Comprehensive characterization of CDK inhibitors using a complete panel of all 20 human cyclin-dependent kinases

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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 30 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, including cancer.

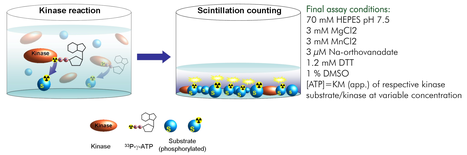
In treatment of cancer, initial results obtained from first and second generation, low specificity CDK inhibitors (e.g., Flavopiridol, Roscovitine, Dinaciclib) have been sobering. But the more recent approval of three CDK4/6 inhibitors for the treatment of certain forms of breast cancer reconfirms CDKs as drug targets in oncology.

The available data strongly suggests that CDK inhibitors require remarkably high specificity towards their respective target CDK(s) 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 31 different Cyclin genes (including 2 pseudogenes) have been identified and experimental data indicates that at least 50-60 different, biologically relevant CDK-Cyclin complexes may exist.

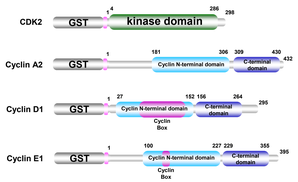
Due to the similarity within the CDK family and the broad spectrum of their physiological functions, selectivity of CDK inhibitors is of pivotal relevance. We report here the set up of biochemical assays for all 20 human CDKs. In total the panel includes 32 active CDK-Cyclin complexes allowing not only evaluation of the selectivity of compounds within the CDK family but also to evaluate the relevance of different cyclins bound to a given CDK with respect to their inhibition by CDK targeting compounds.

Principle of the assay



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] = KM (app.) of respective kinase substrate/kinase at variable concentration

The radiometric, Flashplate® based ^{33}PanQinase 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 reaction stop, all proteins are immobilised on the Flashplate reaction vessel surface and the incorporated radioactivity is measured by scintillation counting



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 a very similar approach.

Results

A: IC_{50} values determined in vitro for 12 small molecule inhibitors using 32 different CDK-Cyclin complexes

IC_{50} >10^{-5} M; IC_{50} 10^{-7}-10^{-8} M; IC_{50} 10^{-6}-10^{-7} M; IC_{50} <10^{-8} M

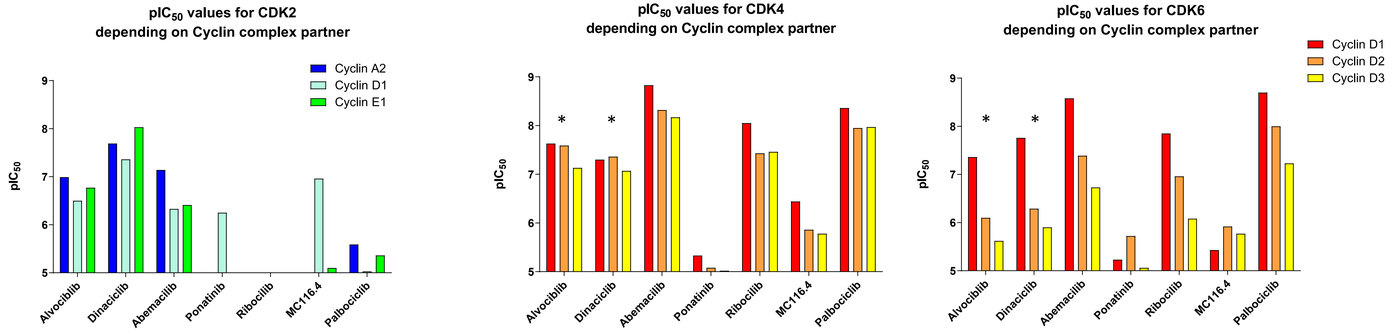
Compound	CDK1/CycA2	CDK1/CycB1	CDK1/CycE1	CDK2/CycA2	CDK2/CycD1	CDK2/CycE1	CDK3/CycC	CDK3/CycE1
	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)
Selicicilb (Roscovitine)	4,24E-06	5,23E-06	2,44E-06	7,66E-07	2,53E-06	2,67E-07	5,71E-06	1,06E-06
OTS964	>1E-05	>1E-05	>1E-05	3,97E-06	>1E-05	2,88E-06	>1E-05	>1E-05
NVP-2	1,72E-06	1,42E-06	8,46E-07	2,64E-06	5,82E-06	2,53E-06	2,04E-06	3,17E-06
Alvocidib (Flavopiridol)	6,02E-08	9,90E-08	2,03E-07	1,02E-07	3,15E-07	1,68E-07	2,04E-07	3,13E-07
Dinaciclib	7,85E-08	1,06E-07	3,55E-08	2,03E-08	4,40E-08	9,44E-09	5,23E-08	8,44E-09
Abemaciclib	1,30E-06	1,38E-06	9,98E-07	7,30E-08	4,70E-07	3,85E-07	2,02E-07	9,40E-07
CCT251545 (HY-12681)	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05
Ponatinib	>1E-05	>1E-05	7,52E-07	>1E-05	5,58E-07	>1E-05	5,57E-07	>1E-05
Ribociclib	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05
MC116.4	>1E-05	>1E-05	2,13E-07	>1E-05	1,09E-07	7,85E-06	1,36E-07	>1E-05
THZ531	>1E-05	>1E-05	5,74E-06	3,70E-06	6,67E-06	1,96E-06	4,24E-06	5,43E-06
Palbociclib	>1E-05	>1E-05	>1E-05	2,54E-06	9,38E-06	4,32E-06	2,32E-06	>1E-05

