Journal of Balkan Science and Technology
journal homepage: www.jbst.nku.edu.tr/

Design Of New Inhibitor Compounds Against SARS-COV-2 Main Protease Using Computer-Assisted Retrosynthetic Methods

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Research Article

Keywords:
SARS-CoV-2
COVID-19
Main protease
Inhibitor design
Retrosynthesis
Molecular docking

Received: 16.01.2025
Accepted: 22.03.2025
Published: 19.05.2025

DOI: 10.55848/jbst.2025.06

ABSTRACT

SARS-CoV-2 is the pathogen that caused the COVID-19 (coronavirus disease 2019) pandemic. While effective vaccines have been developed, there is still an urgent need to develop antiviral drugs for post-infection treatment. This study focuses on the design of novel inhibitory compounds against the SARS-CoV-2 main protease, a key enzyme in viral replication. Reported Mpro inhibitor compounds were fragmented using computer-assisted retrosynthetic methods, and new inhibitors were designed from these fragments following synthesizability rules. The binding affinities of the newly designed compounds were analyzed through docking calculations, and the top 5 compounds showed higher binding affinities than previously described inhibitors, with docking scores ranging from -11.67 to -12.51 kcal/mol. Similarity searches on PubChem revealed that these designed compounds are 83-95% similar to a large number of existing commercially available proprietary compounds, indicating limited novelty but potentially improved synthetic accessibility. This study demonstrates the potential of computer-assisted retrosynthetic design to rapidly generate new, synthetically feasible inhibitor candidates for further optimization and development as SARS-CoV-2 antiviral therapeutics.

1. Introduction

SARS-CoV-2 is the pathogen responsible for the ongoing COVID-19 pandemic, which continues to have a significant impact on global health [1]. While several effective vaccines have been developed, there remains an urgent need for the development of new antiviral drugs to treat post-infection cases [2]. The SARS-CoV-2 main protease (Mpro), also known as 3C-like protease, is a key enzyme involved in viral replication and has been identified as a promising target for antiviral drug development [3-6]. Several studies have reported the design and evaluation of inhibitory compounds against the SARS-CoV-2 main protease [7, 8]. These compounds have shown promising in silico and in vitro results, but there is still a need to develop more potent and synthetically feasible inhibitors. Recent studies have also explored the potential of computer-assisted retrosynthetic methods to design new inhibitor compounds against SARS-CoV-2 proteases[2]. These computational methods, including virtual screening and structure-based drug design, have accelerated the development of potential therapeutic candidates for COVID-19 by prioritizing promising compounds for further experimental validation. Computational retrosynthetic methods have emerged as a powerful tool for de novo design of novel inhibitor compounds against SARS-CoV-2 proteases. These approaches involve the fragmentation of known inhibitor structures and subsequent recombination of the fragments to generate new molecular scaffolds that adhere to synthetic feasibility criteria. This strategy has the potential to rapidly expand the chemical space of potential SARS-CoV-2 inhibitors, ultimately accelerating the development of effective antiviral therapeutics. Significant advancements have been achieved in the development of antiviral agents targeting SARS-CoV-2, with numerous promising compounds described in the scientific literature [9, 10]. Notably, several compounds have been reported as potent inhibitors of the SARS-CoV-2 main protease, including N3, suramin, and ritonavir. N3 is a peptidomimetic inhibitor that was designed based on the substrate-like structure of the Mpro cleavage site. Crystallographic studies have shown that N3 binds tightly to the Mpro active site, making several key interactions that contribute to its high binding affinity and inhibitory potency[11]. Suramin is a polysulfonated naphthylurea compound that has demonstrated inhibitory activity against the SARS-CoV-2 main protease. Several studies have reported the potential of suramin as a therapeutic agent for COVID-19. Suramin has been shown to inhibit the SARS-CoV-2 main protease, as well as other viral proteases, by interfering with protein-protein interactions and disrupting viral entry and replication processes. In addition to its direct antiviral effects, suramin has also been found to modulate the host immune response, potentially mitigating the inflammatory cascade associated with severe COVID-19 [12-14]. Owing to its multi-targeted mechanism of action, suramin has garnered significant interest as a repurposed drug candidate for the treatment of SARS-CoV-2 infection and COVID-19 [15]. Ritonavir, a protease inhibitor used for the treatment of HIV infection, has also been investigated as a potential inhibitor of the SARS-CoV-2 main protease. Several studies have reported the antiviral activity of ritonavir against SARS-CoV-2,

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with the drug showing the ability to inhibit viral replication and reduce viral load in cell-based assays. Ritonavir has been proposed as a repurposed drug candidate for the treatment of COVID-19 due to its known safety profile and previous use in treating other viral infections. Furthermore, clinical trials have been conducted to evaluate the efficacy of ritonavir, often in combination with other antivirals, in the management of SARS-CoV-2 infection and COVID-19. While the results of these studies have been mixed, they highlight the potential of ritonavir as a therapeutic option for COVID-19, particularly in the context of combination therapy targeting multiple stages of the viral life cycle [7, 16-18]. In addition to the previously mentioned inhibitors, other SARS-CoV-2 main protease inhibitors have also been identified through drug discovery efforts. Compounds such as 11a, PF-07321332, and boceprevir have demonstrated potent inhibitory activity against the SARS-CoV-2 Mpro in in vitro and in silico studies. 11a is a peptidomimetic inhibitor that binds tightly to the Mpro active site, disrupting the enzyme's catalytic function [19, 20]. PF-07321332 (nirmatrelvir), also known as Paxlovid, is an orally available Mpro inhibitor that has shown promising results in clinical trials for the treatment of COVID-19 [21-25]. Boceprevir, a protease inhibitor initially developed for the treatment of hepatitis C, has also been investigated as a potential inhibitor of the SARS-CoV-2 main protease [24, 26].

