

Structure-based optimization of LRH-0003 derivatives targeting SARS-CoV-2 Mac1: discovery of L-5-A with improved binding affinity and favorable drug-like properties

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Article

Keywords: SARS-CoV-2, Mac1, nsp3, molecular docking, virtual screening, LRH-0003, L-5-A, ADMET, PLIP

Posted Date: October 6th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11242963/v1>

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Additional Declarations: No competing interests reported.

Abstract

The continuous mutation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) poses a serious challenge to existing antiviral drugs, underscoring the urgent need to develop broad-spectrum antiviral agents targeting conserved regions of the virus. Mac1 (Macrodomain 1) is a highly conserved domain of the SARS-CoV-2 nsp3 protein with ADP-ribose hydrolase activity, playing a key role in viral evasion of the host innate immune response, and has been validated as an antiviral drug target by the international CACHE Challenge. In this study, using LRH-0003 ($IC_{50} = 1.7 \mu M$) as the lead compound and its co-crystal structure with Mac1 (PDB: 5SRY), we performed structure-guided derivative design and two rounds of iterative optimization. Through CB-Dock2 molecular docking, the binding affinities of 16 derivatives were evaluated, and L-5-A—a derivative in which a carboxyl group was introduced at the C6 position of the LRH-0003 core scaffold and the pyrrolidinone ring was expanded to a piperidinone ring—achieved a Vina score of -9.3 kcal/mol, an improvement of 1.1 kcal/mol over the parent compound (-8.2 kcal/mol). PLIP interaction analysis indicated that the improved binding affinity of L-5-A originates from a strong $2.75 \text{ \AA}$ hydrogen bond between the C6 carboxyl group and the backbone NH of PHE156, together with a newly formed aromatic–hydrophobic contact ($3.03 \text{ \AA}$). ADMET prediction showed that L-5-A satisfies all criteria of Lipinski's Rule of Five (0 violations), with predicted GI absorption of “High” and blood–brain barrier permeability of “No”, suggesting favorable oral bioavailability and a low risk of central nervous system side effects. This study provides clear structural guidance for the further optimization of Mac1 inhibitors, and L-5-A, as a novel Mac1 inhibitor candidate with strong binding affinity and favorable drug-likeness, warrants further experimental validation of its antiviral activity.

Introduction

Since its outbreak in late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected hundreds of millions of people worldwide, posing a sustained threat to public health¹. Although the rapid development of vaccines and antiviral drugs has provided important tools for pandemic control, the continuous evolution of the virus—from Alpha and Delta to Omicron and its sublineages—has repeatedly generated new variants, some of which have reduced the efficacy of existing vaccines and drugs². For example, nirmatrelvir, the active ingredient of Paxlovid, targets the active site of the main protease (Mpro); however, mutations at residue E166 have been shown to reduce the potency of nirmatrelvir by up to 3000-fold while preserving substrate cleavage capacity^{3, 4}. In the Paxlovid clinical trial, E166V was the only drug resistance mutation to emerge, observed in three patients treated with Paxlovid⁴. Therefore, identifying conserved viral targets that are less prone to drug resistance mutations and developing novel broad-spectrum antiviral drugs is of significant scientific and clinical value.

Nonstructural protein 3 (nsp3) of coronaviruses is the largest protein encoded by the viral genome, comprising approximately 1,945 amino acids and containing at least 16 functional domains⁵. Among them, Macrodomain 1 (Mac1; also known as the X domain or ADP-ribose phosphatase) has attracted considerable attention in recent years as an antiviral drug target⁶. Mac1 possesses ADP-ribose

hydrolase activity and can remove ADP-ribose modifications from host proteins, thereby suppressing the host innate immune response and helping the virus evade immune clearance^{7, 8}. Genetic studies have shown that inactivation of Mac1 can markedly attenuate viral replication in vivo by enhancing the host innate immune response⁹. A study published on bioRxiv in 2024 provided pharmacological proof of concept, demonstrating that Mac1 is a viable therapeutic target and that its inhibitor AVI-4206 reduced viral load and improved survival in an animal model of SARS-CoV-2 infection⁹. Mac1 is highly conserved among coronaviruses, and viruses lacking Mac1 are almost non-pathogenic in mouse models¹⁰. In 2026, Round 3 of the international CACHE Challenge specifically focused on “Targeting the SARS-CoV-2 Nsp3 Macrodomein,” attracting 23 computational teams worldwide that collectively screened 1,739 candidate ligands¹¹. These advances further substantiate the importance of Mac1 as an antiviral drug target.

