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TARGETING CANCER

Characterization of CDK inhibitors in a biochemical assay using a comprehensive panel of human CDK-Cyclin complexes

Abstract #2801

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Introduction

Members of the family of cyclin dependent kinases (CDKs) have been recognized as pivotal regulators of cell cycle progression for more than 20 years. Concordant to their central role in the control of cell division they have been in the focus of research of proliferation associated diseases ever since, most prominently amongst these cancer.

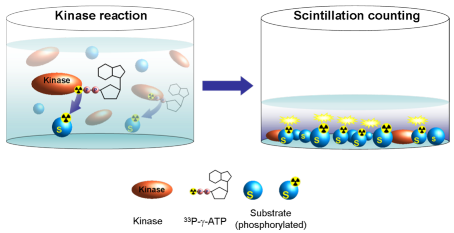
In treatment of cancer, initial results obtained from first and second generation, low specificity CDK inhibitors (e.g. Flavopiridol, Roscovitine, Dinaciclib, AT7519, R547) have been sobering. But the recent approval of the CDK-inhibitor Palbociclib for the treatment of certain forms of breast cancer reconfirms CDKs as drug targets in oncology.

The now available data strongly suggests that CDK inhibitors require very high specificity towards their respective target CDK(s) in order to be effective. However, only a comparably small fraction of the potentially physiologically relevant active CDK complexes have been available for biochemical screening so far.

To date 20 different CDK genes and 17 different Cyclin genes have been identified and experimental data indicates that at least 50-60 different, biologically relevant CDK-Cyclin complexes may exist. Although it is widely accepted for e.g. CDK2 that its biolgocial function is quite distinct when either complexed to Cyclin A or E most inhibitors are classified with a focus on the CDK moiety alone.

By using a broader panel of different Cyclin-complexes for the same CDK the focus of this study was to clarify to what extent the potency of small molecule inhibitors differs when challenged by CDKs complexed to different Cyclins. At the same time the overall CDK specificity for these compounds was evaluated on a panel of 28 CDK-Cyclin complexes containing at least one CDK-Cyclin complex for 15 of the currently described 20 CDK genes.

Principle of the assay



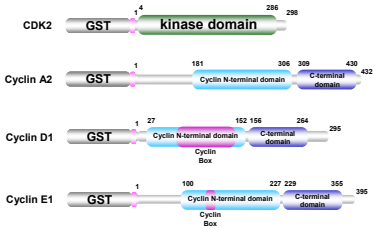
A radiometric, Flashplate® based assay setup was used to determine the phosphorylation of various substrates by CDK-Cyclin complexes.

Kinase and substrate were incubated in presence of ATP containing ^{33}P-γ-ATP as tracer. After the kinase reaction was stopped, the proteins were immobilised on the Flashplate reaction vessel surface and the incorporated radioactivity measured by scintillation counting

Assay Protocol:
in a 96 well Flashplate® mix per well:
-10 µl ATP/^{33}P-γ-ATP mixture
-25 µl reaction buffer
-5 µl compound in 10% DMSO
-10 µl substrate/kinase mix
-incubation 60min, 30°C
-stop by addition of 50µl 2% H_3PO_4
-wash 2x with 200 µl 0.9% NaCl
-analyse plate by scintillation counting

Final assay conditions:
70 mM HEPES pH 7.5
3 mM MgCl2
3 mM MnCl2
3 µM Na-orthovanadate
1.2 mM DTT
1 % DMSO
ATP*, substrate and kinase at variable concentration

*: [ATP] corresponding to the apparent K_M[ATP] of the respective kinase



Schematic overview of example recombinant CDK2 and its associated Cyclins.
CDK2-Cyclin complexes were produced by recombinant expression in insect cells using the Baculo Virus Expression System. Complexes were purified under identical, native conditions.
All further CDK-Cyclin complexes in this study were constructed and purified by basically the same approach.

