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In Silico Investigation Of Pyrimidine-Coumarin Hybrids From The Perspective Of Multi-Target Screening As COX And SARS-Cov-2 Inhibitors

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Abstract: *A chemical database of 15 representative pyrimidine-coumarin derivatives were built which were previously synthesized novel structures, developed and analyzed in-vivo by our research team, exhibited significant analgesic and antipyretic properties. To further explore their potential mechanisms, we conducted molecular docking studies against cyclooxygenase (COX 1 and COX 2) enzymes, which are key mediators of inflammation and pain, as well as COVID-19 target proteins [cathepsin L and main protease (Mpro)] using molecular docking, involved in viral replication and immune response modulation. The docking results demonstrated strong binding affinities, suggesting that these compounds may exert their analgesic and antipyretic effects through COX inhibition while also potentially interacting with viral proteins relevant to COVID-19 treatment comapared to standard drugs. These findings highlight the dual therapeutic potential of the synthesized molecules and warrant further in vitro and in vivo evaluation. It was concluded that in general, pyrimidine-coumarin derivatives scaffolds have the potential of being selective against all the four receptors. Besides, drug-likeness and toxicity issues of the tested ligands were predicted using ADMET lab 2 and ProTox-II web servers.*

Key Words*: pyrimidine-coumarin derivatives; molecular docking; insilico analysis; pharmacokinetic prediction; COVID-19 inhibitors; COX inhibition*

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. I. Introduction

Common physiological reactions to inflammation include pain and fever, which are mostly caused by prostaglandins, which are produced by the enzyme cyclooxygenase (COX). Nonsteroidal anti-inflammatory drugs (NSAIDs) exert their analgesic and antipyretic effects by inhibiting COX-1 and COX-2, key enzymes involved in prostaglandin biosynthesis. A prevalent class of analgesics that target the cyclooxygenase (COX) iso-enzymes are non-steroidal anti-inflammatory drugs (NSAIDs). Around the world, they are employed for a variety of medicinal purposes. They are regarded as one of the best families for treating a variety of conditions, including rheumatism and arthritis, and for their usage as analgesics because of their wide range of pharmacological activities, which include antipyretic, analgesic, and anti-inflammatory effects. Additionally, this family includes aspirin (acetylsalicylic acid), which has been in use for over a century.[1] Although NSAIDs are frequently used to treat acute pain, their usage is constrained by side effects such as platelet inhibition, renal impairment, and gastrointestinal bleeding. Among all pharmacological classes, they have one of the highest related death rates. COX-1 and COX-2 are important mediators of pain and inflammation.[2] Low dosages of aspirin can lower temperature and pain, but greater amounts are needed to have anti-inflammatory benefits. For analgesic and antipyretic properties, it mainly inhibits COX-1; for anti-inflammatory effects, it inhibits COX-2. According to in vitro research, aspirin is quite selective for COX-1.[3] salicylate salts have historically been used as analgesics, antipyretics, and anti-inflammatory medications[4]. The majority of conventional NSAIDs on the market today work by blocking the prostaglandin (PG) G/H synthase enzymes, also referred to as cyclooxygenases which have antipyretic, analgesic, and anti-inflammatory properties.[5]

COVID-19, caused by the new SARS-CoV-2 virus, has triggered a huge global public health disaster. Even though a number of successful vaccines have been created and made available, the ongoing appearance of novel variations continues to be a problem. Significantly, the delta (B.1.617.2) variant spread quickly throughout several continents, and the omicron form seems to be much more contagious despite exhibiting some resistance to current vaccinations. Furthermore, vaccines are not as commonly available or accepted as pharmaceutical therapies, and people with compromised immune systems might not respond well to vaccination. Infection with SARS-CoV-2 increases the transcription of the CTSL gene and the activity of its enzyme. In turn, increased CTSL expression promotes SARS-CoV-2 infection. The delta (B.1.617.2) variant of SARS-CoV-2 has spread quickly across continents, and the omicron variant, a recently emerged SARS-CoV-2 variant, may be

more transmissible than all the previous variants and partially resistant to current vaccines. Despite the implementation of several effective vaccines, new variants of the virus are still emerging.[6] Because of its significance in the viral life cycle and its remarkable conservation among other coronaviruses, the virus's primary protease (Mpro) is another desirable target for inhibitor development.[7,8]

A number of pharmaceutical molecules, such as anti-inflammatory and antiviral medicines with strong binding affinities to various biological targets, can be designed and synthesized using Coumarin[9–15] and pyrimidine[16–19] motifs as a favored scaffold and model framework. Through the use of combinatorial chemistry and a privileged structure approach to drug development, they can be readily altered to meet "the rule of five" of Lipinski and become a drug-like molecule. As antiviral drugs, they are both extensively researched as COX inhibitors, which draws scientists' attention to their potential importance in preventing other viral illnesses.[20]

The encouraging biological results pointed to a possible mechanism involving COX inhibition. Because COX-inhibitors currently have such severe side effects, it is imperative to find newer drug molecules that can effectively reduce inflammation and pain while lowering side effects like cardiac issues. Given the significance of COX inhibition in pain and fever management, the search for novel COX inhibitors with improved efficacy and reduced side effects remains an active area of pharmaceutical research. In our previous study[21], we synthesized a novel series of 4-[4-(6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-chromen-2-ones [5-7(a-e)] (**Figure-1**) and evaluated their in vivo analgesic and antipyretic activities, prompting us to further investigate their binding interactions with COX-1 and COX-2 through molecular docking studies. This study, highlighted their potential as COX inhibitors for pain and fever management.

