Prevention of the Spread of Virus Infection in Global Societies: Anti-SARS-CoV-2 Agents Learned from HIV Researches

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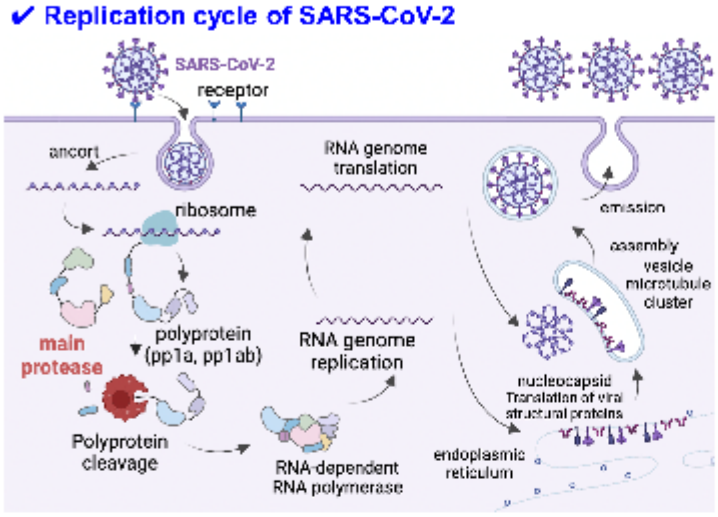
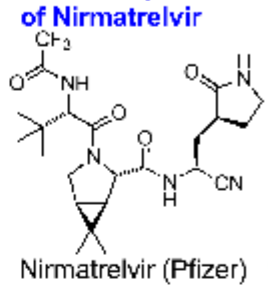
Introduction

More than three years have passed since the beginning of the pandemic of the novel emerging infection disease, COVID-19 which is produced by SARS-CoV-2. Since SARS-CoV-2 main protease (M^{pro}) is essential for viral replication and there is no human enzyme closely homologous with the M^{pro}, it is an important and attractive drug discovery target for treatment of COVID-19. Recently, inhibitors of the SARS-CoV-2 M^{pro}, Nirmatrelvir and Ensitrelvir, were developed. However recent studies warned of the possibility of the emergence of drug-resistant SARS-CoV-2 variants against the Nirmatrelvir therefore development of novel drugs and an increase in the repertory of the drugs are urgently needed. In order to meet this challenge, we previously reported highly potent M^{pro} inhibitors, **TKB198** and **TKB211**, based on YH-53/5h, which was developed by Hayashi et al. in 2013 as a SARS-CoV M^{pro} inhibitor. More recently, our further studies led to the development of more potent inhibitors, **TKB245** and **TKB248**. In this study, we report our structure-activity relationship studies and evaluation of the anti-SARS-CoV-2 activity of derivatives of these inhibitors.

✔ Replication cycle of SARS-CoV-2



✔ Potential problems of Nirmatrelvir



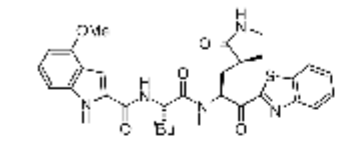
Nirmatrelvir (Pfizer)

Owen, D. R., et al. Science 2021, 374, 1586-1593.

Problems
- antiviral activity
- metabolic stability

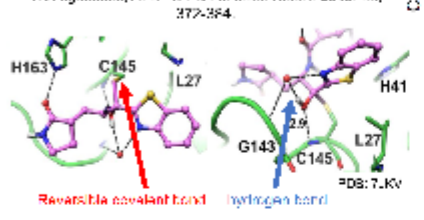
→Solve these problems

✔ YH-53/5h



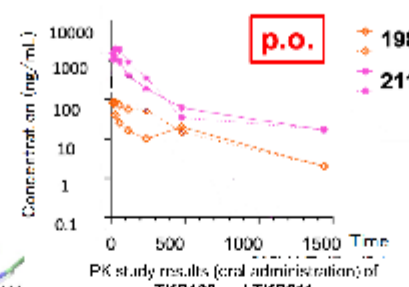
YH-53/5h
(SARS-CoV M^{pro} inhibitor)

Thanigaimalai, P., et al. Eur. J. Med. Chem. 2013, 65, 372-384.

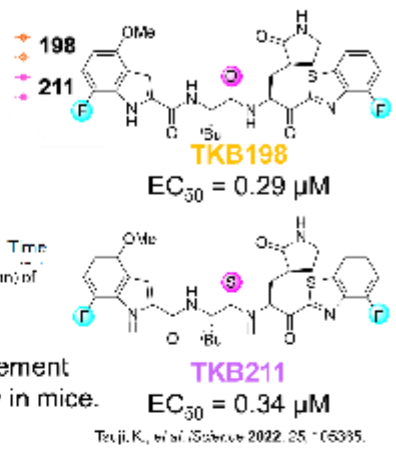


Hatton, S., et al. Mol. Cancer. 2021, 12, 808.

