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MOLECULAR DOCKING BASED *IN-SILICO* APPROACH REVEALS THE POTENTIAL INHIBITORY ACTIVITY OF NSAIDS AGAINST SARS-COV-2 PROTEINS

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Keywords:

NSAID, SARS-CoV-2, Mutation, Molecular docking, Binding affinities, Viral infection

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ABSTRACT: The rapid rate of mutation of the RNA genome of the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is responsible for the emergence of viral variants, leading to the enhanced survivability of the virus. Hence, searching for new drugs that can restrict new viral infections by interacting with wild-type and mutated viral proteins is important. However, new drug development's economic and time-constraining nature makes 'drug repurposing' a more viable solution to address the problem. In this work, we conducted a computational study to screen 23 Non-Steroidal Anti-Inflammatory Drugs (NSAID) interactions with 5 major viral proteins of SARS-CoV-2 that are mainly involved in host infection. Our *in-silico* results establish a database that shows that different NSAID ligands interact with the different viral proteins with good binding affinities. Stabilizing point mutations were introduced within the conserved amino acids involved in ligand-protein interactions. Redocking the NSAID ligands with these mutated viral proteins showed that the NSAID ligands could bind with the mutated and wild-type viral proteins with comparable binding affinities. We conclude that the NSAID ligands could be repurposed as therapeutic drugs against the SARS-CoV-2 virus. Additionally, our work generated a repository that includes binding affinities, possible modes of interaction, and specific interacting residues of the protein (wild-type and mutated) ligand complexes that could be used for future validation studies. Further, our results point to the potential of these drugs to treat other viral infections with similar disease etiology.

INTRODUCTION: Coronaviruses co-exist in the human habitat, with the first global outbreak in 2002 [1]. Currently, the treatment options used to address the disease are limited because of the constantly evolving character of the virus. These viruses have been recorded to infect mammals, and avian species [2].

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To this date, six diverse coronavirus strains have been recorded that show negative implications on humans [3]. The four genera, namely alpha coronavirus, beta coronavirus, gamma coronavirus, and delta coronavirus constitute the Coronavirus family [4].

The severe acute respiratory syndrome coronavirus (SARS-CoV-2) strain of the Coronavirus associated with the lineage A beta coronavirus genera was first identified when a pandemic broke out in 2002 and lasted till 2003 [5]. Again in 2012, a beta coronavirus originating from the lineage C strain led to an outbreak in Saudi Arabia. The disease-causing strain was named Middle East

Respiratory Syndrome Coronavirus (MERS CoV) [6, 7]. The severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) is a novel strain of coronavirus whose outbreak led to a pandemic in the year 2019. The COVID-19 pandemic emerged first in Wuhan city located in the Hubei province of China, in December 2019 [8, 9] and proceeded to spread across the globe leading to a huge number of deaths in several nations. In contrast to the outbreaks in 2002 and 2012 caused by the SARS-CoV and MERS-CoV strains of the coronavirus, the 2019 strain of COVID, SARS-CoV-2 yielded a pandemic because of the enhanced human-to-human transmission. Currently, the treatment options that are being used to address the disease are limited because of the ever-evolving character of the virus. The genetic material characterizing the class of Coronaviruses is single-stranded RNA [10]. Owing to the unstable nature of the RNA, coronaviruses show enhanced rates of mutability [11].

Though COVID-19 treatment has undergone extreme advancements during the previous year, no strict regime can be followed to treat an affected individual. Therefore, there remains a constant need to identify new drugs that could effectively target wild-type and mutated variants. The quickest way to accomplish this is to repurpose the existing and approved drugs to find novel functions against other diseases [12]. Re-purposing of a drug is a time-effective and cost-effective method and can yield highly valuable results with great efficiency [13, 14, 15].

This approach mostly consists of investigating drugs computationally through data-driven approaches and carrying out wet-lab validation upon receiving affirmation through the *in-silico* studies [16, 17]. Therefore, upon studying the SARS-CoV-2 proteins and the symptoms caused by the infection, NSAIDs emerged as a potential choice of drug. The severe symptoms of COVID-19 infections arise from cytokine storm-mediated inflammatory response [18]. Cytokine storms lead to widespread tissue damage resulting in multiorgan failure and death. *In-vitro* studies reveal that the infection caused by SARS CoV-2 shows enhanced levels of COX-2 which plays a pivotal role in coronavirus-related cytokine storm and tissue damage [19]. Thus, targeting COX-2 could be a reasonable approach to combat the disease [20]. Owing to the anti-inflammatory properties of NSAIDs and their known activity against the cyclooxygenase-2 (COX-2) pathway, they appear as potential drugs to engage in the reduction of symptoms [21, 22]. Therefore, screening an array of NSAIDs against the targetable proteins involved in SARS-CoV-2 viral infection can provide insight into the effectiveness of the drugs and prompt future wet lab experimentations for validation.

Various NSAIDs have been shown to be effective against COVID-19 infections such as naproxen [23], ketoprofen [20] and indomethacin when used alone or in combinations [19]. Out of all the drugs, Piroxicam and indomethacin exhibited maximum efficacy with IC_{50} values of 8.21 and 8.51 µM, respectively [24]. Clinical studies involving hospitalized patients have recorded a decreased need for ventilation for patients who had been administered NSAIDs like ibuprofen or naproxen [25]. There have also been conflicting studies on using NSAIDs to treat patients suffering from viral infection. It was shown that the NSAID targets the ACE2 receptor and deteriorates the patient's condition [26]. However, other studies involving ibuprofen and meloxicam show no effect on the levels of ACE2 or the propagation of the viral genetic material [19].

