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Innovative Antiviral Strategy Targeting PL^{pro}: Discovery of Jun12682 and Analysis of Its Antipandemic Effects

Xuan Jia
Tropical Veterinary Medicine Center, Hainan Institute of Tropical Agricultural Resources, Sanya, 572024, China
Corresponding author email: cherryjxuan@gmail.com

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The paper "Design of a SARS-CoV-2 papain-like protease inhibitor with antiviral efficacy in a mouse model," authored by Bin Tan, Xiaoming Zhang, Ahmadullah Ansari, Prakash Jadhav, et al., was published in *Science* on March 29, 2024. The authors are affiliated with Department of Medicinal Chemistry, Ernest Mario School of Pharmacy, Rutgers, the State University of New Jersey, and Department of Physiological Sciences, College of Veterinary Medicine, Oklahoma State University, among others. This research focuses on a key enzyme of SARS-CoV-2, the papain-like protease (PL^{pro}), which plays a crucial role in the virus's replication and immune evasion mechanisms. Using a structure-guided approach, the research team designed a series of non-covalent PL^{pro} inhibitors. These inhibitors specifically target a newly discovered ubiquitin-binding site, Val70Ub, on PL^{pro}, demonstrating their antiviral activity in both in vitro and in vivo models. The lead compound, Jun12682, showed excellent antiviral effects in a mouse model, improving survival rates, and reducing pulmonary viral loads, offering a new strategic direction for the treatment of SARS-CoV-2.

1 Experimental Data Analysis

In the study, the discovery and evaluation of Jun12682 were accomplished through a series of meticulous experimental steps. In vitro experiments demonstrated that Jun12682 has very strong inhibitory activity against PL^{pro}, with an inhibitory constant (Ki) of only 37.7 nanomolar, and an effective concentration for 50% of maximal effect (EC50) value of 1.1 micromolar in the FlipGFP PL^{pro} cell assay, indicating its high efficacy in inhibiting the key enzyme activity of the virus. Further in vivo experiments conducted in a mouse model showed that Jun12682 significantly improved the survival rate of mice infected with SARS-CoV-2, mitigated the virus-induced weight loss, and effectively reduced the viral load in the lungs. These experimental data collectively validate the value of Jun12682 as a potential anti-SARS-CoV-2 therapeutic drug.

Figure 1 displays the X-ray crystal structure of the hybrid covalent inhibitor Jun11313, designed based on XR8-24 and Cp7, with SARS-CoV-2 PL^{pro}. In structure (B), the interactions between Jun11313 and the PL^{pro} binding site are represented by hydrogen bonds (black dashed lines), with the spatial structure of Jun11313 shown in green ball-and-stick model. Figure (C) overlays the structure of PL^{pro} with Jun11313 against the XR8-24 complex, highlighting the amino acid residues related to the binding of both compounds. Structure (D) further presents an overlay of the binding modes of Jun11313, XR8-24, and ubiquitin on PL^{pro}. Structure (E) provides the generic chemical structure of the designed biarylphenyl PL^{pro} inhibitors and highlights key interactions. Lastly, the flowchart (F) shows the optimization process for PL^{pro} inhibitors, with Jun12682 being selected as the leading candidate for in vivo studies.

Figure 2 showcases a series of representative compounds of biarylbenzamide class SARS-CoV-2 PL^{pro} inhibitors. Item A in the illustration represents the positive control GRL0617; items B, C to F are PL^{pro} inhibitors containing thiophene and pyrazole. These compounds were characterized for their half-maximal inhibitory concentration (IC50) and inhibitory constant (Ki) through FRET-based enzymatic activity assays, and their cytotoxicity (CC50)

in Vero E6 cells was evaluated. Moreover, the EC50 values for FlipGFP PL^{pro} were tested using 293T cells transfected with PL^{pro} and FlipGFP. The antiviral activity (EC50) of these compounds against SARS-CoV-2 was assessed in Caco-2 cell lines expressing hACE2 and hTMPRSS2. The figure also provides data on the half-life (T1/2) of PL^{pro} inhibitors in mouse liver microsomes, with all data presented as the mean ± standard deviation of three technical replicates.

