Conformational Adaption May Explain the Slow Dissociation Kinetics of Roniciclib (BAY 1000394), a Type I CDK Inhibitor with Kinetic Selectivity for CDK2 and CDK9

Pelin Ayaz, Dorothee Andres, Dennis A. Kwiatkowski, Carl-Christian Kolbe, Philip Lienau, Gerhard Siemeister, Ulrich Lücking, and Christian M. Stegmann*

ACS Chem. Biol. **2016**, 11, 1710-1719

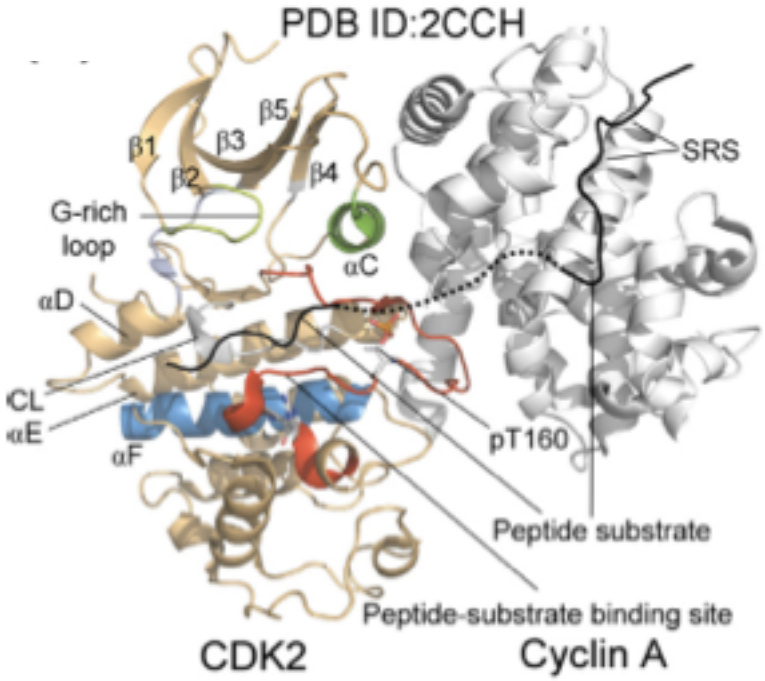
Wipf Group Current Literature

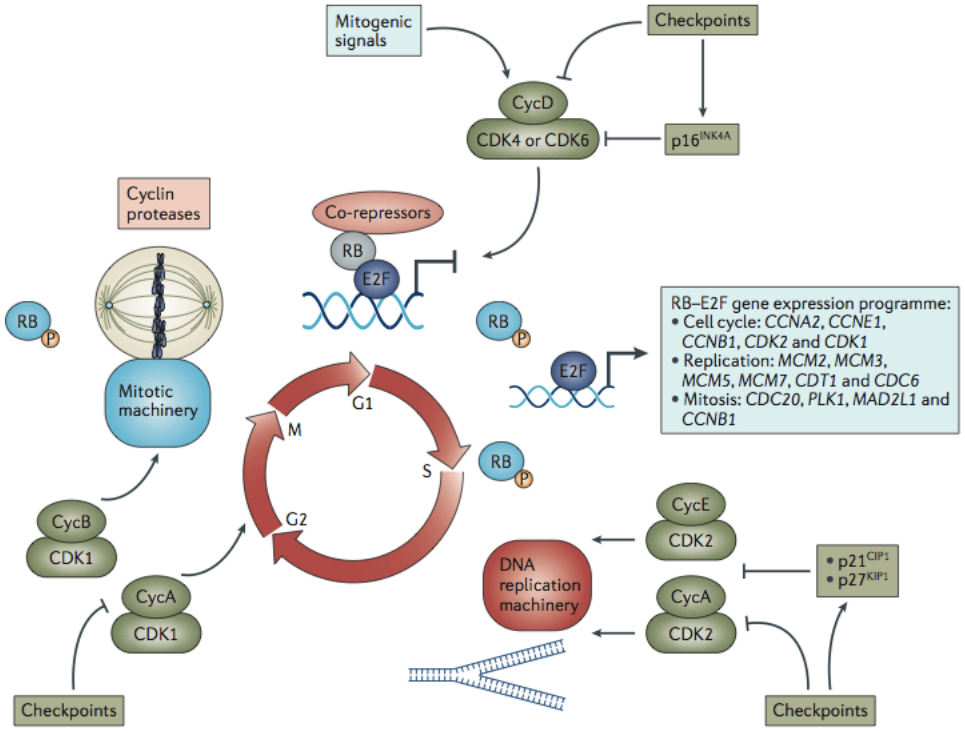
Chaemin Lim

07/09/2016

Cyclin-dependent kinases (CDKs)