We chose 23 NSAIDs which are widely used as anti-inflammatory agents. Besides the diverse array of NSAIDs, the SARS-CoV-2 proteins that have been selected for the computational study and consequent creation of a library were SARS-CoV spike glycoprotein (5WRG), COVID-19 main protease; 3C-like proteinase (6LU7), SARS-CoV-2 RNA-dependent RNA polymerase (6M71), SARS-CoV-2 Nsp9 RNA-replicase (6WXD), SARS-CoV-2 (COVID-19) NSP3 macrodomain (6YWK).

The SARS-CoV-2 proteins were selected based on their pivotal function in causing viral infections. SARS-CoV-2 spike glycoprotein is the surface protein that plays an important role in the infection of the host and consists of S1 and S2 subunits [27]. These two subunits mediate attachment to the host, binding to the receptor, and membrane-membrane fusion. Moreover, the S1 subunit is a determinant of the zoonotic transmission of the virus [28, 29]. The next in consideration was the main protease of COVID-19 abbreviated as M pro. It is a 3C-like protease. The role of M pro is to assist the virus in doubling and transcribing its genetic material [30].

SARS-CoV-2 RNA-dependent RNA polymerase abbreviated as RdRp, also called NSP-12, functions to assist in the transcription and consecutive translation of the virus's genetic material. This protein is a target of remdesivir, a widely used drug for treating COVID patients. Non-structural protein 9 (Nsp9) is an RNA Replicase that mediates virulence and propagation of the viral genetic material [31] and has been investigated to be essential in the infection of human cells [32].

SARS-CoV-2 NSP3 macrodomain protein plays a role in evading the host immune responses by removal of the ADP-ribosylation sites upon infection by the virus [33]. Based on the characteristics mentioned above of the viral proteins, they serve as lucrative targets for drug designing and studying drug-protein interactions. Since COVID-19 infections are associated with inflammation and cytokine storm regulated by the aforementioned proteins, we have chosen an array of Non-Steroidal Anti-Inflammatory drugs (NSAIDs) to find out if they can be effective in treating the infection. Using computational methods, we have built a database comprising data containing information on protein-ligand interactions which could be used to choose possible drug candidates for conducting *in-vitro* and *in-vivo* experiments for further validation. Since this RNA virus is constantly evolving due to stabilising mutations, we have introduced point mutations on the viral proteins and checked their binding affinities with the group of NSAID drugs. Interestingly, we have found that the NSAIDs bind to the wild-type and mutated proteins with comparable binding energies. This work establishes that these NSAID drugs could be effective against the mutated version of the virus. This work further points to the possibility of using these NSAID drugs to combat other viral infections with similar disease etiology.

MATERIALS AND METHODS:

Ligands and Viral Proteins: Twenty-three NSAID ligands commonly used as anti-inflammatory drugs were chosen for our computational studies **Table 1**. The information regarding these ligands, including the names, their PubChem IDs and their 2D chemical structures, was obtained from PubChem and listed in **Table 1**. The SARS-CoV-2 proteins that have been selected for the computational study and consequent creation of a library are denoted in **Table 1.**

Protein Preparation: The 3D conformations of the five major viral proteins associated with the SARS-CoV-2 virus were obtained from the Protein Data Bank [34] **Table 2**. The proteins were edited by removing native ligands using PyMOL (4.6.0) software. Further preparation of the proteins and the ligands was performed using AutoDock Tools [35], a software tool included in the AutoDock Vina Package (4.2.6) [35]. The protein molecules were edited by removing the water molecules since the molecules present in the pocket region often interfere with the docking. Kollman charges and polar hydrogens were inserted into the structures.

Ligand Preparation: 3D conformations of ligand molecules (NSAID drugs) were downloaded from the web-based site called PubChem **Table 1**. The ligand file (.sdf) was converted using PyMOL (4.6.0). Ligand preparation was carried out with the help of AutoDock Tools (1.5.6), wherein Gasteiger charges were attached to the ligand and the hydrogens of non-polar nature were unified.

Molecular docking using Auto Dock Vina (4.2.6): Grid preparation for the protein was done using AutoDock Tools (1.5.6). The grid dimensions were obtained for the different proteins. Then, in-silico docking was done using AutoDock Vina (4.2.6) [35]. Mutated proteins were docked with the 23 NSAIDs implementing similar methods and parameters. Docking tools were set to generate 10 poses for each NSAID ligand to be docked with the binding site of each viral protein. The grid coordinates were set as follows: 5WRG: 190.072, 190.526, 167.976; 6M71: 121.5, 123.272, 127.073; 6LU7: -26.168, 12.544, 59.032; 6YWK: 33.619, 27.159, 26.224; 6WXD: 41.365, -12.508, 16.176 and the grid box size was set at 40 x 40 x 40 for all proteins. Based on the binding affinities and the binding interactions with the targets, the best binding pose for the ligands was selected for further studies. Docking parameters were set to contain 10 binding modes of which the mode that signified the highest binding affinity was considered.

Molecular docking using the DockThor Server: We further used the DockThor server (https:

//www.DockThor.lncc.br/v2/#) to validate our results on the wild type and mutated protein-ligand docking scores obtained from the Auto Dock Vina software [36].

We performed blind docking mode having parameters with grid size set as X=40, Y=40, Z=40, discretization = 0.42 and number of evaluations: 1000000, population size: 750, initial seed value = -1985, number of runs = 24. X, Y and Z coordinates were set at: 121.009, 121.761, 124.98 for 6M71; 40.323, -11.523, 13.8425 for 6WXD; -25.64, 12.819, 57.9365 for 6LU7; 188.446, 193.4115, 169.67 for 5WRG and 30.424, 26.63, 27.6975 for 6YWK.

All these parameters were kept constant during docking studies. After successful docking, binding affinities of the protein-ligand complexes were estimated in Kcal/mol unit.

Induction of Point Mutations on Viral Proteins using the "DynaMut" Server: For introducing point mutations on specific amino acids of viral proteins, the DynaMut server (http:// biosig.unimelb.edu.au/dynamut/) was used. This server could predict all the changes in protein flexibility, rigidifications, stability, interatomic interaction patterns upon comparing point mutated proteins with the wild-type counterparts [37]. This server also provided the mutated protein file (in .pdb format) which we have downloaded and used for our docking studies.