In recent years, multiple Mac1 inhibitors have been reported. Sherrill et al. (2022) identified a series of pyrrolopyrimidine Mac1 inhibitors through high-throughput screening, among which compound 15c exhibited low-micromolar inhibitory activity ($IC_{50} = 6.1 \mu M$) in biochemical assays¹². In 2024, Wazir et al. reported in the Journal of Medicinal Chemistry MDOLL-0229 (compound 27), with an IC_{50} of $2.1 \mu M$, the first small molecule demonstrated to inhibit coronavirus replication in cellular models through Mac1 targeting¹³. MDOLL-0229 showed selectivity toward coronavirus Mac1 proteins, and sequencing of drug-resistant murine hepatitis virus identified mutations in Mac1, further supporting its target specificity. Its co-crystal structure with Mac1 (PDB: 8TV7) provided a structural template for subsequent drug design. In 2026, orally available Mac1 inhibitors such as AVI-4206 and AVI-6451 were successively reported¹⁴. In addition, LRH-0003 is a neutral Mac1 inhibitor whose co-crystal structure with Mac1 has been resolved (PDB: 5SRY, resolution $1.05 \text{ \AA}$)¹⁵. LRH-0003 forms key hydrogen bonds with the oxyanion hole of Mac1 (constituted by the backbone NH of PHE156 and ASP157) through its 1-aminopyrrolidin-2-one group, with an IC_{50} of $1.7 \mu M$ ¹⁶. However, the binding affinity of LRH-0003 still has considerable room for improvement, and its drug-likeness has not been systematically optimized.

In this study, using LRH-0003 as the lead compound and its crystal structure with Mac1 (PDB: 5SRY), we carried out structure-guided derivative design and systematic optimization. We designed four categories of optimization strategies: (1) strengthening the hydrogen-bond network; (2) optimizing π - π stacking and hydrophobic interactions; (3) exploring new interactions; and (4) combined optimization. After two rounds of iterative optimization, we identified L-5-A—a derivative in which a carboxyl group was introduced at the C6 position of the LRH-0003 core scaffold and the pyrrolidinone ring was expanded to a piperidinone ring—achieving a Vina score of -9.3 kcal/mol , an improvement of 1.1 kcal/mol over the parent compound (-8.2 kcal/mol). Meanwhile, L-5-A satisfies all criteria of Lipinski’s Rule of Five (0 violations), with predicted GI absorption of “High” and blood–brain barrier permeability of “No”, suggesting a low risk of central nervous system side effects. Protein–ligand interaction analysis (PLIP) indicated that the improved binding affinity of L-5-A mainly originates from a $2.75 \text{ \AA}$ strong hydrogen bond between the C6 carboxyl group and the backbone NH of PHE156, as well as newly formed aromatic–hydrophobic interactions.

This study not only provides clear structural guidance for the further optimization of Mac1 inhibitors, but also offers a complete research paradigm for structure-based antiviral drug design, spanning “lead compound discovery → rational design → iterative optimization → drug-likeness evaluation.” As a novel Mac1 inhibitor candidate with strong binding affinity and favorable drug-likeness, L-5-A warrants further experimental validation of its antiviral activity through methods such as surface plasmon resonance, enzyme inhibition assays, and cellular assays.

Materials and Methods

1 Viral Sequence Acquisition

The NCBI (National Center for Biotechnology Information) database was used to download the complete genomic nucleotide sequences of the original SARS-CoV-2 strain (Wuhan-Hu-1) and the major variants, serving as the initial data source for this study.

2 Sequence Alignment and Conserved Region Analysis

MEGA (Molecular Evolutionary Genetics Analysis) software was used to perform multiple sequence alignment of the downloaded nucleotide sequences, with the ClustalW algorithm used for global alignment. Based on the alignment results, nucleotide regions that are highly conserved across the different variants were identified as candidate regions for subsequent drug target research. Through multiple sequence alignment, we identified a conserved 104 bp region in the Y domain of nsp3. This region is located near the Mac1 domain of the nsp3 protein; Mac1 is highly conserved among coronaviruses and has been validated as an antiviral drug target by the international CACHE Challenge. On this basis, we selected Mac1 as the target protein for subsequent structural biology studies.

3 Protein Structure and Drug Molecule Acquisition

High-resolution crystal structures of the SARS-CoV-2 Mac1 domain (PDB ID: 5SRY, resolution 1.05 Å) were downloaded from the RCSB PDB (Protein Data Bank) database for subsequent molecular docking and structural analysis. Meanwhile, the three-dimensional structures or SMILES formats of candidate drug molecules for initial screening were downloaded from the PubChem database, including GC376, lopinavir, nirmatrelvir, remdesivir, MDOLL-0229, AVI-4206, and LRH-0003, in order to select the most suitable lead compound for subsequent structural optimization. The selection criteria were based on: (1) reported broad-spectrum antiviral activity in the literature; (2) chemical structure diversity; and (3) known clinical safety data. In addition, the binding of nirmatrelvir and LRH-0003 to Mac1 has been reported in the literature.