✔ Fluorination and thioamidation of the lead compound



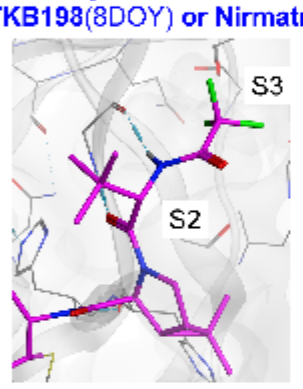
Thioamide bond replacement improved blood stability in mice.



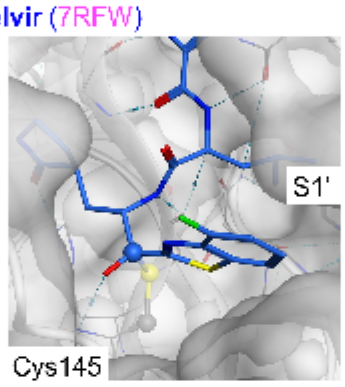
Teji, K., et al. JScience 2022, 25, 105385.

TKB245 & TKB248

✔ Co-crystal structures of SARS-CoV-2 M^{pro} with TKB198(8DOY) or Nirmatrelvir (7RFW)

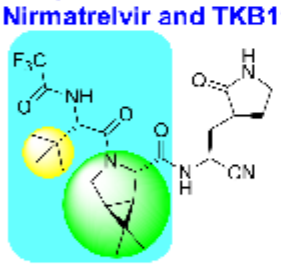


- Nirmatrelvir has numerous interactions in the S2-S3 region of SARS-CoV-2 M^{pro}.

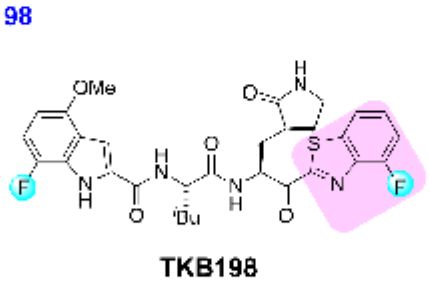


- **TKB198** forms a covalent bond with Cys145, the active center of SARS-CoV-2 M^{pro}. Also, it inactivates the enzymatic activity of M^{pro}.

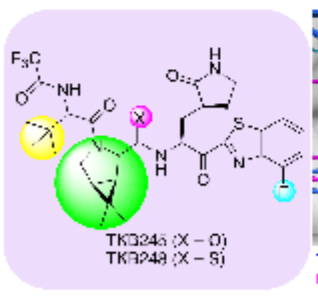
✔ Hybridization of SARS-CoV-2 inhibitors with Nirmatrelvir and TKB198

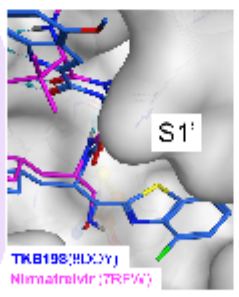


Nirmatrelvir



TKB198





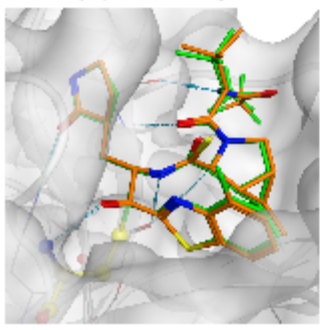
4-F benzothiazole moiety occupies SARS-CoV-2 M^{pro} pocket (S1') effectively.

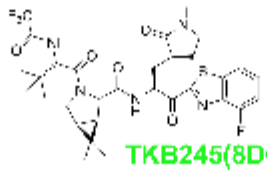
✔ Biological evaluation of TKB245 and TKB248

Compound	EC_{90} (μM)	EC_{95} (μM)	IC_{90} (μM)	IC_{95} (μM)	$T_{1/2}$ (h)	Oral F (%)
YH-53	2.6	9.47	0.13	1.5	0.27	n.d.
TKB198	0.27	0.83	0.023	0.75	0.83	1.98
TKB245	0.03	0.53	0.007	0.14	3.82	48
TKB248	0.22	0.87	0.074	6	4.34	72
Nirmatrelvir	0.94	7.81	0.013	0.77	1.03	56

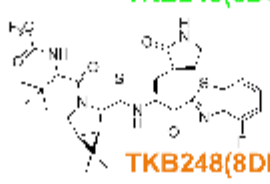
- Oral F = AUC per unit dose after oral administration / AUC per unit dose after intravenous administration.
- PK test was performed using human liver stomatic mice (**TKB245**, **TKB248**, and Nirmatrelvir) and ICR mice (YH-53 and **TKB198**).
- EC_{90}: Drug concentration to inhibit half of the multiplication of SARS-CoV-2.