RESULTS:

Molecular Docking of NSAIDs and Viral Proteins: For this study, we considered 23 NSAIDs commonly used as anti-inflammatory agents **Table 1** and docked them with five SARS-CoV-2 proteins **Table 2** that play a significant role in host infection.

Molecular docking was conducted to determine the binding energies of the protein-ligand complexes that give an idea of the affinity of the ligand for the protein. Enhanced affinity suggests favourable binding.

Docking was carried out using two software tools: DockThor and AutoDock Vina. The results have been graphically represented in **Fig. 1.** Analysis of the data points out that different NSAIDs bind to different viral proteins with varying binding affinities. Co-screening studies were performed with known ligands that have been reported to bind with the viral proteins.

While docking these reference ligands to their respective targets, all the docking parameters were kept constant with the ones chosen for our study.

The co-screening data revealed that the reference ligands show a comparable binding affinity value to most of the protein-ligand complexes screened in this study. This further reveal that the obtained binding scores are significant between the viral proteins and the NSAID ligands. Therefore, this database will assist in the selection of suitable NSAIDs to proceed with further wet-lab experiments to validate the *in-silico* studies.

TABLE 1: LIST OF NSAID LIGANDS, AND RESPECTIVE PUBCHEM IDS

Serial no	Ligand name	PubChem ID
1	Aspirin	2244
2	Celecoxib	2662
3	Diclofenac	3033
4	Diflunisal	3059
5	Etodolac	3308
6	Fenoprofen	3342
7	Fluriboprofen	3394
8	Ibuprofen	3672
9	Indomethacin	3715
10	Ketoprofen	3825
11	Magnesium Salisylate	54684589
12	Mefenamic acid	4044
13	Meloxicam	54677470
14	Nabumetone	4409
15	Naproxen	156391
16	Oxaprozin	4614
17	Piroxicam	54676228
18	Rofecoxib	5090
19	Salsalate	5161
20	Sodium salicylate	16760658
21	Sulindac	1548887
22	Tolmetin sodium	23665411
23	Valdecoxib	119607

TABLE 2: LIST OF PROTEINS OF SARS-COV-2 WITH THEIR PDB IDS

Serial no	PDB ID	Protein name
1	6LU7	COVID-19 main protease
2	5WRG	SARS-CoV spike glycoprotein
3	6YWK	SARS-CoV-2 NSP3 macrodomain
4	6M71	SARS-CoV-2 RNA-dependent RNA polymerase
5	6WXD	SARS-CoV-2 Nsp9 RNA-replicase

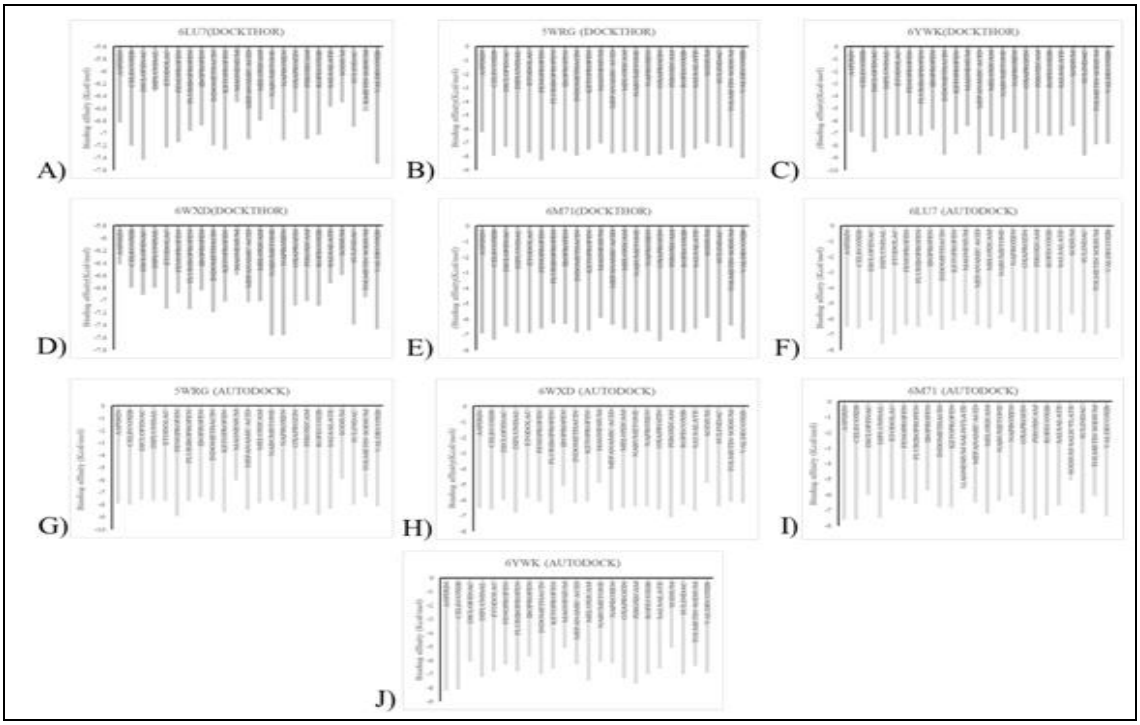


FIG. 1: BINDING AFFINITIES OF ALL 23 NSAID GROUP OF LIGANDS HAVE BEEN ESTIMATED WITH 5 VIRAL PROTEINS USING DOCKTHOR (A-E) AND AUTODOCK VINA (F-J) SOFTWARE. BINDING ENERGY HAS BEEN PLOTTED ALONG Y-AXIS IN KCAL/MOL UNIT

NSAID Binding with Viral Proteins Involves Different Interatomic Interactions: Proteins are characterized by diverse inter-atomic interactions that help confer stability to their structure [38]. Quite similarly, the bonds formed between the protein and the ligands help to stabilize the complex [39]. These bonds include hydrogen bond interactions, van der Waals interactions, electrostatic interactions and others [40]. We have screened the interacting residues involved in the protein-ligand complex using Discovery Studio software that generates a two-dimensional view of the interactions taking place between the protein and the ligand. The visualization of the bonds helped us predict the stability of the complex. All the residue-level interactions have been enlisted in **Table 3**.

