WP3.3. Development of anti-HBV compounds targeting STING protein (Birkuš, Nencka)

Research aims

- Design and synthesis of appropriate precursors for synthesis of CDNs – nucleotide phosphonates and their phosphorylated analogues.
- Development of novel enzyme cyclization approaches towards CDNs.
- Exploration of synthetic pathways to CDNs of solid support.
- Systematic search for small fragment s with significant affinity towards STING protein by NMR-based approaches and development of the obtained small fragments to drug like molecules.
- Virtual screening for small molecular STING agonists and chemical optimization of the obtained hits.
- Antiviral and cytostatic profiling of the obtained compounds.

Research plan and methodology

Our primary objective is synthesis of novel CDNs with increased stability toward hydrolysis by host phosphodiesterases. We will achieve this goal by replacing the phosphodiester bonds in CDNs with phosphonate and/or phosphinate bonds. The main focus of our chemistry efforts will be on preparation of phosphonate intermediates, installation of suitable protection groups and preparation of triphosphate analogues (nucleoside phosphonate diphosphates). The archetypal example of prepared compounds and workflow of chemical approach is depicted on Fig. 1A. The selected derivatives will be optimized based on computational modelling (model of sample derivative is depicted on Fig 1B). We will explore synthesis CDN derivatives based on enzymatic cyclization with bacterial and eukaryotic dinucleotide synthases such as cGAS and chemical cyclization techniques on solid support (LCAA-CPG).

All prepared CDNs will be profiled for affinity toward recombinant STING using calorimetry and/or plasmon surface resonance and their ability to induce cytokines in PBMC or reporter cell line. The conditioned medium from PBMCs treated with the synthetic CDNs will be evaluated for anti-HBV activity in HBV infected PHHs. To better understand SAR, crystal structures of recombinant STING with novel CDNs will be solved. The stability of CDNs will be determined in S9 microsomal fractions derived from liver and intestine and recombinant ENPP1. In order to improve gut absorption, we will prepare prodrugs from one or two lead CDNs.

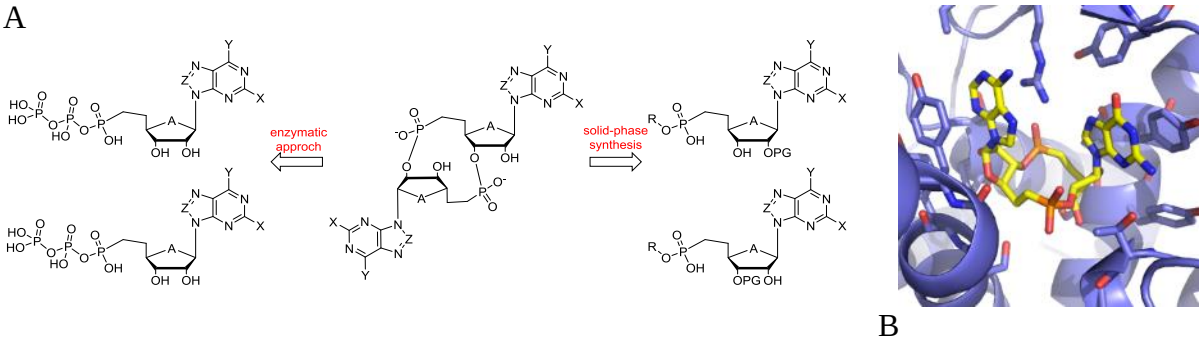

Fig. 1. *A) Example of planned compounds and potential approaches towards CDNs. B) Molecular model of the posphonate-based CDNs bound to STING (based on the structure PDB id: 4LOH)*

The best drug candidates will be selected based on their CaCo cell absorption and stability in primary hepatocyte cell cultures. We will monitor the abilities of the candidates to elicit host innate immunity *in vivo* by measuring plasma levels of induced cytokines and correlate them with their pharmacokinetics in animal models. Ultimately, the drug candidates will be tested for anti-HBV activity in woodchuck model of chronic HBV.

