Nobel Quinazoline Derivatives Targeting SARS-CoV-2 RdRp with In Vitro and In Vivo Efficacy

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BACKGROUND

Current SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) and protease inhibitors reduce viral loads and disease severity in patients with COVID-19. However, drug-resistant variants continue to emerge, highlighting the need for new small-molecule inhibitors. Amodiaquine, an antimalarial drug, has been reported to exhibit antiviral activity against SARS-CoV-2. We previously designed and synthesized novel amodiaquine derivatives with enhanced potency and reduced cytotoxicity (1). Building on these findings, we redesigned the amodiaquine scaffold by replacing its quinoline core with a quinazoline backbone. Using this approach, we synthesized and screened more than 400 derivatives to identify compounds with stronger anti-SARS-CoV-2 activity and improved selectivity.

METHODS

Antiviral Assays: Novel quinazoline derivatives were synthesized based on previously reported procedures (1, 2). Cell-based antiviral assays were performed using VeroE6/TMPRSS2 and HEK293T/ACE2 cells. The cells were mock-infected or infected with SARS-CoV-2 at an MOI of 0.01 in the presence of serially diluted compounds and further incubated. After 3 days, the number of viable cells was quantified using 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT). Viral RNA level in the culture supernatants of infected cells was measured by real-time RT-PCR.

Time-of-Addition Assay: VeroE6/TMPRSS2 cells (2×10^4 cells/well) were incubated at 37°C for 24 hours. The cells were then subjected to viral infection and compound treatment at different time intervals relative to infection. Following these treatments, the cells were incubated for an additional 47 hours. The number of viable cells was measured using the MTT assay.

RdRp Inhibition Assay: The inhibition of SARS-CoV-2 RdRp activity *in vitro* was evaluated according to the previous report (3).

Docking Simulations: Docking simulations were performed using ASEDock (MOLSIS, Inc., Tokyo, Japan) to model the interactions between the compounds and SARS-CoV-2 RdRp (4).

In Vivo Study: BALB/C mice were anesthetized and intranasally inoculated with 1×10^5 $TCID_{50}$ of SARS-CoV-2 (WK-521 strain). The mice were orally administered the compounds at 2.5 mg/kg twice daily. On day 4, the mice were euthanized, and total RNA was extracted from the excised lung tissues. The viral RNA levels were quantified by real-time RT-PCR.

We identified potent and selective **quinazoline** antivirals that inhibit SARS-CoV-2 RNA-dependent RNA polymerase (RdRp), remain effective against variants, and show *in vivo* efficacy, supporting their potential as affordable COVID-19 therapeutics.

RESULTS.

Fig. 1. From quinoline to quinazoline derivatives

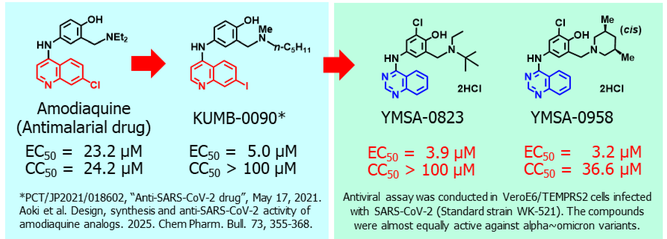


Fig. 2. Anti-SARS-CoV-2 activity of novel quinazoline derivatives

Cell (Assay)	Compound #785	Compound #808	Compound #842
VeroE6/TMPRSS2 (CPE)	**3.3** > 100	**7.6** > 100	**5.0** > 100
VeroE6/TMPRSS2 (RNA)	**1.9**	**1.9**	**2.0**
Vero (RNA)	**1.7**	**5.5**	**1.2**
HEK293T/ACE2 (RNA)	**0.12** > 100	**0.12** 85	**0.31** 14
Various variants (CPE assay in Vero/TMPRSS2 cells)			
Alpha variant (QK002)	**2.7**	**3.8**	**2.6**
Beta variant (TY8-612)	**6.8**	**9.3**	**8.1**
Gamma variant (TY7-501)	**3.5**	**5.4**	**3.4**
Delta variant (TY11-927)	**7.8**	**6.4**	**3.2**
Omicron variant (TY38-873)	**2.3**	**4.6**	**3.7**

The values in the upper row indicate EC_{50} (μM), and the value in the lower row indicate CC_{50} (μM).