TABLE 3: 2D INTERACTIONS OF THE PROTEIN-LIGAND COMPLEX WHERE THE BONDED AMINO ACID RESIDUES AND THE CORRESPONDING NSAIDS HAVE BEEN ENLISTED

	6LU7	5WRG	6YWK	6WXD	6M71
Aspirin	LEU 286, LEU 287, ASN 238, ASP 289, ARG 131, LYS 137, GLU 290	PHE 952, THR 980, ARG 977, ASP 976, THR 980	LYS 9, LYS 9, ALA 21, LYS 28, LYS 31	THR 67, TYR 66, LYS 92, PHE 40, ILE 91 ILE 65, VAL 41, PHE 56	PRO 677, ARG 349, GLU 350, VAL 315, PRO 461, PHE 321
Celecoxib	LEU 286,287; ASN 238, ASP 289, LYS 173, ARG 131, GLU 290	PHE 952, THR 980, ASP 976, THR 980	LYS 19, LEU 169, LYS 29, ALA 21, LYS 28, ASP 22, ASN 0, LYS 31	VAL 41, PHE 56, ILE 65, TYR 66, ILE 91, PHE 40, LYS 92	PRO 677, ARG 349, GLU 350, VAL 315, PRO 461 PHE 321
Diclofenac	GLN 110, ASN 151, PHE 294, ILU 106, VAL104	ARG 977, THR 980, GLY 981	LEU 160, ALA 129, VAL 49, PRO 136	VAL 41, ILE 65, MET 12, ARG 39	ARG 349, ASN 623, PRO 461, PRO 677
Diflunisal	PHE 294, ASP 153, ILE 152, THR 292, THR 111, GLN 110	ARG 977, TYR 738, PHE 952, ASN 951	LYS 31, ASN 87, ALA 21, LYS 28, LYS 29, GLU 170	ARG 39, SER 59, ILE 65, VAL 41	PRO 677, ARG 349, PRO 461, ASN 68
Etodolac	PHE 294, GLN 110, THR 292, THR 111	ASP 976, TYR 738, LEU 983, ARG 977	LEU 10, VAL 165, LEU 0, TYR 161, ASN 0	ARG 39, ILE 65, LYS 58, PHE 40	LEU 122, PHE 396, ASP 390, CYS 395, THR 393, SER 397, LEU 389
Fenoprofen	PRO 293, PHE 294, THR 111, GLU110	PHE 741, THR 943	LEU 169, LYS 19, ALA 21, LYS 28,	ILE 65, PHE 56, ARG 39, MET	ASN 628, PRO 461, ARG 349, CYS 395,

