2020). Several studies were performed to allocate a potential inhibitor for such enzyme and potentially end the viral life in the human body (Kandeel and Al-Nazawi 2020; Peele et al. 2020; Tachoua et al. 2020). Among those studies, most used virtual docking and structure-based analysis (Hall and Ji 2020; Kumar et al. 2020; Rahman et al. 2020; Selvaraj et al. 2020; Yu et al. 2020).

Several natural products were also examined for potential activity against SARS-CoV-2 main protease, with polyphenolic compounds and coumarins showing the highest possible activity (Orhan and Senol Deniz 2020; Owis 2020). One of the famous classes of natural products known for their antiviral activities is coumarins (Mishra et al. 2020). Coumarins or benzopyrenes are compounds initially isolated from certain plant species such as *Umbelliferae* and *Rutaceae* (Stefanachi et al. 2018). Currently, they comprise a stable big group of natural, synthetic compounds with versatile pharmacological activities (Venugopala et al. 2013). They also shredded a high antiviral profile against various viral species, including HIV, influenza, Hepatitis viruses, Enterovirus 71 (EV71), coxsackievirus A16 (CVA16), dengue virus, and chikungunya virus. They exert their activity *via* affecting different targets hindering viral entry, survival, and infection (Mishra et al. 2020). For example, calanolide A,**20** and B, **21**, Fig. 3, isolated from *Calophyllum lanigerum* leaves, are reported for their anti-HIV activity (Kashman et al. 1992; Newman et al. 1998). Their ability to inhibit reverse transcriptase is the mechanism *via* which they introduce their action (Stefanachi et al. 2018). Coumarins were also reported to inhibit HIV protease, integrase, stop viral DNA replication, and introduce viral cell cycle arrest (Hassan et al. 2016). Introducing the 4-phenyl group to coumarin as in mesuol, **9**, and isomesuol, **10**, Fig. 2, isolated from *Marila pluricostata*, also reduced HIV-1 replication (Ryu et al. 2010). Moreover, Glycyrol, **36**, Fig. 3 separated from *Glycyrrhiza uralensis* roots showed an ability to inhibit neuraminidase activity in the influenza virus (Osman 2018). Other synthetic coumarins were found to block replication of H1N1 and H3N2 viruses, probably through a similar mechanism (Shen et al. 2018). The *Glycyrrhiza* compounds Glycerol **36**, Glycirin **15,** and the naturally occurring wedelolactone **37** showed a potent HCV inhibitory activity. Such activity encouraged using coumarin conjugated with purine ribofuranoside for designing potent HCV agents (Hwu et al. 2011). Other coumarin conjugates with uracil, thymine, and guanosine were successful inhibitors of the replication of chikungunya virus (Hwu et al. 2015, 2019). Several synthetic coumarins were promising leads as antivirals against spring viremia of carp virus (Griffin 2020).

Based on the rich literature supporting the presence of coumarins as promising antiviral candidates, herein we report the virtual docking of selected coumarin drugs, including coumarin antibiotics, other famous coumarin drugs (Figs 1 and 2), and some natural coumarins previously reported as antiviral agents (Hassan et al. 2016) (Fig. 3) into the active site of SARS-CoV-2 main protease to introduce coumarins as a potential therapeutic strategy against COVID-19.

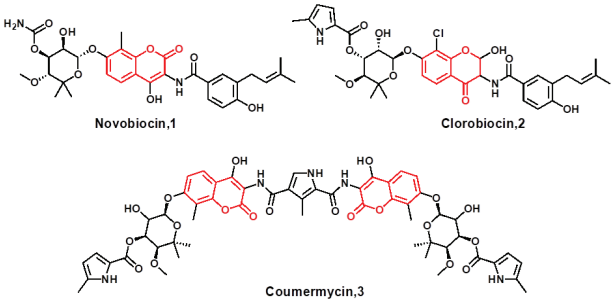

Figure 1. Structure of Aminocoumarin antibiotics Novobiocin, Clorobiocin, and Coumermycin.

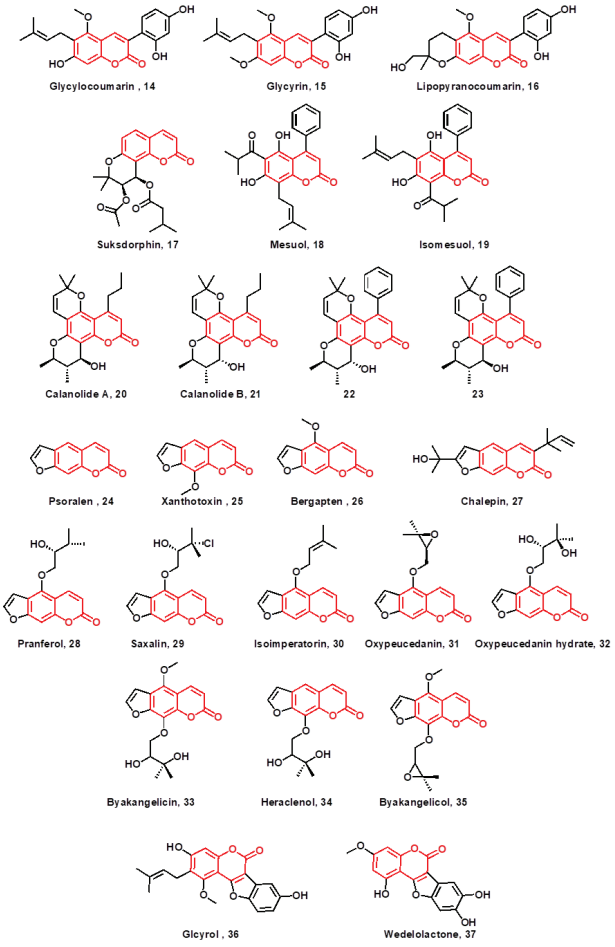

Figure 3. Structure of naturally occurring coumarins **13**–**37** reported possessing antiviral activity.

Materials and methods

Docking study

Enzymes'active sites structure preparation

The crystal structures of the SARS-CoV-2 selected enzymes were downloaded from the Protein databank at https://www.rcsb.org. For M^{pro} enzyme (PDB: 5RH4, 1.34A°), the structure was identified by X-ray diffraction as the crystal structure of SARS-CoV-2 main protease in complex with Z1530425063[3]; for Papain-like protease (PDB: 6wx4, the structure was determined by

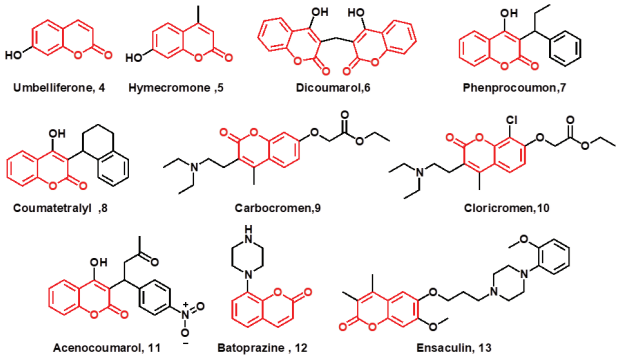

Figure 2. Structure of some coumarin drugs **4**–**13**.

X-ray diffraction as Crystal structure of the SARS CoV-2 papain-like protease in complex with peptide inhibitor VIR251,1.66 Å (Rut et al. 2020)) and RNA dependent RNA polymerase (nsp 12, PDB: 7bv2 as The nsp12-nsp7-nsp8 complex bound to the template-primer RNA and triphosphate form of Remdesivir; 2.5 Å (Yin et al. 2020)). All proteins were prepared by removing water molecules, any additional RNA or non-specific protein structures, followed by protonation and automatic correction to check for any errors in the atom's connection and type using molecule preparation tool found in MOE software. Potential and charges were fixed, and dummy atoms were added instead of legend atoms.

The active site of the used enzyme was prepared, hydrogen atoms were added, charges were fixed, dummy atoms were introduced in ligand position, compounds were docked, and possible interactions with amino acid residues were computed within the active binding site. Poses were studied and selected according to the best energy scores and binding interactions observed.

Database preparation

The 3D structure of the selected coumarin compounds, N3, chloroquine and Remdesivir, were built using a builder interface, energy was minimised to an RMSD gradient of 0.01 kcal/ mol and 0.1 A°. All compounds were added to a database and saved as an mdb file.

Docking of the target molecules to the selected enzymes' binding site

Docking of the selected compounds database to the active site of SARS-CoV-2 selected enzymes was performed using MOE 2014 software via the docking tool, and theinteractions were measured using the reports generated upon using the computing ligand interaction tool present in the MOE software Both the active site and the compound database were opened, the dock tool was initiated with dummy atoms selected as docking site, alpha triangle as the placement methodology, and London dG as the scoring methodology. After docking completion, obtained poses were evaluated and poses with the highest energy scores and best ligand–enzyme interactions were selected and recorded. Poses selected had rmsd values of 0.8–1.3 A.