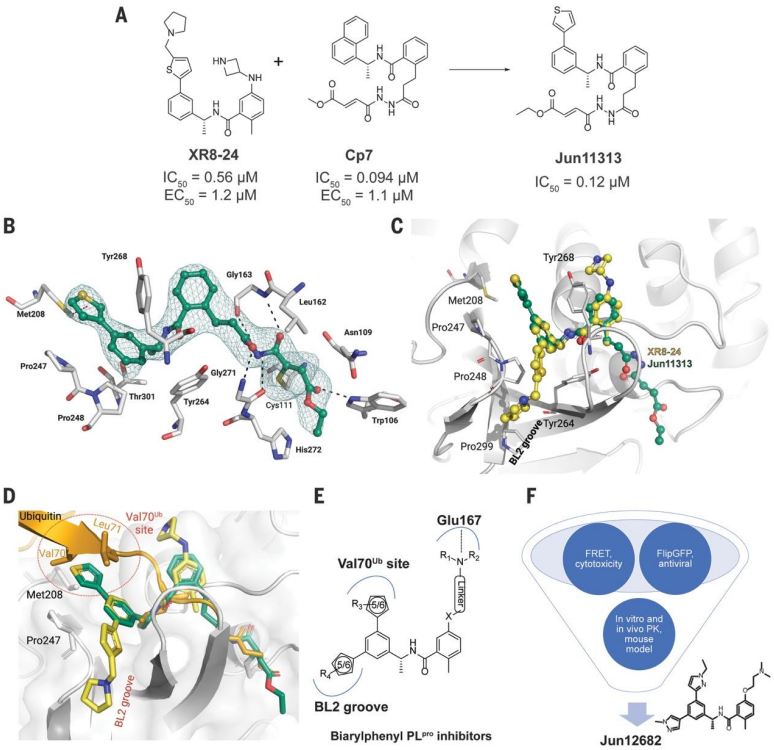

Figure 1 X-ray crystal structure of the covalent inhibitor Jun11313 with SARS-CoV-2 PL^{pro} and structure-based design of biarylphenyl SARS-CoV-2PL^{pro} inhibitors

