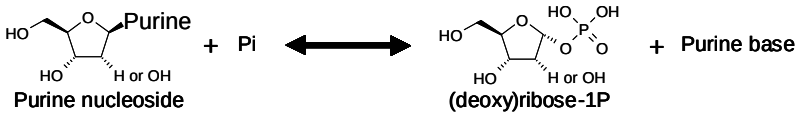
Purine Nucleoside Phosphorylase, a multiple-faced enzyme

Catalytic activity

Purine Nucleoside Phosphorylase (PNP) is involved in salvage pathway of the purine metabolism.

PNP catalyzes the cleavage of the glycosidic bond of ribo- or deoxyribonucleosides, in the presence of inorganic phosphate as a second substrate, to generate the purine base and ribose- or deoxyribose-1-phosphate. The reaction is reversible for natural substrates:



Purine nucleoside (deoxy)ribose-1P

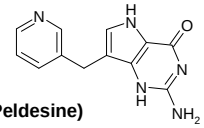
Therapeutic potential of PNP inhibitors

PNP deficiency leads to T-lymphocytopenia, usually with no apparent effects on B-lymphocyte function. These symptoms suggest possible chemotherapeutic applications of potent inhibitors of PNP, as selective immunosuppressive agents, to treat T-cell leukemias or T-cell-mediated autoimmune diseases, such as lupus erythematosus and rheumatoid arthritis[1, 2].

The decrease in plasma and urine levels of urate is an additional symptom of PNP deficiency. PNP inhibitors may contribute to treat hyperuricemic states, such as secondary or xanthine gout.

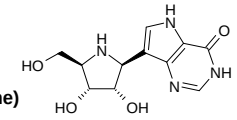
Some PNP inhibitors have undergone clinical trials for the treatment of cutaneous T-cell lymphoma, acute lymphoblastic leukemia (ALL), HIV infections, and psoriasis.

Peldesine (BCX-34) was granted orphan drug status for the treatment of T-cell lymphoma and reached phase III as an immunomodulator for inflammatory autoimmune diseases. It has recently entered clinical trial for HIV infections[3].



BCX-34 (Peldesine)

Forodesine (BCX-1777) has US orphan drug status for the treatment of T-cell non-Hodgkin's lymphoma, including cutaneous T-cell lymphoma (CTCL), chronic lymphocytic leukaemia (CLL) and related leukaemias, including T-cell prolymphocytic leukaemia (PLL), adult T-cell leukaemia and hairy cell leukaemia, and for the treatment of acute lymphocytic leukaemia (ALL). Forodesine was also designed Orphan drug in Europe for ALL in December 2006, and for CTCL in February 2007[4].



BCX-1777 (Forodesine)

PNP inhibitors are also investigated to prevent the cleavage, and the resulting deactivation of Nucleoside Analogues by PNP.

> *Note: Protozoan parasites are auxotrophic for purine and have their own PNPs which have specific activities and properties that differ from the human PNP. Protozoan parasites PNPs are considered to be reasonable target against infection* (e.g. Plasmodium falciparum)[5].

PNP, a threat for therapeutic efficacy of Nucleoside Analogues

In vivo, phosphorolysis is highly favoured over purine nucleoside synthesis and is coupled with two additional enzymatic reactions: oxidation of the liberated purine base by xanthine oxidase (XO) and its phosphoribosylation by hypoxanthine-guanine phosphoribosyltransferase (HGPRT)[6]. Thus, PNP plays a key role in the salvage pathway of the purine metabolism, enabling the cell to utilize purine bases recovered from metabolized purine ribo- and deoxyribonucleosides to synthesize purine nucleotides.