Molecular dynamic simulation study of the docked complexes

Molecular dynamic simulations were done to the most stable complex observed with the three studied enzymes. The MD simulations were executed employing the Desmond simulation package of Schrödinger LLC.23 The NPT ensemble with the temperature 300 K and a pressure 1 bar was applied in all runs. The simulation length was 50 ns with a relaxation time 1 ps for the ligands coumermycin. TIP3P solvent model was applied with an orthorhombic-shaped boundary box. The OPLS-2005 force field was utilised to neutralise the system by adding the Na+ salt. The Protein-ligand system was minimised by a hybrid method of the steepest descent method and LBFGS algorithms.

Results and discussion

Docking study

Docking against SARS-CoV-2 M^{pro} enzyme

The selected coumarins; aminocoumarin antibiotics **1–3** (Fig. 1), coumarin drugs **4–13** (Fig. 2), and natural coumarins with reported antiviral activity **14–37** (Fig. 3) were docked into the active site of SARS-CoV-2 main protease (PDB: 5RH4, 1.34Å) using Molecular Orbital Environment (MOE, 2019) software. The observed binding interactions alongside energy scores for the obtained enzyme compound complexes are listed in Tables 1 and 2.

All data obtained compared to a potent standard M^{pro} inhibitor called **N3**, Fig. 4. **N3** was redocked into the active site of M^{pro,} and the observed interactions were similar to that reported implying the validity of the used method (Baz and Boivin 2019; Jin et al. 2020). Validation of the methodology used was tested via redocking of hydroxychloroquine, a reported inhibitor for M^{pro}. The energy score recorded was -6.78 kcal/mol compared to a reported -6.9 kcal/mol (Baildya et al. 2020). The nearby residues also were similar to that reported (Fig. 4b). The used method showed a potential formation of hydrogen bonding with THR 190, CYS 44, and hydrophobic interactions with GLU 166 and GLN 189 with proximity THR 25, GLN 192, ASN 142, and MET 165. THR 25, MET 49, PHE 140, LEU 141, ASN 142, GLY 143, SER 144, CYS 145, MET 165, HIS 164, GLU 166, GLN 189 were reported residues of proximity (Baildya et al. 2020). The docking was also

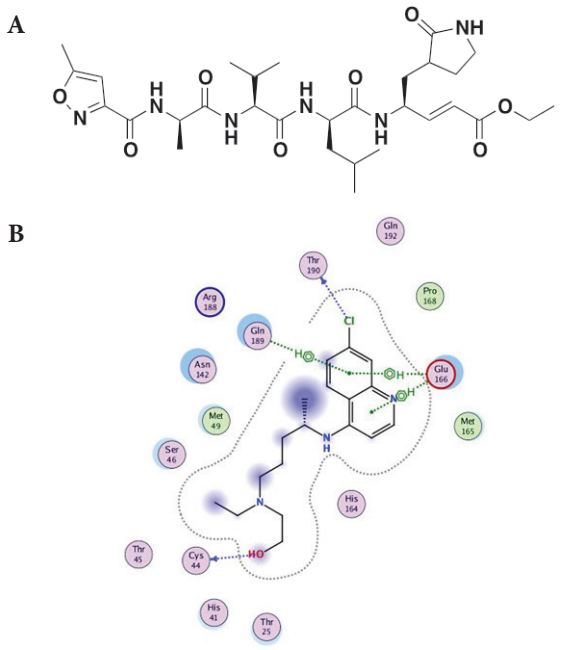

Figure 4. (**A**) Structure of standard inhibitor of SARS-CoV-2 main protease, N3, (**B**) 2D pose for the interaction of hydroxychloroquine into the active site of SARS-Cov-2 main protease enzyme.

Table 1. Energy scores, types of interactions observed for the complexes formed from coumarin drugs **1–13** and N3 with different amino acid residues in the active site of SARS-CoV-2 main protease.

Entry	Compound name	Energy score	Interaction		
1	Novobiocin	-8.17	CYS 145	H-bond	3.47
			THR 190	H-bond	2.89
			MET 165	H-bond	3.51
			THR 26	H-Bond	3.01
			GLN 189	Pi-H	4.12
2.	Clorobiocin	-8.69	CYS 145	H-Bond	3.59
			THR 25	H-bond	2.92
			GLN 189	Pi-H	4.15
3	Coumermycin	-9.30	CYS 145	H-bond	4.31
			THR 24	H-bond	3.01
			GLU 166	H-bond	2.82
			GLY 143	Pi-H	3.83
4	Umbelliferone	-4.62	GLN 192	H-Bond	3.08
			MET 165	Pi-H	4.62
			GLN 189	Pi-H	3.88
			GLN 189	Pi-H	4.13
5	Hymecromone	-4.79	GLN 192	H-acceptor	3.03
			MET 165	Pi-H	4.63
			GLN 189	Pi-H	3.86
			GLN 189	Pi-H	4.14
6	Dicoumarol	-6.34	MET 165	H-bond	3.52
			HIS 163	Pi-H	4.66
			GLN 189	Pi-H	3.56
7	Phenprocoumon	-6.11	CYS 145	H-bond	3.72
			MET 165	Pi-H	4.44
			GLN 189	Pi-H	4.12
8	Coumatetralyl	-5.79	MET 165	H-bond	3.67
			HIS 41	Pi-H	3.69
			GLN 189	Pi-H	3.92
9	Carbocromen	-7.01	CYC 145	H-bond	3.78
			CYS 145	H-bond	3.93
10	Cloricromen	-6.72	MET 165	H-bond	3.90
			GLY 143	H-bond	3.11
11	Acenocoumarol	-7.10	MET 165	H-bond	3.86
			GLN 192	H-bond	3.05
			MET 165	Pi-H	4.57
12	Batoprazine	-5.45	GLU 166	H-bond	3.12
			MET 165	Pi-H	4.58
13	Ensaculin	-7.42	CYS 145	H-bond	3.34
			GLU 166	Pi-H	4.28
	Standard N3 (standard)	**-8.52**	**GLU 166**	**H-bond**	**3.03**
			GLU 166	**H-bond**	**2.75**
			GLN 189	**H-bond**	**2.96**
			GLU 166	**H-bond**	**2.94**
			THR 190	**H-bond**	**3.40**
			GLN 192	**H-bond**	**3.29**
			HIS 41	**Pi-H**	**3.63**

Table 2. Energy scores, types of interactions observed for the complexes formed from natural coumarins **14–37** with different amino acid residues in the active site of SARS-CoV-2 main protease.

Entry	Compound name	Energy score	Interaction		
14	Glyclocoumarin	-6.58	MET 49	H-bond	3.97
			PHE 140	H-bond	2.82
			CYS 145	H-bond	3.11
15	Glycyrin	-6.84	ARG 188	H-bond	2.92
			GLU 166	H-bond	2.81
			GLU 166	Pi-H	4.33
16	Lipopyranocoumarin	-6.70	THR 26	H- bond	3.44
			GLY 143	H-bond	2.85
17	Suksdorphin	-7.05	ASN 142	H-bond	3.23
			HIS 41	Pi-H	4.18
18	Mesuol	-7.61	CYS 145	H-bond	3.48
			GLU 166	H-bond	2.99
			GLN 189	Pi-H	4.09
19	Isomesuol	-7.09	SER 46	H-bond	3.34
			GLY 143	H-bond	3.09
20	Calanolide A	-6.92	GLY 143	H-bond	2.72
			CYS 145	H-bond	3.28
21	Calanolide B	-6.75	CYS 145	H-bond	3.15
			GLY 143	H-bond	2.81
			CYS 145	H-bond	3.28
22	After B	6.69	CYS 145	H-bond	3.04
			CYS 145	H-bond	3.27
23	Same	-7.01	-	-	-
24	Psoralen	-4.79	GLN 192	H-bond	3.05
			GLN 189	Pi-H	3.83
			GLN 189	Pi-H	4.13
25	Xanthotoxin	-5.02	GLY 143	H-bond	2.94
			GLU 166	H-bond	3.00
26	Bergapten	-5.07	GLN 192	H-bond	3.04
			GLU 166	Pi-H	4.11
			GLN 189	Pi-H	3.85
			GLN 189	Pi-H	4.16
27	Chalepin	-6.17	GLU 166	H-bond	2.96
			GLY 143	H-bond	2.82
28	Pranferol	-5.95	HIS 41	H-Pi	4.11
			GLU 166	Pi-H	4.12
			GLN 189	Pi-H	4.37
29	Saxalin	-6.33	HIS 164	H-bond	3.24
			HIS 41	H-Pi	4.07
			GLU 166	Pi-H	3.93
			GLN 189	Pi-H	4.63
30	Isoimperaton	-6.02	HIS 41	H-Pi	4.06
			GLU 166	Pi-H	3.95
			GLN 189	Pi-H	4.48
31	Oxypecedanin	-6.13	HIS 41	H-Pi	4.09
			GLN 189	Pi-H	4.07
32	Oxy hydrate	-6.01	LEU 141	H-bond	2.86
			ASN 142	H-bond	2.72
33	Baykangelicin	-6.79	ASN 142	H-bond	3.39
			CYS 145	H-bond	3.38
			HIS 163	H-bond	2.89
			GLY 143	H-bond	3.21
			HIS 41	Pi-H	3.97
34	Heraclenol	-6.12	ASN 142	H-bond	2.84
			HIS 163	H-bond	3.19
			HIS 41	H-Pi	4.18
			GLN 189	Pi-H	4.30
35	Bayakan gelicol	-6.58	HIS 41	H-Pi	4.39
			GLN 189	Pi-H	4.25
36	Glycerol	-6.73	HIS 164	H-bond	2.94
			GLN 192	H-bond	3.50
			GLU 166	Pi-H	3.72
37	Wedeloactone	-5.78	MET 165	H-bond	3.95
			ARG 188	H-bond	2.82
			GLY 143	H-bond	3.13
			GLU 166	Pi-H	4.52
			GLU 166	Pi-H	3.85
			GLN 189	Pi-H	4.24