These newly identified inhibitors, along with the previously discussed compounds, highlight the progress made in the development of antiviral therapeutics targeting the SARS-CoV-2 main protease. The continued exploration of diverse chemical scaffolds and the application of computational design strategies, such as the retrosynthetic fragment analysis described in this study, hold promise for the rapid generation of novel, synthetically feasible inhibitor candidates for further optimization and development as SARS-CoV-2 antiviral therapeutics.

In this study, we present the design of novel inhibitory compounds against the SARS-CoV-2 main protease using computer-assisted retrosynthetic methods. Existing some Mpro inhibitor compounds reported in the literature were fragmented, and new Mpro inhibitors were designed from these fragments based on synthesizability rules. The molecular interactions of the newly designed compounds with Mpro were analyzed by docking calculations, and their binding affinities were evaluated. Furthermore, in order to demonstrate the synthesis potential of these novel inhibitor candidate compounds, similarity searches were performed on PubChem [27].

2. Material and Method

2.1. Retrosynthetic fragment analysis and design of a new compound library

The first step of this study was to identify some existing Mpro inhibitor compounds reported in the literature. These existing inhibitors were subjected to computer-assisted retrosynthetic analysis and molecular fragments were generated. Then, using this fragment library, new molecules were obtained in accordance with chemical synthesizability rules. These procedures were performed using the RECAP Analysis and RECAP Synthesis modules of the Molecular Operating Environment (MOE2018) software [28]. The objective of RECAP Analysis is to systematically fragment molecules within a database by applying simplified retrosynthetic rules. This process facilitates the generation of fragments, enabling the collection of statistical data on their occurrence and characteristics. Subsequently, the RECAP Synthesis module can recombine these fragments in a stochastic manner to produce novel chemical structures that are both synthetically feasible and chemically diverse.

The Retrosynthetic Combinatorial Analysis Procedure (RECAP) is a computational methodology designed to fragment molecules by cleaving bonds that are considered likely to be reconstructed using reliable and widely utilized chemical reactions. This technique builds upon the frameworks established by Lewell (1998) and Fechner (2006) [29, 30]. Each resulting fragment is uniquely encoded using the extended SMILES (Simplified Molecular Input Line Entry System) notation, as introduced by Weininger (1988), to preserve the chemical environment and context of the cleavage sites [31].

When applied to compound libraries, RECAP enables the systematic collection of fragment-based statistical data. These fragments can then be recombined through a RECAP Synthesis approach, facilitating the creation of novel chemical structures and advancing the development of innovative compounds. RECAP Analysis and RECAP Synthesis methodology are detailed in the manuals of MOE software.

2.2. Virtual screening

Virtual screening of the de novo designed new compound library against SARS-CoV-2 main protease was performed. In order to perform these calculations quickly and within a reasonable time frame, standard fast docking procedures were performed, where the protein structure was treated as rigid and the bonds of the ligands as flexible. At the end of this process, the top 100 compounds with the highest docking scores and most favorable binding positions were identified to guide more precise protein-ligand interaction studies.

2.3. Induced-fit docking calculations

To further refine the evaluation of the protein-ligand interactions, we conducted induced-fit docking calculations on the top 100 compounds from the initial virtual screen. Induced-fit docking is advantageous in modeling flexibility in both the protein and the ligand during the binding process, providing a more accurate prediction of the binding modes and affinities.

Protein flexibility was accounted for by defining that the receptor was flexible within a certain region around the ligand binding site. The molecular flexibility algorithm, available in the MOE software, was used for these induced-fit docking calculations, allowing simultaneous optimization of both the protein and the ligand structure.

The crystal structure of the SARS-CoV-2 Mpro in complex with an inhibitor (PDB ID: 7BQY) was retrieved from the Protein Data Bank and utilized as the target for the docking studies [11, 32]. As a result of these calculations, potential Mpro inhibitors were identified by examining the docking scores, docking energies and protein-ligand binding poses of the compounds. Detailed methodology for induced-fit docking calculations can be found in our previous work [33-39].

2.4. Similarity search and analysis

To further evaluate the novelty and potential patentability of the top-ranked compounds from the induced-fit docking, a similarity search was performed in the PubChem chemical database. The goal was to identify how similar the newly designed compounds were to existing registered molecules. Similarity was evaluated based on the Tanimoto coefficient, a widely used metric for comparing molecular fingerprints.

3. Results and Discussion

3.1. Retrosynthetic fragment analysis and new compound design

Existing SARS-CoV-2 Mpro inhibitor compounds reported in the literature were subjected to retrosynthetic fragmentation using the RECAP Analysis module of MOE. This process generated a library of molecular fragments that were subsequently recombined using the RECAP Synthesis module to produce new, synthetically feasible Mpro inhibitor candidates [7, 40].

Key steps in the retrosynthetic analysis and new compound design process included:

1) Identification of existing Mpro inhibitor compounds from the literature [8, 40]. Some previously reported Mpro inhibitors are given in Table 1.

Table 1. Some identified Mpro inhibitors and calculated docking scores (E_score, kcal/mol).