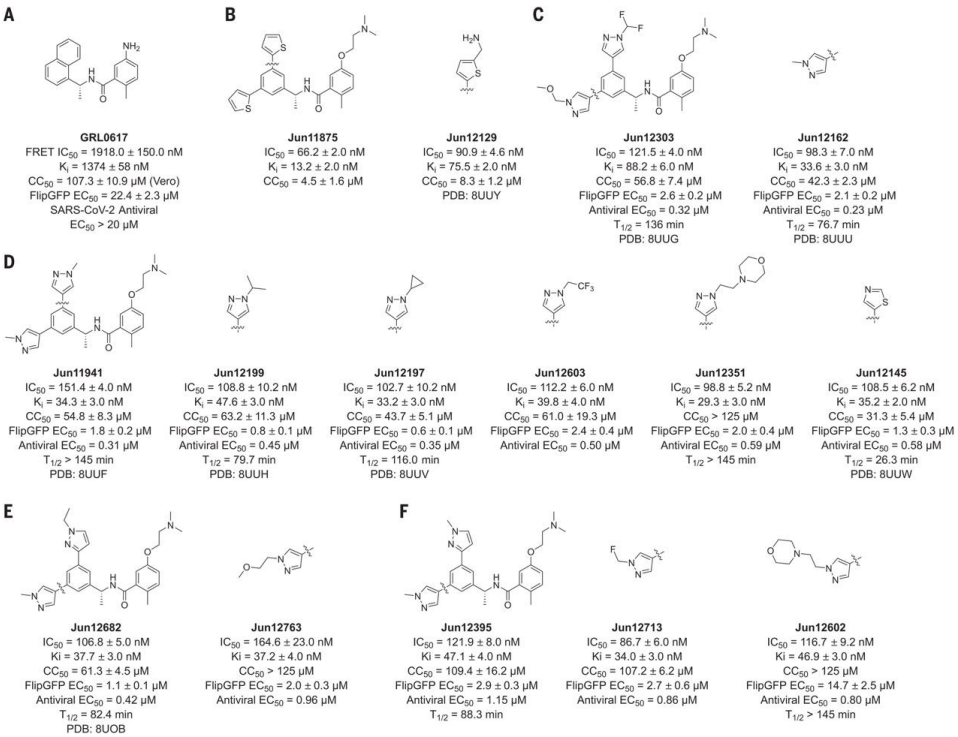

Figure 2 Representative biarylbenzamide series of SARS-CoV-2 PL^{pro} inhibitors

Figure 3 displays the antiviral activity of PL^{pro} inhibitors against different variants of SARS-CoV-2 and the mechanistic studies of Jun12682. Figures A, B, and C show the inhibitory effects of nirmatrelvir, Jun11941, and Jun12682 on the original WA1 strain, the Delta variant, the Omicron variant, and resistant strains, respectively. Jun12682 exhibits significant antiviral activity against all viral strains, with particularly strong effects against the Omicron variant. Figures D to G present the quantitative results of Jun12682 inhibiting SARS-CoV-2 PL^{pro} enzymatic activity, including the inhibition of ubiquitin (Ub-AMC) and interferon-stimulated gene 15 (ISG15-AMC) hydrolysis, demonstrating its potent inhibitory action. Figures H and I are screening for inhibition against human proteases USP7 and USP14, where Jun12682 shows selective inhibition of PL^{pro} over USP7 and USP14. Figure J evaluates the impact of Jun12682 and the control GRL0617 on the stability of SARS-CoV-2 PL^{pro} through differential scanning fluorimetry, indicating that Jun12682 can effectively stabilize the PL^{pro} protein. Overall, these data suggest that Jun12682 is a potent SARS-CoV-2 PL^{pro} inhibitor with potential as a new antiviral drug.

Figure 4 illustrates the X-ray crystal structures of SARS-CoV-2 PL^{pro} with multiple biarylbenzamide inhibitors, with a particular emphasis on the atomic model of Jun12682 at the PL^{pro} binding site. This model reveals the hydrogen bonds (black dashed lines), van der Waals contacts (red dashed lines), and π-π interactions (light green dashed lines) between Jun12682 and PL^{pro}. Additionally, for each compound, the inhibitory constant Ki from FRET enzymatic assays and the antiviral activity EC50 value against SARS-CoV-2 in Caco-2 cells are provided. The "polder map" of the inhibitors, displayed as a gray mesh, reveals the precise morphology of binding to PL^{pro}, confirming the potential efficacy of these molecules in blocking viral functions.

Figure 5 presents the pharmacokinetic (PK) characteristics of PL^{pro} inhibitors in vitro and in vivo and the in vivo antiviral efficacy of Jun12682. Figures A and B show the plasma concentration of different compounds in mice. Notably, Jun12682 exhibits favorable pharmacokinetic properties after oral administration (Figure C). Figure D summarizes the in vitro pharmacokinetic parameters of Jun12682, such as microsomal stability and CYP inhibition. Figures E to G and H to J designed treatment experiments for 5 days and 3 days, respectively, to evaluate the efficacy of Jun12682 against the SARS-CoV-2 N501Y variant infection in a mouse model, including changes in body weight and survival rates. Mice treated with Jun12682 showed lower viral loads (Figure K), reduced pathological damage (Figures N and O), and decreased levels of inflammatory factors (Figure M) compared to untreated mice. These results collectively suggest that Jun12682 has potential as an oral antiviral drug against SARS-CoV-2.

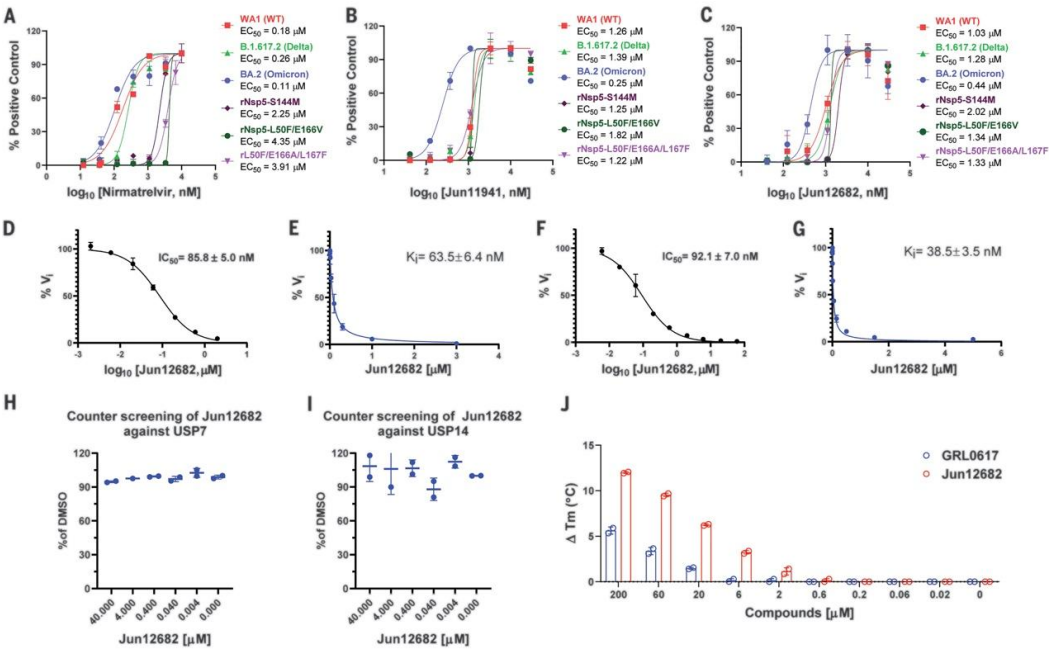

Figure 3 Antiviral activity of PL^{pro} inhibitors against SARS-CoV-2 variants and mechanistic studies of Jun12682