Results

A: IC_{50} values determined in vitro for 8 small molecule inhibitors using 28 different CDK-Cyclin complexes

IC_{50} >10^{-6} M | IC_{50} >10^{-7} M | IC_{50} >10^{-8} M | IC_{50} <10^{-8} M

Compound ID	CDK1/CycA2 IC50 (M)	CDK1/CycB1 IC50 (M)	CDK1/CycE1 IC50 (M)	CDK2/CycA2 IC50 (M)	CDK2/CycD1 IC50 (M)	CDK2/CycE1 IC50 (M)	CDK3/CycC IC50 (M)
Flavopiridol	2,20E-07	2,71E-07	2,53E-07	7,48E-08	7,27E-07	5,47E-07	1,21E-06
Roscovitine	9,43E-06	>1E-05	6,77E-06	3,76E-07	3,18E-06	4,05E-07	>1E-05
Palbociclib	>1E-05	>1E-05	>1E-05	4,15E-06	5,35E-06	9,68E-06	>1E-05
Sorafenib	>1E-05	>1E-05	>1E-05	>1E-05	1,16E-06	>1E-05	7,48E-06
MER128	2,63E-08	4,88E-08	2,50E-08	3,44E-09	5,96E-08	1,23E-08	1,17E-08
Dinaciclib	3,13E-07	3,18E-07	7,63E-08	4,11E-08	1,34E-07	2,07E-08	2,86E-07
Ponatinib	>1E-05	>1E-05	3,67E-06	2,59E-06	1,59E-07	>1E-05	2,41E-06
MER151	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05

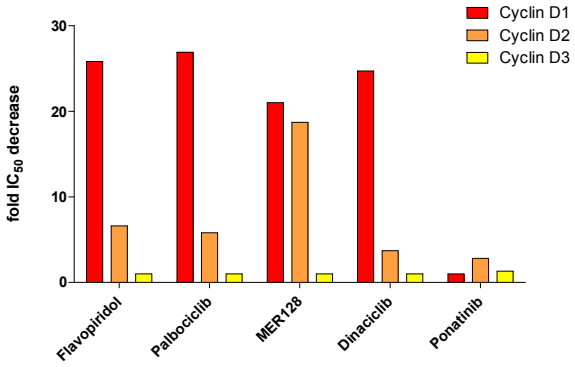
Compound ID	CDK6/CycD2 IC50 (M)	CDK6/CycD3 IC50 (M)	CDK7/CycH/MAT1 IC50 (M)	CDK8/CycC IC50 (M)	CDK9/CycK IC50 (M)	CDK9/CycT1 IC50 (M)	CDK12wt/CycK IC50 (M)
Flavopiridol	9,05E-07	5,99E-06	9,65E-07	4,59E-08	1,45E-08	5,98E-09	1,60E-07
Roscovitine	>1E-05	>1E-05	1,44E-06	>1E-05	2,46E-06	4,14E-07	1,49E-06
Palbociclib	1,18E-08	6,80E-08	>1E-05	>1E-05	5,69E-07	3,89E-07	4,60E-06
Sorafenib	>1E-05	>1E-05	>1E-05	5,72E-08	7,84E-06	>1E-05	>1E-05
MER128	2,92E-07	5,46E-06	1,55E-06	2,15E-09	2,26E-09	3,00E-10	1,05E-08
Dinaciclib	3,97E-07	1,47E-06	2,17E-07	2,88E-06	5,16E-09	1,59E-09	6,80E-09
Ponatinib	2,63E-06	5,68E-06	>1E-05	1,44E-08	1,34E-06	2,35E-06	1,09E-06
MER151	>1E-05	>1E-05	>1E-05	9,93E-09	1,00E-05	4,77E-06	>1E-05

Compound ID	CDK3/CycE1 IC50 (M)	CDK4/CycD1 IC50 (M)	CDK4/CycD2 IC50 (M)	CDK4/CycD3 IC50 (M)	CDK5/p25NCK IC50 (M)	CDK5/p35NCK IC50 (M)	CDK6/CycD1 IC50 (M)
Flavopiridol	1,11E-06	1,03E-07	4,34E-08	1,60E-07	3,02E-07	2,86E-07	2,32E-07
Roscovitine	1,71E-06	>1E-05	>1E-05	>1E-05	1,01E-06	7,71E-07	>1E-05
Palbociclib	>1E-05	1,36E-08	2,19E-08	1,15E-08	6,91E-06	3,63E-06	2,83E-09
Sorafenib	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05
MER128	1,08E-08	4,61E-07	7,08E-07	9,12E-07	2,03E-08	1,49E-08	2,60E-07
Dinaciclib	1,98E-08	1,36E-07	1,37E-07	1,41E-07	2,35E-08	1,61E-08	5,94E-08
Ponatinib	>1E-05	7,06E-06	>1E-05	2,00E-06	>1E-05	>1E-05	7,24E-06
MER151	>1E-05	>1E-05	>1E-05	5,11E-06	>1E-05	>1E-05	>1E-05

