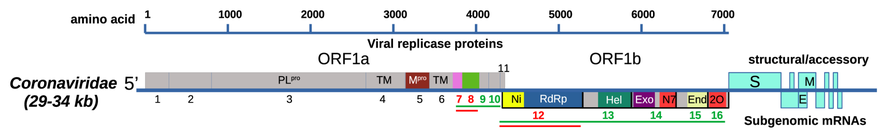
A Favipiravir analogue and chain terminator active against SARS-CoV-2

Ashleigh Shannon[1], Véronique Fattorini[1], Benedikt Ganter[2], Franck Touret[3], Aurélie Chazot[1], Bruno Coutard[3], Karine Alvarez[1], Chris Meier[2], Barbara Selisko[1] & Bruno Canard[1]

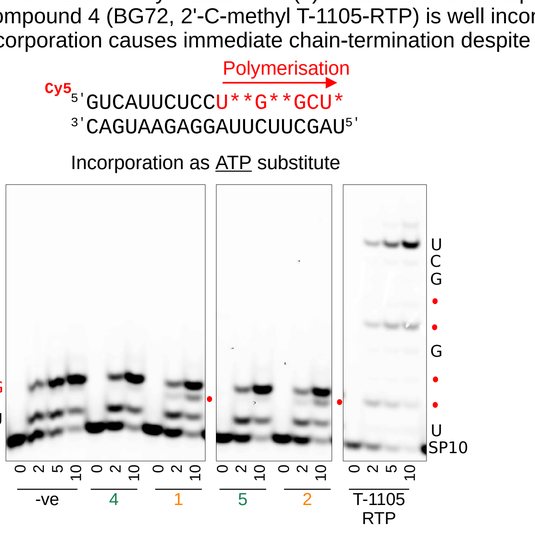
[1]*AFMB, CNRS, Aix-Marseille University, UMR 7257, Case 925, 163 Avenue de Luminy, 13288 Marseille Cedex 09, France.*
[2]*University of Hamburg, Faculty of Sciences, Department of Chemistry, Organic Chemistry, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany.*
[3]*Unité des Virus Émergents (UVE: Aix-Marseille University - IRD 190 - Inserm 1207 - IHU Méditerranée Infection), Marseille, France.*

INTRODUCTION

- Coronaviruses (CoV) - large genome +RNA viruses (~ 30 kb)
  2 large ORFs = non-structural replicase enzymes
  3' subgenomic mRNAs = structural/accessory proteins

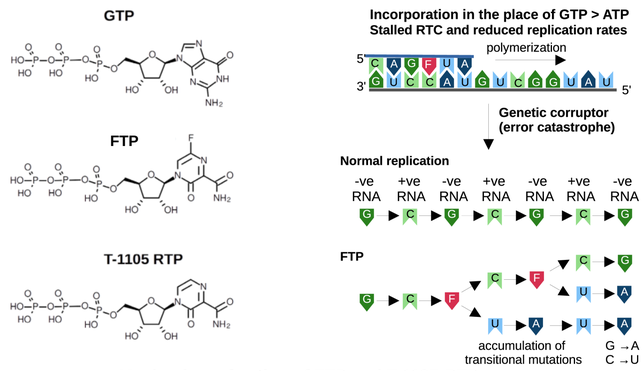


Schematic of coronavirus genome. *PL^{pro} and M^{pro} = viral proteases, TM = transmembrane domain. Ni =* Nidovirus RdRp-Associated Nucleotidyltransferase (NiRAN), RdRp = RNA dependent RNA polymerase, Hel = helicase, Exo = exonuclease, N7 and 2O = methyltransferases, End = endonuclease



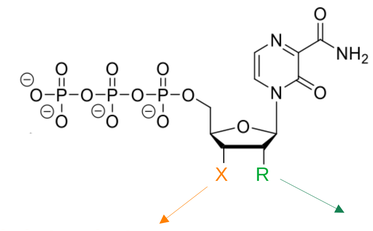
Minimal Replication Transcription Complex (RTC). *Viral RdRp (nsp12), and cofactors nsp7 + nsp8*

Favipiravir-ribose-triphosphate (FTP) and non-fluoronated T-1105-RTP
- Purine analogues - target viral RTC for incorporation into viral RNA
- Slow down RNA synthesis and cause viral mutagenesis (1)