- A group of serine/threonine kinases that can modify various protein substrates involved in cell cycle progression.
- As their name suggests, CDKs require the presence of cyclins to become active.
- All eukaryotes have multiple cyclins, each of which acts during a specific stage of the cell cycle.
- CDK4 and CDK6 regulate entry into cell cycle / CDK1 and CDK2 operate primarily in M phase and S phase

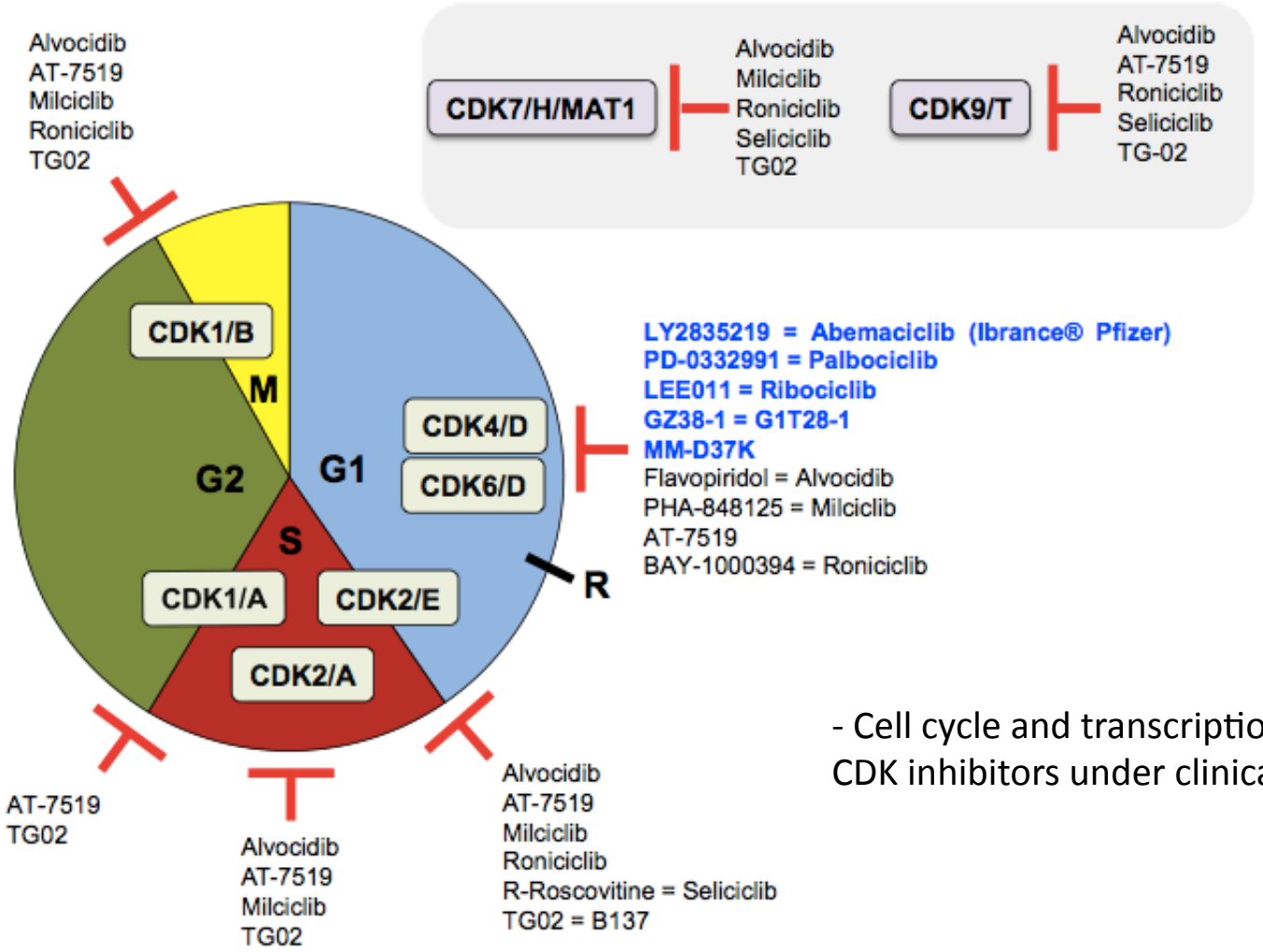




Pharmacological Res. **2016**, *107*, 249.
Nature Reviews Drug discovery, **2015**, *14*, 130.

Cell cycle kinases as therapeutic targets for cancer

- Loss of cell-cycle control and increase resistance to apoptosis are major hallmarks of cancer.
- Cancer cells exhibit dyregulated cell division along with the formation of abnormal chromosome numbers.