mol	mol_id	name	E_score
O=C(N[C@H](C(=O)N[C@H](/C=C/[C@@H](OCc1ccccc1)O)C[C@H]1C(=O)NCC1)CC(C)C)[C@@H](NC(=O)[C@@H](NC(=O)c1noc(C)c1)C)C(C)C	23	n3	-11.63
S(=O)(=O)([O-])c1c2c(NC(=O)c3cc(NC(=O)c4cc(NC(=O)Nc5cc(C(=O)Nc6c(C)ccc(C(=O)Nc7c8c(S(=O)(=O)[O-])cc(S(=O)(=O)[O-])cc8c(S(=O)(=O)[O-])cc7)c6)ccc5)ccc4)c(C)cc3)ccc(S(=O)(=O)[O-])c2cc(S(=O)(=O)[O-])c1	33	suramin	-11.30
O=C(OCc1scnc1)N[C@H]([C@@H](O)C[C@@H](NC(=O)[C@@H](NC(=O)N(Cc1nc(C(C)C)sc1)C)C(C)C)Cc1ccccc1)Cc1ccccc1	29	ritonavir	-10.85
[O+H2][Co-3]1234[n+]5c6c(n([C@@H]7[C@H](O)[C@@H]([C@@H](CO)O7)OP(=O)([O-])O[C@H](C)CNC(=O)CC[C@]7(C)[C@@H](CC(=O)N)[C@@H]8[C@@]9(C)[C@](CC(=O)N)(C)[C@H](CCC(=O)N)C(C(C)=C%10[C@](CC(=O)N)(C)[C@H](CCC(=O)N)C(N1%10)=C)C=1C(C)(C)[C@H](CCC(=O)N)C(=C(C)C7=[N+]28)[N+]3=1)=[N+]49)c5)cc(C)c(C)c6	19	hydroxocobalamin	-10.50
FCC(=O)[C@@H](NC(=O)[C@@H](NC(=O)[C@@H](NC(=O)[C@@H](NC(=O)OCc1ccccc1)CC(=O)OC)CCC(=O)OC)C(C)C)CC(=O)OC	36	z-devd-fmk	-10.38
S(=O)(=O)(NC(=O)[C@]12NC(=O)[C@H]3[C@H](C(=O)N(C)CCCC/C=C\[C@@H]1C2)C[C@H](Oc1c2c(c(C)c(OC)cc2)nc(-c2scc(C(C)C)n2)c1)C3)C1CC1	32	simeprevir	-10.26
S(=O)(=O)(C(C)(C)C)CC1(NC(=O)N[C@H](C(C)(C)C)C(=O)N2[C@@H](C(=O)N[C@@H](C(=O)C(=O)NC3CC3)CCCC)[C@H]3C(C)(C)[C@H]3C2)CCCCC1	24	narlaprevir	-9.96
O=C(OC(C)(C)C)NC=1C(=O)N([C@H](C(=O)N[C@H]([C@@H](O)C(=O)NCc2ccccc2)C[C@H]2C(=O)NCC2)CC2CC2)C=CC=1	3	13b	-9.80
P(=O)(OCC(=O)[C@@H](NC(=O)[C@@H](NC(=O)c1[nH]c2c(c(OC)ccc2)c1)CC(C)C)C[C@H]1C(=O)NCC1)([O-])[O-]	26	pf-07304814	-9.50
O=C(N[C@@H](C(C)(C)C)C)C(=O)N1[C@H](C(=O)N[C@@H](C(=O)C(=O)N)CC2CCC2)[C@H]2C(C)(C)[C@H]2C1)NC(C)(C)C	6	boceprevir	-9.21
O=C(N[C@H](C=O)C[C@H]1C(=O)NCC1)[C@@H](NC(=O)c1[nH]c2c(c1)cccc2)CC1CCCCC1	1	11a	-9.01
O=C(N[C@H](C(=O)CO)C[C@H]1C(=O)NCC1)[C@@H](NC(=O)c1[nH]c2c(c(OC)ccc2)c1)CC(C)C	25	pf-00835231	-8.86
O=C(OCc1ccccc1)N[C@@H](C(=O)N[C@@H](C(=O)C(=O)NCc1ncccc1)CCC)CC(C)C	7	calpain_inh12	-8.66
S(CC[C@@H](NC(=O)[C@H](NC(=O)[C@H](NC(=O)C)CC(C)C)CC(C)C)C=O)C	8	calpain_inh2	-8.52
Fc1cc(C[C@H](NC(=O)c2[nH]c3c(c2)cccc3)C(=O)N[C@H](C=O)C[C@H]2C(=O)NCC2)ccc1	2	11b	-8.46
S(=O)(=O)([O-])[C@@H](O)[C@@H](NC(=O)[C@@H](NC(=O)OCc1ccccc1)CC(C)C)C[C@@H]1C(=O)NCC1	18	gc-376	-8.44
O=C(OCc1ccccc1)N[C@H](C(=O)N[C@H](C=O)C[C@@H]1C(=O)NCC1)CC(C)C	17	gc-373	-8.43
O=C([O-])[C@@H]1[C@@H](O)[C@H](O)[C@@H](O)[C@H](Oc2c(O)c([O-])c3C(=O)C=C(c4ccccc4)Oc3c2)O1	5	baicalin	-7.74
FCC(=O)[C@@H](NC(=O)[C@H](NC(=O)OCc1ccccc1)Cc1ccccc1)C	37	z-fa-fmk	-7.70
O=C1c2c(C(=O)C=3OC(C)(C)[C@H](O)[C@@H](NCc4ccccc4)C1=3)cccc2	10	cay-10581	-7.34
Clc1cc2[n+H]ccc(N[C@H](CCC[N+H](CCO)CC)C)c2cc1	20	hydroxychloroquine	-7.27
Clc1cc2[n+H]ccc(N[C@H](CCC[N+H](CC)CC)C)c2cc1	11	chloroquine	-7.00
Cl[C@@H]1/C(=N/CCN2CCOCC2)/C(=O)c2ncccc2C1=O	12	da-3003-1	-6.82
O=C1N(Cc2ccccc2)C(=O)SN1c1c2c(ccc1)cccc2	34	tideglusib	-6.70
FC(F)(F)c1ccc(C2=NN(C)C3=NC(=O)N(C)C(=O)C3=N2)cc1	35	walrycin_b	-6.64
S(SC(=S)N(CC)CC)C(=S)N(CC)CC	14	disulfiram	-6.57
O=C1[C@@H](c2cc(O)c(O)c(O)c2)Oc2c(c([O-])cc(O)c2)C1=O	22	myricetin	-6.57
FC=1C(=O)[N-]C(=O)N(C(=O)NCCCCCC)C=1	9	carmofur	-6.44
O=C1c2c([O-])c([C@H](O)C/C=C(\C)/C)cc([O-])c2C(=O)C=C1	31	shikonin	-6.42
O=C1[C@@H](c2cc(O)c(O)cc2)Oc2c(c([O-])c(O)c(O)c2)C1=O	28	quercetagetin	-6.35
O=C1c2c([O-])c(O)c(O)cc2OC(c2ccc(O)cc2)=C1	30	scutellarein	-6.18
S(=O)(=O)(N)c1c2C(=O)c3c(c([O-])ccc3)C(=O)c2ccc1	21	LLL-12	-6.13
O=C1[C@H](O)[C@@H](c2cc(O)c(O)c(O)c2)Oc2c1c([O-])cc(O)c2	13	dihydromyricetin	-6.11
O=C1c2[n+](-c3c1cccc3)ccc1c2[nH]c2c1cccc2	16	fascaplysin	-6.09
O=C1c2c([O-])c(O)c(O)cc2OC(c2ccccc2)=C1	4	baicalein	-5.92
S(Sc1[n+H]cc[nH]1)[C@H](CC)C	27	px-12	-5.74
O=C1N(c2ccccc2)[Se]c2c1cccc2	15	ebselen	-5.65