Mechanism of action of FTP and T-1105-RTP

Aim: Study effect of ribose modifications on activity of T-1105-RTP



Obligate chain terminators

1 - 3'-deoxy (BG82)
2 - 3'-fluoro (BG-AW-19)
3 - 3'-azido (BG-AS-13)

Non-obligate chain terminators

4 - 2'-C-methyl (BG72)
5 - 2'-fluoro-2'-C-methyl (BG-AW-18)

2' and 3' modifications of T-1105-RTP

SUMMARY

- Favipiravir is an antiviral nucleotide analogue prodrug that is ribophosphorylated in the infected cell and well incorporated by the viral RNA-dependent RNA polymerase of SARS CoV-2 and of other RNA viruses.
- To change its (mainly) mutagenic mode of action (MoA) we explore ribose modifications of a non-fluorinated Favipiravir-ribose-triphosphate (RTP)
- The presence of a 2'C-methyl-group allows incorporation instead of GTP, leads to chain termination and shows a slight protective effect against proof reading excision by the viral exonuclease compared with the parent compound.
- Nevertheless, different prodrug forms do not show a higher antiviral effect in SARS CoV-2 infected cells.

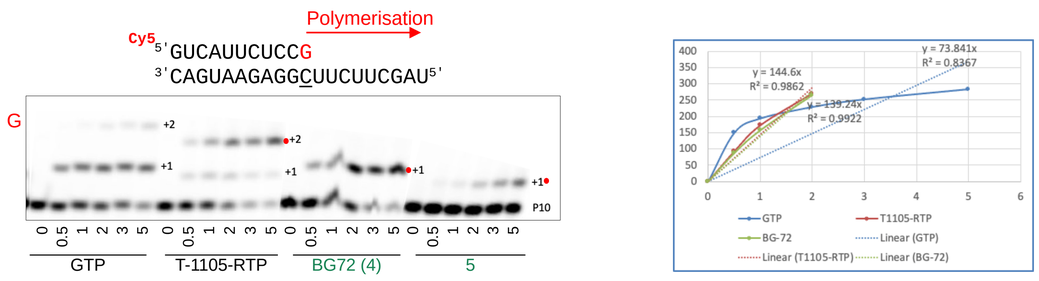
RESULTS

INCORPORATION OF ANALOGUES IN THE PLACE OF ATP AND GTP

- SARS- CoV-2 RTC does not efficiently incorporate T1105-RTP analogues with modifications at the 3' position (compounds 1-3)
- 2'-fluoro-2'-C-methyl T1105-RTP (5) is also not well incorporated
- Compound 4 (BG72, 2'-C-methyl T-1105-RTP) is well incorporated as a GTP, but not ATP substitute
- Incorporation causes immediate chain-termination despite presence of 3'OH

Incorporation of T-1105-RTP and modified analogues in the place of ATP (left) or GTP (right). *Incorporation of analogues shown with red dot. Mismatched nucleotide indicated with red letter, correct nucleotide in black*

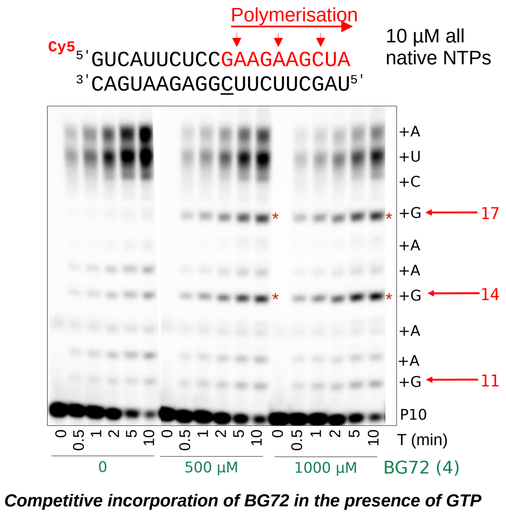
- BG72 (compound 4) incorporation speed is comparable with T-1105-RTP (initial velocity)



Single nucleotide incorporation of GTP, T-1105-RTP, BG72 and AW-18 (left). *Calculated initial velocities are: T1105-RTP = 110.2 +/- 43.4, BG72 = 119.8 +/- 51.5, BG-AW-18 (5) = 2.3 +/- 1.8*

