Targeting cellular and viral enzymes involved in hemorrhagic fever virus replication using new small molecule broad-spectrum inhibitors

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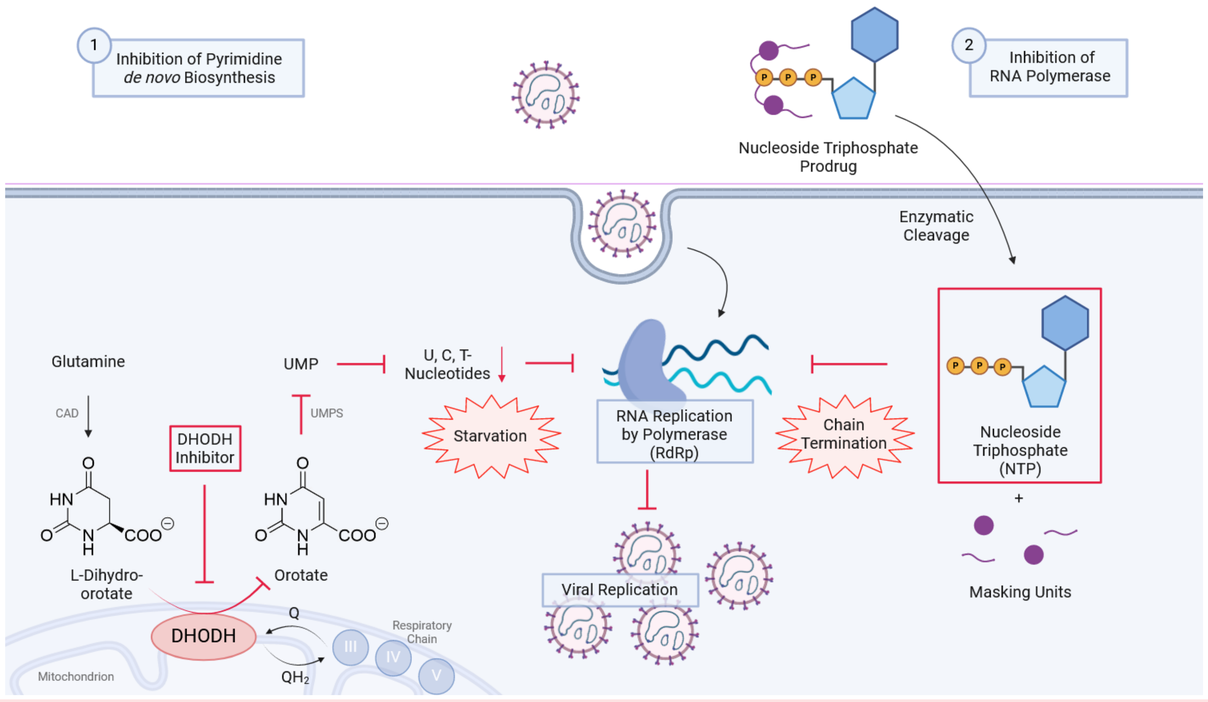
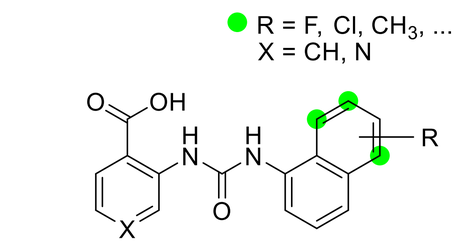