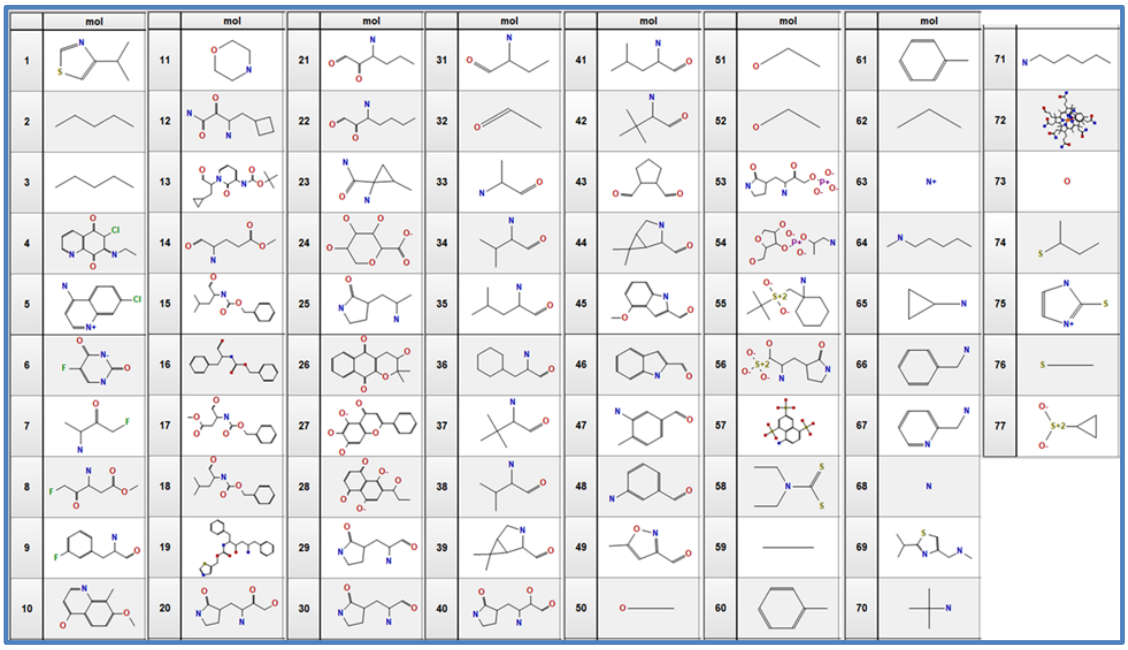
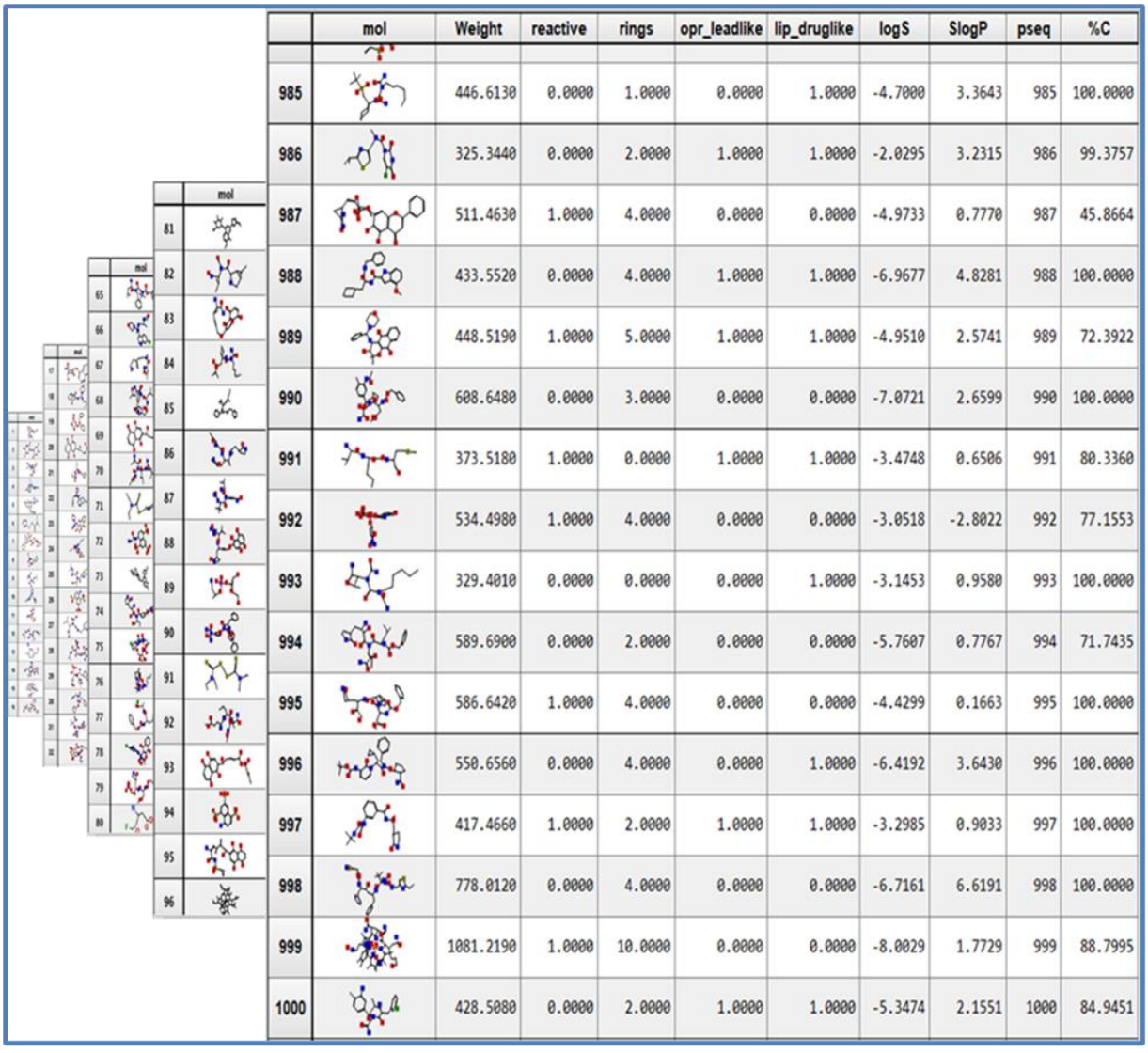


