Allosteric inhibitors targeting 5’-nucleotidases to restore the antitumor therapy efficacy.

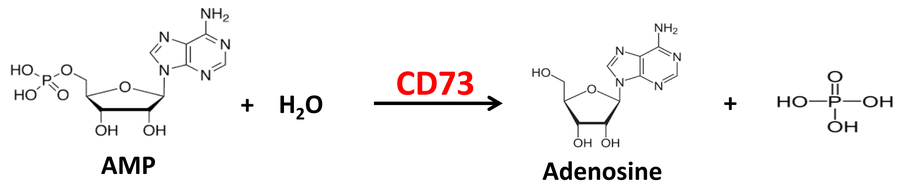
A. Braka[1], R. Rahimova[1], S. Peyrottes[2], L. P. Jordheim[3] and L. Chaloin[1]

[1] Institut de Recherche en Infectiologie de Montpellier (IRIM), UMR9004 - CNRS - Université Montpellier, 1919 route de Mende, 34293 Montpellier cedex 5, France.
[2] Institut des Biomolécules Max Mousseron (IBMM), UMR 5247 CNRS – Université Montpellier - ENSCM, Campus Triolet, Place Eugène Bataillon, 34095 Montpellier cedex 5, France.
[3] Université Lyon 1, Centre de Recherche en Cancérologie de Lyon, INSERM U1052, CNRS UMR 5286, Centre Léon Bérard, F-69008 Lyon, France.

Background

In humans, eight 5'-nucleotidases catalyze the hydrolysis of nucleoside 5'-monophosphates into their respective nucleosides in order to regulate the intracellular / extracellular nucleotide pools. Two main enzymes, cytosolic 5’-nucleotidase II (**cN-II**) and ecto-5’-nucleotidase (**CD73**) have been identified as main therapeutic targets in cancer. Indeed, cN-II is involved in resistance to **hematological cancer** treatments while CD73-generated adenosine was shown to be a potent suppressor of the anticancer immune response in **breast, colorectal, bladder, pancreas and ovarian** cancers. CD73 is overexpressed in cancerous and endothelial cells and promotes tumor growth, cell invasion and tumor metastasis through the action of adenosine binding to P1 receptors (immune cells).

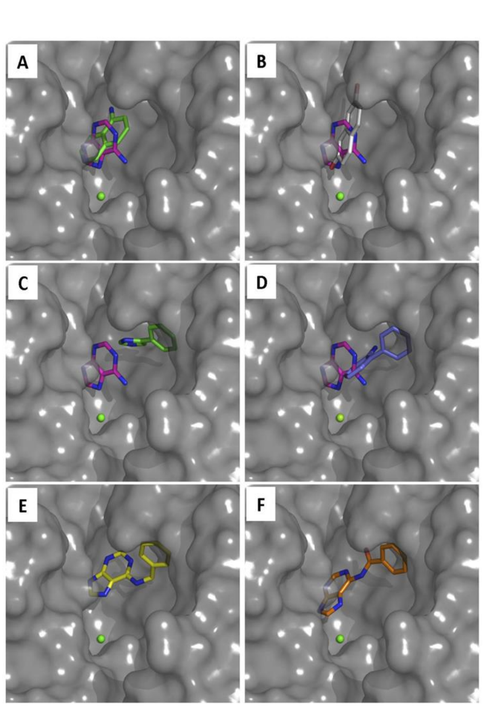
Nucleoside-monophosphate ⇄ (Nucleotidases / Kinases) Nucleoside + Phosphate