validated by following up with the MD simulation study mentioned later.

Data showed that most of the tested drugs could form stable complexes with the active site of SARS-CoV-2 main protease with energy scores ranging from -9.30 to -4.62 compared to -8.5 for N3, the standard ligand for M^{pro} enzyme. As in Umbelliferone, **4**, a primary coumarin nucleus fits into the receptor with potential binding with Methionine 165 and Glycine 189, 192 residues found in the active site actively pointing at the potential of coumarins to bind to M^{pro}. More stable complexes were observed with extended coumarins such as the NMDA receptor antagonist, Ensaculin, **13** with an energy score of -7.42, and potential binding with the essential cysteine residue CYS 145. Similarly, Acenocoumarol, **11**, and Carbocromen, **9**, also showed relatively stable complexes with energy scores of -7.1 and -7.01 and showing interactions with MET 165 and CYS 145, respectively, Table 1. The most stable complexes were observed with larger structures of aminocoumarin antibiotics, suggesting a possible high activity against SARS-CoV-2. Three aminocoumarin antibiotics were examined: Novobiocin, Clorobiocin, and Coumermycin. They fit into the N3 binding pocket and show stable complexes with energy scores of -8.17 for Novobiocin, -8.69 for Clorobiocin, and the most stable complex was observed with Coumermycin showing higher stability (-9.30) even than that observed with N3. They all showed potential binding interaction with the CYS 145 residue in M^{pro} active site; Table 1, Figures 5 and 6. Coumermycin also showed additional hydrogen bonding with THR 24, GLU 166, and one hydrophobic interaction with GLY 143 residues found in the active site, Fig. 6. Though Novobiocin showed more interactions (4 hydrogen bonds and one hydrophobic interaction, Table 1), the energy score suggests a more stable complex with Coumermycin. These data suggest the relative importance of the molecular size of the coumarin derivative in such settings. Surface mapping of M^{pro} active site reflected stable areas where bonding is formed with Coumermycin,**3**, Fig. 6A.

Previous reports suggested an antiviral activity of coumarin antibiotics. Novobiocin suppresses the replication of cytomegalovirus (Sekiguchi and Shuman 1997) and is used successfully in experiments to inhibit Zika virus with an EC_{50} of 25 μg/ml (Baz and Boivin 2019). It also blocks vaccinia viral assembly and morphogenesis and shows excellent activity against the herpes virus (Palu et al. 1986)., Ferrazzi et al. reported the antiviral activity of the Coumermycin, which is a carbohydrate with two coumarin groups, showing that Coumermycin inhibits the DNA polymerase activity resulting in suppressing the replication of herpes simplex virus type 1 (Palu et al. 1986; Ferrazzi et al. 1988). Reports also mentioned its ability to inhibit murine retrovirus replication (Varnier et al. 1984). Later evidence supported its potential use against HIV though the mechanism was not understood at that time (G. Tachedjian 1990). It was also used to control the African Swine Fever Virus (ASFV) (Coelho and Leitao 2020). Current docking results go following these previous data

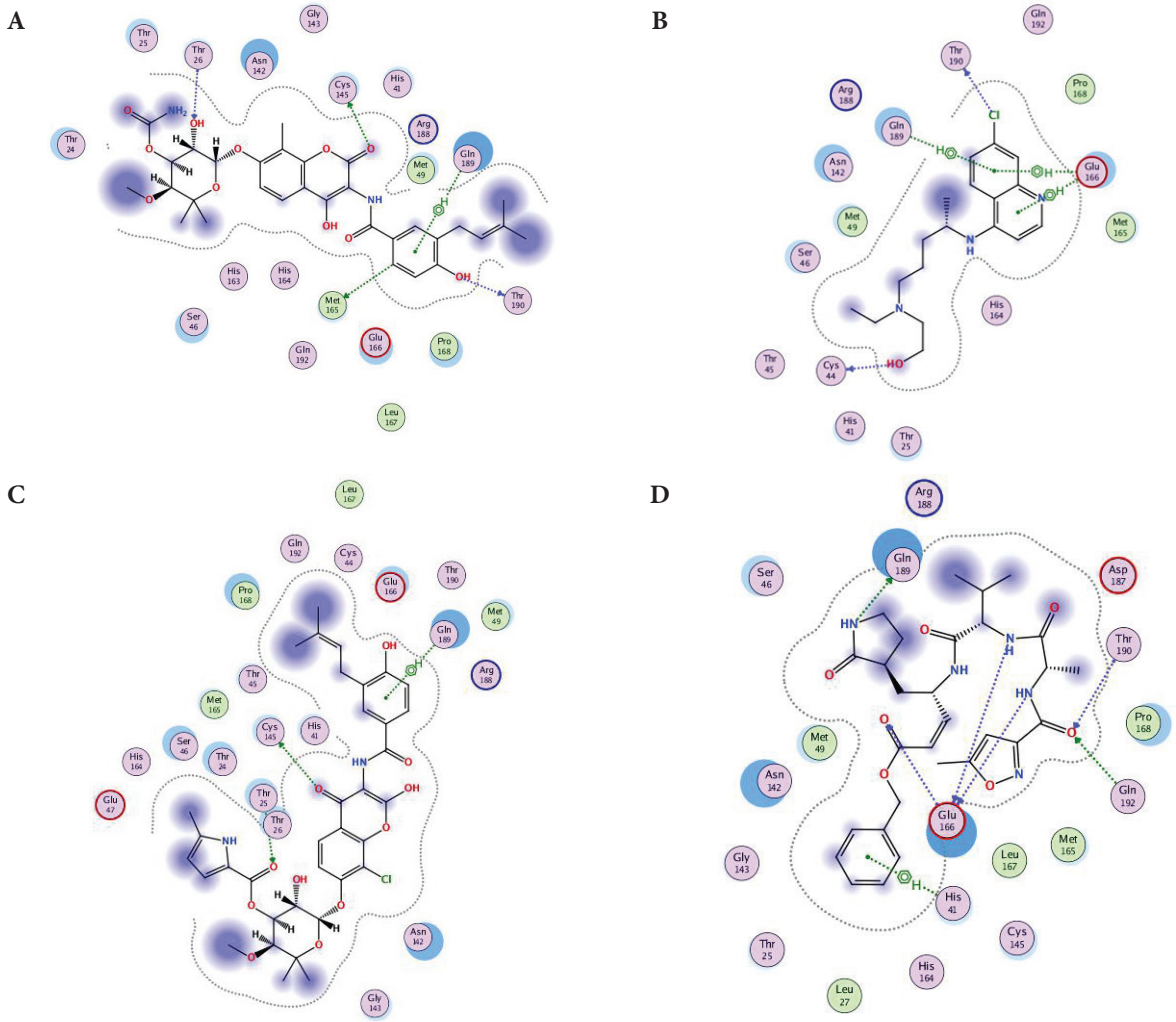

Figure 5. 2D poses of (**A**) Novobiocin; (**C**) Clorobiocin; (**B**) hydroxychloroquine; (**D**) N3; docked into the active site of SARS-CoV-2 main protease.