Fig. 1 Molecular fragments used for de novo compound design.



	mol	Weight	reactive	rings	opr_leadlike	lip_druglike	logS	SlogP	pseq	%C
985		446.6130	0.0000	1.0000	0.0000	1.0000	-4.7000	3.3643	985	100.0000
986		325.3440	0.0000	2.0000	1.0000	1.0000	-2.0295	3.2315	986	99.3757
987		511.4630	1.0000	4.0000	0.0000	0.0000	-4.9733	0.7770	987	45.8664
988		433.5520	0.0000	4.0000	1.0000	1.0000	-6.9677	4.8281	988	100.0000
989		448.5190	1.0000	5.0000	1.0000	1.0000	-4.9510	2.5741	989	72.3922
990		608.6480	0.0000	3.0000	0.0000	0.0000	-7.0721	2.6599	990	100.0000
991		373.5180	1.0000	0.0000	1.0000	1.0000	-3.4748	0.6506	991	80.3360
992		534.4980	1.0000	4.0000	0.0000	0.0000	-3.0518	-2.8022	992	77.1553
993		329.4010	0.0000	0.0000	0.0000	1.0000	-3.1453	0.9580	993	100.0000
994		589.6900	0.0000	2.0000	0.0000	0.0000	-5.7607	0.7767	994	71.7435
995		586.6420	1.0000	4.0000	0.0000	0.0000	-4.4299	0.1663	995	100.0000
996		550.6560	0.0000	4.0000	0.0000	1.0000	-6.4192	3.6430	996	100.0000
997		417.4660	1.0000	2.0000	1.0000	1.0000	-3.2985	0.9033	997	100.0000
998		778.0120	0.0000	4.0000	0.0000	0.0000	-6.7161	6.6191	998	100.0000
999		1081.2190	1.0000	10.0000	0.0000	0.0000	-8.0029	1.7729	999	88.7995
1000		428.5080	0.0000	2.0000	1.0000	1.0000	-5.3474	2.1551	1000	84.9451

Fig. 2 A library of newly designed compounds created with the RECAP Synthesis procedure.

2) Fragmentation of these inhibitors using RECAP Analysis to generate a library of molecular building blocks (Fig. 1).

3) Recombination of the fragments using RECAP Synthesis to design new compounds adhering to synthetic feasibility rules.

Through the retrosynthetic fragmentation and subsequent recombination of known Mpro inhibitors, we generated a library of 1000 new potentially synthesizable compounds (Fig. 2).

3.2. Virtual screening and induced-fit docking calculations

The newly designed compounds were evaluated through virtual screening and induced-fit docking calculations against the crystal structure of the SARS-CoV-2 Mpro. The top 100 compounds with the highest docking scores from the initial virtual screen were selected for further analysis using the induced-fit docking protocol.

The induced-fit docking results revealed that several of the newly designed compounds exhibited superior Mpro binding affinities compared to previously reported inhibitors. According to induced-fit docking calculations, the top 10 compounds showing the highest binding affinity towards Mpro are given in Fig. 3. Specifically, the docking scores of the top 5 compounds ranged from -11.67 to -12.51 kcal/mol, indicating stronger binding interactions than the original inhibitor N3 (-10.8 kcal/mol) [40] (in this study N3: -11.63 kcal/mol) and other literature compounds (Table 1). In this study, the crystal structure 7BQY.PDB was used as the Mpro protein model. Virtual screening and docking calculations were performed on this model. This crystal structure also contains the N3 reference inhibitor bound to the protein. To compare the molecular interactions of the newly designed compounds with Mpro, the Mpro-N3 molecular interaction in the 7BQY crystal structure was reconstructed with MOE software and given in Fig. 4. The protein-ligand molecular interactions of the top three designed compounds showing the highest binding affinity towards Mpro are given in Fig. 5.

3.3. Similarity search and analysis

To assess the novelty and potential patentability of the top-ranked compounds from the induced-fit docking analysis, a similarity search was performed in the PubChem chemical database. This analysis revealed that the newly designed compounds exhibited 83-95% similarity with a large number of already registered compounds. In the PubChem chemical database, similarity searches based on Tanimoto-based 2D fingerprint similarity searches revealed 122 compounds with 90% similarity to hit-compound-1, 79 compounds with 83% similarity to hit-compound-2 and 265 compounds with 95% similarity to hit-compound-3.

While the high similarity to existing molecules suggests a lack of true novelty, it also indicates that the designed compounds may be synthetically accessible, as they are structurally related to known chemical entities. The similar compounds identified in the similarity search could potentially serve as starting points for further optimization and lead development efforts.