This phosphorolysis reaction of purine nucleosides by PNP has a direct impact on the therapeutic efficacy of Nucleoside Analogues. Antitumour or antiviral nucleoside analogues are likely to be cleaved by PNP before being phosphorylated by the cell nucleoside kinases and converted to the active nucleotide form. For instance, 2',3'-dideoxyguanosine (ddG)[7], 9-β-D-arabinofuranosyl guanine (AraG)[8] as well as one of its produg, Nelarabine (Arranon®, GSK)[9], which is intracellularly converted to AraG by Adenosine deaminase (ADA), are PNP resistant nucleoside analogues, whereas 2',3'-dideoxyinosine (ddI)[10] is easily cleaved *in vivo* by PNP.

Since acyclonucleoside analogues are particularly resistant to cleavage by PNP though phosphorylated by viral thymidine kinases (TK), they are generally considered as excellent candidates as antiviral agents (*e.g.* aciclovir, ganciclovir)[11].

> *Note that Ganciclovir is not only PNP resistant, but is also a PNP inhibitor.*

PNP, a tool for enzymatic synthesis of Nucleoside Analogues

The reversible reaction catalyzed by PNP can be favorably exploited to synthesize nucleoside analogues, especially when chemical synthesis is difficult to prepare and / or gives low yields.

(coming soon, "Transribosylation by PNP")

1. S. Hikishima *et al.* (2007): **Synthesis and biological evaluation of 9-deazaguanine derivatives connected by a linker to difuoromethylene phosphonic acid as multi-substrate analogue inhibitors of PNP**, *Bioorg. Med. Chem. Lett.* 17(15) 4173–4177
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3. http://clinicaltrials.gov/ct2/show/NCT00002237
4. http://www.medscape.com/viewarticle/555215
5. K. Chaudhary *et al.* (2006): ***Toxoplasma gondii* Purine Nucleoside Phosphorylase Biochemical Characterization, Inhibitor Profiles, and Comparison with the *Plasmodium falciparum* Ortholog**, *J. Biol. Chem.* 281(35), 25652-25658
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11. D. Shugar (1999): **Viral and host-cell protein kinases: enticing antiviral targets, and relevance of nucleoside, and viral thymidine, kinases**, *Pharmacol. Ther.* 82(2-3), 315-335