The search for novel therapeutic agents with dual or multi-target potential also has gained significant attention in drug discovery. Given the increasing evidence of shared inflammatory pathways between fever, pain, and viral infections, we hypothesized that these chromen-2-one derivatives might exhibit additional therapeutic benefits as potential inhibitors of key viral proteins involved in SARS-CoV-2 pathogenesis. In this study, we report detailed multitarget molecular docking simulations of our previously synthesized compounds against COVID-19 and COX target proteins, aiming to explore their binding affinities and mechanisms. This computational approach serves as a preliminary step towards repurposing analgesic and antipyretic scaffolds for antiviral drug development emphasizing their potential as multifunctional therapeutic agents. This study also presents docking analyses, correlating computational findings with previously observed biological activities, to propose these compounds as potential candidates for further development.

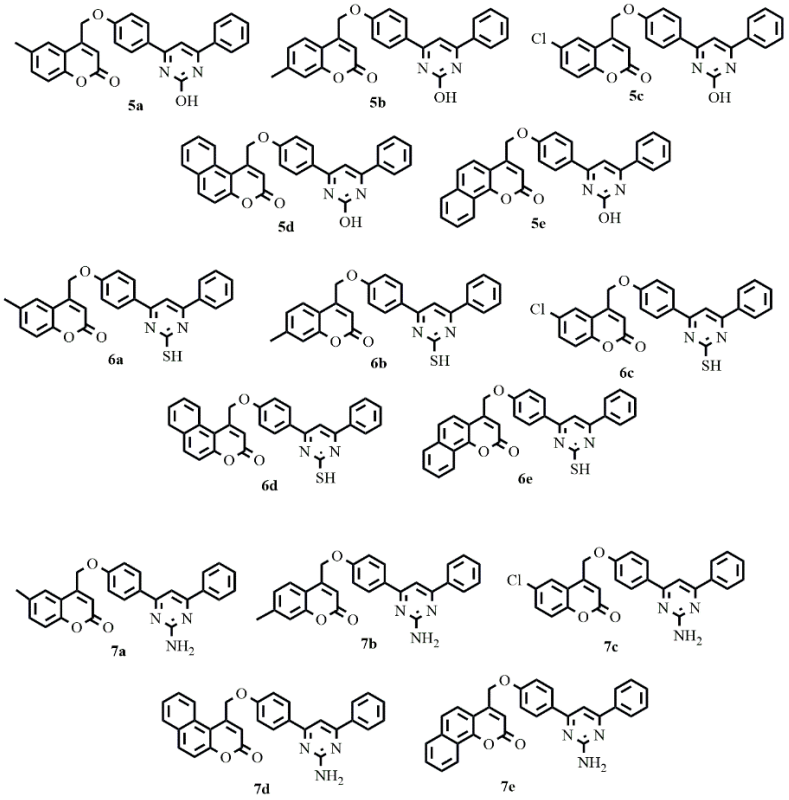

Figure-1 Structures of pyrimidine-coumarin analogs [(5-7)a-e]

II. Materials and Methods

2.1. Preparation of ligands

ChemDraw software was used to prepare the ligand structures (**Figure-1**). For a subsequent docking step, the produced ligands were stored as SDF and PDBQT extensions.

2.2. Protein selection, molecular docking

The crystal structures of proteins were acquired from the RCSB Protein Data Bank (PDB) based on resolution and R factors specifically 3KK6[22], 3LN1[23], 2XU3 and 6M0K[24]. The proteins were made ready using AutoDock Tools v.1.5.7. to remove unwanted co-crystallized molecules, to add charges (Gaussian, Gasteiger), and to incorporate polar hydrogens. Docking was achieved using AutoDock Vina [25]. Using PyMOL and Discovery Studio, post-docking analysis of protein-ligand interactions was performed. Our research team has already published the synthetic procedure and characterisation of pyrimidine-coumarin derivatives, and they are accessible in the literature..[21]

2.3. *Grid parameter setting and docking calculation*

The active site of the proteins under study was the focal point of grids of appropriate sizes with default spacing that included all ligand atom types. The supporting information lists the parameters that were used in sizing the grid box. Root mean square deviation (rmsd) was used to cluster the docking data, and free energy of binding was used to rank them.

2.4. Druglikeness and toxicity properties

The ligands were predicted for their druglikeness using SwissADME[26]. Using the ProTox-II[27] program, the toxicity profile of each ligand was determined.