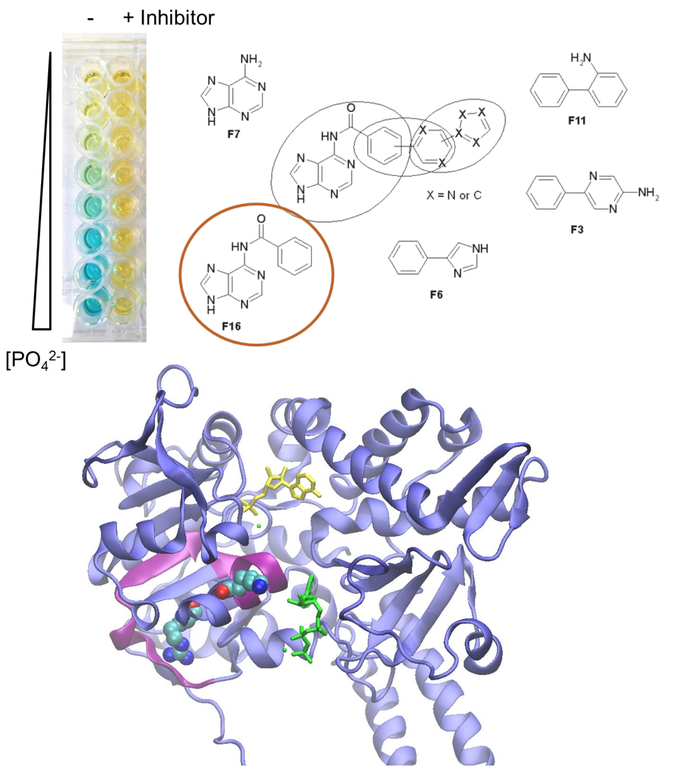


For instance, the treatment of acute myeloid leukemia (**AML**) with cytarabine led to a worse patient outcome when **cN-II** was over-expressed [1]. Moreover, an enzyme hyperactivity was recently correlated with resistance to several chemotherapies in relapsed patients [2-3]. On the other hand, the inhibition of **CD73** activity either by siRNA, substrate analogs or mAb led to tumor regression and restore the immune response through ATP signaling [4-6].

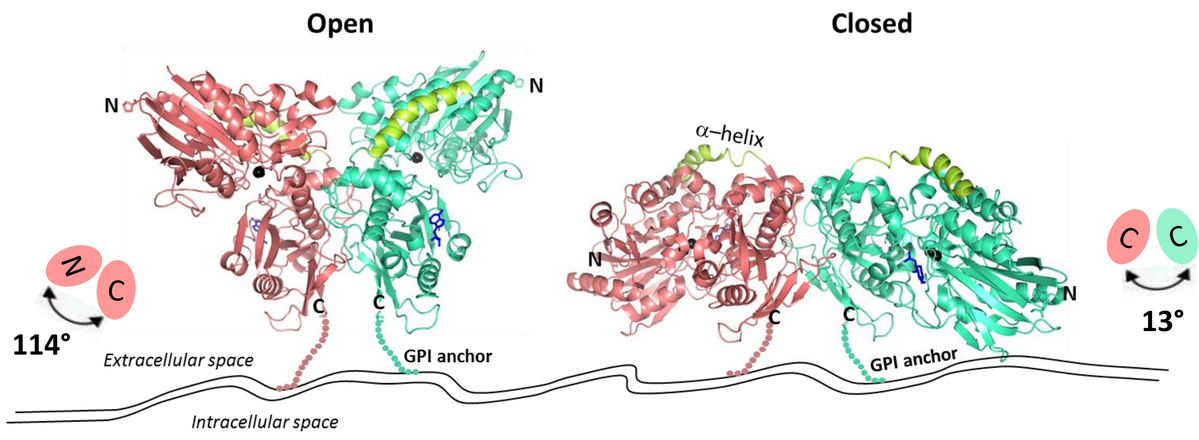
Fragment-Based Drug Design to inhibit cN-II [7]

A **FBDD** approach was applied to design potent and non-competitive inhibitors against this enzyme. Starting with 300 fragments, a **NMR** screening using the purified recombinant enzyme was carried out and hit fragments were evaluated for their inhibitory activity. A fragment growing strategy guided by molecular docking (to avoid overlapping fragments) allowed to propose new molecules resulting in the assembly of three hit fragments.