COMPETITION WITH NATIVE NUCLEOTIDES

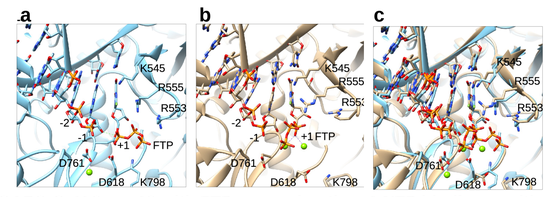
- BG72 chain-terminated incorporation products seen at positions 14 and 17, but not 11 (sequence dependent)
- GTP preferred ~250–520 fold over BG72



Competitive incorporation of BG72 in the presence of GTP

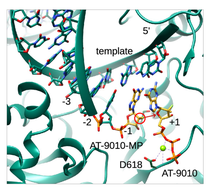
STRUCTURAL IMPACT OF RIBOSE MODIFICATION

- modified favipiravir base causes variable nucleotide positioning in active-site



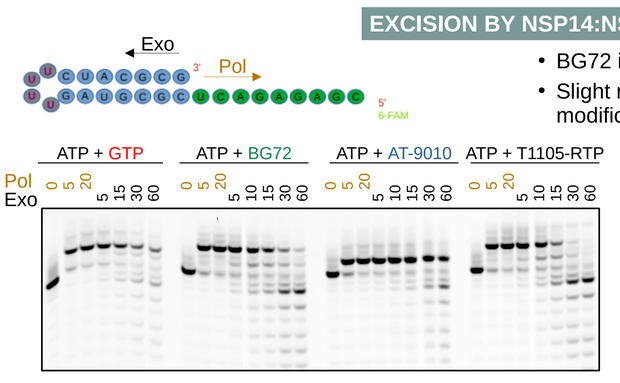
SARS-CoV-2 nsp12 with FTP occupying the +1 NTP binding position in the pre-incorporated state (a = 7CTT, b= 7AAP, c= superposition)

- Ribose modification likely prevents alignment of next incoming NTP, causing chain termination (like bemnifosbuvir triphosphate (AT-9010) and sofosbuvir (2))

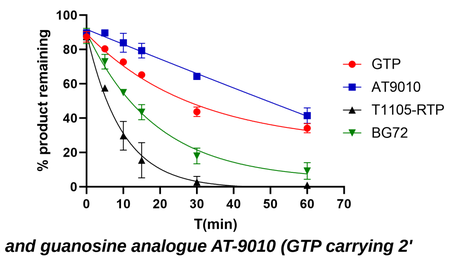


2' methyl group on AT-9010 causes chain-termination through misalignment of incoming NTP. PDB 7ED5

EXCISION BY NSP14:NSP10 COMPLEX

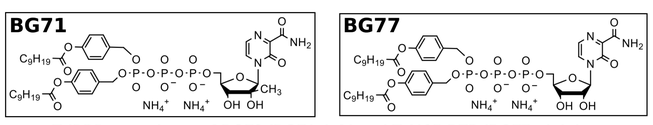


- BG72 is excised by nsp14:nsp10 proofreading complex
- Slight resistance compared to T-1105-RTP, showing ribose modification plays a role in excision ability



Incorporation (Pol) and excision (Exo) of control native NTP, T1105-RTP, BG72, and guanosine analogue AT-9010 (GTP carrying 2' fluoro & C-methyl groups at the ribose)

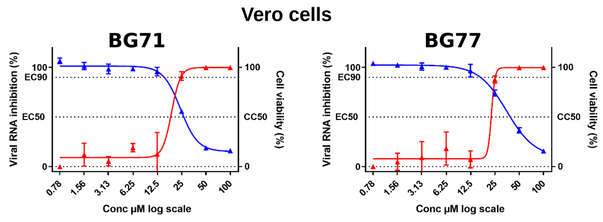
ANTIVIRAL ACTIVITY IN CELLS



TriPPPro prodrugs of BG72 (BG71, left) and T-1105-RTP (BG77, right)