III. Results and Discussion

3.1. Molecular docking

Predicting the probable binding geometries of a possible ligand of a known three-dimensional structure with a target protein is the main goal of molecular docking. A series of pyrimidine-coumarin analogs [(5-7)a-e] were examined *in silico* in this study to illustrate their potential binding energies and ways of interaction with four crystal structures, COX-1 (PDB code: 3KK6), COX-2 (PDB code: 3LN1), cathepsin- L (PDB code: 2XU3) and main protease of SARS-CoV-2 (PDB code: 6M0K) (**Table-1**).

Ligands	Receptors (KCal/mol)			
	Cathepsin L (2XU3)	Main protease (6M0K)	COX-1 (3KK6)	COX-2 (3LN1)
5a	-10.8	-8.8	-10.6	-11.4
5b	-10.5	-8.8	-10.2	-11.6
5c	-10.7	-8.8	-10.7	-9.8
5d	-11.6	-9.1	-11.2	-11.0
5e	-11.1	-9.4	-10.8	-11.1
6a	-10.4	-7.9	-11.1	-11.4
6b	-10.4	-8.8	-11.4	-10.4
6c	-10.3	-8.2	-10.4	-9.8
6d	-11.1	-8.6	-12.0	-10.0
6e	-10.6	-9.3	-11.0	-11.8
7a	-10.9	-8.5	-11.2	-9.8
7b	-10.6	-8.9	-11.7	-10.0
7c	-10.8	-8.2	-10.3	-11.6
7d	-11.8	-9.4	-10.9	-12.5
7e	-11.1	-9.3	-11.8	-12.9
Aspirin (Cox2)	----	----	----	-7.2
Analgin (Cox1)	----	----	-6.5	----
Nirmatrelvir	-8.5	-7.4	----	----

Table 1 Selected Binding Energies of the pyrimidine-coumarin analogs [(5-7)a-e]

According on the results of the docking screening, targeting COX-1 and COX-2 receptors, three strongly binded analogs (5d, 6d and 7e) and (5b,6e and 7e) were chosen to characterize the binding mechanism with 3KK6 (COX-1) and 3LN1 (COX-2). **(Table-2 & 3).** The 3D and 2D diagrams show how the inhibitors interacts with the surrounding residues of COX-1 and COX-2 receptors (**figure-2 & 3).** The docking findings were imported into PyMOL and Discovery Studio Visualizer to achieve this.

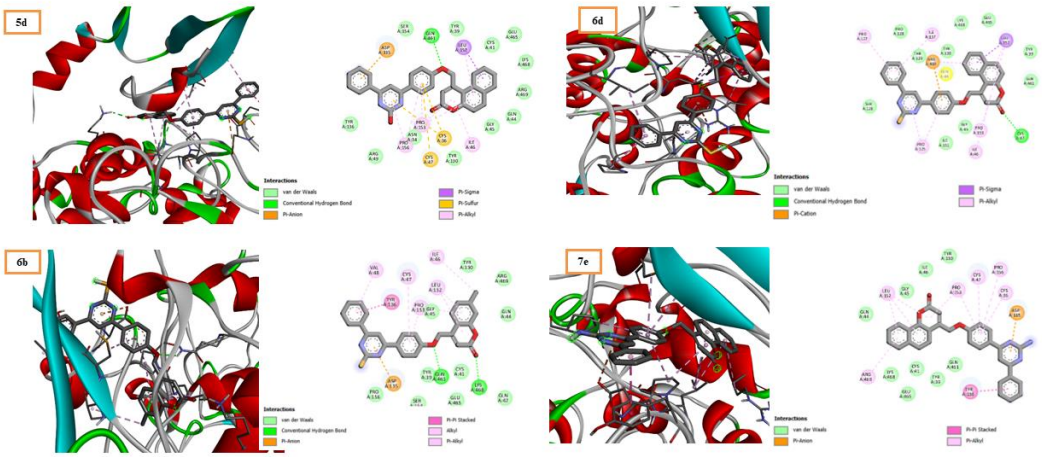

Figure-2 Representation of 3D & 2D interaction between selected strong nonbonding interaction of pyrimidine coumarin analogs with COX-1 receptor.