Compound	CDK4/CycD1	CDK4/CycD2	CDK4/CycD3	CDK5/p25NCK	CDK5/p35NCK	CDK6/CycD1	CDK6/CycD2	CDK6/CycD3
	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)
Selicicilb (Roscovitine)	>1E-05	>1E-05	>1E-05	1,47E-06	4,51E-07	>1E-05	>1E-05	>1E-05
OTS964	5,36E-06	7,55E-06	>1E-05	8,55E-06	2,98E-06	>1E-05	>1E-05	>1E-05
NVP-2	1,21E-06	3,57E-06	8,79E-06	3,93E-06	1,36E-06	2,32E-06	>1E-05	>1E-05
Alvocidib (Flavopiridol)	2,32E-08	2,59E-08	7,41E-08	2,92E-07	8,67E-08	4,41E-08	7,91E-07	2,39E-06
Dinaciclib	4,96E-08	4,40E-08	8,45E-08	2,09E-08	4,21E-09	1,75E-08	5,09E-07	1,25E-06
Abemaciclib	1,48E-09	4,81E-09	6,80E-09	1,13E-06	2,42E-07	2,64E-09	4,12E-08	1,86E-07
CCT251545 (HY-12681)	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05
Ponatinib	4,64E-06	8,40E-06	9,45E-06	>1E-05	>1E-05	5,91E-06	1,90E-06	8,69E-06
Ribociclib	8,94E-09	3,68E-08	3,43E-08	>1E-05	>1E-05	1,41E-08	1,09E-07	8,39E-07
MC116.4	3,61E-07	1,38E-06	1,67E-06	>1E-05	>1E-05	3,73E-06	1,21E-06	1,68E-06
THZ531	>1E-05	>1E-05	>1E-05	6,99E-06	2,78E-06	>1E-05	>1E-05	>1E-05
Palbociclib	4,41E-09	1,11E-08	1,08E-08	4,91E-06	1,48E-06	1,99E-09	1,00E-08	5,93E-08