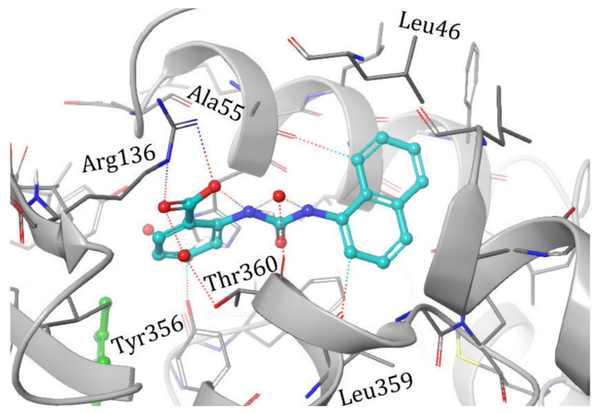
Introduction

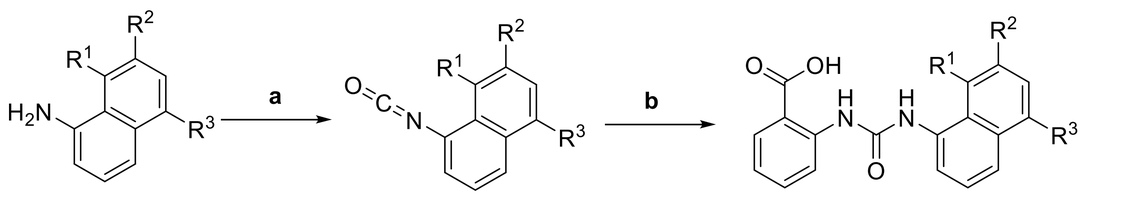
- Viral hemorrhagic fevers (Ebola Virus, Lassa Virus, Dengue Fever) are a great threat to global health due to their high infectiousness, fatality rate, risk of imported infections and lack of proper treatment
- Therefore, the development of broad-spectrum therapeutics to improve the preparedness for future outbreaks of viral hermorrhagic fevers is of great importance
- This projects aim is to delevop:
  (I) small molecule inhibitors of the host cell enzyme Dihydroorotate Dehydrogenase (DHODH) to reduce the amount of natural pyrimidine nucleosides in infected cells
  (II) nucleoside triphosphate analogues which inhibit the viral RNA-dependend RNA-polymerase (RdRp) which is a key enzyme in the viral replication in infected cells
- Synergistic effect by combining both inhibitors: Reduced production of pyrimidines upon DHODH inhibition leads to an increase of incorporation of RdRp targeting NTP analogues

Synthesis of DHODH inhibitors



IC_{50}(*h*DHODH) = 81 nM



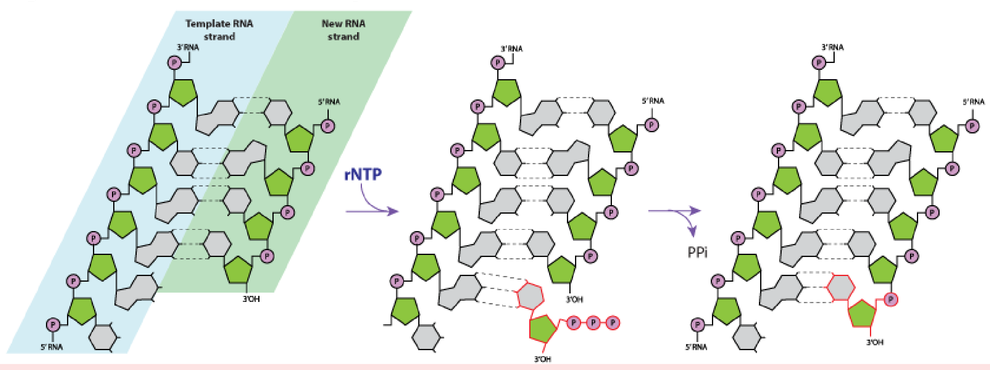


[a] 1.1 eq. Et_3N, 0.50 eq. triphosgene, THF, reflux, 4 h
[b] 1.5 eq. anthranilic acid, THF, 50 °C, o.n., **28 – 99 %** (over 2 steps).

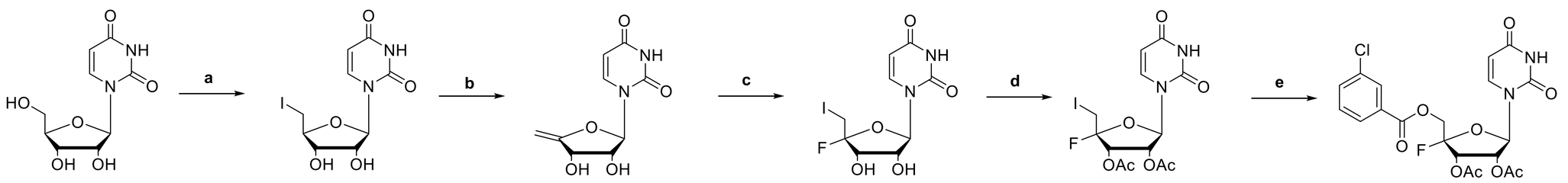
Evaluation of DHODH inhibitors

- membrane permeability (PAMPA) → Increased properties for 7-methyl compound
- kinetic solubility
- metabolic stability (S9) → Decreased properties for 8-methyl compound
- hDHODH inhibition
- logD → Other compounds need to be evaluated