Drugs	Bond Category	Interaction type	Distance (Å)	Residues in contact
5d	Hydrogen Hydrophobic Hydrophobic Miscellaneous Hydrophobic Miscellaneous Miscellaneous Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrophobic	CH PA PA PSu PA PSu PSu PA PA PA PA PA PS	2.46 3.59 4.23 5.43 4.97 5.06 5.62 4.82 4.80 5.38 4.05 4.71 3.73	ASP135, PRO156, PRO103, ASN34, CYS36, CYS47, ILE46, GLN461, LEU152 (CHAIN-A)
6d	Hydrophobic Hydrophobic Hydrophobic Hydrophobic Electrostatic Hydrophobic Hydrophobic Hydrogen Hydrophobic Hydrophobic Hydrophobic Hydrophobic	PA PA PA PA PCat PA PA CH PA PA PS PA	4.63 5.21 4.18 5.11 3.88 4.33 5.44 2.36 5.49 4 .80 3.95 4.96	PRO127, ILE137, PRO125, ARG469, PRO153, ILE46, CYS47, LEU152 (CHAIN-A)
7e	Hydrophobic Electrostatic Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrophobic	PPS PAn PA PA PA PA PA PA PA PA PA	3.99 4.78 5.43 4.82 5.32 4.71 4.41 5.45 5.30 4.97 5.04	PRO153, ARG469, PRO156, CYS47, LEU152, TYR136, ASP135, CYS36 (CHAIN-A)

Table-2 Selective Nonbonding interactions data of pyrimidine-coumarin analogs [(5-7)a-e] with COX-1(3KK6) receptor. CH = Conventional Hydrogen Bond; A = Alkyl; PA = Pi-Alkyl; PS = Pi-sigma; PDH = Pi-Donor Hydrogen Bond; PPS = Pi-Pi Stacked; PPT = Pi-Pi *T*-shaped; PSu= Pi-Sulfur; PAn= Pi-Anion; PAn= Pi-Cation

Based on docking studies, the results showed that 5d, 6d and 7e are the most promising ligands, which bound more stronger with COX-1 (-11.2, 12.0, -11.8 kcal/mol) and 5b, 6e and 7e bound more stronger with COX-2 (-11.6, 11.8, -12.9 kcal/mol) respectively via many hydrophobic bonding ,hydrogen, electrostaticc and miscellaneous nonbonding interactions than standard inhibitors analgin (-6.5 kcal/mol) and aspirin (-7.2 kcal/mol). The docked poses clearly show that the drug molecules bind within the active site of the COX-1 and COX-2 macromolecular structures **(Figure-2 & 3).** The parent molecules 6d and 7e found to be strongly binded

to respective receptors COX-1 and COX-2 with the lowest binding energies among all other ligands, exhibited interactions with the key residues PRO127, ILE137, PRO125, ARG469, PRO153, ILE46, CYS47, LEU152 and ARG455, ARG29, LEU158, PRO139, CYS21, CYS32, PRO140, ALA142 respectively. Interaction of 6d with CYS47 and interaction of 7e with CYS32 through hydrogen bonding within a very closer bond distances 2.36 Å and 3.66 Å respectively. The interactions of analogs (5d, 6d and 7e) and (5b, 6e and 7e) with the COX-1 and COX-2 represented in (**Table 2 & 3)**. Docking tests revealed that all of the compounds could serve as possible agents for the construction of pharmaceuticals for COX inhibition, and the analogs with the lowest binding energy value among all those investigated may be the best potential COX inhibitors. All docking scores are detailed in the supporting documentation.

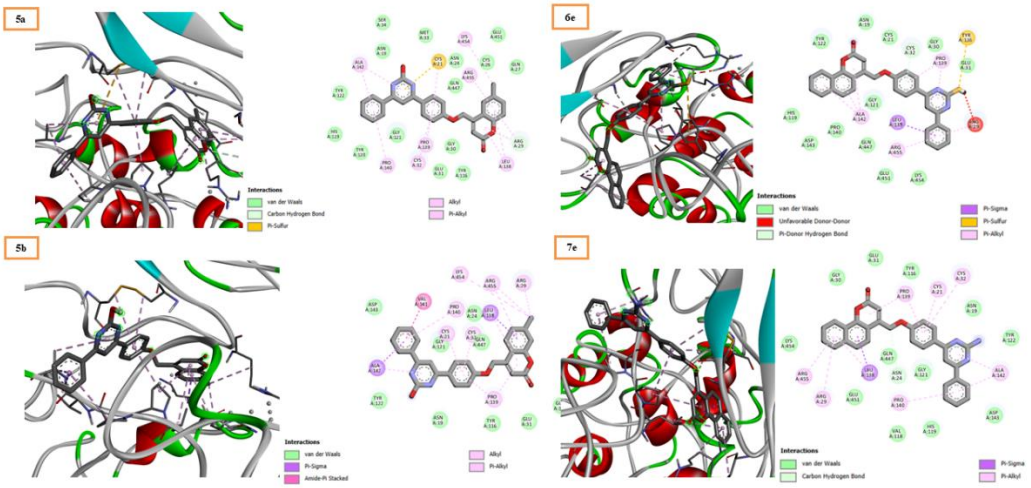

Figure-3 Representation of 3D & 2D interaction between selected strong nonbonding interaction of pyrimidine coumarin analogs with COX-2