Compound	CDK7/CycH/MAT1	CDK8/CycC	CDK9/CycK	CDK9/CycT1	CDK10/CycQ	CDK11B/CycK	CDK12/CycK	CDK13/CycK
	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)
Selicicilb (Roscovitine)	1,84E-06	>1E-05	1,19E-06	1,10E-06	>1E-05	>1E-05	3,77E-06	2,26E-06
OTS964	>1E-05	>1E-05	1,13E-06	1,30E-06	1,37E-06	3,69E-08	9,05E-06	5,88E-06
NVP-2	>1E-05	>1E-05	2,50E-09	2,59E-09	5,27E-08	6,25E-06	5,57E-08	9,87E-08
Alvocidib (Flavopiridol)	1,13E-06	5,80E-08	5,41E-09	4,08E-09	8,27E-08	3,55E-06	1,55E-07	1,43E-07
Dinaciclib	1,79E-07	4,63E-06	4,40E-09	4,33E-09	4,16E-08	7,85E-06	9,09E-09	8,87E-09
Abemaciclib	4,46E-06	1,53E-06	1,61E-08	3,24E-08	3,71E-07	3,94E-06	5,71E-07	3,44E-07
CCT251545 (HY-12681)	>1E-05	3,81E-08	2,96E-06	3,76E-06	>1E-05	>1E-05	>1E-05	>1E-05
Ponatinib	>1E-05	4,83E-08	2,84E-06	2,59E-06	1,14E-07	1,35E-06	2,13E-06	1,74E-06
Ribociclib	>1E-05	>1E-05	7,50E-07	1,15E-06	>1E-05	>1E-05	>1E-05	>1E-05
MC116.4	>1E-05	3,08E-08	3,18E-06	3,10E-06	2,76E-07	6,82E-07	>1E-05	6,30E-07
THZ531	2,22E-06	>1E-05	1,17E-06	1,01E-06	>1E-05	>1E-05	1,86E-07	>1E-05
Palbociclib	>1E-05	>1E-05	2,63E-07	5,77E-07	2,43E-06	>1E-05	7,35E-06	8,69E-06

Compound	CDK14/CycY	CDK15/CycA2	CDK16/CycY	CDK17/p35NCK	CDK18/CycY	CDK19/CycC	CDK20/CycH	CDK20/CycT1
	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)	IC50 (M)
Selicicilb (Roscovitine)	6,94E-06	3,82E-06	3,21E-06	>1E-05	2,63E-06	>1E-05	6,50E-06	4,89E-06
OTS964	>1E-05	>1E-05	4,54E-06	8,90E-06	>1E-05	>1E-05	>1E-05	>1E-05
NVP-2	>1E-05	2,03E-06	3,12E-06	4,51E-06	6,73E-06	>1E-05	1,08E-06	2,12E-07
Alvocidib (Flavopiridol)	1,82E-07	1,96E-08	6,40E-08	2,63E-06	1,80E-07	5,44E-08	1,29E-06	8,06E-07
Dinaciclib	3,63E-08	4,36E-08	1,66E-07	1,30E-06	1,51E-07	3,24E-06	5,76E-06	2,06E-07
Abemaciclib	7,97E-07	1,22E-07	9,29E-08	1,91E-07	5,01E-07	1,45E-06	1,38E-08	3,33E-08
CCT251545 (HY-12681)	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	1,19E-07	>1E-05	>1E-05
Ponatinib	1,67E-06	>1E-05	>1E-05	3,27E-06	4,30E-06	5,35E-08	>1E-05	6,45E-06
Ribociclib	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	>1E-05	9,14E-06
MC116.4	2,43E-06	>1E-05	1,54E-06	2,61E-06	4,73E-07	6,26E-08	>1E-05	>1E-05
THZ531	1,38E-07	8,31E-06	8,79E-06	8,81E-06	9,01E-06	>1E-05	5,04E-06	2,95E-06
Palbociclib	>1E-05	>1E-05	1,97E-06	1,44E-06	>1E-05	>1E-05	7,10E-07	8,40E-07

B:



Comparison of the inhibition of selected CDKs complexed to different Cyclins by different small molecule inhibitors. pIC50 values were used for better visualization of the differences in inhibtion potency. Cyclin dependent differences in inhibitor potency were also variable for different compounds: While e.g. Alvocidib and Dinaciclib dependent inhibition of CDK4 was not significantly influenced by the type of D-type cyclin present, in the case of CDK6 a strong Cyclin-dependency was observed (marked by asteriks in the above panel).

Summary

➢32 different kinase complexes have been compared for inhibition by 12 small molecule inhibitors
➢inhibition profiles matched and expanded literature data
➢for several CDKs (CDK2, CDK3, CDK6) significant differences in inhibitor potency were observed dependent on the Cyclin complex partner
➢IC_{50} values were shifted up to 70fold depending on the Cyclin-complex partner for some CDKs
➢Cyclin effects were partially comparable, partially different for different inhibitors and CDKs

Outlook

➢further expansion of the CDK-Cyclin panel to cover all complexes so far shown to form physiologically relevant functional entities

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