4 Molecular Docking

The CB-Dock2 online platform was used for protein–ligand molecular docking. This platform calculates binding affinity based on the AutoDock Vina engine and outputs the Vina score (unit: kcal/mol) as the binding energy assessment metric. Default parameters were used during docking, and the three-dimensional structures of ligands were generated from SMILES strings. To validate the reliability of the docking method, the co-crystallized ligand LRH-0003 of 5SRY was used as a positive control for redocking; the consistency between the calculated and crystallographic conformations confirmed the validity of the docking parameters. The binding mode of this positive control served as the reference standard for the docking scores and binding poses of all subsequent derivatives. The platform was used to score the seven initially screened drugs (GC376, lopinavir, nirmatrelvir, remdesivir, MDOLL-0229, AVI-4206, LRH-0003), and LRH-0003 (– 8.2 kcal/mol) was selected as the best-performing lead compound for subsequent derivative design. In the derivative design stage, the platform was again used to score and screen all derivatives. Redocking of the co-crystallized ligand LRH-0003 of 5SRY (PDB ligand code RIW / Enamine ID Z5265428218) as a positive control yielded a Vina score of – 7.9, validating the reliability of the docking method. To further confirm the robustness of the docking parameters, repeated docking was performed using independently generated molecular conformations (Vina score: –8.2); the two results were highly consistent, indicating that the docking method is stable and reliable. After redocking of the positive control, the deviations of key hydrogen-bond distances from the crystal structure were all less than 0.3 Å, and the binding poses were highly consistent, demonstrating the reliability of the docking method.

5 Visualization and Structural Analysis

PyMOL software was used for the visualization of the protein three-dimensional structure and molecular docking results. PyMOL was used to visually analyze the docked conformations, measure hydrogen-bond lengths, and design drug derivatives based on the binding pose and interacting residues. The NRGSuite-Qt plugin was used to assist in analyzing non-covalent interactions at the protein–ligand binding interface and to generate three-dimensional binding mode diagrams, visually displaying the binding conformation of the ligand molecule in the Mac1 active site. The Consensus Force Field (CF) score produced by this plugin is based on a different force field than the Vina score and can serve as a cross-reference to mutually corroborate the trends of binding affinity, which we used to confirm the pharmacological activity of the target drugs. In addition, the three-dimensional docking mode diagrams generated by the plugin were further used for protein–ligand interaction analysis.

6 Derivative Design

The design of the derivatives was based on the binding mode revealed by the crystal structure of the LRH-0003–Mac1 complex (PDB: 5SRY). Based on the binding poses and key interacting residues obtained from the preceding docking, the following four categories of optimization strategies were designed:

- (1) Strengthening the hydrogen-bond network: introduction of a hydroxyl group ($-OH$) or amino group ($-NH_2$) at the C4 position of the pyrrolidinone ring;
- (2) Optimizing π - π stacking and hydrophobic interactions: introduction of a methoxy group ($-OCH_3$) at the C8 position of the pyridoindole core; shifting the fluorine position ($7-F \rightarrow 6-F$); expanding the five-membered pyrrolidinone ring to a six-membered piperidinone ring;
- (3) Exploring new interactions: introduction of an ethylamine side chain ($-CH_2CH_2NH_2$) or carboxyl group ($-COOH$) at the C6 position of the core;
- (4) Combined optimization: combining the most effective modifications from the above strategies (hydroxyl + methoxy dual modification; amino + six-membered ring dual modification).

Based on the docking results of the first-round optimization, the most promising structural scaffolds were selected for second-round focused optimization. The main strategies included: the combination of C6-COOH with the piperidinone ring; the combination of C6-COOH with the F position shift; the triple modification of piperidinone + F position shift; substitution of the amino group by the carboxyl group; and the shift of the carboxyl position ($C6 \rightarrow C7$). Through two rounds of iterative optimization, the candidate molecule with the optimal binding energy was ultimately selected as the basis for subsequent research.

7 ADMET Prediction

The SWISS-ADME online tool (<https://www.swissadme.ch/>) was used to predict the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of the candidate drug molecules. The key metrics evaluated included molecular weight (MW), lipophilicity (consensus LogP), hydrogen-bond donors (HBD), hydrogen-bond acceptors (HBA), topological polar surface area (TPSA), and gastrointestinal (GI) absorption. Drug-likeness was assessed using Lipinski's Rule of Five, and the number of violations was counted.

8 Protein–Ligand Interaction Analysis

The PLIP (Protein–Ligand Interaction Profiler) online platform was used to analyze the non-covalent interactions between proteins and ligands. The analysis covered hydrogen bonds, hydrophobic interactions, and π - π stacking, with the following thresholds: the distance threshold for hydrogen bonds was set to 3.5 Å, and that for hydrophobic interactions to 4.0 Å. The PLIP analysis results were used to identify the key residues involved in binding and the types of interactions.

Results

1 Derivative Design and Molecular Docking Results

First, based on the binding pose of the co-crystallized complex Z5265428218 of 5SRY as the criterion, several currently mainstream drugs targeting the nsp3 protein of SARS-CoV-2 were selected and subjected to blind docking with CB-Dock2, yielding a set of Vina scores consistent with the positive-control binding pose, as shown below. The two molecules with the highest scores were selected for the subsequent research stage, and the remaining molecules were excluded.