PNP - Purine Nucleoside Phosphorylase

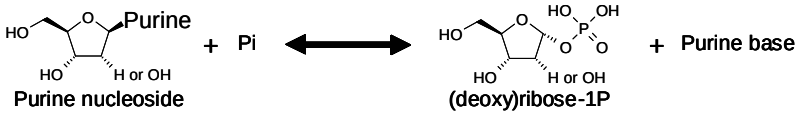
Human, recombinant expressed in E. coli

E.C. 2.4.2.1

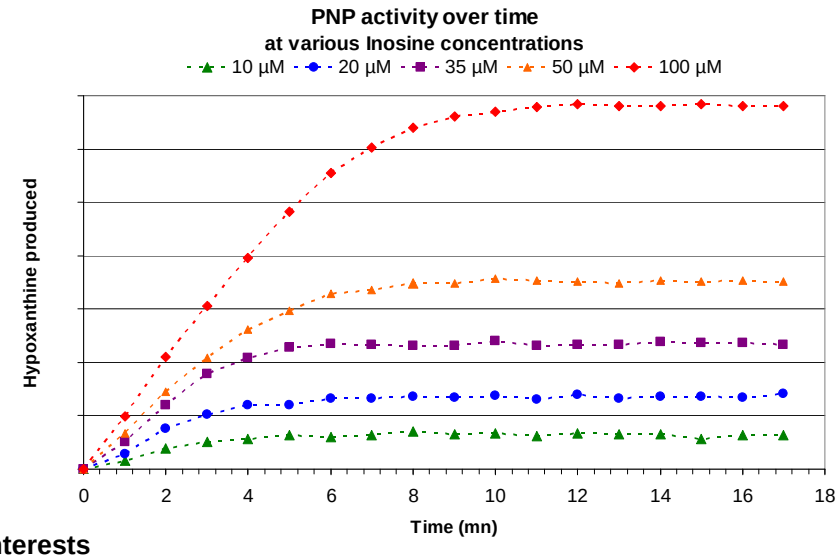
Description

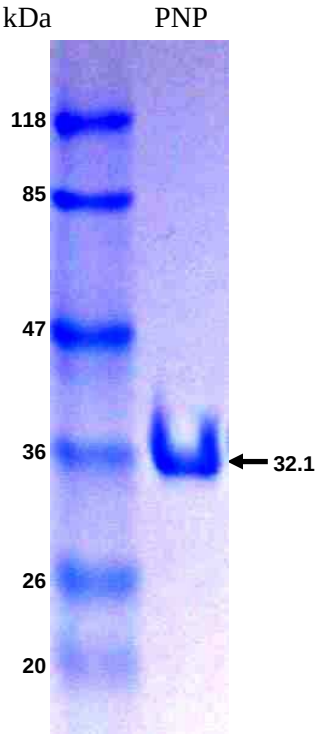
Metabolic function
Purine Nucleoside Phosphorylase (PNP) is involved in salvage pathway of the purine metabolism.

Catalytic activity
PNP catalyzes the cleavage of the glycosidic bond of ribo- or deoxyribonucleosides, in the presence of inorganic phosphate as a second substrate, to generate the purine base and ribose- or deoxyribose-1-phosphate. The reaction is reversible for natural substrates:



NOVOCIB's PNP is a human recombinant Purine Nucleoside Phosphorylase expressed in *E. coli*. It has an apparent molecular weight of 32.12 kDa.





Interests

PNP inhibition
Several PNP inhibitors have been developed to treat cancer, viral infection and auto-immune diseases. **(see sheet # IVS-Nov2, "PNP Inhibition - In vitro Screening Assay" for further details)**

PNP, a threat for therapeutic efficacy of Nucleoside Analogues
PNP's activity *in vivo* can be responsible for the cleavage and the subsequent deactivation of Nucleoside Analogues, thus unable to be phosphorylated by nucleoside kinases. The resistance to cleavage by PNP is worth being investigated to increase the therapeutic efficacy of Nucleoside Analogues. **(see sheet # NCR-Nov2, "PNP Cleavage activity - Nucleoside Resistance Assay" for further details)**

PNP, a tool for enzymatic synthesis of Nucleoside Analogues
PNP can be exploited for the reversible reaction that it catalyzes to synthesize nucleoside analogues, for instance with potential antiviral and antineoplastic activities, especially when chemical synthesis is difficult to prepare and / or gives low yields.
(coming soon, "Transribosylation by PNP" for further details)

NOVOCIB has cloned and purified a human recombinant Purine Nucleoside Phosphorylase (PNP) and has developed a range of related PRECICE® services.

PNP Services

- **PNP Inhibition - *In Vitro* Screening Assay**
- **PNP Cleavage activity - Nucleoside Resistance Assay**
- ***(coming soon)* Transribosylation by PNP**

PNP Inhibition - *In vitro* Screening Assay

IMPORTANT: Client-specified alterations can be accommodated.

Aim: To screen compounds for their abilities to inhibit human PNP *in vitro*.

Therapeutic potential of PNP inhibitors

PNP deficiency leads to T-lymphocytopenia, usually with no apparent effects on B-lymphocyte function. These symptoms suggest possible chemotherapeutic applications of potent inhibitors of PNP, as selective immunosuppressive agents, to treat T-cell leukemias or T-cell-mediated autoimmune diseases, such as lupus erythematosus and rheumatoid arthritis[1, 2].
The decrease in plasma and urine levels of urate is an additional symptom of PNP deficiency. PNP inhibitors may contribute to treat hyperuricemic states, such as secondary or xanthine gout.
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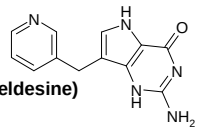

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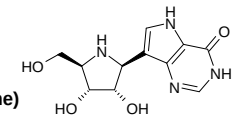