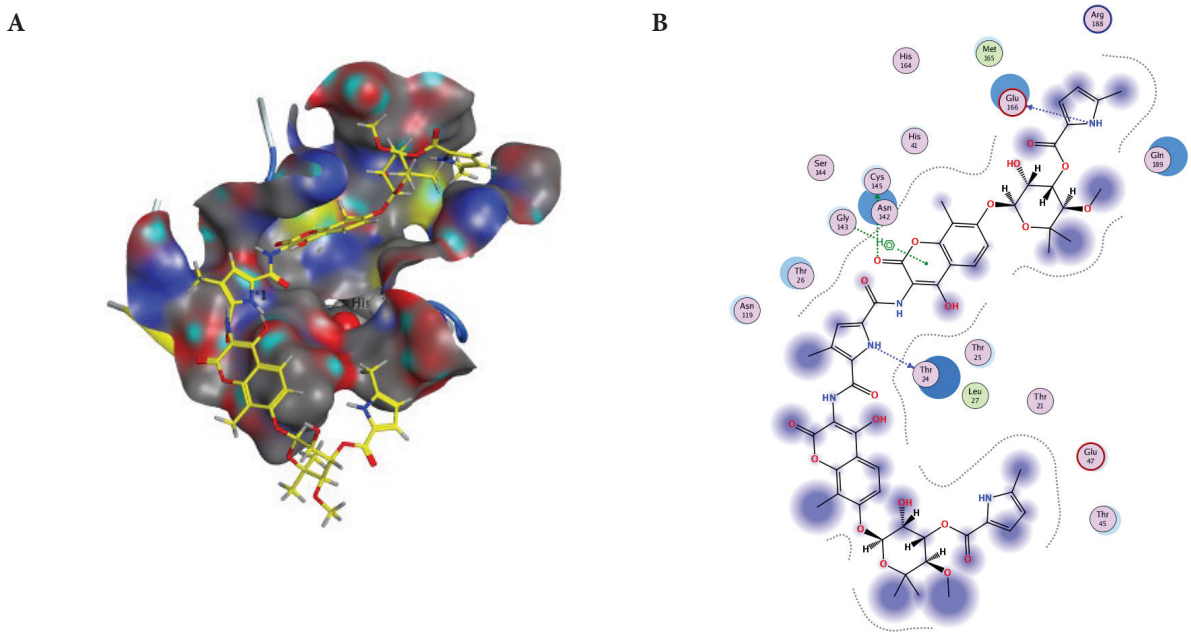

Figure 6. (**A**) surface map (**B**) 2D poses showing ligand interactions of Coumermycin docked into the active site of SARS-CoV-2 main protease (PDB: 5rh4).

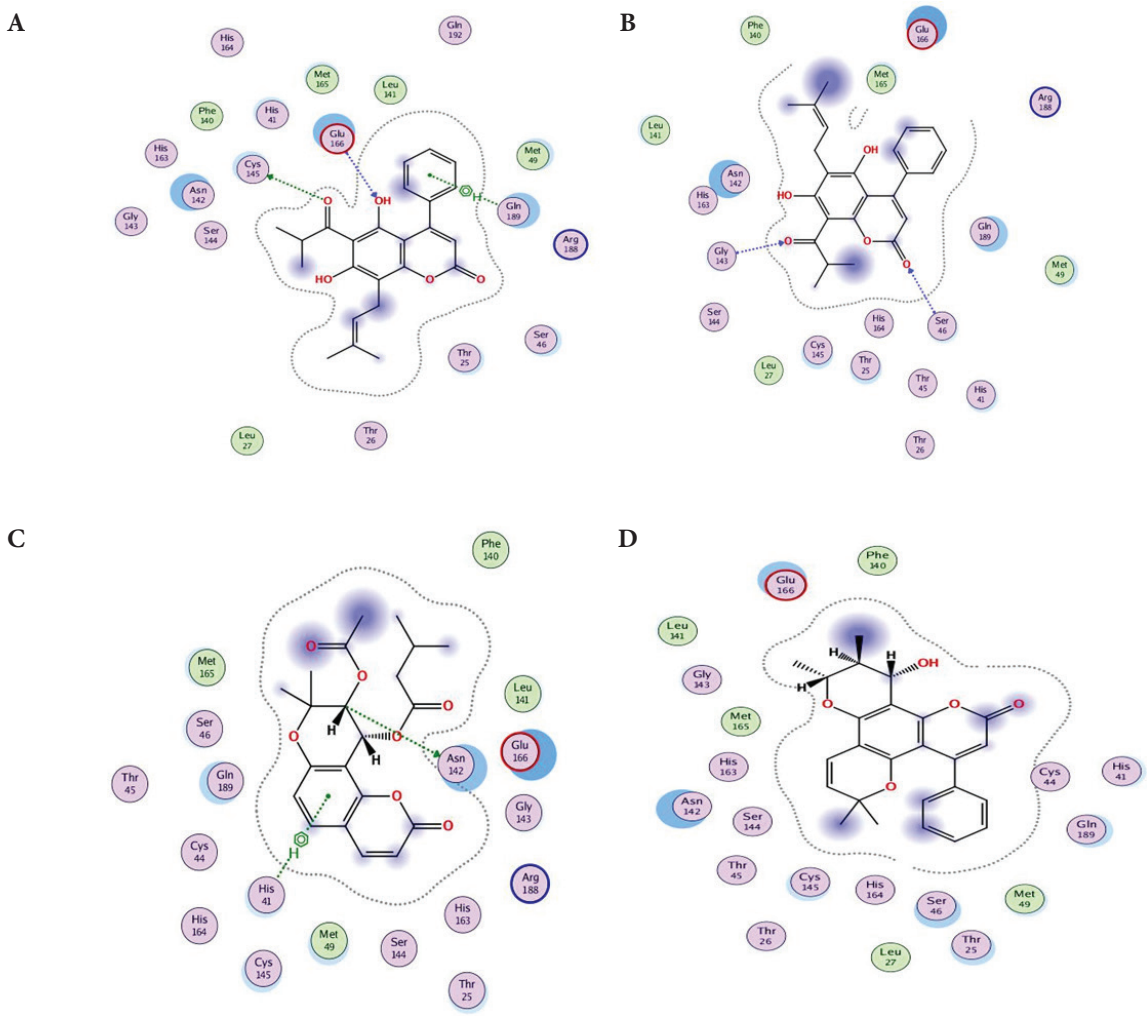

Figure 7. 2D poses of (**A**) Mesuol; (**B**) Isomesuol; (**C**) suksdorphin; (**D**) Calanolide; docked into the active site of SARS-CoV-2 main protease (PDB: 5rh4).

suggesting the possibility of using these drugs in the fight against COVID-19 worldwide struggle. Moreover, the high safety profile of Coumermycin also encourages taking these studies for further steps into *in vitro* testing to prove the anti-SARS-CoV-2 activity.

Meanwhile, docking naturally found coumarins revealed a formation of less stable complexes (energy scores of -4.79 to -7.61; Table 2). 4-Phenyl coumarins isolated from *Marila pluricostata*; Mesuol; **18** and Isomesuol; **19** reported earlier for their anti-HIV activity; showed the highest potential activity against M^{pro}. Mesuol **18** formed the most stable complex, possibly binding with CYS 145, GLU 166, and GLN 189; Fig. 7A. Switching the two-side chain into Isomesuol decreased the complex stability, and the number of observed interactions decreased with the disappearance of the CYS 145 interaction, Fig. 7B. The four tested Calanolides **20**–**23** also form relatively stable complexes, mainly binding with CYS 145 or GLY 143. Glycyrin **15** and Baykangelicin **33** also showed relatively stable complex with the binding pocket with potential binding with ARG 188, GLU 166, GLU 166 for Glycyrin and ASN 142, CYS 145, HIS 163, GLY 143 for Baykangelicin. Smaller sized coumarins gave far less stable complexes with the order of compound potential activity is **24**, **25**, **26**, **37**, **28**, **32**, **30**, **34**, **31**, **27**, **29**, **35**, **36**, **14**, **22**, **16**, **21**, **33**, **15**, **20**, **23**, **17**, **19** and **18**, Table 2.