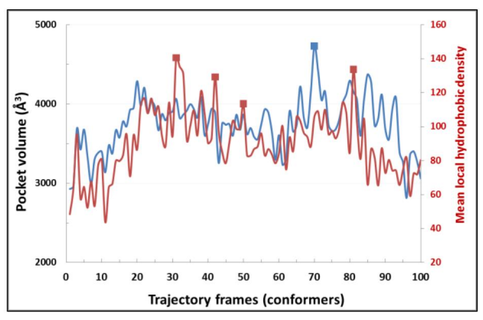


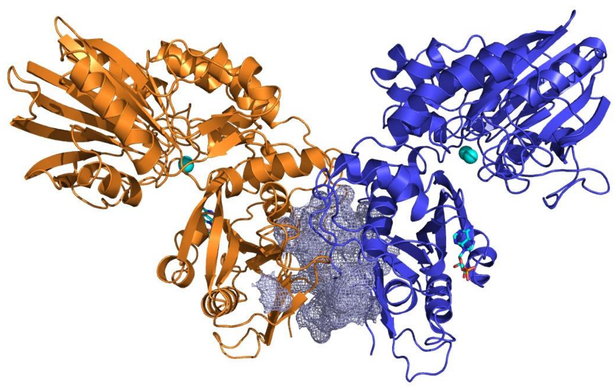
Virtual screening to inhibit CD73 [8]



Selection of the target cavity for virtual screening

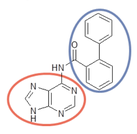
Druggable cavities detection and determination of their volume changes during the molecular dynamics simulation (performed to reproduce the large domain motions occurring during the enzymatic reaction) and selection of five conformers for screening.





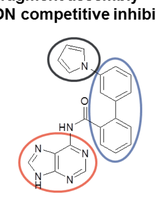
Kinetics studies for inhibition mode determination of fragment assemblies