Table 1
Docking scores of the initially screened drugs targeting SARS-CoV-2 nsp3 (CB-Dock2, blind docking).

Original ID / Code	Docking Score (kcal/mol)	Subsequent Processing
Z5265428218	-7.9	Not advanced to subsequent optimization
GC376	-7.8	Not advanced to subsequent optimization
Lopinavir	-7.9	Not advanced to subsequent optimization
Nirmatrelvir	-8.1	Advanced to optimization stage
Remdesivir	-5.2	Not advanced to subsequent optimization
MDOLL-0229	-5.8	Not advanced to subsequent optimization
LRH-0003	-8.2	Advanced to optimization stage

Then, we performed derivative design. Based on the data obtained in the previous step, we selected LRH-0003 as the basis for our subsequent research.

2 Binding Mode Analysis of LRH-0003 with Mac1

The binding mode of LRH-0003 with 5SRY was analyzed in PyMOL using the NRGSuite-Qt plugin (Fig. 1), which shows the key interacting residues.

The analysis indicated that the shortest distance between LRH-0003 and the backbone NH of PHE156 is 2.5 Å, and that to the side chain of ASP157 is 2.6 Å, both below 3.0 Å, indicating the presence of strong hydrogen-bond interactions. A total of 15 key amino acid residues were identified within 5 Å of the ligand (Table 2), predominantly hydrophobic residues (alanine, valine, leucine, isoleucine, proline), suggesting that hydrophobic interactions are one of the main driving forces stabilizing the binding of LRH-0003 to Mac1.

Table 2
Key interaction metrics of the LRH-0003–Mac1 binding mode.

Shortest distance between ligand and PHE156 (Å)	2.5
Shortest distance between ligand and ASP157 (Å)	2.6
Residues within 5 Å of the ligand	Chain A: ALA21, ASP22, ILE23, VAL24, GLY48, VAL49, ALA52, PRO125, LEU126, ALA129, ALA154, VAL155, PHE156, ASP157, LEU160

With respect to this result, we can make the following analysis. Analysis of the ligand–protein interaction revealed that “the shortest distance between LRH-0003 and the backbone NH of PHE156 is 2.5 Å, and that to the side chain of ASP157 is 2.6 Å, both below 3.0 Å, indicating the presence of strong hydrogen-bond interactions. A total of 15 key amino acid residues were identified within 5 Å of the ligand (Table 2), predominantly hydrophobic residues (alanine, valine, leucine, isoleucine, proline), suggesting that hydrophobic interactions are one of the main driving forces stabilizing the binding of LRH-0003 to Mac1.”

3 Derivative Design and Docking Optimization

Accordingly, we performed targeted derivative design.

We conducted two rounds of iterative optimization to obtain the optimal design results.

In the first round of derivatives, the hydrogen-bond enhancement strategy (L-1-A, L-1-B) did not significantly change the binding affinity (both – 8.2 kcal/mol). In the π – π stacking optimization strategy, introduction of a methoxy group at the C8 position of the core (L-2-A) decreased the binding energy to – 7.6 kcal/mol, whereas expansion of the pyrrolidinone to a piperidinone (L-2-C) improved the binding energy to – 8.3 kcal/mol. In the exploration of new interactions, introduction of a carboxyl group at the C6 position of the core (L-3-B) yielded a binding energy of – 8.4 kcal/mol, an improvement of 0.2 kcal/mol over the parent compound.

Based on the first-round results, we combined the C6 carboxyl group with the piperidinone ring to design L-5-A. This molecule achieved a Vina score of – 9.3 kcal/mol, an improvement of 1.1 kcal/mol over the parent LRH-0003 (– 8.2 kcal/mol), the best performance among all derivatives (Table 3). This suggests that C6-COOH and the piperidinone ring may act synergistically and warrants further validation.

Table 3
Vina scores of the LRH-0003 derivatives.

Compound	Modification	Vina score (kcal/mol)
LRH-0003 (parent)	—	-8.2
L-1-A	Pyrrolidinone C4-OH	-8.2
L-1-B	Pyrrolidinone C4-NH ₂	-8.2
L-2-A	Core C8-OCH ₃	-7.6
L-2-B	F: 7→6	-8.2
L-2-C	Pyrrolidinone → piperidinone	-8.3
L-3-A	Core C6-CH ₂ CH ₂ NH ₂	-7.5
L-3-B	Core C6-COOH	-8.4
L-4-A	-OH + -OCH ₃	-7.9
L-4-B	Piperidinone + -NH ₂	-8.3
L-5-A	C6-COOH + piperidinone	-9.3
L-5-B	C6-COOH + F: 7→6	-8.1
L-5-C	C6-COOH + piperidinone + F: 7→6	-8.3
L-5-D	-COOH on piperidinone ring	-8.7
L-5-E	C6-COOH + hydroxyl	-8.4
L-5-F	Core C7-COOH	-8.5

4 Protein–Ligand Interaction Analysis (PLIP)

To elucidate the structural basis for the improved binding affinity of the optimal derivative L-5-A and the reasons for the reduced activity of the low-activity derivative L-2-A, we used PLIP to compare the interaction modes of the parent LRH-0003, the representative low-activity molecule L-2-A, and the optimal molecule L-5-A (Table 4).