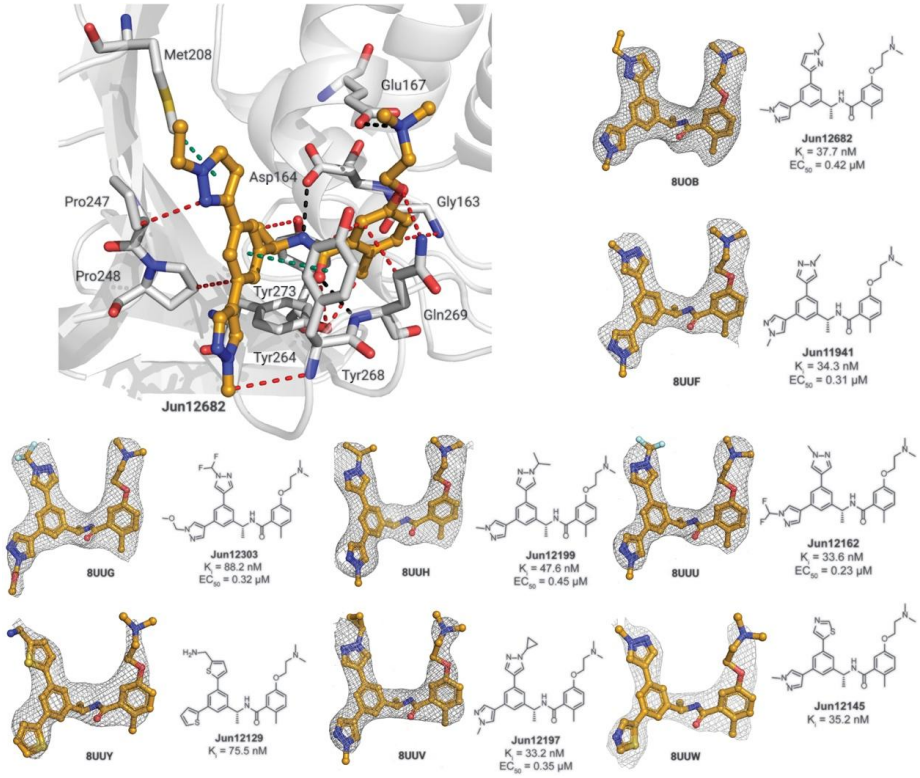

Figure 4 X-ray crystal structures of SARS-CoV-2 PL^{pro} with biarylphenyl PL^{pro} inhibitors

2 Analysis of Research Findings

In this study, the research team made a breakthrough confirmation that the papain-like protease (PL^{pro}) of SARS-CoV-2 is an effective drug target. Through detailed structural analysis, they discovered the Val70Ub site on PL^{pro} for the first time, suggesting its potential as a drug-binding site. This discovery provides a foundation for designing novel antiviral drugs. Using a structure-guided approach, the team successfully developed a series of non-covalent PL^{pro} inhibitors targeting this new site. Among these inhibitors, Jun12682 was particularly noted for its exceptional efficacy. It not only demonstrated potent PL^{pro} inhibitory activity in vitro but also proved to effectively inhibit SARS-CoV-2 replication in a mouse model, showing its great potential as a therapeutic drug. The discovery of Jun12682 validates the strategy of effectively blocking SARS-CoV-2 replication by targeting the Val70Ub site on PL^{pro}, and it opens new directions for future drug development against SARS-CoV-2 and potential variants of coronaviruses. This breakthrough holds significant importance for current COVID-19 treatment research and offers new insights and strategies for the global scientific community in exploring more effective methods for viral treatment.

3 Evaluation of the Research

This study, by integrating advanced methods from structural biology and medicinal chemistry, successfully identified Jun12682 as a potential drug candidate against the SARS-CoV-2 virus, demonstrating the research team's exceptional capability in scientific innovation. Through precise analysis of the structure and function of the PL^{pro} enzyme, the research not only deepened our understanding of viral biology but also showed how to translate complex scientific knowledge into practical therapeutic strategies. The discovery of Jun12682 validates the feasibility, both theoretically and practically, of therapies targeting the SARS-CoV-2 PL^{pro} enzyme, offering new hope and possibilities for combating the current pandemic and potential future coronavirus variants.