In summary, this study demonstrates a computer-assisted retrosynthetic approach to rapidly generate new, potentially synthesizable inhibitor candidates targeting the SARS-CoV-2 main protease. The top-ranked compounds showed superior predicted binding affinities compared to previously reported Mpro inhibitors, though their novelty is limited by their high similarity to existing registered compounds. These results provide a foundation for further medicinal chemistry optimization and development of Mpro-targeted antiviral therapeutics against COVID-19.

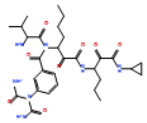
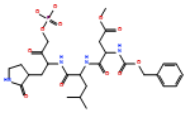
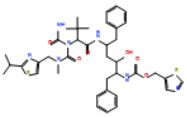
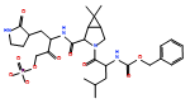
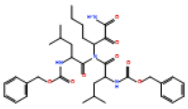
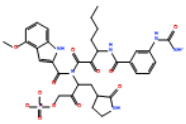
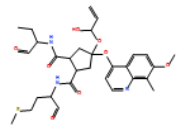
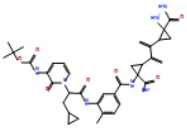
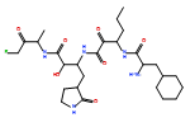
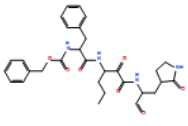
	Structure	mol id	E score
1		24	-12.51
2		19	-12.16
3		21	-11.85
4		39	-11.83
5		41	-11.67
6		9	-11.47
7		75	-11.44
8		3	-11.37
9		8	-11.28
10		33	-11.21

Fig. 3 The top 10 compounds from the newly designed compounds with the highest binding affinity to Mpro.

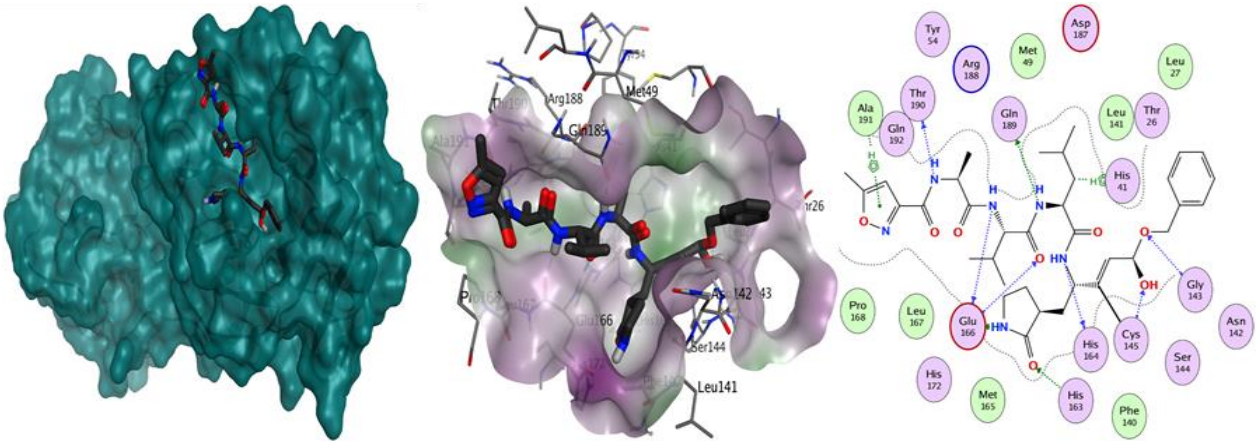


Fig. 4 Molecular interactions of the N3 model inhibitor with SARS-CoV-2 Mpro in the crystal structure 7BQY.PDB, generated with the MOE software.