In parallel, we will perform an extensive search for non-nucleotide agonists based on virtual screening and NMR based fragment screening. We will use ligands from Maybridge database (approx. 50 thousand

compounds) or alternatively from ZINC Lead Now database (approx. 3 million compounds). All screened compounds will be processed and optimized by semiempirical methods (PM6 or PM7) using MOPAC2016 software and docked into available X-ray structures using AutoDock Vina.[1] Furthermore, the screen based on the STD (saturation transfer difference) NMR experiment will be used for the identification of small-fragments and the detailed analysis of STD enhancement will allow for identification of important binding determinants within obtained ligands. The hits from both virual and fragment screening will be tested for their binding sites using protein-detected NMR experiments. Suitable binders will undergo more detailed structural characterization either by X-crystallography or NMR spectroscopy and further optimized by means of modern medicinal chemistry techniques. We will use feedback from the NMR and X-ray studies to enhance performance of the docking procedure by flexibilization of appropriate residues with a significant impact on the selected binding site and optimized structures of the designed small-molecule agonists of STING.

Stability of STING agonists in biologic fluids and their delivery into immune cells represent major obstacles in their clinical development. We want to address the former issue by synthesis of novel phosphonate analogues of cyclic dinucleotides which have not been exemplified in the scientific and patent literature to our knowledge. With exception of few patent examples, little has been published regarding prodrugs of CDNs. We will spend significant effort to prepare novel liphophilic prodrugs of CDNs which will enable their delivery into immune cells. This should ultimately result in significant improvement of the activity of CDNs. Lastly, our approach has been endorsed by our collaborator Gilead Sciences.

The members of the family of the pattern recognition receptors, to which STING belongs, are currently being evaluated in many clinical trials to treat chronic hepatitis B (TLR7, TL8, RIG-I)[1], and cancer (TLR4, TLR7, 8, 9)[2]. Similarly, STING agonists are in Ph1 clinical trials[3] to treat cancer and are being considered for the development to treat chronic hepatitis B[1.] Moreover, they are also being developed as adjuvants for vaccines[1]. Therefore, novel STING agonists stable toward hydrolysis by phosphoesteases as proposed in our application have potential to address many unmet clinical needs.

1. Chang, J.; Guo, J.T., Treatment of chronic hepatitis B with pattern recognition receptor agonists: Current status and potential for a cure. *Antiviral Res.* 2015, 121,152-159.

2. Iribarren, K.; Bloy, N.; Buqué, A.; Cremer, I.; Eggermont, A.; Fridman, W.H.; Fucikova, J.; Galon, J.; Špíšek, R.; Zitvogel, L.; Kroemer, G.; Galluzzi, L., Trial Watch: Immunostimulation with Toll-like receptor agonists in cancer therapy. *Oncoimmunology* 2016, 5(3), e1088631.

3. https://clinicaltrials.gov/ct2/show/NCT02675439

Research schedule

2018-2022

- Design and synthesis of phosphonate/phosphinate analogs of CDNs
- Profiling of biological and biochemical activity of CDNs
- Crystallography of CDNs with STING
- Computational modelling of CDNs bound to STING

2018-2019

- Expression of recombinant STING, ENPP1 and bacterial and eukaryotic cyclic dinucleotide synthases
- Development of cell-based reporter assays to monitor activity of CDNs
- Enzymatic synthesis of CDNs from nucleoside triphosphate precursors

2019-2022

- Synthesis of prodrugs of one or two CDN leads

- S9 stability profiling of CDNs and their prodrugs, PK/PD of drug candidates in animal models and anti HBV activity in woodchuck model Design and synthesis of novel small-molecule agonists of STING based on virtual screening and fragment based hits.

Publications and patents

Publications (Jimp)

	Jimp
2017	0
2018	2
2019	2
2020	2
2021	3
2022	3
Total	12

Journal of Medicinal Chemistry
ACS Chemical Biology
Antimicrobial Agents and Chemotherapy
Plos One
Bioorganic and Medicinal Chemistry
Organic and Biomolecular Chemistry
Journal of Organic Chemistry
ChemBioChem
J Virology
J Hepatology
Molecular Pharmacology

Patents and patent applications

	Patents (granted)	International patent applications (filed)
2017	0	0
2018	0	0
2019	1	1
2020	1	1
2021	0	0
2022	0	0
Total	2	2

We expect IP protection in the following areas:
Novel STING agonist based on CDNs.
Small molecular STING agonists.

Cooperation with foreign institutions

- The intermediates of the synthesis will be screened for antiviral activities at KU Leuven in a broad panel of RNA, DNA and retro-viruses.
- Gilead Sciences will perform pharmacokinetic and pharmacodynamic profiling of lead compounds in animal models