Drugs	Bond Category	Interaction type	Distance (Å)	Residues in contact
5b	Hydrophobic	PA	5.14	ALA142, VAL141, PRO140, CYS21, CYS32, PRO139, LEU138, LYS454, ARG455, ARG29 (CHAIN-A)
	Hydrophobic	APS	4.37	
	Hydrophobic	PA	4.69	
	Hydrophobic	PA	3.47	
	Hydrophobic	PA	5.00	
	Hydrophobic	PA	5.15	
	Hydrophobic	PA	4.40	
	Hydrophobic	PA	5.02	
	Hydrophobic	PA	5.24	
	Hydrophobic	PS	3.68	
	Hydrophobic	A	4.67	
	Hydrophobic	A	5.08	
	Hydrophobic	A	4.51	
6e	Hydrophobic	PA	4.62	ALA142, CYS32, PRO139, LEU138, ARG455, ARG29, GLU31, TYR116 (CHAIN-A)
	Hydrophobic	PA	5.16	
	Hydrophobic	PA	3.99	
	Hydrophobic	PA	5.40	
	Hydrophobic	PA	4.39	
	Hydrogen	PDH	2.78	
	Hydrophobic	PA	4.74	
	Hydrophobic	PA	5.05	
	Hydrophobic	PA	4.10	
	Hydrophobic	PA	5.10	
7e	Hydrophobic	PA	5.30	ARG455, ARG29, LEU158, PRO139, CYS21, CYS32, PRO140, ALA142 (CHAIN-A)
	Hydrophobic	PA	4.94	
	Hydrophobic	PA	4.02	
	Hydrophobic	PA	5.04	
	Hydrophobic	PA	5.39	
	Hydrophobic	PA	4.65	
	Hydrogen	CaH	3.66	
	Hydrophobic	PS	3.82	
	Hydrophobic	PA	5.10	
	Hydrophobic	PA	4.79	
	Hydrophobic	PA	5.30	

Table-3 Selective Nonbonding interactions data of pyrimidine-coumarin analogs [(5-7)a-e] with COX-2 receptor.. CH = Conventional Hydrogen Bond; A = Alkyl; PA = Pi-Alkyl; PS = Pi-sigma; PDH = Pi-Donor

Hydrogen Bond; PPS = Pi-Pi Stacked; PPT = Pi-Pi *T*-shaped; PSu= Pi-Sulfur; PAn= Pi-Anion; PAn= Pi-Cation; APS=Amide Pi Stacked; CaH=Carbon-Hydrogen bond

Further in this investigation, series of pyrimidine-coumarin analogs were studied *in silico* to highlight their possible binding energy and interaction modes against two crystal structures, main protease of SARS-CoV-2 (PDB code: 6M0K) (**Tables-4**) and cathepsin- L (PDB code: 2XU3) (**Table-5**). From the outcomes obtained from docking studies, targeting two receptors, three strongly binded analogs (5e, 6e and 7e) and (5d, 6d and 7d) were chosen to characterize the binding mechanism with 6M0K (M^{pro}) and 2XU3 (CTSL). The 3D and 2D diagrams show how the inhibitors interact with the surrounding residues of M^{pro} and CTSL receptors (**figure-4 & 5)** which were attained by importing the docking results into PyMOL and Discovery Studio Visualizer.

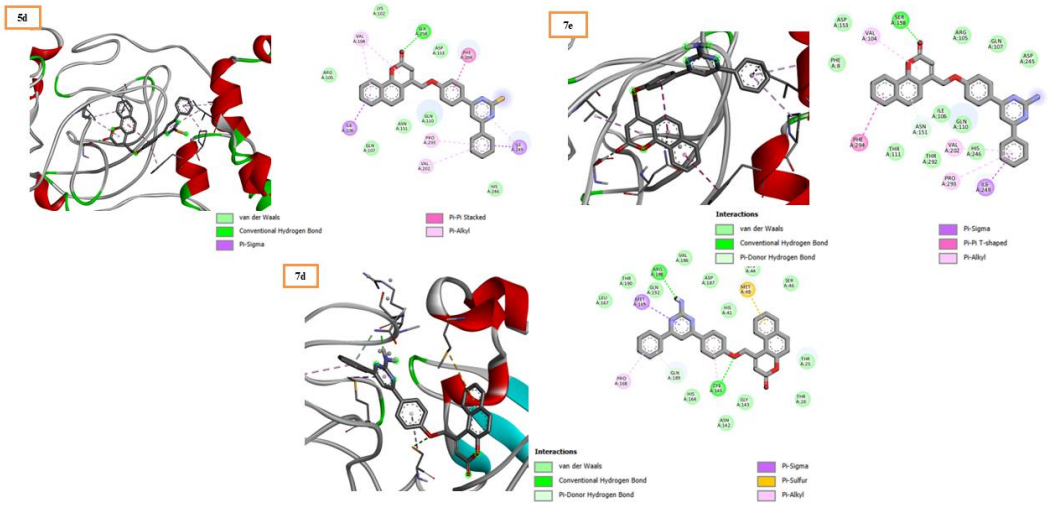

Figure-4 Representation of 3D & 2D interaction between selected strong nonbonding interaction of pyrimidine coumarin analogs with Mpro