Docking against SARS-CoV-2 PL^{pro} enzyme

The studied compounds were docked against the active site of papain-like protease (PL^{pro}) (PDB:6wx4). Docking against the active site of papain-like protease resulted in the formation of relatively stable complexes. The highest Amino coumarin antibiotics **1**–**3** and ensaculin **13** were the most potentially active inhibitors of the enzyme. They showed highly stable complexes with energy scores of -7.19, -7.54, -9.42 and -7.29 kcal/mol, respectively. Coumermycin showed the highest energy score with the highest number of possible interactions, including hydrogen bonds with ASP 164 and LYS 157 and various hydrophobic interactions with HIS 89, TYR 264, ASP 108, LEU 162, and TYR 268, Table 3, Fig. 8. Other coumarin drugs demonstrated less stable complexes with energy scores of -4.38 to -6.64 kcal8/mol, Table 3. A similar docking

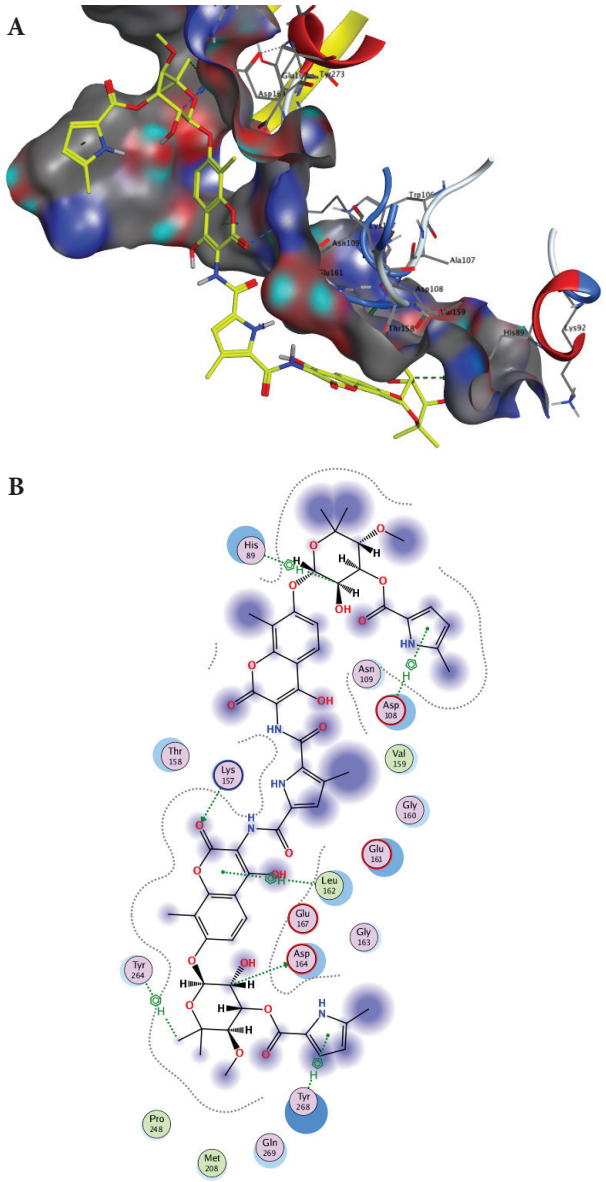

Figure 8. (**A**) surface map (**B**) 2D poses showing ligand interactions of Coumermycin; docked into the active site of SARS-CoV-2 papain-like protease (PDB: 6wx4).

was obtained for a virtual docking study for novobiocin against the active site of papain-like protease (Mitra et al. 2020), supporting the potential role of coumarin antibiotic hypothesised in the current study. An independent report also suggested using Coumermycin A1 to treat COVID-19 via blocking SARS-CoV-2 papain-like protease (PL^{pro}). They reported the ability of coumermycin A1 to bind to PL^{pro} with an energy binding score of -12 kcal/mol(ARULANANDAM 2020) according to data obtained in the current research.

Naturally occurring coumarins studied also yielded relatively stable complexes with the active site of PL^{pro}. Mesoul **18** and Isomesoul **19** formed the most stable complexes formed with energy scores of 6.12 -6.05 kcal/mol, Table 3. Collectively the ability of the two natural products to inhibit the two proteases strongly supports their potential use in the fight against SARS-Cov-2.

Table 3. Energy scores, types of interactions observed for the complexes formed from coumarin drugs **1–13 and 17–19** with different amino acid residues in the active site of SARS-CoV-2 papain-like protease.

Entry	Compound name	Energy score	Interaction		
1	Novobiocin	-7.19	TYR 268	H-bond	2.84
			LEU 162	H-bond	3.13
			LEU 162	Pi-H	3.83
2	Clorobiocin	-7.54	GLU 161	H-Bond	3.32
			LYS 157	H-bond	3.25
			TYR 264	H-bond	3.20
			LYS 157	Pi-cation	3.81
			LEU 162	Pi-H	3.89
3	Coumermycin	-9.42	ASP 164	H-bond	3.28
			LYS 157	H-bond	3.18
			HIS 89	H-Pi	4.18
			TYR 264	H-Pi	4.35
			ASP 108	Pi-H	3.86
			LEU 162	Pi-H	4.20
			TYR 268	Pi-H	3.57
4	Umbelliferone	-4.38	ASP 302	H-Bond	3.33
			ARG 166	H-bond	3.14
5	Hymecromone	-4.61	-	-	-
6	Dicoumarol	-5.85	TYR 264	H-bond	2.96
			ASP 164	Pi-H	4.09
7	Phenprocoumon	-5.57	PRO 247	Pi-H	4.41
8	Coumatetralyl	-5.36	ASP 164	Pi-H	4.66
9	Carbocromen	-6.41	PRO 248	Pi-H	3.60
			TYR 268	Pi-H	3.97
10	Cloricromen	-6.82	TYR 264	H-Pi	4.09
			TYR 268	Pi-H	3.87
11	Acenocoumarol	-6.64	ASP 164	H-bond	3.40
			TYR 264	H-bond	3.40
			PRO 247	Pi-H	4.45
			TYR 268	Pi-H	3.48
12	Batoprazine	-5.41	ARG 166	H-bond	3.29
			TYR 273	H-bond	2.76
13	Ensaculin	-7.29	TYR 264	H-Pi	3.96
			GLN 269	Pi-H	4.21
			GLN 269	Pi-H	3.95
17	Suksdorphin	-5.92	TYR 268	H-bond	3.28
			TYR 264	H-Pi	3.73
18	Mesuol	-6.12	ASP 164	Pi-H	3.65
19	Isomesuol	-6.05	LYS 157	H-bond	3.25
			ASP 164	Pi-H	3.60

Docking against SARS-CoV-2 RdRp enzyme

The studied compounds were also docked against the active site of RNA-Dependent RNA (RdRP) (PDB:7bv2). The docking methodology was validated via redocking the co-crystallised RdRp inhibitor Remedisivir with data obtained similar to that reported for Remedisivir (Rut et al. 2020). The results showed that compounds **1–13**, **18**, **19** and **33** demonstrated the most stable complexes and were reported in Table 4. Results showed that coumermycin **3** successfully fit into the Remedesivir binding pocket, forming hydrogen bonds with ASP 760 and neighbouring ARG 553 or ARG 555 residues. An -10.33 kcal/mol energy score implied a highly stable complex formation between compound **3** and amino acid residues present in the RdRP active site. The stable complex formed lacked the metal interactions formed by Remedisivir, Table 4, (Suppl. material 1). Unfortunately, the other coumarin-carrying drugs did not show similar stable complexes with energy scores ranging from -4.8 to

Table 4. Energy scores, types of interactions observed for the complexes formed from coumarin drugs 1–13, 18, 19, 33 and Remedisivir with different amino acid residues in the active site of SARS-CoV-2 RNA-dependant RNA polymerase (PDB: 7bv2).

Entry	Compound name	Energy score	Interaction		
1	Novobiocin	-4.80	ASP 618	H-bond	2.81
			LYS 551	H-bond	3.04
			ARG 553	H-bond	3.33
			MG 1005	Metal	2.05
2-	Clorobiocin	-4.97	ASP 623	H-bond	3.17
			ARG 553	H-bond	3.27
			MG 1004	Metal	2.05
3-	Coumermycin	-10.33	ASP 760	H-bond	3.08
			ASP 618	H-bond	2.99
			ASP 623	H-bond	3.70
			ARG 555	H-bond	3.43
			ARG 555	H-bond	3.21
			ARG 555	H-bond	3.20
			ARG 569	H-bond	3.22
			LYS 551	H-bond	2.32
			LYS 551	H-bond	3.22
4	Umbelliferone	-4.91	ARG 553	H-Bond	3.41
5	Hymecromone	-5.15	SER 682	Pi-H	3.81
6	Dicoumarol	-5.86	ARG 555	H-bond	3.26
7	Phenprocoumon	-5.71	SER 682	Pi-H	3.94
8	Coumatetralyl	-6.01	SER 682	Pi-H	3.75
			SER 682	Pi-H	4.45
9	Carbocromen	-5.72	SER 682	Pi-H	3.98
10	Cloricromen	-5.06	ASN 691	H-bond	2.99
			ARG 553	H-bond	3.13
			ARG 553	H-bond	3.13
11	Acenocoumarol	-5.63	ARG 555	H-bond	3.00
12	batoprazine	-5.41	MET 542	H-bond	3.90
			ARG 553	H-bond	3.29
13	Ensaculin	-6.81	SER 681	H-bond	3.43
			SER 682	H-bond	3.31
33	Bayakangelicin	-8.15	ARG 553	H-bond	3.02
			Mg 1004	Metal	2.25
			MG 1004	Metal	2.31
			MG 1004	Metal	2.11
			ARG 555	Pi-H	4.02
18	Mesuol		ARG 553	H-bond	3.04
			ARG 553	H-bond	2.93
			MG 1004	Metal	1.98
19	Isomesuol	-7.79	ARG 553	H-bond	2.94
			MG 1004	Metal	2.02
	Remdesivir	-9.85	ASP 760	H-bond	2.77
			ARG 553	H-bond	3.37
			ARG 553	H-bond	3.04
			MG 1004	Metal	2.15
			MG 1005	Metal	2.18
			MG 1004	Metal	2.10
			MG 1005	Metal	2.04
			MG 1005	Metal	2.18
			MG 1005	Metal	2.04
			ARG 555	Pi-cation	3.48

-6.8 kcal/mol compared to -9.3 for Remedisivir. Natural products **14**–**37** also demonstrated an excellent fit to the same active site with energy scores of -5.2 to -8.1 kcal/mol. The compounds were most stable. Complexes formed were reported in Table 4 for compounds **33**>**19**>**18**. Compound **33** showed a hydrogen bond formed with ARG 553, metal interactions with Mg ion ana hydrophobic interaction with ARG 555 similar to that observed with remedesivir. Mesuol **18** and Isomesoul **19** consistently demonstrated reliable interactions against this third protein, Table 4, reflecting their potential use in the fight against COVID-19.