✔ Co-crystal structures of SARS-CoV-2 M^{pro} with TKB245 or TKB248





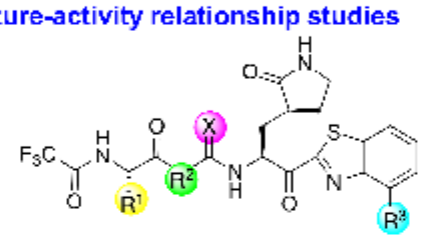
TKB245(8DOX)



TKB248(8DPR)

Results & Discussion

✔ Structure-activity relationship studies



Compound	R^1	R^2	R^3	X	EC_{50} (μM)	CC_{50} (μM)
TKB245			F	O	0.012	> 100
TKB248			F	S	0.31	> 100
TKB269			F	O	0.36	> 100
TKB255			F	O	0.095	> 100
TKB263			F	O	2.4	> 100
TKB253			F	O	0.25	> 100
TKB267			F	O	2.8	> 100
TKB265			F	O	0.59	> 100
TKB286			Cl	O	0.019	> 100
TKB287			Br	O	0.063	> 100

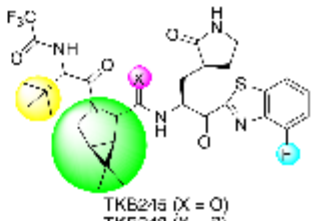
✔ Short summary

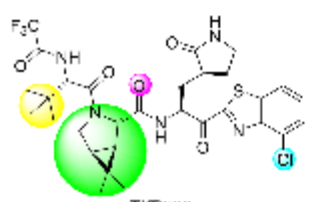
R^1 : L-*tert*-Leu residue is suitable.

R^2 :  is the best amino acid among the tested derivatives.

R^3 : F and Cl is tolerable.

TKB245 and **TKB248** are still the most promising compounds. In addition, we found **TKB286**, which the activity is comparable to that of **TKB245**.





✔ Antiviral activity in various target cells

Cell line	TKB245	TKB248	Nirmatrelvir	Ensitrelvir fumarate
	EC_{50} (μM)			
VeroE6	0.03	0.22	1.02	0.11
VeroE6 + 2 μM CP100356	0.001	0.03	0.025	-
HeLa-ACE2-TMPRSS2	0.021	0.16	0.21	0.2
A549-ACE2-TMPRSS2	0.0027	0.199	0.017	-

- CP100356 is P-glycoprotein inhibitor

✔ Stability against acid treatment

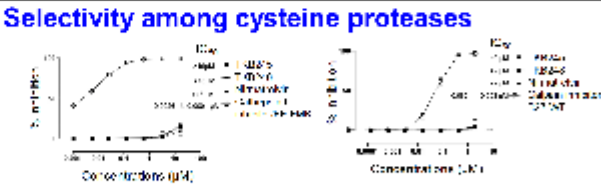
Compound	EC_{50} (μM)		CC_{50} (μM)
	acid (-)	acid (+)	
TKB245	0.074	0.087	>100
TKB248	0.31	0.32	>100
Nirmatrelvir	1.4	2.5	>100

- The acid (-) means test compounds dissolved in PBS without acid treatment.
- The acid (+) means test compounds treated with 0.1 M HCl aq. in PBS, incubated for 1h at 37 °C, and then neutralized with 0.1 M NaOH aq. in the mixture.

✔ Antiviral activity against mutant strains

Compound	EC_{50} (μM)				
	WK-521	α/BA.1	α/BA.2	α/BA.5	α/BA.2.75
TKB245	0.03	0.014	0.052	0.016	0.049
TKB248	0.22	0.282	0.16	0.066	0.43
Nirmatrelvir	0.94	1.047	1.3	1.126	0.965

✔ Selectivity among cysteine proteases



- In vitro enzyme assays with human cathepsin L (left) and human calpains (right).
- Human cathepsin L inhibitor screening and human calpain activity assay kit are designed to measure human cysteine protease activity and identify inhibitors of these enzymes.

✔ Evaluation of potential immune response

No significant cytotoxicity was observed in hPMBC and MOLT-4 which are important components of the human immune response. MT-2 cells (a T cell line derived from normal human cord leukocytes by co-culturing it with human leukemic T-cells) also showed no cytotoxicity.

Higashi-Kuwata, N., et al. Nat. Commun. 2023, 14, 1059.

Teji, K., et al. J. Med. Chem. 2023, 66, 13516-13529.

Conclusion & Acknowledgement

As the results of the SAR studies of **TKB245**, a 4-fluoro or 4-chlorobenzothiazole ketone was suitable as the P1' unit. Biological evaluation demonstrated that **TKB245** and **TKB248** showed preferable acid stability in the test conditions and no significant cytotoxicity in hPMBCs, MOLT-4 cells and MT-2 cells.

These data supported that **TKB245** and **TKB248** can be orally administrable drug candidates for COVID-19 therapy as M^{pro} inhibitors.

We deeply thank to Prof. Yoshio Hayashi and Dr. Sho Konno from Tokyo University of Pharmacy and Life Sciences for the valuable discussions.