Fig. 3. Time-of-addition experiments

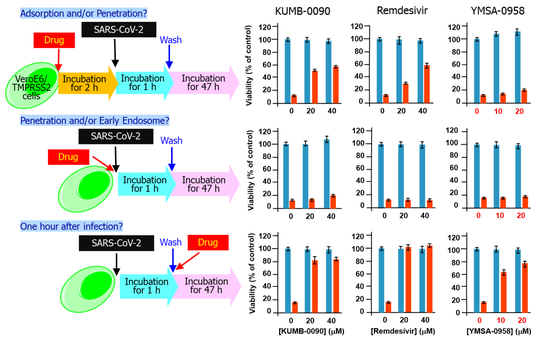


Fig. 4. Inhibition of SARS-CoV-2 RdRp

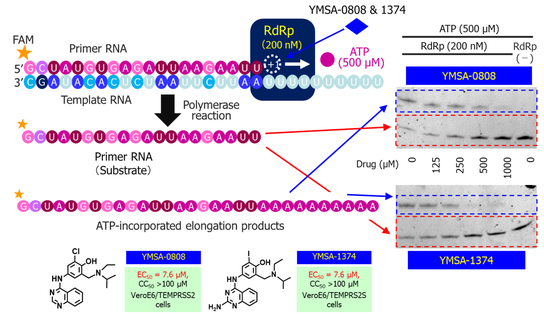


Fig. 6. In vivo efficacy (SARS-CoV-2-infected mice)

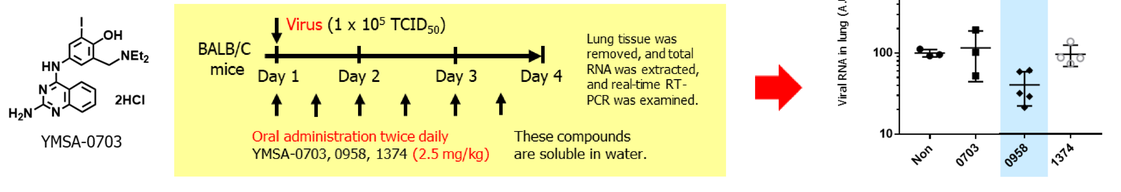
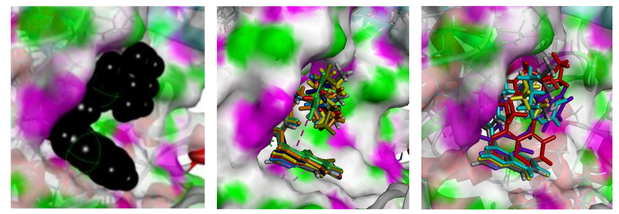


Fig. 5. Docking simulation study with SARS-CoV-2 RdRp



YMSA-0958 | Active compounds | Inactive compounds

CONCLUSIONS

We have identified a new class of potent and selective quinazoline derivatives that inhibit SARS-CoV-2 RdRp and retain antiviral activity against multiple viral variants. One of these compounds demonstrated significant anti-SARS-CoV-2 efficacy in a mouse infection model. Unlike nucleoside analogues, these compounds belong to a distinct chemical class and may be effective against drug-resistant strains. Their low molecular weight and short synthetic route make them promising candidates for combination therapy with existing antivirals. Taken together, these features support their potential as affordable and globally accessible therapeutic options for treating SARS-CoV-2 infection.

ADDITIONAL KEY INFORMATION

References

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Conflict of Interest

The authors declare no conflict of interest.

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