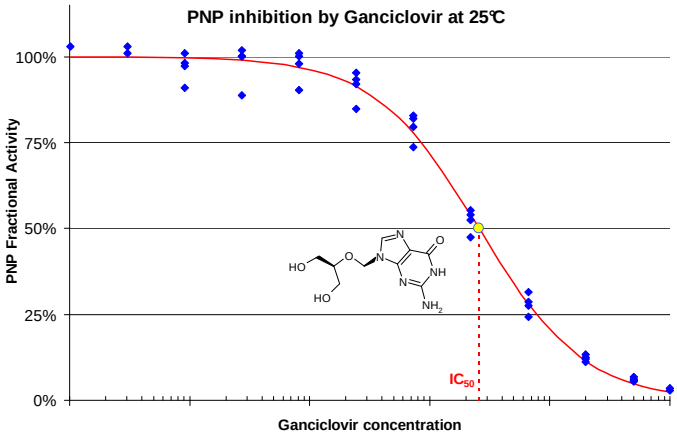
BCX-1777 (Forodesine)

PNP inhibitors are also investigated to prevent the cleavage, and the resulting deactivation of Nucleoside Analogues by PNP.
(see sheet # NCR-Nov2, "PNP Cleavage activity - Nucleoside Resistance Assay" for further details)

> *Note: Protozoan parasites are auxotrophic for purine and have their own PNPs which have specific activities and properties that differ from the human PNP. Protozoan parasites PNPs are considered to be reasonable target against infection (*e.g. Plasmodium falciparum)[5].

Description of the *In vitro* screening assay

PNP enzyme used in the assay is a human recombinant PNP, cloned by **NOVOCIB** from human cells, expressed in *E. coli*, and produced and purified in **NOVOCIB**'s laboratory. **(see sheet # E-Nov 2 for further information)**
PNP purification is controlled before every assay by SDS-PAGE. Protein concentration is measured by Bradford method. PNP specific activity is then determined - 1 unit of PNP catalyzes the cleavage of 1 µmole of inosine per minute at pH 8.0 at 25 °C



Procedure
NOVOCIB has developed a spectrophotometric procedure to directly follow the PNP phosphorolytic reaction on inosine (IR).
The assays are performed at 25°C or 37°C in 200µl o f reaction buffer on 96-well microplate. Pipetting is done by a Multiprobe® II Robotic Liquid Handling System (Packard BioScience). Ganciclovir is used as positive control for PNP inhibition.
Replicate assays: One point is defined as a well per compound and per concentration tested. *In vitro* PNP Inhibition Screening Assays are usually performed in duplicate (2 wells per compound and per concentration). Both negative and positive controls are done in duplicate. Triplicates are available upon request.
Every assay, from one to 90 points, is done with one negative control, *i.e.* without inhibitor, and two positive controls containing Ganciclovir as a PNP inhibitor. Controls are done in duplicate. If an additional microplate is needed, it includes the three controls (in duplicate, *i.e.* 6 wells).

Confirmation by HPLC:
For every positive assay, an HPLC (Agilent 1100 series) control of PNP inhibition is performed by measuring inosine (IR) and Hypoxanthine (Hx) concentrations in the assay and in comparison with negative and positive controls.

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4. http://emea.europa.eu/pdfs/human/comp/opinion/45260406en.pdf
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PNP Cleaving activity - Nucleoside Resistance Assay

IMPORTANT: Client-specified alterations can be accommodated.

Aim: To evaluate the resistance of Nucleoside Analogues to cleavage by human PNP.

Nucleoside Analogues deactivation by PNP
In vivo, phosphorolysis is highly favoured over purine nucleoside synthesis and is coupled with two additional enzymatic reactions: oxidation of the liberated purine base by xanthine oxidase (XO) and its phosphoribosylation by hypoxanthine-guanine phosphoribosyltransferase (HGPRT)[1]. Thus, PNP plays a key role in the salvage pathway of the purine metabolism, enabling the cell to utilize purine bases recovered from metabolized purine ribo- and deoxyribonucleosides to synthesize purine nucleotides.