Table 4
Comparison of PLIP interactions among LRH-0003, L-2-A, and L-5-A.

Feature	LRH-0003 (parent)	L-2-A (low-activity)	L-5-A (optimal)
Vina score (kcal/mol)	-8.2	-7.6	-9.3
Number of hydrogen bonds	3	2	4
Shortest hydrogen-bond distance	3.24 Å (VAL49)	2.74 Å (LEU126)	2.75 Å (PHE156)
Aromatic–hydrophobic contact	None	None	Yes (3.03 Å)
Number of hydrophobic interactions	5	5	5

The parent LRH-0003 forms three hydrogen bonds, from the backbone NH of VAL49 (3.24 Å), the backbone NH of LEU126 (3.28 Å), and the backbone NH of PHE156 (4.08 Å); the hydrogen bond with PHE156 has a longer distance (4.08 Å) and is thus weaker. Hydrophobic interactions involve five residues: VAL49, LEU126, VAL155, PHE156, and LEU160.

The low-activity derivative L-2-A forms only two hydrogen bonds, from the backbone NH of ILE23 (3.30 Å) and the backbone NH of LEU126 (2.74 Å); hydrophobic interactions involve five residues: VAL49, LEU126, ALA129, PHE156, and LEU160. Notably, L-2-A completely loses the direct hydrogen bond with PHE156, which may be the main reason for the decrease of its binding energy to - 7.6 kcal/mol.

L-5-A forms four hydrogen bonds, from the backbone NH of ILE23 (3.37 Å), the backbone NH of VAL24 (3.92 Å), the backbone carbonyl of ALA154 (3.70 Å, as acceptor), and the backbone NH of PHE156 (2.75 Å). Among them, the hydrogen bond between the backbone NH of PHE156 and the carboxyl group has a distance of 2.75 Å, markedly shorter than the corresponding distance in the parent compound (4.08 Å), restoring and strengthening the hydrogen-bond interaction with PHE156. In addition, L-5-A forms a close hydrophobic contact of 3.03 Å with PHE156, together with five hydrophobic interactions.

The three-way comparison demonstrates that the hydrogen bond with the backbone NH of PHE156 is a key factor in maintaining high binding affinity. L-2-A loses the hydrogen bond with PHE156, leading to decreased activity, whereas L-5-A restores a strong hydrogen bond with PHE156 (2.75 Å) through the introduction of the C6 carboxyl group and gains a new 3.03 Å aromatic–hydrophobic contact, forming a multi-mode synergistic stabilization network of “hydrogen bond + hydrophobic contact” and achieving a binding energy of - 9.3 kcal/mol. This explains the effectiveness of the combined C6-COOH + piperidinone modification and provides clear structural guidance for subsequent derivative design.

Key Findings Summary

Table 5
Key findings summary.

Molecule	Interaction with PHE156	Vina score (kcal/mol)	Description
Parent LRH-0003	Weak hydrogen bond (4.08 Å)	−8.2	Baseline
L-2-A (low-activity)	None	−7.6	Lost the key interaction
L-5-A (optimal)	Strong hydrogen bond (2.75 Å) + aromatic–hydrophobic contact (3.03 Å)	−9.3	Multi-mode synergy, optimal

5 ADMET Prediction and Drug-Likeness Assessment

To verify the safety and industrial synthesis feasibility of L-5-A, SWISS-ADME was used to perform ADMET prediction on the parent LRH-0003 and L-5-A to assess their drug-likeness.

Table 6
ADMET prediction and drug-likeness parameters of LRH-0003 and L-5-A.

Parameter	LRH-0003 (parent)	L-5-A	Acceptable range
Molecular weight (MW)	285.28	343.31	< 500
Consensus LogP	2.18	1.30	< 5
Hydrogen-bond donors (HBD)	2	2	≤ 5
Hydrogen-bond acceptors (HBA)	4	6	≤ 10
TPSA	73.91 Å ²	100.35 Å ²	< 140
GI absorption	High	High	—
Blood–brain barrier permeability	Yes	No	—
Lipinski violations	0	0	≤ 1
Bioavailability score	0.55	0.56	—

Data source: SWISS-ADME online prediction tool (<https://www.swissadme.ch/>).

L-5-A has a molecular weight of 343.31 Da, a consensus LogP of 1.30, two hydrogen-bond donors, six hydrogen-bond acceptors, and a topological polar surface area (TPSA) of 100.35 Å². All parameters meet the standard ranges of Lipinski’s Rule of Five, with zero violations. GI absorption is predicted to be “High,” with a bioavailability score of 0.56.