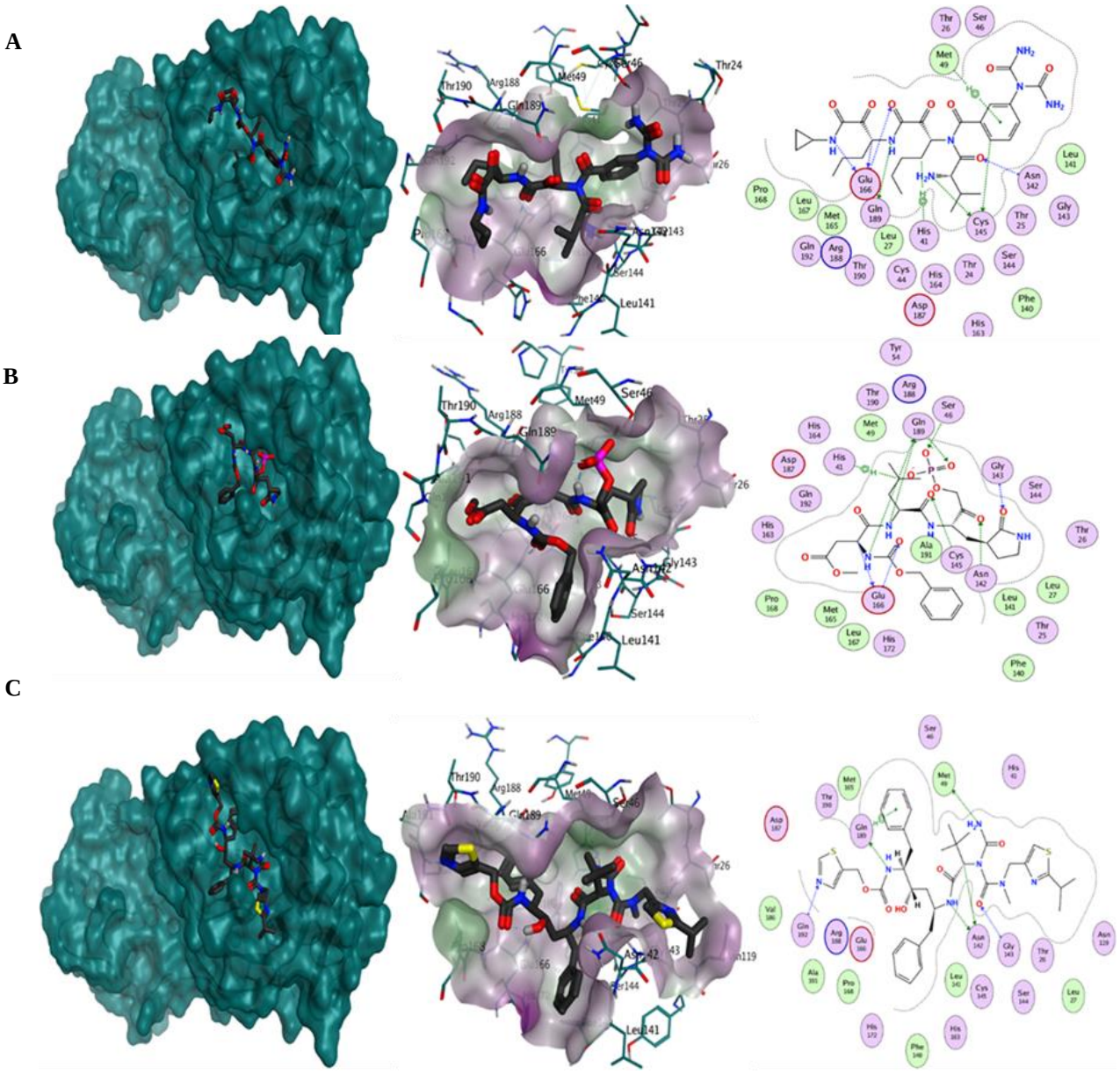


Fig. 5 Molecular interactions of hit compounds with Mpro. **A:** Hit compound-1 (mol id: DC222_1_24); **B:** A: Hit compound-2 (mol id: DC813_2_19); **C:** Hit compound-3, id: DC998_3_21.

4. Conclusion

This research has effectively illustrated the potential of computer-aided retrosynthetic analysis and fragment recombination approaches in designing novel inhibitor compounds targeting the SARS-CoV-2 main protease. The top-ranked compounds identified through our virtual screening and induced-fit docking approaches exhibited superior binding affinities compared to previously reported Mpro inhibitors, with docking scores ranging from an impressive -11.67 to -12.51 kcal/mol.

While the high similarity (83-95%) of the designed compounds to existing registered molecules suggests limited true novelty, this computational methodology has proven highly effective in rapidly generating synthetically feasible inhibitor candidates for further optimization and development as SARS-CoV-2 antiviral therapeutics. The identification of similar commercially available compounds also indicates promising paths forward tofor lead development and optimization efforts.

The structural insights gained from the docking poses and binding interactions of the top compounds with Mpro can greatly inform future medicinal chemistry efforts targeting this essential viral enzyme. Additionally, the retrosynthetic fragmentation and recombination strategy demonstrated here has broader applicability beyond this specific study, and could potentially be extended to the design of inhibitors against other protein targets of interest.

Future work will focus on the experimental validation of the top computationally-derived inhibitor candidates, as well as iterative rounds of structure-based optimization to improve potency, selectivity, and overall drug-like properties. Continued development of these Mpro inhibitors holds great potential to yield novel antiviral therapeutics to combat the ongoing COVID-19 pandemic, as well as future coronavirus outbreaks.

Declaration

Author Contribution: Conceive– D.C.; Design– D.C.; Experimental Performance, Data Collection and Processing– D.C.; Analysis and Interpretation– D.C.; Literature Review– D.C.; Writer– D.C.; Critical Review– D.C.

Conflict of interests: The author has declared no conflict of interest.

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References

[1] Chen, K.Y., et al., A highly sensitive cell-based luciferase assay for high-throughput automated screening of SARS-CoV-2 nsp5/3CLpro inhibitors. Antiviral Res, 2022. 201: p. 105272.

[2] Kalita, P., T. Tripathi, and A.K. Padhi, Computational Protein Design for COVID-19 Research and Emerging Therapeutics. ACS Cent Sci, 2023. 9(4): p. 602-613.

[3] Li, Q. and C. Kang, Progress in Developing Inhibitors of SARS-CoV-2 3C-Like Protease. Microorganisms, 2020. 8(8).

[4] He, J., et al., Potential of coronavirus 3C-like protease inhibitors for the development of new anti-SARS-CoV-2 drugs: Insights from structures of protease and inhibitors. Int J Antimicrob Agents, 2020. 56(2): p. 106055.

[5] Ullrich, S. and C. Nitsche, The SARS-CoV-2 main protease as drug target. Bioorg Med Chem Lett, 2020. 30(17): p. 127377.

[6] Huang, Y., et al., Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin, 2020. 41(9): p. 1141-1149.

[7] Bolcato, G., et al., Targeting the coronavirus SARS-CoV-2: computational insights into the mechanism of action of the protease inhibitors lopinavir, ritonavir and nelfinavir. Sci Rep, 2020. 10(1): p. 20927.

[8] Zhang, L., et al., Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved alpha-ketoamide inhibitors. Science, 2020. 368(6489): p. 409-412.

[9] Katre, S.G., et al., Review on development of potential inhibitors of SARS-CoV-2 main protease (M(Pro)). Futur J Pharm Sci, 2022. 8(1): p. 36.

[10] Agost-Beltran, L., et al., Advances in the Development of SARS-CoV-2 Mpro Inhibitors. Molecules, 2022. 27(8).

[11] Jin, Z., et al., Structure of M(pro) from SARS-CoV-2 and discovery of its inhibitors. Nature, 2020. 582(7811): p. 289-293.

[12] Boniardi, I., et al., Suramin inhibits SARS-CoV-2 nucleocapsid phosphoprotein genome packaging function. Virus Res, 2023. 336: p. 199221.

[13] Kwon, P.S., et al., Suramin binds and inhibits infection of SARS-CoV-2 through both spike protein-heparan sulfate and ACE2 receptor interactions. Commun Biol, 2023. 6(1): p. 387.