4 Conclusions

Jun12682, as a novel PL^{pro} inhibitor, has shown significant anti-SARS-CoV-2 activity both in vitro and in vivo, offering not only a new potential means to combat the COVID-19 pandemic but also new directions and strategies for the development of therapeutic drugs for coronavirus diseases. This work highlights the importance of PL^{pro} as a drug target and demonstrates the power of structure-guided design methods in modern drug discovery.

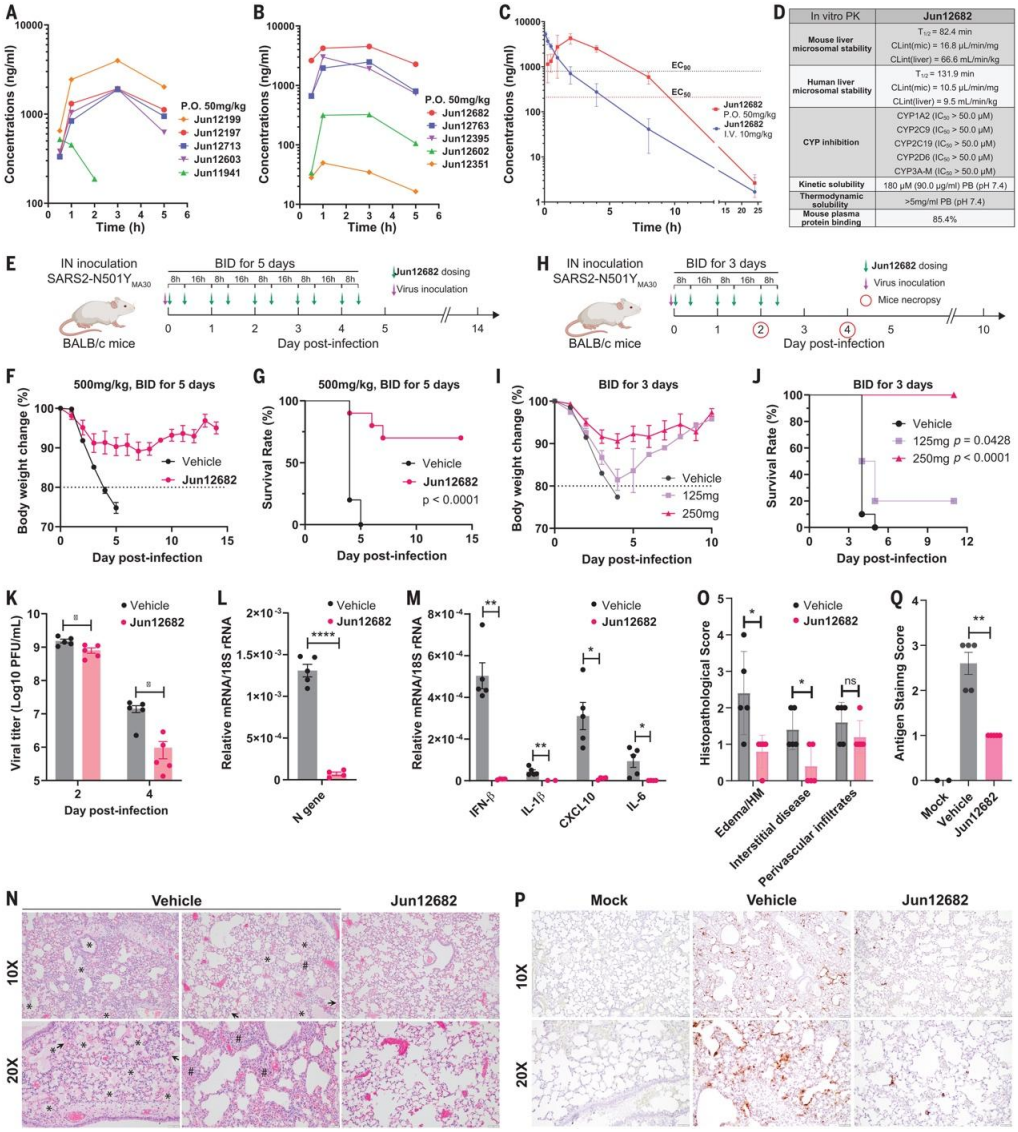

Figure 5 In vitro and in vivo PK profiling of PL^{pro} inhibitors and in vivo antiviral efficacy of Jun12682

5 Access the Full Text

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Acknowledgments

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