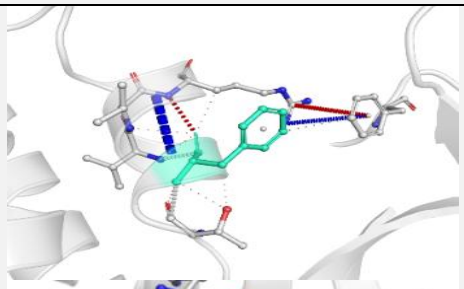
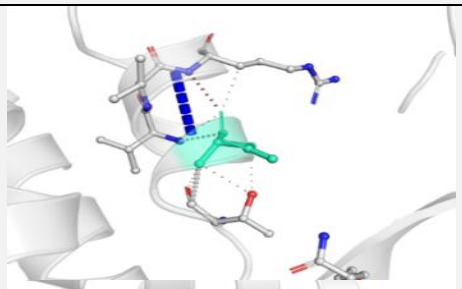
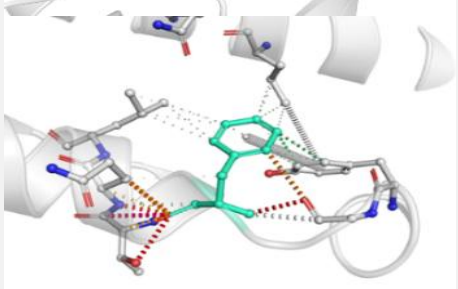
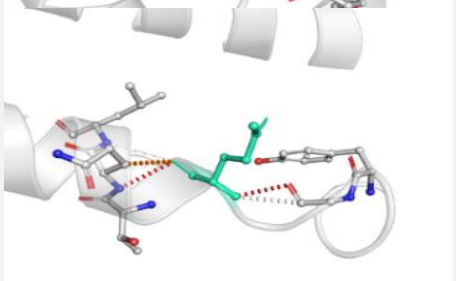
			LYS 31, ASP 22 ASN 87	12, THR 67, VAL 41	PRO 677
Fluriboprofen	ILE 106, THR 292, THR 111, PHE 294, GLN 110	PHE 741, ALA 940, THR 943, GLN 744, ALA 940	LEU 169, LYS 19, ALA 21, GLU 26, LYS 31, LYS 29, LYS 28, ASP 22	ILE 65, ARG 39, VAL 41, THR 67, PHE 56, MET 12	VAL 844, ILE 548, ALA 840, PHE 441, ARG 858, ASP 845, VAL 848, LEU 854
Ibuprofen	ASP 295, GLN 110, THR 292, THR 111, PHE 294	PHE 741, TYR 989, ALA 940, THR 943	LYS 31, ASP 22, LYS 28, ALA 21	PHE 40, VAL 41, ILE 65, PHE 56	VAL 848, PHE 441, VAL 844, ARG 858, ASP 845, ILE 548, TYR 546
Indomethacin	ASP 153, PHE 294, ASP 295, THR 292	ARG 977, GLY 981	LEU 10, TYR 161, VAL 165, LEU 169, GLU 170, LYS 29	VAL 41, THR 67, PHE 56, SER 59, ILE 65	THR 252, SER 255, PHE 321, PRO 323, ARG 249, PRO 461
Ketoprofen	ILE 106, ASN 151, PHE 294, SER 158	PHE 741, TYR 989, ALA 940, THR 943	LYS 19, ALA 21, LYS 29, ASN 87, LYS 28	ILE 65, SER 59, ARG 39, ILE 91, VAL 41	THR 394, PRO 461, CYS 395, PRO 677, LYS 676
Magnesium Salisylate	THR 111, GLN 110, ASN 151, ASP 295, THR 292	PHE 741, THR 988	ALA 21, LYS 28, ASP 22, ASN 20, LYS 31	ARG 39, ILE 65, PHE 56, MET 12, SER 59	VAL 166, PRO 620, ASP 164, SER 795, LYS 798
Mefanamic Acid	PHE 294, ILE 106, VAL 104, THR 111, GLN 110	PHE 741, ALA 940, SER 985, THR 988	ALA 21, LYS 28, LYS 31, LYS 29	MET 12, ARG 39, PHE 56, SER 59, PRO 57, VAL 41, ILE 65	ASP 845, VAL 844, ARG 858, ILE 847, VAL 848, LEU 854
Meloxicam	ASP 197, ARG 131, ASP 269	PHE 952, ASP 976, ARG 977, THR 980	LYS 163, ASN 159, LEU 160	MET 12, ARG 39, ILE 91, VAL 41	PRO 322, ARG 249, PHE 321, THR 319, THR 252, PRO 461
Nabumetone	PHE 294, GLN 110, THR 111	PHE 741, THR 988	LYS 163, VAL 63, ASN 59	ARG 39, SER 59, MET 12, ILE 65	VAL 315, PRO 461, ARG 349
Naproxen	PHE 294	PHE 741, THR 943, THR 988, SER 985	LEU 169, ALA 21, LYS 31, LYS 28, ASP 22	VAL 41, PHE 56, ILE 65, MET 12, ARG 39	LEU 854, ARG 858, VAL 848, ILE 548, VAL 844, PHE 441, ASP 845
Oxaprozin	VAL 104, GLN 110, THR 111, THR 292	LEU 983, THR 980, PHE 952	LYS 163, ASN 59, ASP 157, LEU 160, VAL 63	VAL 41, PHE 56, ARG 39, MET 12, ILE 65	PRO 323, PRO 677, ASN 628, ARG 349, PRO 461, CYS 395
Piroxicam	ASP 197, LYS 137, ASP 189, THR 199, ARG 131	TYR 738, THR 980, ASP 976, ARG 977	LEU 69, LYS 29, LYS 28, ASN 20, ALA 21, LYS 31, LYS 19	ARG 39, ILE 65	ASN 459, PRO 677, PRO 461, ARG 349, VAL 315
Rofecoxib	PHE 294, LYS 102, VAL 104	ARG 747, THR 988, GLU 939, ALA 940	ASP 22, ALA 21, LYS 29, LYS 31	ILE 65	ARG 349, PHE 396, PRO 461, ASN 628, PRO 677, CYS 395
Salsalate	THR 292, PHE 294, THR 111, ILE 106, VAL 104	ALA 940, LEU 944, PHE 741	LYS 28, ASP 22, ASN 87, ASN 2, LYS 31	SER 59, MET 12, ARG 39, PHE 56	PRO 677, ARG 349, PRO 461, GLU 350, ASN 459
Sodium Salisylate	THR 292, ASP 295, THR111	THR 988, PHE 741, SER 985, GLN 984	LYS 28, ALA 21, LYS 31, ASP 22	PHE 56, ARG 39, ILE 65, MET 12, SER 59	ARG 349, PRO 461, ASN 628, GLU 350
Sulindac	PHE 294, GLN 110, ASP 153, ASN 151, THR 292	ASP 976	TYR 161, LYS 29, LYS 19, ALA 21, LEU 169, GLU 170	PHE 56, VAL 41, THR 67, ILE 65, ARG 39	ASN 628, PRO 461, PRO 677, ARG 349, PRO 323
Tolmetin Sodium	ASN 151, ILE 106, THR 111, GLN 110	PHE 741, LEU 983, THR 980, ARG 977, ASP 976	VAL 63, LYS 163, TYR 152, ASN 4	VAL 41, MET 12, ARG 39, ILE 65, PHE 56	VAL 315, PRO 677, ARG 457, ASN 49, PRO 461, ARG 349
Valdecoxib	ASP 153, PHE 294, VAL 104	GLN 936, ARG 996, ARG 747, LEU 745, THR 988	ASP 22, LEU 169, LYS 19, ALA 21, LYS 31, ASN 87, LYS 28	ILE 65, ARG 39, VAL 41, ILE 91, THR 67	PRO 677, CYS 395, ASN 628, LEU 460, PRO 461, ARG 349

