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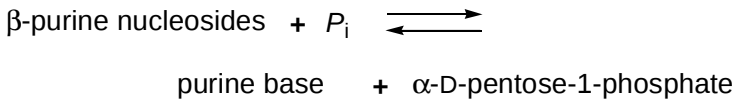
- Kandydat musi uzyskać zgodę przyszłych opiekunów przed rozmową kwalifikacyjną. Kontakt dr hab. Agnieszka Bzowska tel. 22 554 0789, e-mail: abzowska@biogeo.uw.edu.pl

Synteza, badania biofizyczne i biologiczne nukleozydów i nukleotydów purynowych – potencjalnych leków immunosupresyjnych i przeciwnowotworowych

Synthesis, biophysical and biological studies of purine nucleosides and nucleotides with potential medical applications as immunosuppressive and anticancer drugs

Phosphorylation and dephosphorylation of biological molecules including proteins, sugars, and nucleosides is one of the most fundamental processes in the mammalian organism. Molecular design to regulate the activity of enzymes which catalyze the phosphorylation/dephosphorylation processes has a bright future in the development of functional molecules as therapeutic reagents and biological tools [1]. Purine nucleoside phosphorylase (PNP, E.C. 2.4.2.1), a nucleoside processing enzyme, is ubiquitous and essential in providing precursors for RNA and DNA synthesis and energy metabolism [2]. PNP catalyzes the reversible phosphorolytic cleavage of the glycosidic bond of ribo- and deoxyribonucleosides, in the presence of inorganic orthophosphate (Pi) as a second substrate, to generate purine bases and ribose(deoxyribose)-1-phosphate (Scheme 1).



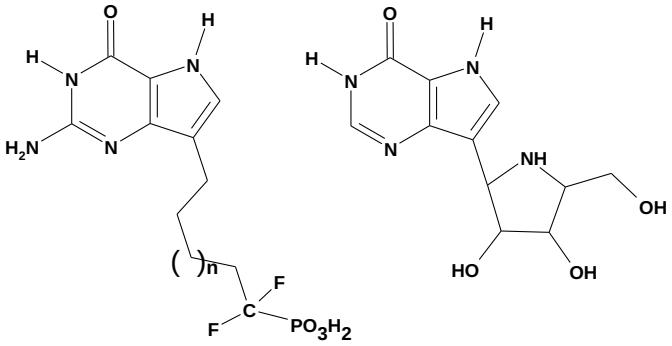
Scheme 1. PNP-catalyzed reversible phosphorylation of purine nucleosides

In mammals, PNP is crucial for the proper functioning of cellular immune systems, since PNP deficiency in humans leads to the impairment of T-cell function, usually with no apparent effects on B-cell function [2,3]. Consequently, PNP inhibition has become a target for drug design on selective immunosuppressive agents and anti cancer drugs (against T-cell cancers). In the 34 years since the genetic deficiency of PNP has been discovered [3], great efforts have been made to design inhibitors of PNPs with potential medical applications [see 2]. However, only the transition state analogue of human PNP, immucillin-H (trade name BCX-1777), has reached phase I/II clinical trials against human T-cell leukemia [4].

PNP accomplishes the reversible phosphorylation of purine nucleosides via a ternary complex of the enzyme, nucleoside, and orthophosphate. Compounds that contain covalently linked elements of both substrates (nucleoside and orthophosphate) in their structure are expected to act as a "multi-substrate analogue" inhibitor of PNP. Therefore, a number of metabolically stable acyclic nucleotides containing a purine and phosphate-like moiety connected by a linker have been synthesized [5]. Of the PNP inhibitors reported, 9-(5′,5′-difluoro-5′-phosphonopentyl)-9-deazaguanine (DFPP-DG; Scheme 2) and its analogues desigend by our group in cooperation with the group of prof. Tsutomu Yokomatsu (Tokyo, Japan) and syntesized by the group of prof. Yokomatsu, belong to the most potent and structurally simple multi-substrate analogue inhibitors of PNP with dissociation constant in the pM range [6].

However, despite the excellent *in vitro* PNP inhibitory potential of DFPP-DG and analogues, no differences were observed between the effects on the growth of tumour cells sensible to inhibition of PNP activity, such as human adult T-cell leukaemia and lymphoma cells, and other leukaemia and lymphoma cells of B-cell, or non-T-, non-B-cell lineages [7]. Poor effects on lymphoma and leukaemia cell proliferation are most probably due to a poor ability of the compounds to penetrate cell membranes. For that reason, our future studies will be directed to synthesis of pro-drug of DFPP-DG to improve its cell penetration. The

second approach is based on our previous biochemical and biophysical studies of some acyclonucleosides and nucleotides and biophysical studies of the molecular mechanism of the enzyme [see e.g. 8]. Although those that were synthesized and characterized show inhibition constants in the μM range, their structure have a big potential for further modifications that are expected to improve interactions with the active site of the mammalian PNP. Synthesis of new analogues can be performed in the group of dr. B. Žinić, which deals with the synthesis of small molecules belonging to groups of nucleobase and nucleotide derivatives, possessing biological activity with potential beneficial action in treatment of diseases like chronic inflammation and cancers.[9] Due to the potential medical applications of the inhibitors, hence some patent potential, their structures are not given here.



Scheme 2. Structure of DFPP-DG and analogues: n = 1, DFPP-DG; n = 2, homo-DFPP-DG; n = 3, 6C-DFPP-DG (left panel); and structure of the transition state analogue inhibitor immucillin H (right panel).

The goal for the PhD student would be to synthesize at least one of the new inhibitors, characterize its interactions with the mammalian purine nucleoside phosphorylase. Similar studies except of the chemical step would be done by a PhD student also for those inhibitors that will be synthesized in the group of prof. Tsutomu Yokomatsu.

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