[14] Salgado-Benvindo, C., et al., Suramin Inhibits SARS-CoV-2 Infection in Cell Culture by Interfering with Early Steps of the Replication Cycle. Antimicrob Agents Chemother, 2020. 64(8).

[15] Eberle, R.J., et al., The Repurposed Drugs Suramin and Quinacrine Cooperatively Inhibit SARS-CoV-2 3CL(pro) In Vitro. Viruses, 2021. 13(5).

[16] Hung, Y.P., et al., Oral Nirmatrelvir/Ritonavir Therapy for COVID-19: The Dawn in the Dark? Antibiotics (Basel), 2022. 11(2).

[17] Kieck, D., L. Mahalick, and T.T. Vo, Medication-Related Problems Identified and Addressed by Pharmacists Dispensing COVID-19 Antivirals at a Community Pharmacy. Pharmacy (Basel), 2023. 11(3).

[18] Markowitz, M., et al., A preliminary study of ritonavir, an inhibitor of HIV-1 protease, to treat HIV-1 infection. N Engl J Med, 1995. 333(23): p. 1534-9.

[19] Dai, W., et al., Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. Science, 2020. 368(6497): p. 1331-1335.

[20] Gossen, J., et al., A Blueprint for High Affinity SARS-CoV-2 Mpro Inhibitors from Activity-Based Compound Library Screening Guided by Analysis of Protein

Dynamics. ACS Pharmacol Transl Sci, 2021. 4(3): p. 1079-1095.

[21] Owen, D.R., et al., An oral SARS-CoV-2 M(pro) inhibitor clinical candidate for the treatment of COVID-19. Science, 2021. 374(6575): p. 1586-1593.

[22] Costacurta, F., et al., A comprehensive study of SARS-CoV-2 main protease (Mpro) inhibitor-resistant mutants selected in a VSV-based system. PLoS Pathog, 2024. 20(9): p. e1012522.

[23] Bege, M. and A. Borbas, The Design, Synthesis and Mechanism of Action of Paxlovid, a Protease Inhibitor Drug Combination for the Treatment of COVID-19. Pharmaceutics, 2024. 16(2).

[24] Gammeltoft, K.A., et al., Substitutions in SARS-CoV-2 Mpro Selected by Protease Inhibitor Boceprevir Confer Resistance to Nirmatrelvir. Viruses, 2023. 15(9).

[25] Li, J., et al., Structural Basis of the Main Proteases of Coronavirus Bound to Drug Candidate PF-07321332. J Virol, 2022. 96(8): p. e0201321.

[26] Gohl, M., et al., From Repurposing to Redesign: Optimization of Boceprevir to Highly Potent Inhibitors of the SARS-CoV-2 Main Protease. Molecules, 2022. 27(13).

[27] PubChem Compound Database. National Center for Biotechnology Information. Accessed 03.01.2025.

[28] Molecular Operating Environment (MOE), 2019.01; Chemical Computing Group ULC, 1010 Sherbrooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2019. Feb. 01, 2019. [Online]. Available: https://www.chemcomp.com/.

[29] Lewell, X.Q., et al., RECAP--retrosynthetic combinatorial analysis procedure: a powerful new technique for identifying privileged molecular fragments with useful applications in combinatorial chemistry. J Chem Inf Comput Sci, 1998. 38(3): p. 511-22.

[30] Fechner, U. and G. Schneider, Flux (1): a virtual synthesis scheme for fragment-based de novo design. J Chem Inf Model, 2006. 46(2): p. 699-707.

[31] Weininger, D., SMILES, a chemical language and information system. 1. Introduction to methodology and encoding rules. Journal of Chemical Information and Computer Sciences, 1988. 28(1): p. 31-36.

[32] Liu, X., Zhang, B., Jin, Z., Yang, H., Rao, Z., THE CRYSTAL STRUCTURE OF COVID-19 MAIN PROTEASE IN COMPLEX WITH AN INHIBITOR N3 at 1.7 angstrom. 2020.

[33] I.I. Ozturk, S.Y., M. Gürgan, D. Ceyhan, C.N. Banti, S.K. Hadjikakou, M. Manoli, E. Moushi, A.J. Tasiopoulos,, Synthesis, characterization and biological evaluation of novel antimony(III) iodide complexes with tetramethylthiourea and N-ethylthiourea. Inorganica Chimica Acta, 2019. 491: p. 14-24.

[34] Özdemir M, K.B., Ceyhan D, Bulut M, Yalcin B. , In silico, 6LU7 protein inhibition using dihydroxy-3-phenyl coumarin derivatives for SARS-CoV-2. Journal of the Turkish Chemical Society Section A: Chemistry, 2020. 7(3): p. 691-712.

[35] Ozturk, I.I., Yarar, S., Gürgan, M., Ceyhan, D., Panagiotou, N., Tasiopoulos, A. J., … Aral, C, Novel binuclear antimony(III) halide complexes of 5-methoxy-2-mercaptobenzimidazole: synthesis, structural characterization, and biological studies. Journal of Coordination Chemistry, 2020. 73(3): p. 485–505.

[36] GERÇEK, Z., CEYHAN, D., and ERÇAĞ, E. , Synthesis and molecular docking study of novel COVID-19 inhibitors. Turkish Journal of Chemistry, 2021. 45(3): p. 704-718.

[37] Ozturk, I.I., et al., Structural and biological features of bismuth(III) halide complexes with heterocyclic thioamides. Journal of Molecular Structure, 2021. 1227.

[38] Ozdemir, M., et al., Design and in silico study of the novel coumarin derivatives against SARS-CoV-2 main enzymes. J Biomol Struct Dyn, 2022. 40(11): p. 4905-4920.

[39] Özdemir, M., et al., 7,8-Dihydroxycoumarin derivatives: In silico molecular docking and in vitro anticholinesterase activity. Journal of Molecular Structure, 2023. 1274.

[40] Andi, B., et al., Hepatitis C virus NS3/4A inhibitors and other drug-like compounds as covalent binders of SARS-CoV-2 main protease. Sci Rep, 2022. 12(1): p. 12197.