Collectively, the literature supported the obtained results; virtual docking suggested an anti-protease role against SARS-Cov-2 M^{pro} for coumarins found in *Salvadora persica* (Ferrazzi et al. 1988). Flavonoids isolated can bind successfully to the **N3** binding site with the ability to bind to CYS 145, as observed in the current study. Binding energies were similar to those surveyed here, ranging from -7.4 to -8 kcal/mol (Bhuiyan et al. 2020). Additionally, 17 coumarin derivatives demonstrated stable interaction against RdRp with binding affinities of less than -10 and regular interactions were made with ASP 623, THR 556, LYS 621, and Pro 620 residues (Ozdemir et al. 2020). Other natural and synthetic coumarins were studied and showed similar capabilities in inhibiting SARS-Cov-2 main protease. They were theoretically bound to GLY 143 and GLN 198 with hydrogen bonds while forming hydrophobic interactions with ASN 142, CYS 145, HIS 164 and MET 165 (Chidambaram et al. 2020). The same set of amino acid residues was embodied in the binding poses obtained in the current study. On the experimental level, a coumarin derivative isolated from the Marine Sponge *Axinella cf. corrugata* inhibited M^{pro} of the earlier version of SARS viral, inhibiting its replication (de Lira 2007). In addition, another *in silico* study showed that novobiocin has a potential inhibition against RdRp (Wu et al. 2020a), and Coumarins isolated from *A. keiskei* showed inhibitory effects against the SARS-CoV M^{pro} and PL^{pro} (Park et al. 2016).

Molecular dynamics (MD) simulation

MD simulation is an in-silico method commonly used to study the dynamic behaviour and stabilisation of the protein and ligand complex during different conditions (Mirza and Froeyen 2020). The molecular dynamics simulation of the docked Coumermycin as the most stable complex formed with the three studied enzymes was done. MD simulation was employed to validate the docking study. It also further evaluated protein ligands stability. TIP3P solvent model was applied with an orthorhombic-shaped boundary box. The OPLS-2005 force field was used to neutralise the system by adding the Na+ salt. The Protein-ligand system was minimised by a hybrid method of the steepest descent method and LBFGS algorithms. The MD simulation was conducted at 50 ns of initial confirmation of the three complexes obtained after the docking of coumermycin with the three studied enzymes by Desmond. Coumermycin complexes were selected as they were the most stable complexes formed throughout the whole study. Results of MD were documented by reporting RMSD, RMSF, Rg, SASA and protein-ligand interactions.

RMSD

The configuration and vigorous properties of protein/ligand complexes during the simulation time of 50 ns were studied as the backbone RMSDs. The RMSD was calculated as the mean distance of complexes between atoms presents in the spine and is obtained from the following equation (Liu and Kokubo 2017).

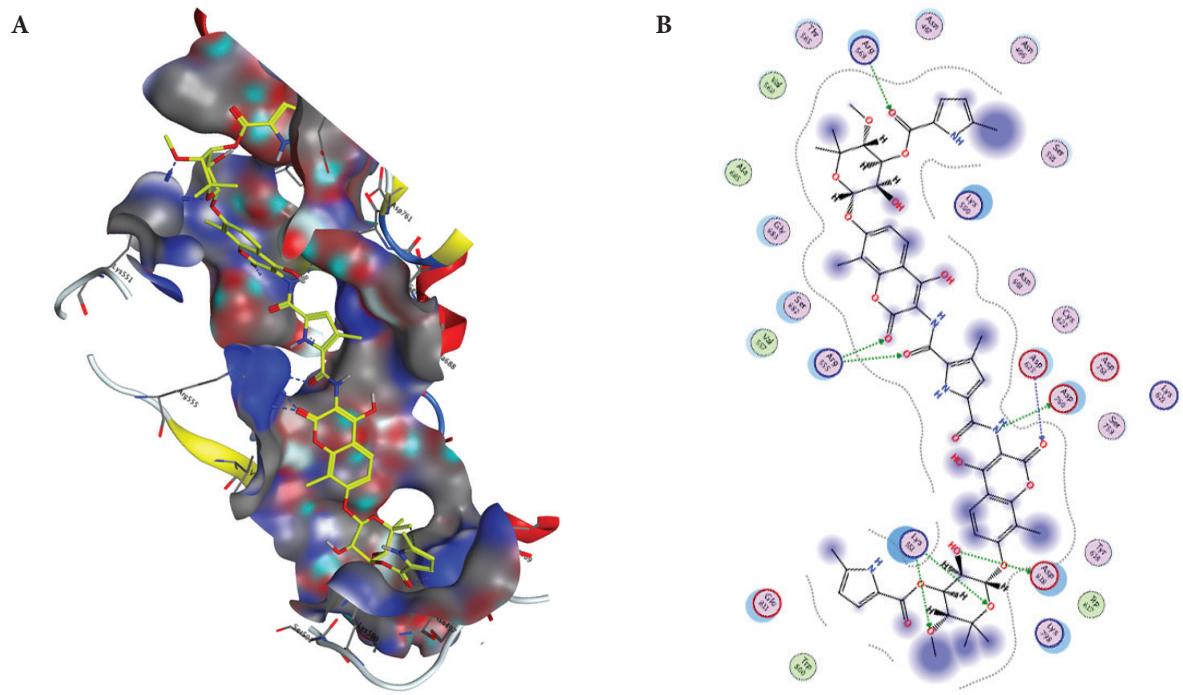

Figure 9. (**A**) surface map (**B**) 2D poses showing ligand interactions of Coumermycin; docked into the active site of SARS-CoV-2 RNA-dependent RNA polymerase (PDB: 7bv2).

$$\underline{RMSD} = \frac{1}{N}\sum_{i=0}^{N}\delta\frac{2}{i=0} \qquad 1).$$

In equation (Al-Khafaji et al.), the N is the complete no. of atoms present in an equation, and d indicates the subsequent distance of particles between the N pairs. The backbones RMSD of the three complexes are shown in Fig. 10.

The RMSD of the complex of Coumermycin/M^{pro} detected a minimal deviation at 0.05 nm from 5 to 35 ns. After 35ns, the complex showed stabilisation during the 50 ns MD simulation.

Similarly, the RMSD of complex Coumermycin/PL^{pro} represented a minor deviation at 0.05 nm from 1 to 5 ns, and it stabilised throughout the 50 ns of simulation. The RMSD of complex Coumermycin/RdRp was steady in 50 ns simulation from 0 to 20 ns, with a slight deviation of 0.015 nm observed throughout the simulation. While overall, the simulation was kept stable throughout the 50 ns simulation. Initially, the RMSD values increased steadily and stayed converged over the simulation time for the three complexes studied (Fig. 10). It is worth mention-