Compound ID	CDK12 R722C/CycK IC50 (M)	CDK13/CycK IC50 (M)	CDK16/CycY IC50 (M)	CDK17/p35NCK IC50 (M)	CDK19/CycC IC50 (M)	CDK20/CycH IC50 (M)	CDK20/CycT1 IC50 (M)
Flavopiridol	1,59E-07	8,43E-07	1,66E-07	2,56E-06	5,27E-07	4,55E-06	2,60E-06
Roscovitine	3,36E-06	5,35E-06	3,22E-06	8,35E-06	>1E-05	>1E-05	8,68E-06
Palbociclib	5,82E-06	>1E-05	2,08E-06	1,65E-06	1,86E-06	1,32E-06	1,26E-06
Sorafenib	>1E-05	>1E-05	9,00E-06	6,24E-06	>1E-05	>1E-05	>1E-05
MER128	1,31E-08	3,93E-08	1,49E-07	9,68E-08	6,31E-09	8,59E-10	2,32E-09
Dinaciclib	8,13E-09	6,30E-08	2,23E-07	3,17E-07	6,15E-06	3,18E-06	2,86E-06
Ponatinib	1,63E-06	3,28E-06	>1E-05	8,88E-07	7,01E-07	3,05E-06	>1E-05
MER151	>1E-05	>1E-05	>1E-05	>1E-05	4,67E-06	2,39E-06	>1E-05

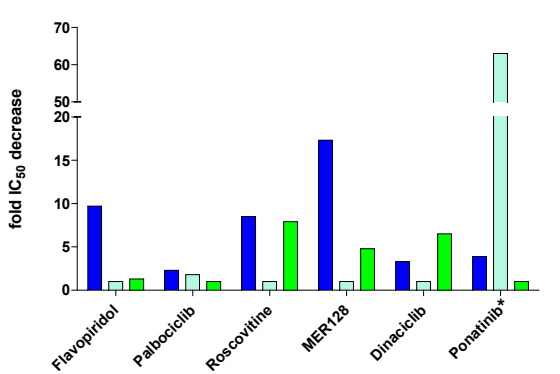
B:

Change of IC_{50} values for CDK6 depending on Cyclin complex partner



C:

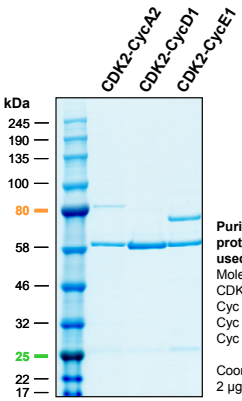
Change of IC_{50} values for CDK2 depending on Cyclin complex partner



CDK inhibition was normalized to the value of the complex weakest inhibited by the respective inhibitor and plotted relative to this value.

*: values for CDK2-CycE1 and Ponatinib are minimum values as inhibition of this complex was >10^{-5} M

D:



Purified recombinant CDK2-Cyclin proteins as example of the material used in this study.
Molecular weights of the proteins:
CDK2 : 60,222 Da
Cyc A2: 74,928 Da
Cyc D1: 59,995 Da
Cyc E1: 72,041 Da

Coomassie stained SDS-PA gel
2 µg total protein per lane

Summary

- 27 different kinase complexes and one mutant have been compared for inhibition by 8 small molecule inhibitors
- inhibition profiles matched and expanded literature data
- for several CDKs (CDK2, CDK3, CDK6, CDK9) significant differences in inhibitor potency were observed dependent on the Cyclin complex partner
- Cyclin dependent IC_{50} variations ranged from 5 to >60 fold
- Cyclin effects were partially comparable, partially different for different inhibitors

Outlook

- completion of CDK panel by set up of in-vitro assays for CDK10, CDK11, CDK14*, CDK15 and CDK18* (*available shortly)
- further extension of the biochemical CDK-Cyclin complex assay panel according to literature data