Compared with the parent LRH-0003, the introduction of the carboxyl group in L-5-A slightly increased the molecular weight (285 → 343 Da) and raised the TPSA (~ 85 → 100.35 Å²), which nevertheless

remains below the 140 Å² threshold for oral absorption. LogP decreased from ~ 1.5 to 1.30, indicating that the carboxyl group increased the hydrophilicity of the molecule. Although these changes shift the physicochemical properties of L-5-A to some extent, it still fully satisfies Lipinski's Rule of Five, suggesting that the molecule has good oral bioavailability potential.

In addition, L-5-A shows no predicted inhibitory activity against the five major CYP enzymes CYP1A2, 2C19, 2C9, 2D6, and 3A4, indicating a low risk of drug–drug interactions.

Discussion

In this study, targeting the SARS-CoV-2 Mac1 domain and using LRH-0003 as the lead compound, we ultimately identified L-5-A as the final drug molecule through two rounds of structure-guided derivative design and iterative optimization. This molecule achieved a Vina score of – 9.3 kcal/mol, an improvement of 1.1 kcal/mol over the parent LRH-0003 (– 8.2 kcal/mol), indicating a relatively marked improvement in binding affinity. Meanwhile, L-5-A satisfies all criteria of Lipinski's Rule of Five (0 violations), with predicted GI absorption of “High” and blood–brain barrier permeability of “No”; while maintaining favorable drug-likeness, L-5-A has a low risk of central nervous system penetration, with safety superior to the parent LRH-0003.

PLIP interaction analysis revealed the structural basis for the improved binding affinity of L-5-A. Compared with the parent LRH-0003, after the introduction of the carboxyl group at the C6 position, L-5-A forms a strong 2.75 Å hydrogen bond with the backbone NH of PHE156, markedly shorter than the hydrogen-bond distance of PHE156 in the parent compound (4.08 Å), indicating stronger affinity of the molecule for its target. In addition, L-5-A forms a 3.03 Å aromatic–hydrophobic contact with PHE156, creating a multi-mode synergistic stabilization network of “hydrogen bond + hydrophobic contact.” In contrast, the low-activity derivative L-2-A completely loses the direct hydrogen bond with PHE156 due to the C8 methoxy modification, and its binding energy decreases to – 7.6 kcal/mol. This comparison clearly demonstrates that the hydrogen bond with the backbone NH of PHE156 is the key factor in maintaining high binding affinity, and that the introduction of the C6 carboxyl group successfully restores and strengthens this key interaction.

Compared with known Mac1 inhibitors, L-5-A exhibits certain competitive advantages. MDOLL-0229 (IC₅₀ = 2.1 µM) is the first small molecule demonstrated to inhibit coronavirus replication in cellular models by targeting Mac1¹³, whereas LRH-0003 has an IC₅₀ of 1.7 µM¹⁶. Although the binding affinity data in this study are based on computational prediction rather than experimental measurement, the Vina score of L-5-A (– 9.3 kcal/mol) is markedly improved over both the parent LRH-0003 (– 8.2 kcal/mol) and the co-crystallized ligand Z5265428218 (– 7.9 kcal/mol), suggesting that it may possess stronger enzyme inhibitory activity, which requires experimental verification. In addition, the molecular weight (343 Da) and LogP (1.30) of L-5-A are both within the ideal range for oral drugs, whereas MDOLL-0229 has a relatively high molecular weight (393.5 g/mol) and LogP (~ 3.5); L-5-A may therefore have certain advantages in drug-likeness.

This study has several limitations. First, all binding affinity data are based on molecular docking predictions (AutoDock Vina) and have not yet been validated experimentally (e.g., surface plasmon resonance, isothermal titration calorimetry). The scoring error of AutoDock Vina is generally within ± 1.0 – 1.5 kcal/mol; therefore, although the 1.1 kcal/mol difference is of reference value, it should be interpreted with caution. Second, molecular dynamics simulations were not performed for L-5-A in this study, and its binding stability under dynamic conditions remains unclear. Third, no in vitro antiviral activity assays were performed; whether L-5-A can inhibit SARS-CoV-2 replication at the cellular level still requires experimental verification.

Future work should proceed along the following directions: (1) validate the direct binding affinity of L-5-A to Mac1 through surface plasmon resonance (SPR) or isothermal titration calorimetry (ITC) experiments; (2) perform molecular dynamics simulations to evaluate the binding stability of L-5-A in solution; (3) evaluate the anti-SARS-CoV-2 activity and cytotoxicity of L-5-A at the cellular level; and (4) carry out further chemical modification of L-5-A to explore the structure–activity relationships of its derivatives.