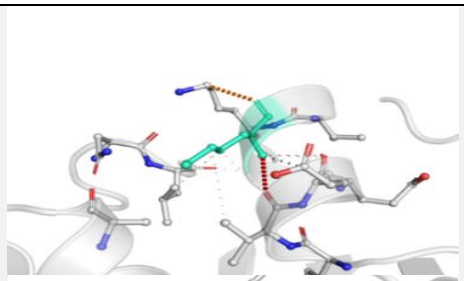
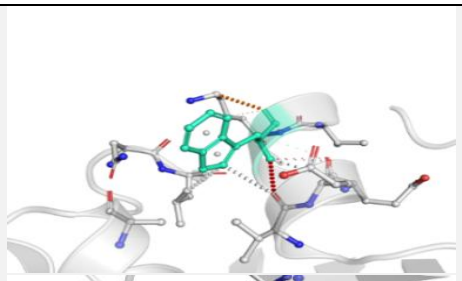
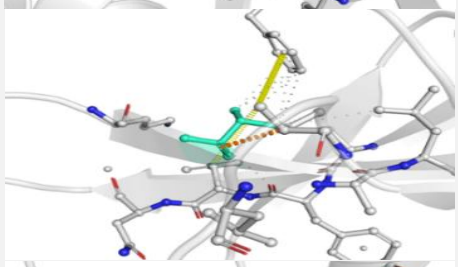
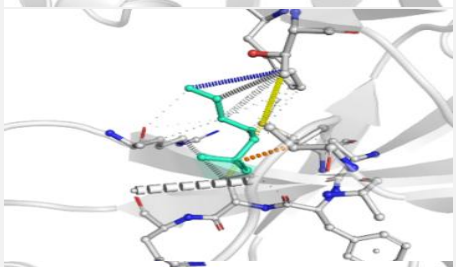
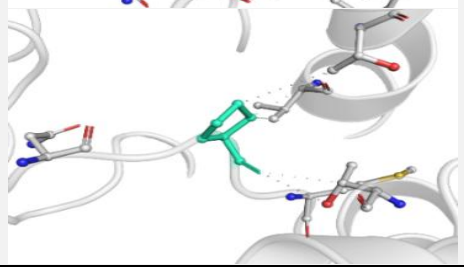
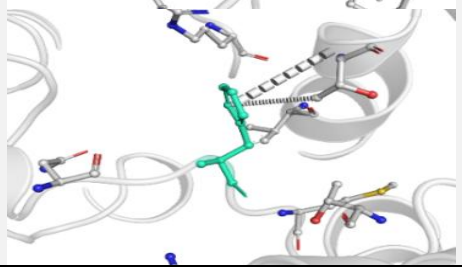
Induced Point Mutations Stabilize Viral Proteins: Viruses are characterized by an evolving and adaptive nature [41, 42]. The main factor which is responsible for this rapid adaptive and emerging nature of viruses is the frequent mutation rate of their genomes [43]. This reflects on the variation and often enhanced stability of viral proteins, which is beneficial for the survival of viruses [44]. Point mutations on viral proteins were induced computationally using the "DynaMut" server to check the effect of mutations on viral proteins. This server estimates the difference between the predicted and actual values of binding energy (ΔΔG). This definition of ΔΔG can vary across different methods. For our analysis, the DynaMut software defined ΔΔG ≥ 0 as stabilizing and ΔΔG < 0 as destabilising mutations [37]. For our work, we selected those point mutations in the **Table 2,** which stabilized the viral proteins. Significant residues that participate in protein-ligand interaction were targeted and modified. The induced point mutations and their consequent effects on flexibility, stability and other parameters were estimated and have been enlisted in **Table 4**. From **Fig. 2**, it is observed that the induced point mutations further stabilize the protein structures by accordingly altering the flexibility or rigidity of the overall protein structure. When point mutations were introduced in the protein, the interatomic interactions were altered compared to the wild type, as shown in **Table 5**.

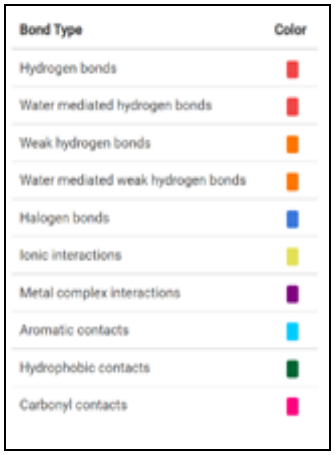
TABLE 4: EFFECTS OF INTRODUCED POINT MUTATIONS OF DESIGNATED AMINO ACIDS ON THE STABILITY OF THE VIRAL PROTEINS

Protein name	PDB ID	Wild type residue	Residue no	Mutated residue	Chain	Type	ΔΔG (Kcal/mol)	ΔΔS VibENCoM (Kcal/mol)
COVID-19 Protease	6LU7	PHE(F)	294	ASP(A)	A	STABLE	0.085	0.453
COVID-19 spike glycoprotein	5WRG	PHE(F)	741	GLU(E)	A	STABLE	1.771	-4.039
COVID-19 NSP3 macrodomain	6YWK	LYS(K)	28	TRP(W)	C	STABLE	0.48	-0.109
COVID-19 Nsp9 RNA-replicase	6WXD	VAL(V)	41	LYS(K)	A	STABLE	0.214	-0.261
COVID-19 RNA polymerase	6M71	PRO(P)	461	HIS(H)	A	STABLE	1.599	-5.144

TABLE 5: CHANGES IN INTERATOMIC INTERACTIONS DUE TO THE INDUCED POINT MUTATIONS ON THE SPECIFIC VIRAL PROTEINS HAVE BEEN DEPICTED. THE DIFFERENT COLOUR CODES REPRESENTING DIFFERENT TYPES OF INTERATOMIC INTERACTIONS BETWEEN THE MUTATED AS WELL AS WILD-TYPE RESIDUES ARE INDICATED IN THE LEGEND BOX

