Structural studies of human purine nucleoside phosphorylase and cyclin-dependent kinase 2 inhibitors

S. Djukic[1], J. Skácel[1], J. Brynda[1,2], P. Pachl[1], T. Vučková, M. Fábry [2], M. Rumlová[3], T. Bílek[1,3], J. Voldřich[1,3], H. Mertlíková-Kaiserová[1], Z. Janeba[1], J. Škerlová[1], M.Peřina[4], R.Jorda[4], V. Kryštof[4], P. Řezáčová[1,2]

[1]Institute of Organic Chemistry and Biochemistry, AS CR, Prague 6, Czech Republic

[2]Institute of Molecular Genetics, AS CR, Prague 4, Czech Republic

[3]University of Chemical Technology, Prague 6, Czech Republic

[4]Department of Experimental Biology, Faculty of Science, Palacky University Olomouc, Czech Republic

Stefan.dukic@uochb.cas.cz

Purine nucleoside phosphorylase (PNP) represents one of the key enzymes of the purine salvage pathway, which is considerably more energy-efficient than *de novo* pathway. It hydrolyses ribose from inosine and guanosine in the presence of an inorganic phosphate, producing hypoxanthine and guanin which can then be recycled trough the salvage pathway or be further degraded to uric acid. PNP's activity is increased during processes which require rapid cell division and proliferation, which makes it a target in treatment of different types of cancer, autoimmune and other conditions in human, as well as treatment for different parasitic diseases such as tuberculosis (caused by *Mycobacterium tuberculosis)* where PNP is essential during transition from latent to active infection. Both human and Mtb PNP are trimers with three active sites. Even though there is a small sequence similarity, overall fold and active site are conserved which presents a challenge in design of selective inhibitors [1,2].

Cyclin-dependent kinase 2 (CDK2) is a Ser/Thr protein kinase that is active during G1 and S phase of the cell cycle and works as check point control. During the G1 phase of the cell cycle, it is activated by binding to cyclin E and in S phase by binding to cyclin A [3]. It is dispensable in healthy cells, as other CDKs can take over its role, but it is essential for proliferation of cancer cells. This makes CDK2 an interesting target in discovery of anticancer compounds [4].

We utilize X-ray crystallography in the structure-based drug discovery approach.

Enzymes were prepared by heterologous expression in *E. coli* and purified in high yields and purity necessary for crystallographic studies. Crystallization conditions for all three enzymes were identified through wide screening and optimization. Diffraction data have been collected on BL14.1 at the BESSY II electron storage ring operated by the Helmholtz-Zentrum Berlin and crystal structures were determined at high resolution (Figure1).

The knowledge of binding properties of these inhibitors will provide us crucial information which will be used to further optimize affinity and selectivity of both PNP and CDK2 inhibitors.

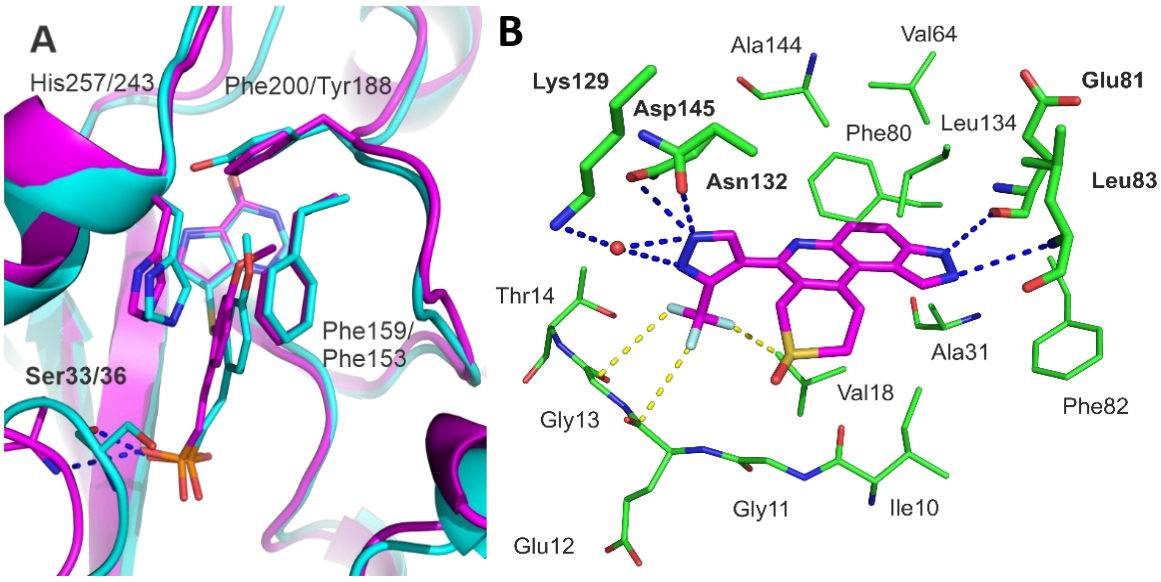

Figure1: A- Overlay of structures of human PNP (magenta) and Mtb PNP (cyan) in complex with one of the inhibitors. **B-** Active site of CDK2 in complex with an inhibitor [5]

1. Nyhan, W. L. Encyclopedia of life sciences 2005, John Wiley & Sons, Ltd.

2. Bzovska, A, Kulikovska, E., Shugar, D. Pharmacology and Therapeutics., 88 (3) 2000, 349-425.

Echalier A, Endicott JA, Noble ME: Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics 2015, 1804 (3): 511–9

4. Wood DJ et al. Cell Chemical Biology. 2018; 26 (1): 121–130.e5.

5.Dayal N et al. J Med Chem 2021; https://doi.org/10.1021/acs.jmedchem.1c00330