RNA Synthesis of the RdRp

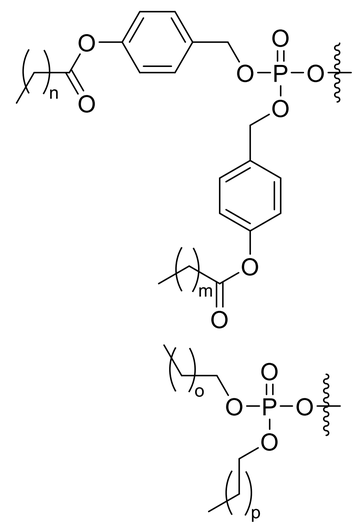


Exemplary synthesis of 4'-Fluorouridine-triphosphate

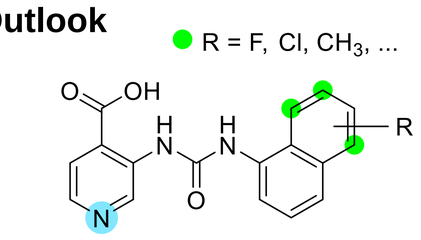


[a] i) 1.50 eq. TPP, 1.50 eq. imidazole, THF, 0 °C, 30 min. ii) 1.10 eq. $I_{2(THF)}$, rt, o.n., **75 %**. [b] 1.50 eq. MeONa, MeOH, 67 °C, 4 h, **quant**. [c] 1.20 eq. TREAT-HF, 1.20 eq. NIS, ACN, 0 °C, 2 h, **39 %**. [d] 3.00 eq. Acetic acid anhydride, 3.00 eq. Et_3N, cat. 4-DMAP, CH_2Cl_2, rt, 3 h, **76 %**. [e] i) 2.44 eq. Bu_4NH*SO_4, 2.55 eq. 3-chloroperbenzoic acid, 2.20 eq. K_2PO_4, H_2O, rt, 20 min. ii) 1.00 eq. nucleoside, 5.00 eq. *m*-CPBA, H_2O/CH_2Cl_2, rt, o.n., **67 %**. [f] 60.0 eq. NH_3, MeOH, rt, 1.5 h, **72 %**. [g] 28.0 eq. $POCl_3$, TMP, 0 °C, 7 h, **39 %**. [h] 10.0 eq. TFAA, 12.0 eq. Et_3N, ACN, 10 min, rt. ii) 5.00 eq. NMI, 10.0 eq. Et_3N, ACN, 10 min, rt. iii) 3.00 eq. $Bu_4N*H_3P_2O_7$, ACN, 1 h, rt, **55 %**.

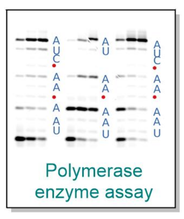
Possible masking units

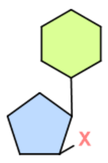


Outlook

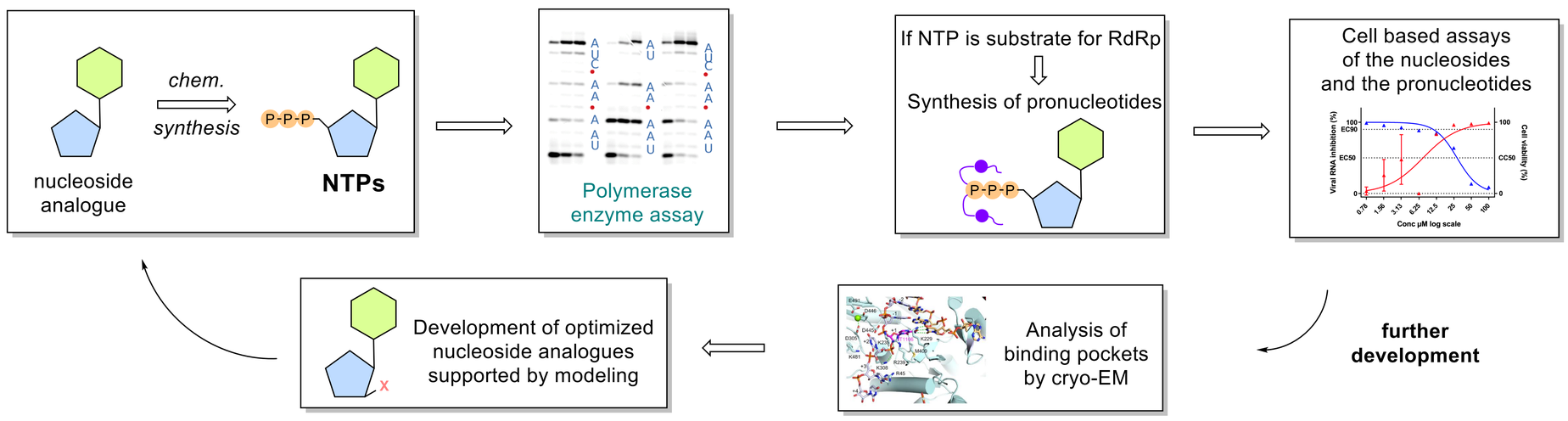


evaluation modification





Design of nucleoside triphosphate-based inhibitors of RNA-dependend RNA-polymerase of haemorrhagic fever viruses



References

[1] Laubach, N. C., "Development of Novel Inhibitors of Human Dihydroorotate Dehydrogenase as Antiviral Drug Candidates - Design, Synthesis and Pharmacological Evaluation," *Dissertation*, **2022**, University of Hamburg.
[2] Souriman, J., Plemper, R. K., Painter, G. R., et al., "4′-Fluorouridine is an oral antiviral that blocks respiratory syncytialvirus and SARS-CoV-2 replication", *Science* **2022**, *375*, 161-167.
[3] Meier, C., Jia, X., Schols, D., "Lipophilic Nucleoside Triphosphate Prodrugs of Anti-HIV Active Nucleoside Analogas Potential Antiviral Compounds", *Adv Sci* **2023**, *10*, 1-14.

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