PDB ID	Mutation	Wild	Mutated
6LU7	F 294 D		
5WRG	F 741 E		

6YWK	K 28 W		
6WXD	V 41 K		
6M71	P461 H		



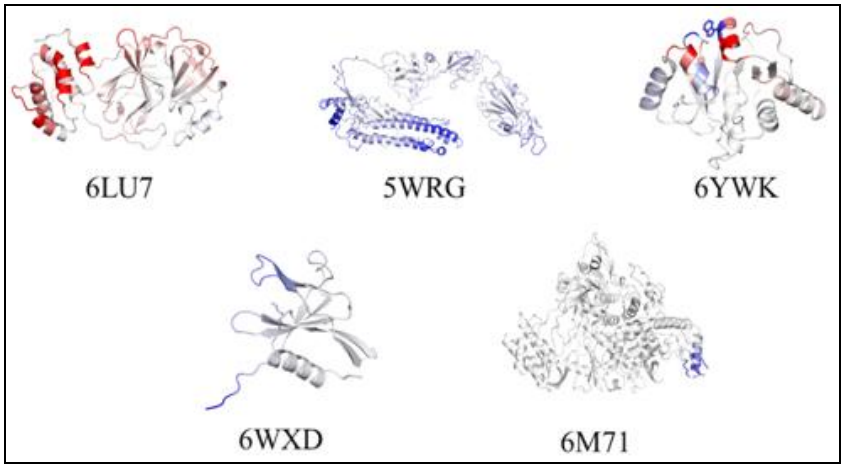


FIG. 2: CHANGES IN PROTEIN STABILITY, FLEXIBILITY, AND RIGIDIFICATION IN VIRAL PROTEINS AFTER INTRODUCING SPECIFIC POINT MUTATIONS. AMINO ACIDS ARE COLOURED ACCORDING TO THE VIBRATIONAL ENTROPY CHANGE UPON MUTATION. BLUE COLOUR REPRESENTS RIGIDIFICATION OF THE STRUCTURE AND RED COLOUR REPRESENTS A GAIN IN FLEXIBILITY

Induced Mutations do not Affect the Binding Affinity of the Protein-ligand Complex: To assess whether the NSAID drugs that show strong binding affinities against wild-type viral proteins could be effective against their mutated versions, redocking were conducted using the same software wherein the binding affinity values of the NSAIDs and the mutated viral proteins were generated (keeping the binding parameters same). It was observed that the induced point mutations did not significantly alter the binding affinities of the protein-ligand interactions since the values of the binding affinities of the protein-ligand complexes in both wild-type and mutated viral proteins were comparable as determined using AutoDock vina and DockThor software **Fig. 3**.

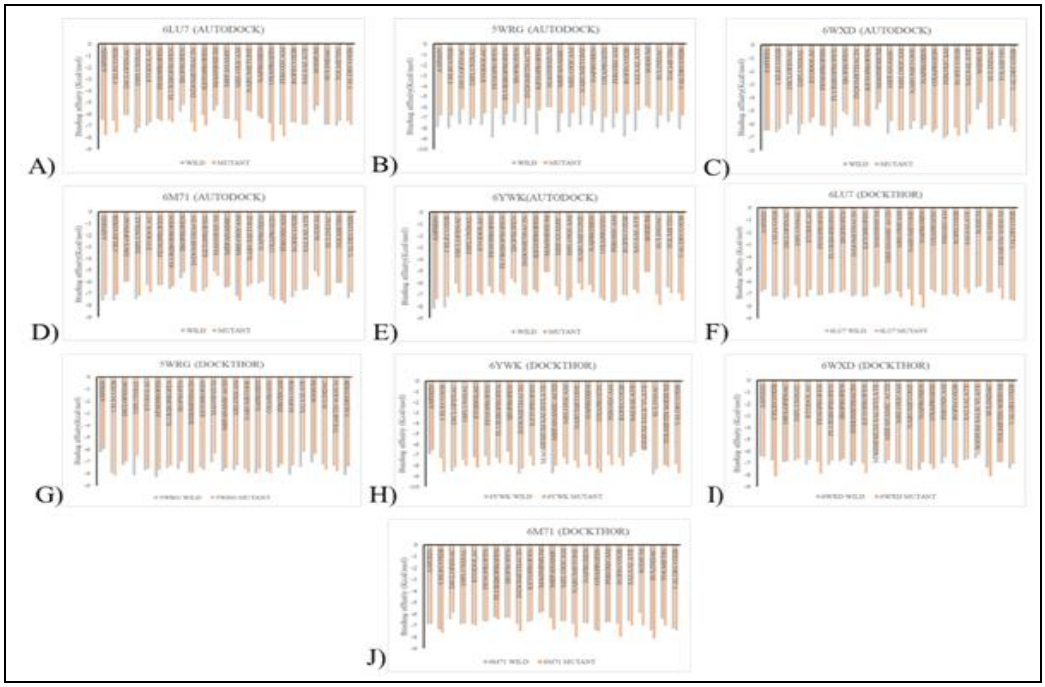


FIG. 3: BINDING AFFINITIES OF THE PROTEIN-LIGAND COMPLEXES FOR BOTH WILD-TYPE AS WELL AS MUTATED VIRAL PROTEINS HAVE BEEN ESTIMATED USING AUTODOCK (A-E) AND DOCKTHOR (F-J) SERVERS.BINDING ENERGY HAS BEEN PLOTTED ALONG Y-AXIS IN KCAL/MOL UNIT.BINDING ENERGY HAS BEEN PLOTTED ALONG Y-AXIS IN KCAL/MOL UNIT