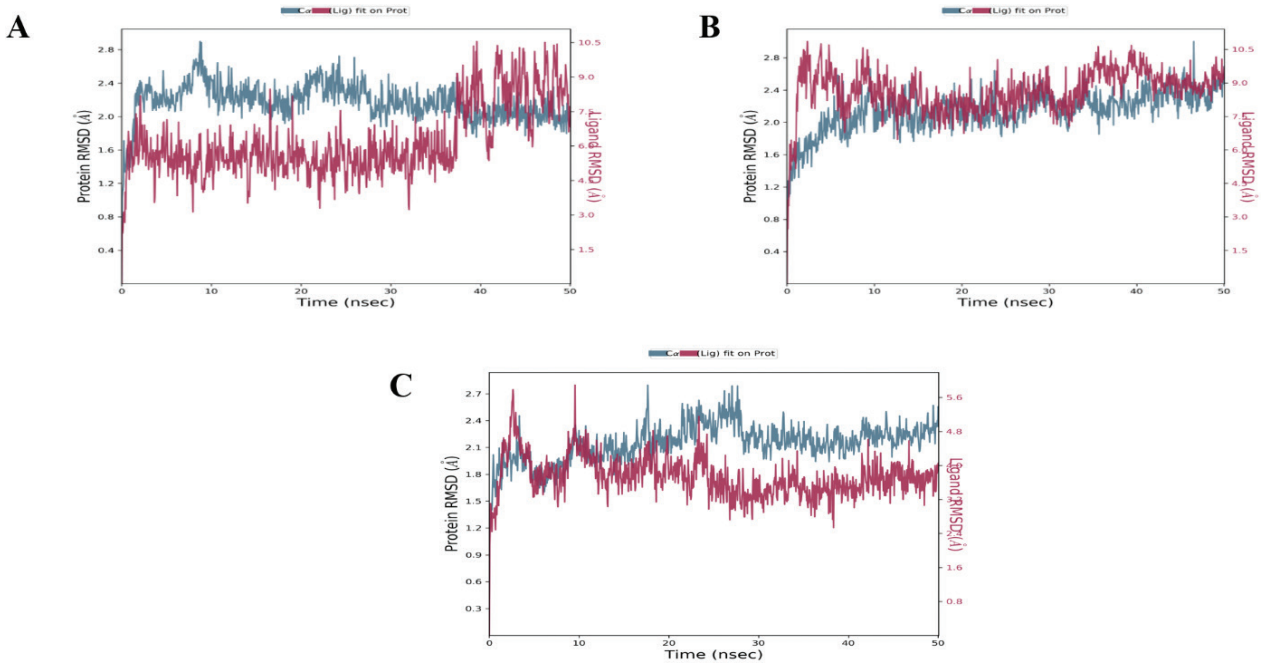

Figure 10. The RMSD plot of Coumermycin complex with SARS-CoV-2 (**A**) Mpro (**B**) PLpro (**C**) RdRp, at 50 ns simulation.

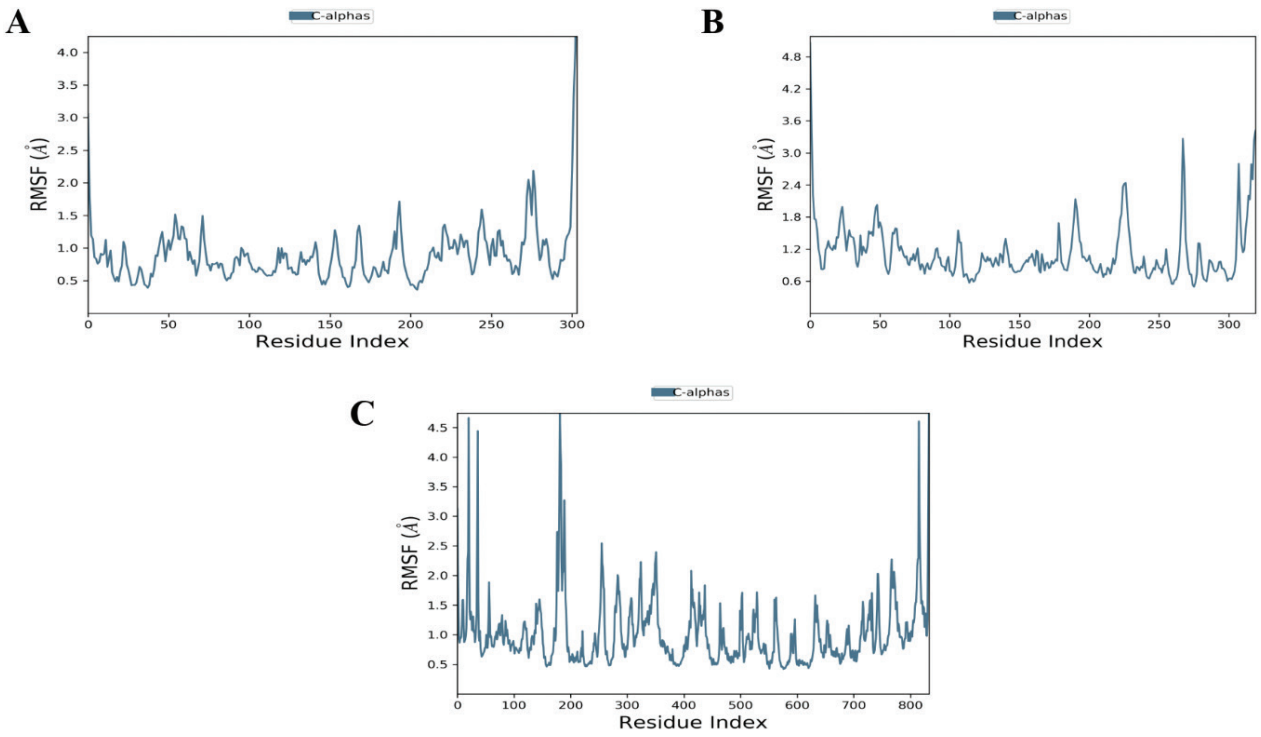

Figure 11. The RMSF plot of Coumermycin complex with SARS-CoV-2 (**A**) Mpro (**B**) PLpro (**C**) RdRp, at 50 ns simulation.

ing; all three complexes had RMSD descriptors that did not exceed 2.5 Å, which ascertains the rigid conformation of the formed complexes.

RMSF

The RMSF calculated the flexibility of common protein and showed an unpaid parameter to examine residual protein's flexibility over the simulation era (Alamri et al. 2020; Chinnasamy et al. 2020). The RMSF of complexes solvent-accessible surface area were observed between the range of 0.5 ± 1.8 Å, 0.6 ± 2.4 Å and 0.5 ± 1.5 Å. The RMSF plot of the complex with both proteases showed minor variations in both complexes. Simultaneously, the RdRp complex showed a higher fluctuation at the C and N terminals of protein (Fig. 11). During the simulation α, the β elements of the secondary structure make the protein structure more rigid.

The radius of gyration (Rg) is a framework for assessing the biological molecule's nature and stability during MD time by calculating the macromolecule's structures (L and Soliman 2016). The Rg values show the MRSD of an atom distance from the common centre of mass. The Rg may also be used to measure whether the complex can stay folded during MD simulation. The Rg values through the simulation at 50 ns of the Coumermycin/M^{pro}, PL^{pro} and RdRp complexes were 0.96 nm ± 1.12 nm, 0.96 nm ± 1.12 nm, 1.05 nm ± 1.14 nm, respectively, that confirming the structures had entered a steady-state. The average Rg values of the three complexes stayed reasonably constant for the 50 ns, suggesting a robust folded arrangement. (Fig. 12).

SASA

SASA or solvent-accessible surface area is a method that is used to calculate the water-accessible area of macromolecules (6). Monitoring the SASA value is a crucial method to estimate the conformational changes that result from dynamic interactions. The estimated average range of SASA values of three complexes solvent-accessible surface area for 50 ns simulation was between the 5 ± 10 nm^2, 5 ± 10 nm^2, 4.5 ± 6 nm^2, respectively. These findings indicated no improvements were found in all three systems' usability regions during 50 ns simulation time. Consequently, the relative constancy of our protein/ligand complexes has been derived from the SASA analysis (Fig. 13). It is worth mentioning that the SASA profile for the RdRp complex was lower than that observed with the other 2 proteins.

Protein-Ligand interaction analysis

Atomic-level knowledge is crucial to forecast the binding pocket of Coumermycin to the target protein's binding site and to validate docking data obtained earlier. The different intermolecular interactions such as hydrogen bonds, water bridges, hydrophobic and ionic interactions were investigated over 50 ns of MD simulation studies for critical mode evaluation. The study stated that the Coumermycin complex with SARS-CoV-2 M^{pro} made strong hydrogen bonding with the amino acid's residues THR 24, THR 25, THR 26, ASN 142, GLU 166, and GLN 189. It also formed strong water bridges interaction with amino acid residues CYC 22, CYS 24, THR 26, HIS 41, ASN 142, HIS 163, GLU 166, and GLN 189. The amino acid's residues GLU 47, HIS 164, GLU 166, and LEU 167 showed the hydrophobic interaction (Fig. 14A, Suppl. material 1). Similarly, the report stated that the Coumermycin/PL^{pro} showed the hydrogen bonding with amino acid's residues ASN-88, HIS 89, TRP 106, ASP 108, VAL 159, GLU 161, TYR 268, and CYS 270.