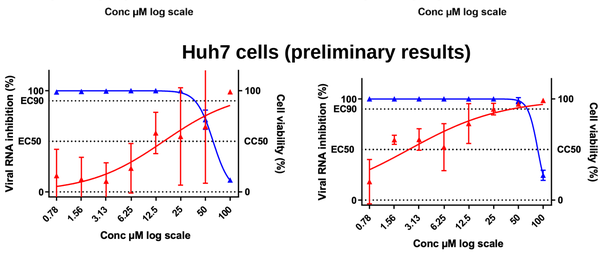
- TriPPPro forms(3) of BG72 and T-1105-RTP seem to be more potent and less toxic in Huh7.5 cells than in Vero cells resulting in an increased Selectivity Index (SI)
- Remdesivir-like and sofosbuvir-like forms of BG72 in Huh7.5 cells are less active but also less toxic than the TriPPPro form
- For T1105-RTP, remdesivir-like and sofosbuvir-like forms are equally active and less toxic than the TriPPPro form
- Overall, T1105-RTP seems to be a more potent antiviral in SARS-CoV-2 infected cells than its analog carrying a 2'C-methyl group (BG72)

Vero cells



	BG71	BG77	Remdesivir
EC_{50} (µM)	18.8 ± 0.6	20.3 ± 3.2	2.5 ± 0.4
CC_{50} (µM)	26.8 ± 0.2	36.4 ± 12.5	n.a.
SI	1.4	1.8	n.a.

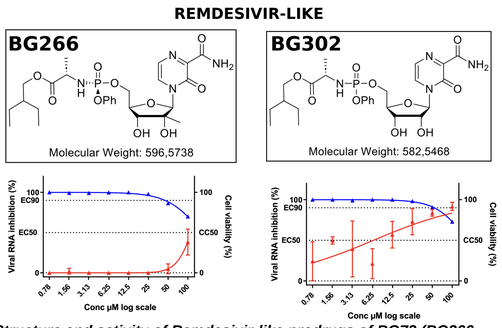
Huh7 cells (preliminary results)



	BG71	BG77	Remdesivir
EC_{50} (µM)	15.6	2.35	0.011
CC_{50} (µM)	62.1	86	n.a.
SI	3.98	36.6	n.a.

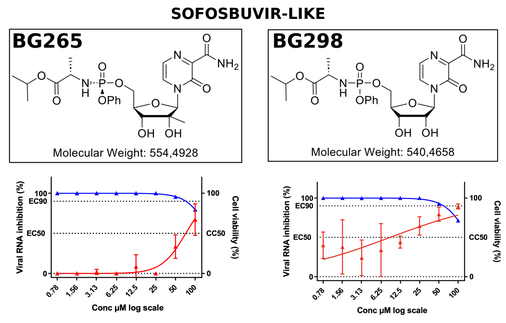
Huh7 cells (preliminary results)

REMDESIVIR-LIKE



Structure and activity of Remdesivir-like prodrugs of BG72 (BG266, left) and T-1105-RTP (BG302, right). Viral RNA inhibition shown in red, cell viability in blue. *BG266 = EC_{50} >100 µM CC_{50} >100 µM. BG302 = EC_{50} 6.5 µM CC_{50} >100 µM*

SOFOSBUVIR-LIKE



Structure and activity of Sofosbuvir-like prodrugs of BG72 (BG265, left) and T-1105-RTP (BG298, right). Viral RNA inhibition shown in red, cell viability in blue. *BG265 = EC_{50} 71.8 µM CC_{50} >100 µM. BG298 = EC_{50} 8.6 µM CC_{50} >100 µM*

CONCLUSIONS

- Introducing a 2' C-methyl group on T-1105-RTP (BG72) changes it's mechanism of action from a viral mutagen to a chain terminator.
- Despite the modification, it can still be efficiently incorporated in the place of GTP, discrimination values however are relatively high.
- Compared with T-1105-RTP, It is less sensitive to proofreading activity of the viral exonuclease.
- Optimisation of prodrug forms is currently underway, preliminary tests showed that 2' C-methyl T-1105-RTP seems to be consistently less efficient than T-1105-RTP.
- Initial studies suggest that modification of the alpha phosphate group may increase activity, potentially by further reducing excision.

(1) Shannon et al. (2020) Rapid incorporation of Favipiravir by the fast and permissive viral RNA polymerase complex results in SARS-CoV-2 lethal mutagenesis. *Nature Communications* 11:4682
(2) Shannon, et al. (2022) A dual mechanism of action of AT-527 against SARS-CoV-2 polymerase. *Nature Communications* 13 :621.
(3) Jia, et al. (2021) Improving properties of the nucleobase analogs T-705/T-1105 as potential antiviral. *Annual Reports in Medicinal Chemistry* 57