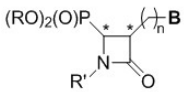
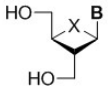
Objectives

The project aims at studies on scope and limitations of the Kinugasa reaction between phosphonylated nitrones and nucleobase-derived alkynes and includes the optimization of reaction conditions for the synthesis of new 4-phosphonylated β-lactams of the general formula **1** as well as studies of their antiviral as well as anticancer properties (ca. 50 new 4-phosphonylated β-lactams). These compounds can be regarded as azetidine analogues of nucleotides from an oxetanocin group **2** of well-documented antiviral activity. Moreover, since a β-lactam moiety is present within the structure of designed compounds **1**, antibacterial screening of a few structurally diversified compounds is justified.



1

n = 0, 1, 2
R = alkyl
R' = Me, Bn
B = natural nucleobase or its mimietic



2

B = Ade, Thy, Gua, *etc.*
X = O, CH_2

The novelty of this proposal is based on the idea that the installation of a dialkoxyphosphonyl group at C4 in the structure of β-lactam-containing phosphonate analogues of nucleosides **1** allows to avoid the first and the most difficult intracellular phosphorylation step and at the same time this group provides a sufficient bioavailability of the designed compounds. Although a few examples of nucleoside analogues containing the azetidine ring instead of a sugar moiety have been reported in the literature, nucleotide analogues having a 4-(phosphonyl)azetidinone framework have not been obtained so far. For this reason we proposed the original approach as an alternative to the reactions previously applied, which relies on the application of N-substituted C-(dialkoxyphosphonyl)nitrones in Kinugasa reaction with respective functionalized alkynes.

The project objectives will be attained by:

1. detailed study on the influence of major parameters (structure of starting materials and their molar ratios, selection and amounts of catalysts, solvents, temperature) on the outcome of the Kinugasa reaction;
2. optimization of the conditions of the Kinugasa reaction followed by the preparation of pure samples of designed β-lactams **1** (ca. 30–40 mg of each);
3. evaluation of antiviral activity (as EC_{50}) of the synthesized compounds toward a broad spectrum of DNA and RNA viruses (more than 20 viruses) in the prestigious European research center Rega Institute for Medical Research in Leuven, Belgium;
4. simultaneously with antiviral activity studies cytotoxic properties of the synthesized compounds (determination of MCC or CC_{50}) towards the uninfected cell lines will be established;
5. cytostatic activity of the tested compounds (expressed as IC_{50}) will be screened on the following tumor cell lines: murine leukemia (L1210), human T-cell leukemia (CEM) and cervical cancer (HeLa);
6. studies on structure-activity (antiviral and anticancer) relationships of 4-phosphonylated β-lactams **1**.

New methods for the synthesis of 4-phosphonylated analogues of nucleosides containing β-lactams moiety of general formula **1** will be elaborated which will require optimization of the reaction conditions for the synthesis of N-substituted C-(phosphonyl)nitrones. Studies on structure-activity relationship will be carried out. We hope that in the end of the project new lead structures will be found in this new class of nucleotide analogues. Results of this studies will be published in scientific journals in the field of organic and medicinal chemistry as well as presented on the conferences.

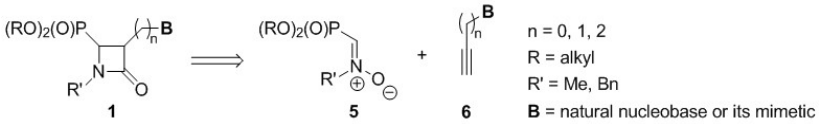
Significance

The commonly applied antiviral drugs have significant limitations, and for many viruses effective medications have not been discovered so far. The issue is highly complicated by the possibility of rapid mutations of viruses. Despite several well-documented achievements, a search for new compounds endowed with antiviral activity belongs to very important areas of research in medicinal chemistry.
Studies on structural analogues of nucleosides and nucleotides proved to be one of the most efficient approaches to the discovery of new antiviral chemotherapeutics. The recognition of their mode of action, which in most cases relies on interfering in the DNA and RNA chain elongation, resulted in spectacular acceleration of research on this group of compounds. In addition to acyclic nucleotide analogues which are the most important group of antiviral drugs (e.g. acyclovir, adefovir, tenofovir, and cidofovir), the other active derivatives having hetero(cyclic) moieties, not only five- but also three- and four-membered rings, instead of a sugar unit, have been obtained. Four-membered rings mimicking a sugar moiety are present in oxetanocins which constitute an outstanding group of compounds with well-documented antiviral activity. While oxetanocin A, isolated from natural sources, inhibits infection caused by HIV-1, its thymidine analogue A-473209 exhibits activity against HSV-1, HSV-2 and VZV viruses. During this project new 4-phosphonylated nucleoside analogues of a general formula **1** will be obtained, as analogues of oxetanocins. We expect the discovery of compounds with unique biological properties (antiviral and anticancer) which in the future will allow for their future therapeutic applications.

Work plan

Achievement of the project objectives requires studies on scope and limitations in the application of phosphonylated nitrones **5** in the Kinugasa reaction with nucleobase-derived alkynes **6**, elaboration of the optimized procedure for the synthesis of 4-phosphonylated analogues of nucleosides containing the β-lactam ring of the general formula **1** and finally preparation of lactams **1** using N-substituted C-phosphonylated nitrones **5** and the respective alkynes **6** derived from natural, modified nucleobases and

mimetics of nucleobases.



At least four C-phosphonylated nitrones **5** will be employed, and structural variations include substituents at the dialkoxyphosphoryl group (problem of bioavailability) as well as substituents at the nitrogen atom (R′ = Me, Bn – lipophilicity). At least 15 alkynes **6**, derivatives of natural nucleobases (e.g. adenine, uracil, thymine, cytosine) and nucleoside analogues (e.g. 5-bromouracil, quinazolin-2,4-dione (benzuracil), 6-chloropurine, 2-amino-6-chloropurine, azauracil) as well as selected substituted derivatives of ethynylbenzene (as non-polar isosters of nucleobases) will be employed.
At least 30-40 mg of each final compound of high purity will be obtained.
Antiviral and anticancer screening will be performed at Catholic University of Leuven (the Rega Institute for Medical Research) at the Professors Balzarini and Schols groups as a continuation of our ongoing collaborations lasting almost five years. Studies on structure-activity relationship will be performed based on collected data of biological activity.