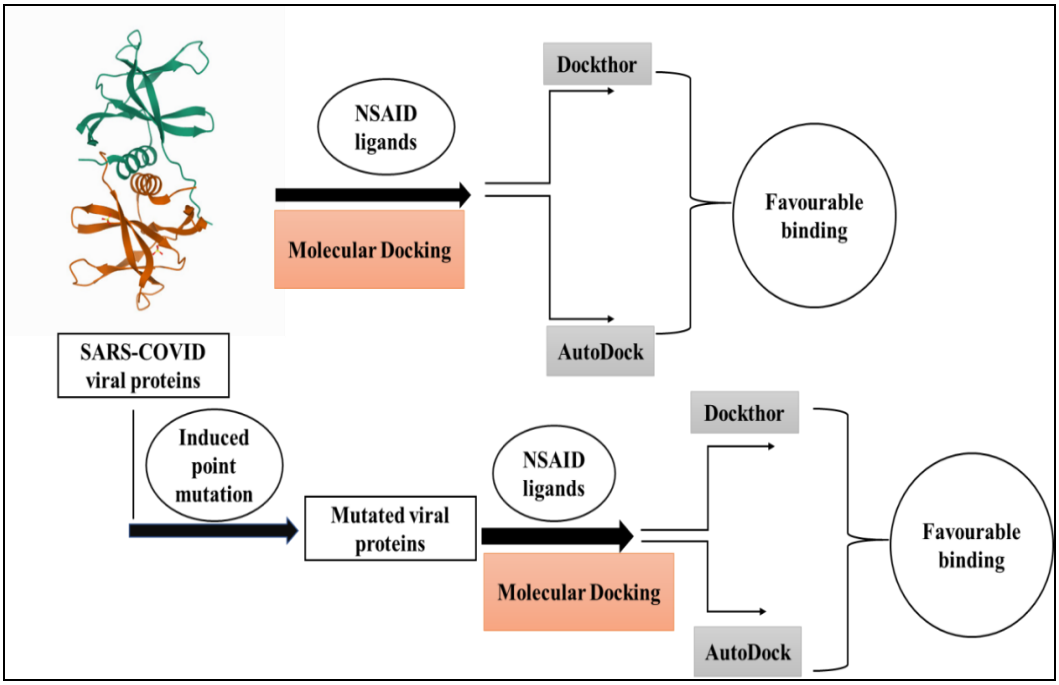


FIG. 4: SCHEMATIC REPRESENTATION OF THE WORK FLOW

DISCUSSION: The COVID-19 outbreak has become a global pandemic and has infected over 110 million people to date and caused a mortality rate of over 2.4 billion. Viruses are highly adaptive in nature and are constantly evolving for better survival. Mutation rates in RNA viruses are higher compared to DNA viruses [45]. Due to their higher mutability rates, different variants of the same virus emerge within a very short time span [46]. Developing vaccines against these RNA-viral proteins remains challenging for scientists [47]. Thus, searching for novel anti-viral drugs that target these viral proteins and their mutated versions is crucial.

Since COX-2 levels are elevated as a result of SARS CoV-2 infection and this protein plays a pivotal role in coronavirus-related cytokine storm and tissue damage [19], in our work, we tried to assess whether NSAIDs could be repurposed for the treatment of SARS-CoV-2 infections. Using "DockThor" and "AutoDock Vina," we showed that 23 NSAIDs interact with five viral proteins with a significant binding energy score, ensuring a favourable complex formation [48].

Viral proteins rapidly mutate for better survival of the virus. Anti-viral ligands specifically target and interact with the active site of the viral proteins in a well-directed residue-specific manner [49]. Due to the higher mutation rates, anti-viral agents often lose their binding specificity to the viral proteins and this could cause new viral outbreaks. Computational mutagenesis studies have been extremely valuable in deciphering various functional aspects of the interaction of SARS-CoV-2 proteins with other proteins and ligands. Induced point mutations on SARS-CoV-2 spike protein were shown to modulate the affinity towards the ACE-2 receptor of the lung epithelial cells in humans [50, 51].

Induction of point mutations on serine 477 of the SARS-CoV-2 spike glycoprotein altered its binding affinity with the human ACE-2 receptor, suggesting this residue's important role in complex formation [52]. Multiple point mutations on the spike glycoprotein of SARS-CoV-2 affected the dynamic stability and overall protein dynamics [53]. Some reports also suggested that specific point mutations in Nsp-10 and Nsp-16 proteins of SARS-CoV-2 have severe impacts on protein dynamicity and stability [54]. One crucial aim of our study is to determine whether the selected NSAID ligands could bind to the wild-type viral proteins and the mutated proteins with comparable binding affinities. This would help the drug to retain the antiviral potential overshadowing the ever-changing nature of the virus. To check whether a mutation in viral proteins causes any significant changes in the affinity of the protein-ligand complex, we computationally introduced some point mutations in those amino acids residue, which was crucial in protein-ligand interactions **Fig. 4**.

Our results reveal that the induced point mutations on the viral proteins did not significantly alter the binding affinity of the drug with the mutated protein when compared to the wild-type **Fig. 3**. Therefore, these NSAID group of drugs could serve as robust anti-viral agents for treating SARS-CoV-2 and SARS-CoV-2 variant mediated viral infections. Additionally, a library comprising data on protein-ligand interactions using computational methods has been created, which will help conduct *in-vitro* and *in-vivo* experiments to validate the *in-silico* data.

In a nutshell, our study shows that 23 NSAIDs interact with 5 SARS-CoV-2 viral proteins with significant binding energy scores thus ensuring a favourable complex formation. The introduction of specific point mutations on the viral proteins was shown to stabilize the proteins by accordingly altering the flexibility or rigidity of the overall protein structure. The induced point mutations on the viral proteins did not significantly alter the binding affinity of the mutated protein with ligands as compared to the wild-type **Fig. 3**.

This attribute would help the drug retain the anti-viral potential overcoming the frequent mutability of the virus. Therefore, these NSAID groups of drugs could serve as robust anti-viral agents for treating SARS-CoV-2 mediated viral infections and possibly for treating other viral infections with similar pathophysiological etiology.

Our comprehensive database on the drug-protein interactions would be useful to researchers for further validating this computational study in the *in vitro* and *in vivo* systems.

Ethics Approval and Consent to Participate Human and Animal Rights: Not applicable

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Authors' Contributions: The main framework of the project, docking using DockThor, Mutation generation, and stability determination, has been performed by AM, docking using AutoDock Vina has been performed by AB. Conceiving of the project and finalization of the manuscript has been done by SG and RS.SG and RS are the respective corresponding authors of this manuscript. All authors approved the publication of the manuscript.

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