This phosphorolysis reaction of purine nucleosides by PNP has a direct impact on the therapeutic efficacy of Nucleoside Analogues. Antitumour or antiviral nucleoside analogues are likely to be cleaved by PNP before being phosphorylated by the cell nucleoside kinases and converted to the active nucleotide form. For instance, 2',3'-dideoxyguanosine (ddG)[2], 9-β-D-arabinofuranosyl guanine (AraG)[3] as well as one of its produg, Nelarabine (Arranon®, GSK)[4], which is intracellularly converted to AraG by Adenosine deaminase (ADA), are PNP resistant nucleoside analogues, whereas 2',3'-dideoxyinosine (ddI)[5] is easily cleaved *in vivo* by PNP.

Since acyclonucleoside analogues are particularly resistant to cleavage by PNP though phosphorylated by viral thymidine kinases (TK), they are generally considered as excellent candidates as antiviral agents (*e.g.* aciclovir, ganciclovir)[6].

> *Note that Ganciclovir is not only PNP resistant, but is also a PNP inhibitor.*

NOVOCIB's has developed a PNP enzymatic assay which consists in evaluating the PNP phosphorolysis activity on Nucleosides Analogues in comparison with natural purine nucleoside substrates.

Description of the Nucleoside Resistance assay
The assays are performed at 25°C or 37°C in 200µl o f reaction buffer (lower volumes are available if compound saving is a constraint) on 96-well microplate. Pipetting is done by a Multiprobe® II Robotic Liquid Handling System (Packard BioScience).

Inosine is used as a positive control and adenosine as a negative control.

> *Note that if inosine is a natural substrate of PNP and is consequently actively cleaved by a wide range of PNPs, adenosine resists to Human PNP phosphorolytic activity but is easily cleaved by other PNPs, such as E.coli PNP for instance.*
> ***This is why we consider as a decisive advantage to evaluate the cleavage resistance of Nucleoside Analogue using Human PNP.***

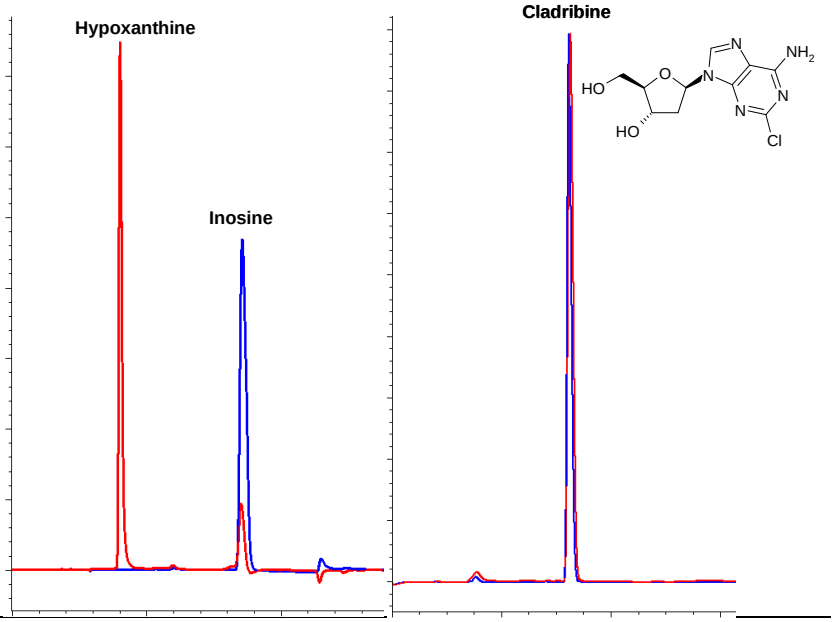
Identification and quantification of the purine nucleosides and the related purine bases produced by Human PNP cleaving activity are performed by HPLC.

Superposed HPLC spectra of two Nucleoside Resistance assays:30 mn incubation at 25°C

Left: Inosine cleavage by Human PNP

Right: Cladribine (deoxyadenosine analogue) resistance to cleavage by Human PNP.

without Human PNP
with Human PNP.



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