Drugs	Bond Category	Interaction type	Distance (Å)	Residues in contact
5e	Electrostatic Electrostatic Electrostatic Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrophobic	PAn PCat PAn AA PA PA PPT PPT	3.91 4.62 3.87 2.84 5.09 4.83 4.92 4.86	TYR237, LEU287, LEU286, ARG131, GLU290, ASP289, THR199 (CHAIN-A)
6e	Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrogen Hydrophobic Hydrophobic Hydrophobic	PA PA PS PA PPS PPT PS CH PA PA PA	5.25 5.28 3.57 5.11 3.90 4.70 3.98 2.20 5.44 5.42 3.98	ILE106, VAL104,SER158, PHE294, PRO293, VAL202, ILE249 (CHAIN-A)
7e	Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrogen	PA PA PS PPT PPT PD PA CH	5.16 5.21 3.67 5.06 5.06 3.81 4.71 2.16	VAL104,SER158, PHE294, PRO293,ILE249 (CHAIN-A)

Table-4 Selective Nonbonding interactions data of pyrimidine-coumarin analogs [(5-7)a-e] with Main protease. CH = Conventional Hydrogen Bond; A = Alkyl; PA = Pi-Alkyl; PS = Pi-sigma; PDH = Pi-Donor Hydrogen Bond; PPS = Pi-Pi Stacked; PPT = Pi-Pi *T*-shaped; PSu= Pi-Sulfur; PAn= Pi-Anion; PAn= Pi-Cation; AA=Acceptor-Acceptor; PD=Pi donor.

Based on docking studies, the results showed that 5e, 6e and 7e are the most promising ligands, which bound more stronger with M^{pro} (-9.4, 9.3, -9.3 kcal/mol) and 5d, 6d and 7d bound more stronger with CTSL (-11.6, 11.1, -11.8 kcal/mol) respectively via many hydrophobic bonding, hydrogen, electrostatic and miscellaneous nonbonding interactions than standard inhibitor Nirmatrelvir (-7.4 kcal/mol and -8.5 kcal/mol). The docked poses clearly show that the drug molecules bind within the active site of the receptors **(Figure-4 & 5.)**

Drugs	Bond Category	Interaction type	Distance (Å)	Residues in contact
5d	Hydrophobic Hydrogen Hydrophobic Miscellaneous Hydrophobic Hydrophobic Hydrogen Miscellaneous Hydrophobic Hydrophobic	PS CH PPT PPS PPS PPS CH PSu PA PA	3.84 1.93 5.02 5.93 3.76 4.11 2.11 5.58 4.59 4.73	TRP193, TRP189, HIS163, LEU144,CYS25, GLN19, GLY23, ALA138, ASP162 (CHAIN-A)
6d	Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrophobic Hydrophobic Miscellaneous Hydrogen	PPT PPS PPS PPS PA PA PSu CH	5.07 3.83 4.24 4.77 4.55 5.35 5.47 2.26	TRP193, TRP189, HIS163, LEU144, CYS25, GLN19, ALA138 (CHAIN-A)
7d	Hydrophobic Hydrophobicl Hydrogen Hydrophobic Hydrogen Miscellaneous Hydrophobic Hydrophobic Hydrophobic	PS PPT CH PA CH PSu PPT PA PA	3.92 5.08 3.78 4.13 2.13 5.56 5.36 4.75 4.55	TRP193, TRP189, HIS163, LEU144,CYS25, GLN19, GLY23, ALA138 (CHAIN-A)

Table-5 Selective Nonbonding interactions data of pyrimidine-coumarin analogs [(5-7)a-e] with Cathepsin-L. CH = Conventional Hydrogen Bond; A = Alkyl; PA = Pi-Alkyl; PS = Pi-sigma; PDH = Pi-Donor Hydrogen Bond; PPS = Pi-Pi Stacked; PPT = Pi-Pi *T*-shaped; PSu= Pi-Sulfur; PAn= Pi-Anion; PAn= Pi-Cation.

The parent molecules 5e and 7d found to be strongly binded to respective receptors M^{pro} and CTSL with the lowest binding energies among all other ligands, exhibited interactions with the key residues (YR237, LEU287, LEU286, ARG131, GLU290, ASP289, THR199) and (TRP193, TRP189, HIS163, LEU144, CYS25, GLN19, GLY23, ALA138) respectively. Minimum binding energy value among all the studied analogs may be the best potential SARS-CoV-2 inhibitors, docking studies showed that all the molecules could function as potential agents for the establishment of drugs for SARS-CoV-2 inhibition. Docking scores of all the synthesised molecules are given in detail in supporting information.