Conclusion

In this study, targeting the SARS-CoV-2 Mac1 domain and using LRH-0003 as the lead compound, we successfully identified L-5-A—a derivative in which a carboxyl group was introduced at the C6 position of the LRH-0003 core scaffold and the pyrrolidinone ring was expanded to a piperidinone ring—through two rounds of structure-guided derivative design and iterative optimization. L-5-A achieved a Vina score of -9.3 kcal/mol, an improvement of 1.1 kcal/mol over the parent LRH-0003 (-8.2 kcal/mol), and satisfies all criteria of Lipinski's Rule of Five (0 violations), with predicted GI absorption of “High” and blood–brain barrier permeability of “No.” PLIP interaction analysis indicated that the improved binding affinity of L-5-A mainly originates from the strong 2.75 Å hydrogen bond between the C6 carboxyl group and the backbone NH of PHE156, as well as newly formed aromatic–hydrophobic interactions. As a novel Mac1 inhibitor candidate with strong binding affinity and favorable drug-likeness, L-5-A warrants further experimental validation of its antiviral activity.

Declarations

Ethics approval and consent to participate

Not applicable. This study did not involve human participants, human data, or animal experiments.

Consent for publication

Not applicable. This manuscript does not contain any individual person's data in any form.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files. The protein structure data used in this study are publicly available from

the RCSB Protein Data Bank (PDB ID: 5SRY). The computational tools used are publicly accessible online platforms (CB-Dock2, SWISS-ADME, PLIP) and open-source software (PyMOL, MEGA).

Competing interests

The author declares that he has no competing interests.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author Contribution

Li ZX conceived and designed the study, performed all computational experiments (sequence alignment, molecular docking, ADMET prediction, and PLIP analysis), analyzed the data, and drafted the manuscript. The author read and approved the final manuscript.

Acknowledgements

The author thanks his academic advisors for their guidance and support throughout this research project. The author also acknowledges the developers of the open-source and freely accessible computational tools used in this study: CB-Dock2, SWISS-ADME, PLIP, PyMOL, and MEGA.

Data Availability

All data supporting the findings of this study are available within the paper and its supplementary information files. The protein structure used for molecular docking (PDB ID: 5SRY) is publicly available from the RCSB Protein Data Bank (<https://www.rcsb.org/>). The SARS-CoV-2 sequence data used for initial screening were obtained from the NCBI Virus database (<https://www.ncbi.nlm.nih.gov/labs/virus/>). All computational tools used in this study are publicly accessible online platforms (CB-Dock2, SWISS-ADME, PLIP) and open-source software (PyMOL, MEGA), as described in the Methods section. No new datasets were generated during the current study.