2-fragment assembly
Competitive inhibition



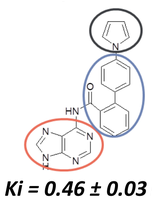
Ki = 0.95 ± 0.17 mM

3-fragment assembly
NON competitive inhibition

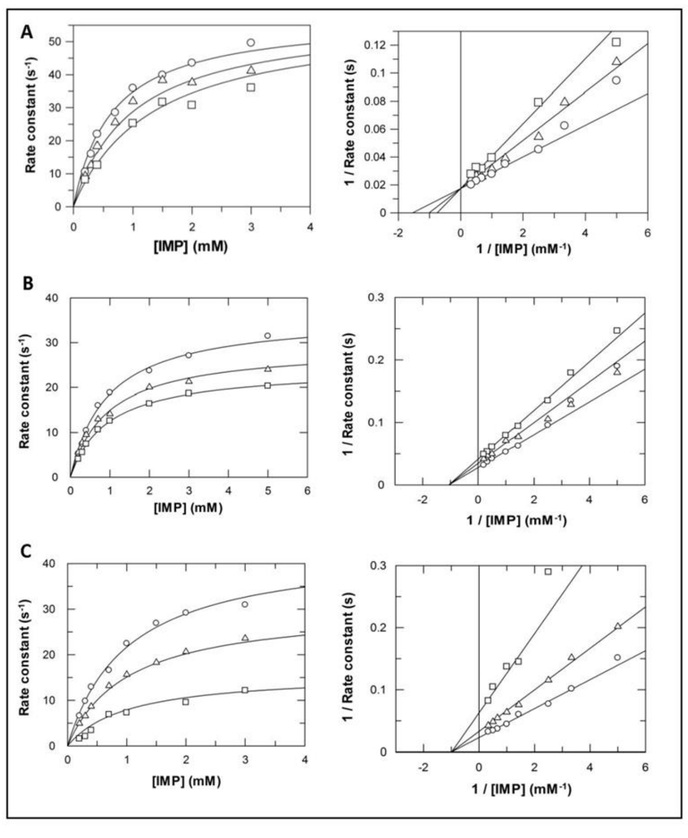


Ki = 1.65 ± 0.1 mM

3-fragment assembly
NON competitive inhibition

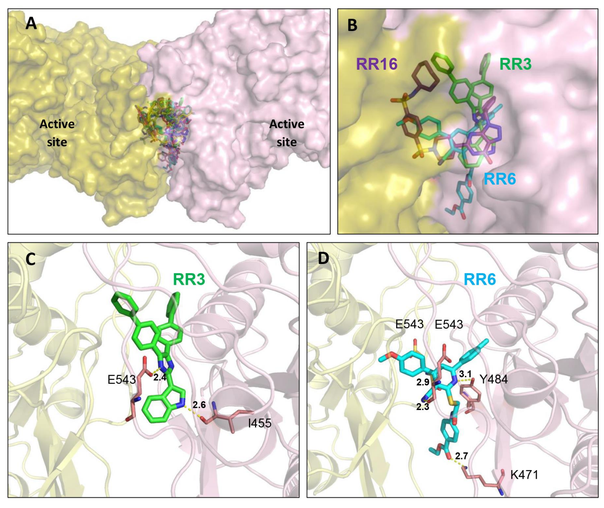


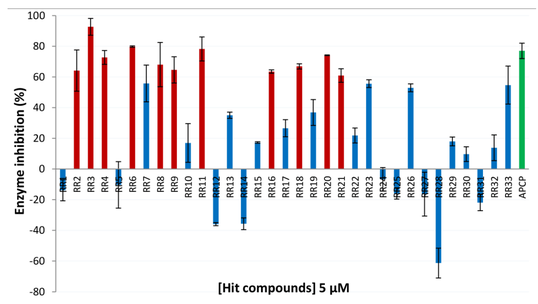
Ki = 0.46 ± 0.03 mM



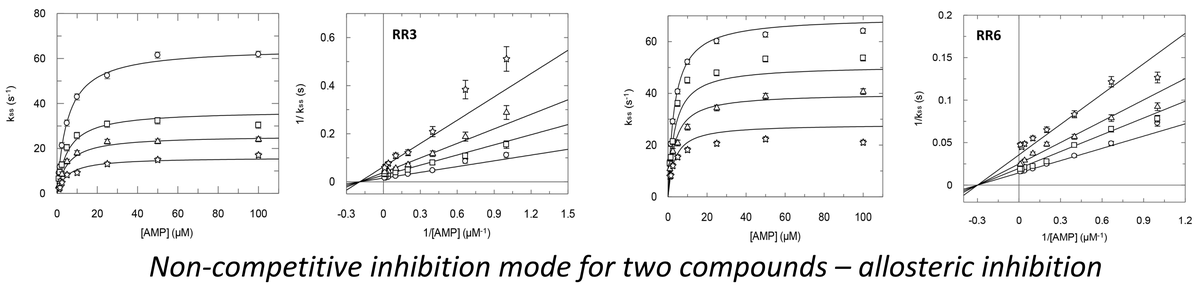
Hit compounds validation and *in vitro* characterization

Virtual screening by molecular docking was performed by using a chemical library of 320,000 drug-like compounds. Structure-activity relationships and kinetics to decipher the mechanism of inhibition for the most active compounds (see below for **RR3** and **RR6**).





In vitro evaluation of CD73 inhibition by hit compounds using the recombinant purified enzyme. Kinetic characterization of two inhibitors



Non-competitive inhibition mode for two compounds – allosteric inhibition

Conclusions

- Discovery of **new cN-II inhibitors** exhibiting non-competitive inhibition mode resulting in the assembly of three fragments (Ki below 0.5 mM) as compared with previous inhibitors (AdiS, Ki = 2 mM).
- 11 hit compounds found to inhibit the **CD73** enzyme activity. The kinetics studies confirmed an allosteric inhibition through a non-competitive inhibition mode with K_i values in the **low micromolar range**.
- Lead optimization of hit compounds by using bioinformatics approaches: scaffold hopping & pharmacophore models to improve potency and water-solubility. Crystallization trials with CD73 and lead compounds and *in vivo* validation.

References:
*1) Galmarini C.M., et al. (**2002**) **British J. of Haematology**, 117, 860-868*
*2) Tzoneva G., et al. (**2013**) **Nat. Med.** 19, 368-371*
*3) Meyer J. A., et al. (**2013**) **Nat. Genet.** 45, 290-294*
*4) Stagg J., et al. (**2010**) **Proc. Natl. Acad. Sci. USA** 107, 1547-1552*
*5) Zhi X., et al. (**2007**) **Clin. Exp. Metastasis** 24, 439-448*
*6) Bhattarai S. et al. (**2015**) **J Med. Chem.** 58, 6248-6263*
*7) Marton Z., et al. (**2015**) **J. Med. Chem.** 58, 9680-9696*
*8) Rahimova R., et al. (**2017**) **PLoS Comp. Biol.** (in press)*

We are grateful to our financial supports and ChemAxon for academic licensing of MarvinSuite