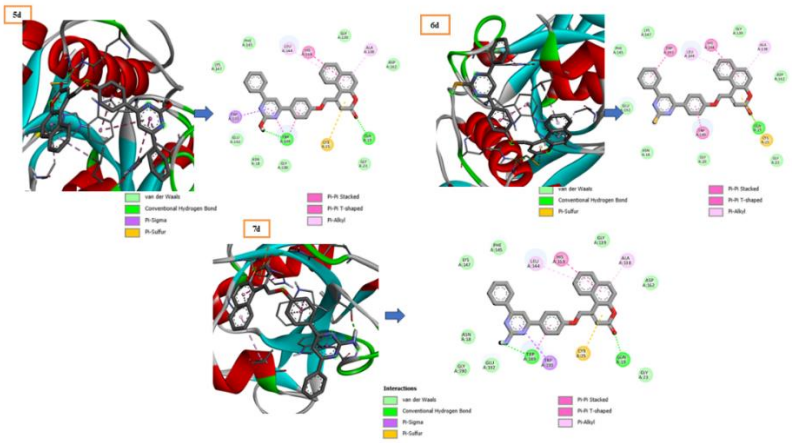

Figure-5 Representation of 3D & 2D interaction between selected strong nonbonding interaction of pyrimidine coumarin analogs with CTSL

Comparing the analyzioed pyrimidine-coumarin analogs [(5-7)a-e] to the standard medication Nirmatrelvir, it is evident that the all analogs bind strongly within the active sites of the CTSL and main protease of SARS-CoV-2, preventing the virus's protein mutation by reducing viral replication. The synthetic compounds may be able to interact spontaneously within the binding site of the SARS-CoV-2 main protease and cathepsin-L, according to the binding affinities. Docking studies revealed that all of the molecules could serve as potential agents for the development of COVID-19 drugs, and the analogs with the highest negative minimum binding energy value among all the studied may be the best potential SARS-CoV-2 inhibitors. Docking scores of all the synthesised molecules are given in detail in supporting information.

3.2. Structure-activity relationships (SARs)

The benzene ring in the structure of the pyrimidine-coumarin analogs [(5-7)a-e] gave the molecule a high electron density, as shown by the highest negative binding score. The addition of groups -CH3 and -Cl generated some changes in binding affinities, but the modification of 5,6- and 7,8-benzo groups coupled with an aromatic ring boosted the binding affinity, according to the results. These results unequivocally demonstrate that aromatic substituents can readily improve the binding ability of all the analogs because of their high electron density.

Summary of SARs are mentioned in **Table-6**. The inclusion of a lactone carbonyl group at the second position of the basic coumarin structure can also be attributed to the lower binding energy and higher interactions of the analogs. In summary, the presence of aromatic groups favor interactions with essential residues, but the amount and location of hydrophobic and hydrogen bonds are crucial for determining the inhibitory potential. In vitro investigations should be conducted to comprehend the anti-SARS-CoV-2 capabilities of the pyrimidine-coumarin analogs, which may be feasible agents against COX receptors and SARS-CoV-2 suppression.

Receptor	Ligand ID	Substituted groups at R and functional group	Binding Energy (kcal/mol)
COX-1	5d	5,6-Benzo: OH	-11.2
	6d	5,6-Benzo: SH	-12.0
	7e	7,8-Benzo: NH_2	-11.8
COX-2	5b	7-CH_3: OH	-11.6
	6e	7,8-Benzo: SH	-11.8
	7e	7,8-Benzo: NH_2	-12.9
Cathepsin- L	5d	5,6-Benzo: OH	-11.6
	6d	5,6-Benzo: SH	-11.1
	7d	5,6-Benzo: NH_2	-11.8
Main protease	5e	7,8-Benzo: OH	-9.4
	6e	7,8-Benzo: SH	-9.3
	7e	7,8-Benzo: NH_2	-9.3

Table 6 Summary of Docking Results and SAR study

3.3. ADME properties prediction

The SwissADME [26] web server was used to assess the pharmacokinetic (ADME) characteristics of the bioactive substances in this series. The computed values for several physicochemical and ADME descriptors, as well as the log P, Ghose, Veber, Lipinski, Muegge, and Bioavailability score and leadlikeness violations, are shown in **Table 7**. Several characteristics of the chemicals employed in this study were determined to ensure absorption, distribution, metabolism, excretion, and toxicity using the SwissADME. The pyrimidine-coumarin analogs [(5-7)a-e], the hydrogen-bond acceptor (NHBA $\leq$ 10), and the hydrogen-bond donor (NHBD $\leq$5) were discovered to have rotatable bond numbers (NRB $\leq$10). The analogs have molecular weights (MW $\leq$ 500) below 500. Since none of the analogs broke Lipinski's rule of five, they were anticipated to be orally active drugs with good gastrointestinal absorption.