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Figures

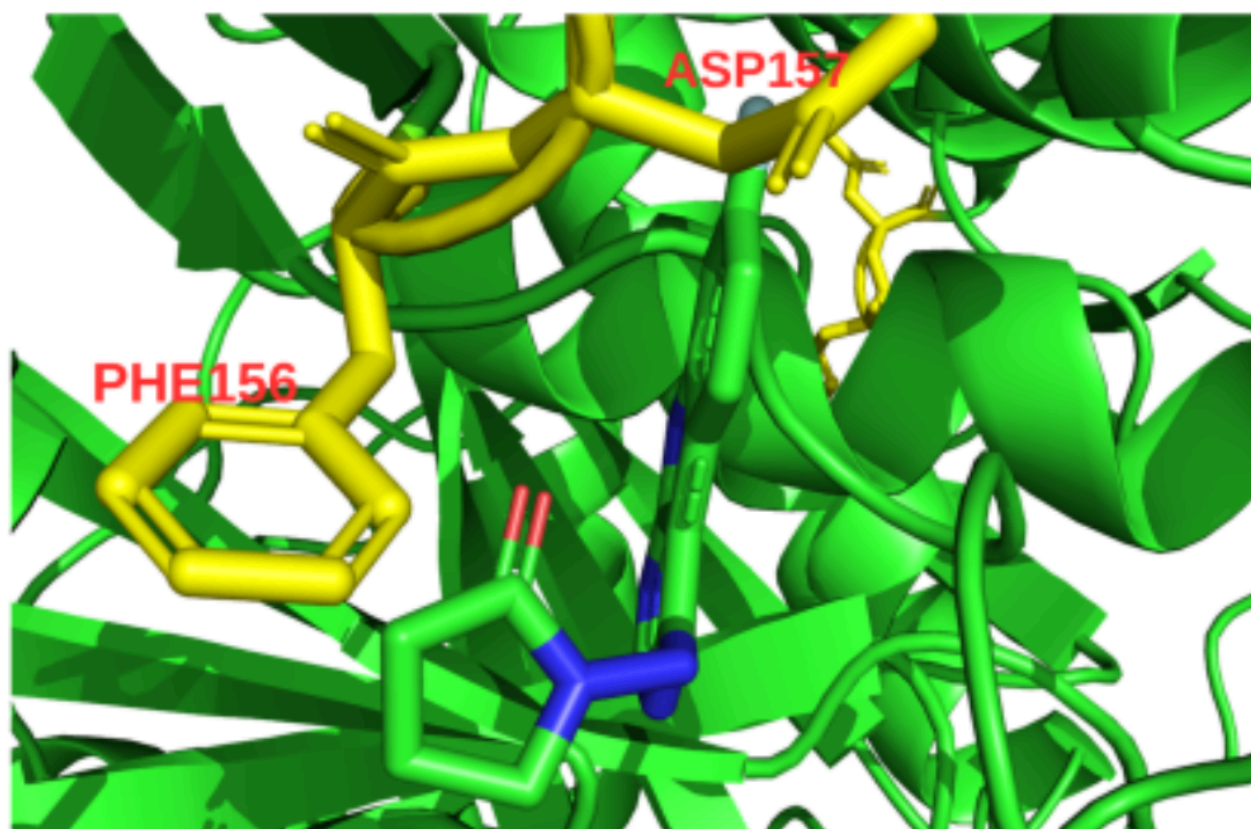


Figure 1

Binding mode of LRH-0003 with Mac1. LRH-0003 is shown as a green stick model; the key residues PHE156 and ASP157 are displayed as yellow stick models, and residue names are labeled in red.

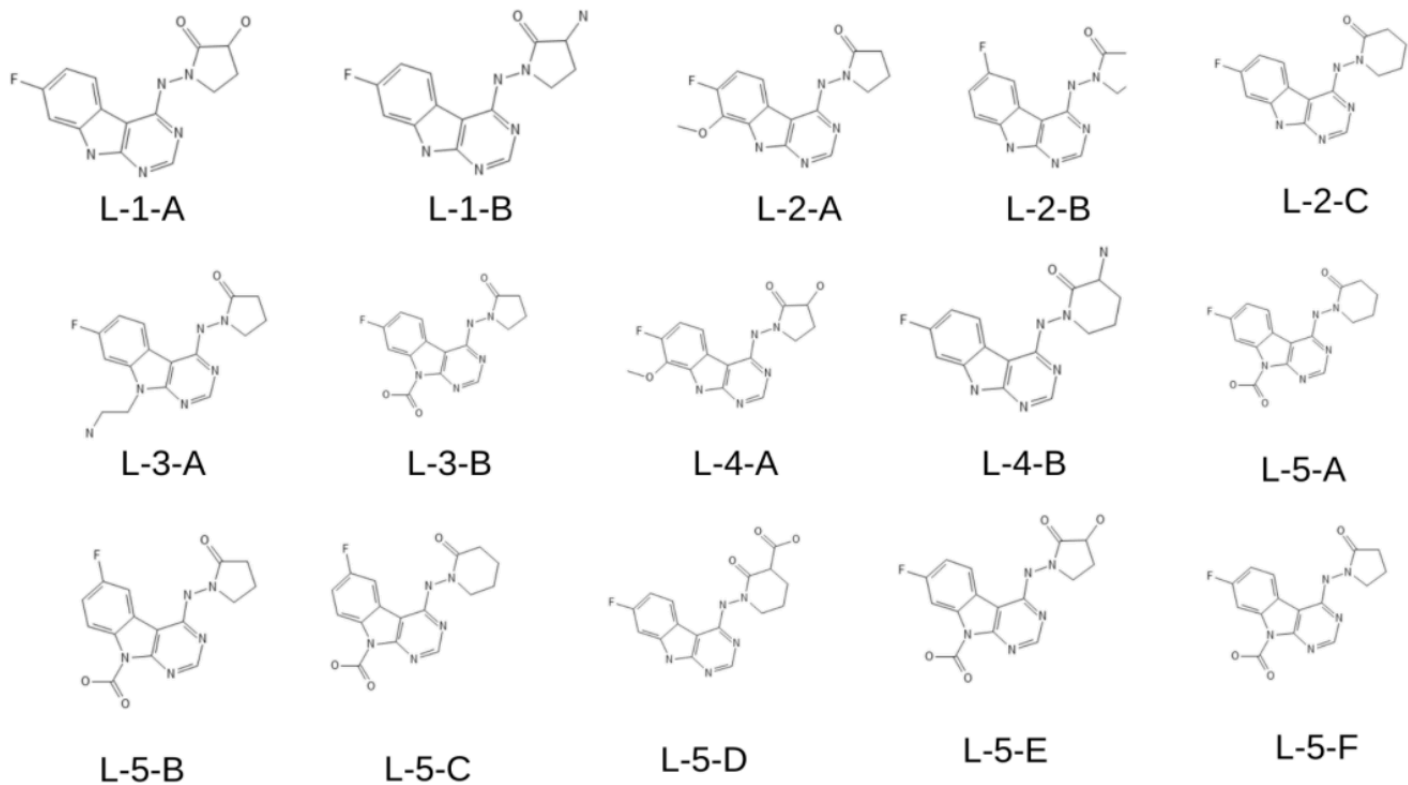


Figure 2

Chemical structures of the LRH-0003 derivatives. The structural formulas were generated by PubChem.