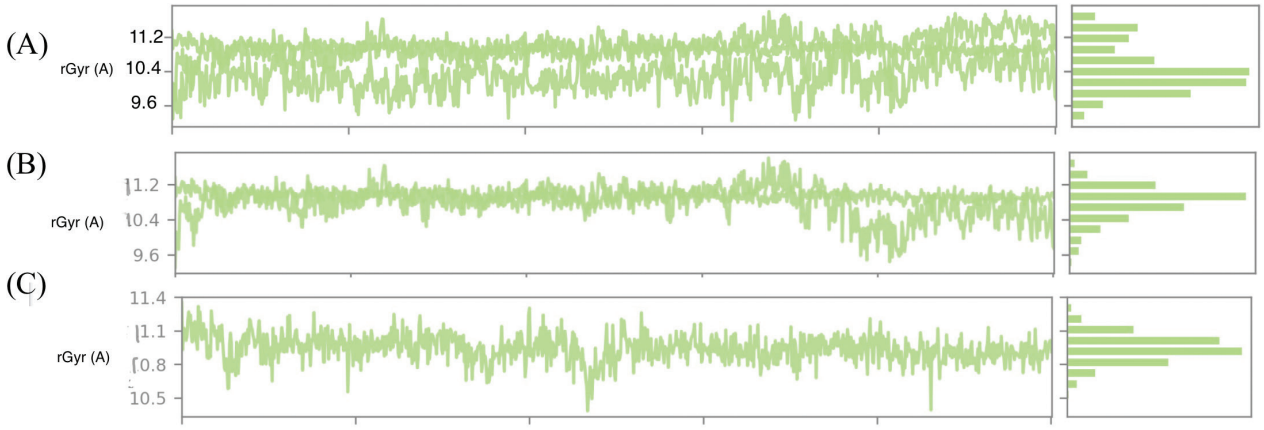

Figure 12. The time frame of evolution against the radius of gyration (Rg) of Coumermycin complexes with SARS-CoV-2 (**A**) Mpro (**B**) PLpro (**C**) RdRp, during 50 ns MD simulation.

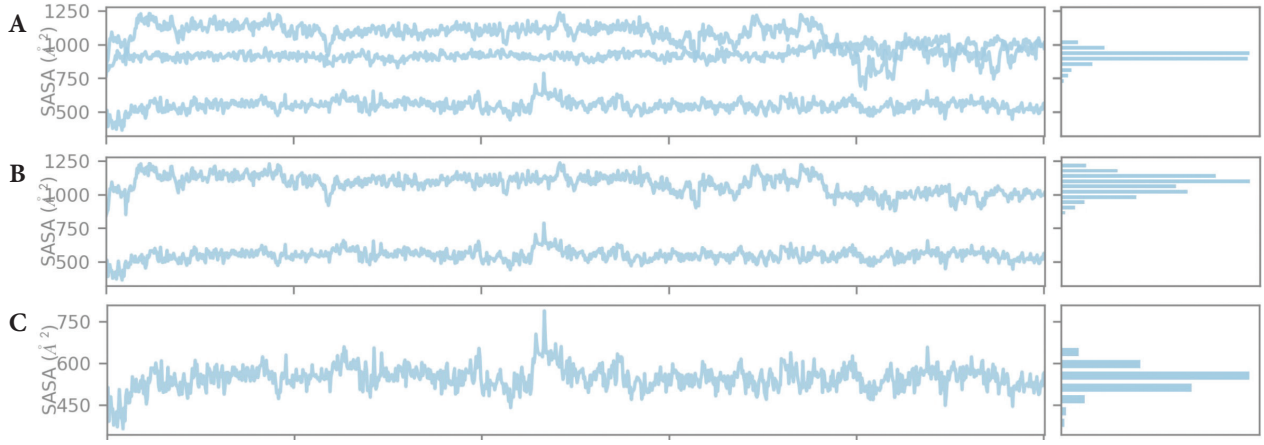

Figure 13. The time frame of evolution against SASA of Coumermycin complexes with SARS-CoV-2 (**A**) Mpro (**B**) PLpro (**C**) RdRp, during 50 ns simulation.

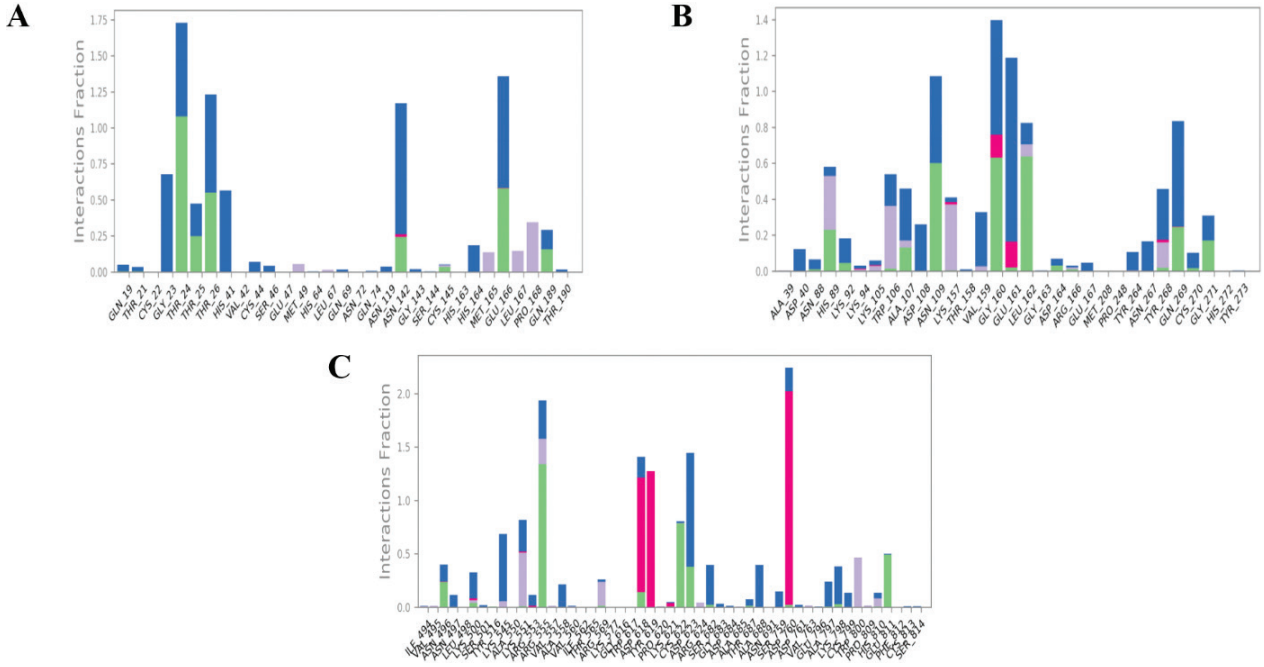

Figure 14. Protein interaction analysis. The green colour = hydrogen bonding, pink color = ionic interaction, grey colour = hydrophobic interaction and blue colour = water bridges showed in Coumermycin complexes with SARS-CoV-2 (**A**) Mpro (**B**) PLpro (**C**) RdRp during 50 ns MD simulations.

The Coumermycin/PL^{pro} formed ionic interaction interactions with the amino acid's VAL 159 and GLY 160. Further, the amino acid's residues ALN 39, LYS 105, ASN 109, and ASN 267 were also implied in hydrophobic interaction with Coumermycin/PL^{pro} (Fig. 14B, Suppl. material 1). The study showed that the Coumermycin/RdRp demonstrated strong hydrogen bonding with the amino acid's residues VAL 495, AR 553, TRP 617, LYS 621, CYS 622, and HIS 810. Along with this, Coumermycin/RdRp formed strong ionic interaction with amino acid residues TRP 617, ASP 618, and SER 759. The amino acid's residues ALA 550, THR 565, and CYS 799 were involved in hydrophobic interaction (Fig. 14C, Suppl. material 1).

Conclusions

The emergence of the COVID-19 pandemic introduced many global economic and health challenges. The starvation for a remedy for attacking SARS-CoV-2, the microorganism causing the pandemic, remains a priority till the current time. Drug repurposing could provide an answer for such a challenge providing safe and well-studied remedies. The present study introduces aminocoumarin antibiotics as potential drugs for treating COVID-19. Theoretically, these drugs can potentially stop viral growth via interfering in the SARS-CoV-2 main protease enzyme, papain-like protease, and RNA-Dependent RNA polymerase enzymes activities. Molecular simulations also supported the use of Coumermycin against SARS-CoV-2 different enzymes as it formed a stable, rigid complex with the studied enzymes. Though coumarins offer a safe pool of compounds of a potential multitarget antiviral activity, the demand for extensive biological studies remains required to support the hypothesised activity. Still, the study offers a solid lead supported by previous use of these agents as antiviral agents.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary material 1

Figures S1–S10

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Data type: Images (pdf. file)

Explanation note: Molecular docking and Dynamic simulations study for repurposing of multitarget Coumarins against SARS-CoV-2 main protease, papain like protease and RNA-Dependent RNA polymerase.

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