Molecules	Formulae	MW g/mol	NRB	NHBA	NHBD	log P	BAS	SA	LV
5a	$C_{27}H_{20}N_2O_4$	436.46	5	6	1	4.62	0.55	3.60	0
5b	$C_{27}H_{20}N_2O_4$	436.46	5	6	1	4.62	0.55	3.46	0
5c	$C_{26}H_{17}ClN_2O_4$	456.88	5	6	1	5.16	0.55	3.70	0
5d	$C_{30}H_{20}N_2O_4$	472.49	5	6	1	5.18	0.55	3.70	0
5e	$C_{30}H_{20}N_2O_4$	472.49	5	6	1	5.18	0.55	3.70	0
6a	$C_{27}H_{20}N_2O_3S$	452.53	5	5	0	5.18	0.55	3.51	0
6b	$C_{27}H_{20}N_2O_3S$	452.53	5	5	0	5.19	0.55	3.51	0
6c	$C_{26}H_{17}ClN_2O_3S$	472.94	5	5	0	5.38	0.55	3.37	0
6d	$C_{30}H_{20}N_2O_3S$	488.56	5	5	0	5.78	0.55	3.61	0
6e	$C_{30}H_{20}N_2O_3S$	488.56	5	5	0	5.73	0.55	3.61	0
7a	$C_{27}H_{21}N_3O_3$	435.47	5	6	1	4.48	0.55	3.58	0
7b	$C_{27}H_{21}N_3O_3$	435.47	5	6	1	4.51	0.55	3.58	0
7c	$C_{26}H_{18}ClN_3O_3$	455.89	5	6	1	4.69	0.55	3.46	0
7d	$C_{30}H_{21}N_3O_3$	471.51	5	6	1	5.02	0.55	3.68	0
7e	$C_{30}H_{21}N_3O_3$	471.51	5	6	1	5.06	0.55	3.68	0

Table 7: SwissADME predicted pharmacokinetic profile for pyrimidine-coumarin analogs [(5-7)a-e]
MF – molecular formula; MW – molecular weight; NRB – number of rotatable bonds; HBA – number of H-bond acceptors; NHBD – number of H-bond donors; log P – partition coefficient between n-octanol and water; BAS – bioavailability score; SA – synthetic accessibility; LV – Lipinski's violation.

3.4. Prediction of toxicity

Since predicting a compound's toxicity is a crucial stage in creating novel medication candidates, in silico toxicity studies were employed since they are quicker and less costly than in vitro testing on cell lines and in vivo testing on animals. Additionally, it can aid in drastically lowering the quantity of animals employed in experimental tests. **Table-8** presents the findings of a study on toxicity prediction for the pyrimidine-coumarin analogs [(5-7)a-e] in addition to ADME prediction. This task was done using an internet tool ProTox-II[27]. While the majority of the compounds were anticipated to be inactive in terms of cytotoxicity, hepatotoxicity, and cardiotoxicity, all of the compounds were projected to be class IV in terms of toxicity and to have mild neurotoxicity and respiratory toxicity.

Molecules	Toxicity Class	Cyto-toxicity	Predicted LD50 (mg/kg)	Hepato-toxicity	Neuro-toxicity	Immuno toxicity	Respiratory toxicity	Cardio-toxicity
5a	4	-ve	500	-ve	+ve	-ve	+ve	-ve
5b	4	-ve	315	-ve	+ve	-ve	+ve	-ve
5c	4	-ve	420	-ve	+ve	-ve	+ve	-ve
5d	4	-ve	1500	-ve	+ve	-ve	+ve	-ve
5e	4	-ve	1500	+ve	-ve	-ve	+ve	-ve
6a	4	-ve	420	+ve	-ve	-ve	+ve	-ve
6b	4	-ve	1500	+ve	-ve	-ve	+ve	-ve
6c	4	-ve	1500	+ve	-ve	-ve	+ve	-ve
6d	4	-ve	1500	+ve	-ve	-ve	+ve	-ve
6e	4	-ve	1500	+ve	+ve	-ve	+ve	-ve
7a	4	-ve	420	+ve	+ve	-ve	+ve	-ve
7b	4	-ve	420	+ve	+ve	-ve	+ve	-ve
7c	4	-ve	1000	+ve	+ve	-ve	+ve	-ve
7d	4	-ve	1500	+ve	+ve	-ve	+ve	-ve
7e	4	-ve	1500	+ve	+ve	-ve	+ve	-ve

Table-8: ProTox-II predicted pharmacokinetic profile for pyrimidine-coumarin analogs.
{+ve=active; -ve=Inactive}

IV. Conclusion

Pyrimidine-coumarin analogs [(5-7)a-e] were tested *in silico* for their pharmacokinetic properties and were validated as having druglike nature. All the structures displayed strong binding interactions on SARS-CoV- and COX receptors, based on the computational analysis. The molecular docking studies revealed that

substitutions such as aromatic ring, benzo and lactone oxygen on the coumarin ring exhibited the most favorable interactions across all the targeted receptors. These modifications enhanced binding affinity and stability, suggesting their crucial role in receptor-ligand interactions indicating the analogs are exceptional candidates for further *in vitro/in vivo* studies. This insight provides a valuable framework for designing novel coumarin-based derivatives with improved pharmacological potential.

Credit authorship contribution statement

S.S.B.: Data validation, writing–original draft, methodology and performed the computational studies. **K.M.H.**: Research Supervision and Validation of work.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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