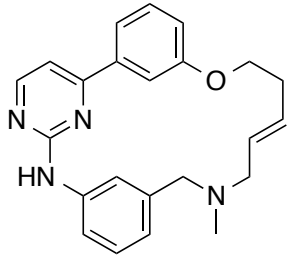


- Cell cycle and transcription regulation CDK inhibitors under clinical evaluation.

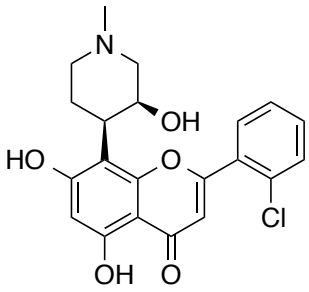
Bioorg. Med. Chem. Lett. **2015**, *25*, 3420.

3

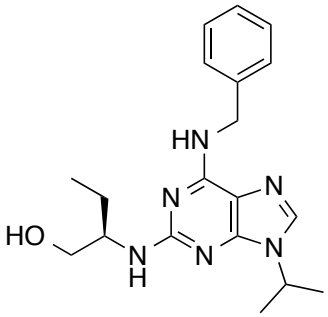
CDK inhibitors under clinical evaluation



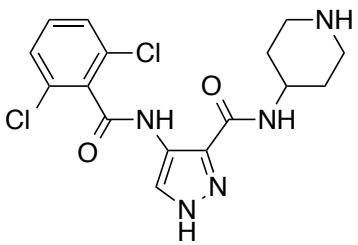
TG02
(SB-137)
CDK 1, 2, 7, 9
phase I



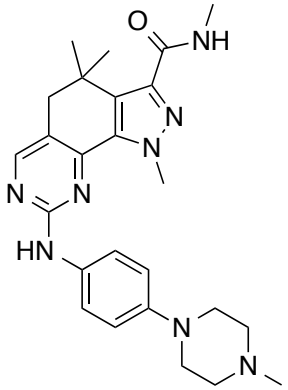
Flavopiridol
(Alvocidib)
CDK 1, 4, 9
phase II



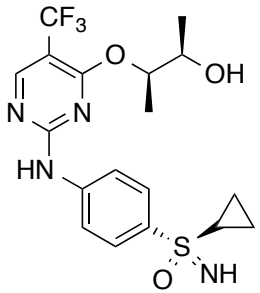
R-Roscovitine
(Seliciclib)
CDK 2, 7, 9
phase II



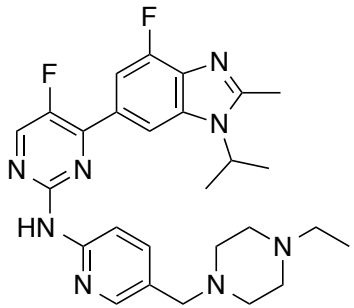
AT-7519
CDK 2, 9
phase II



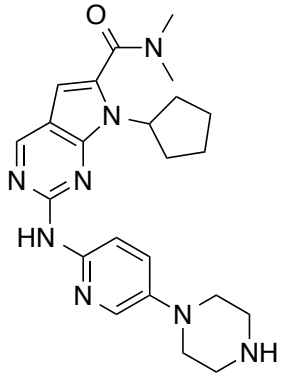
PHA-848125
(Milciclib)
CDK 2
phase II



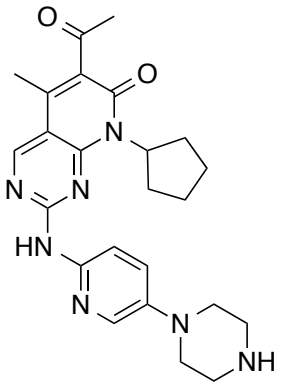
BAY-1000394
(Roniciclib)
CDK 1, 2, 4, 7, 9
phase II
-> Development of BAY1000394
has been terminated
by Bayer (June 29, 2016)



LY2835219
(Abemaciclib)
CDK 4, 6
phase III



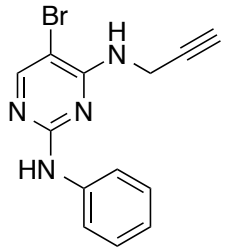
LEE-011
(Ribociclib)
CDK 4 and 6
Phase III



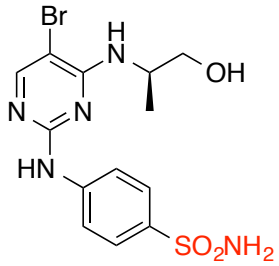
PD-0332991
(Palbociclib
Ibrance®, Pfizer)
CDK 4 and 6
FDA approved, Feb. 2015

Bioorg. Med. Chem. Lett. **2015**, *25*, 3420.
clinicaltrials.gov

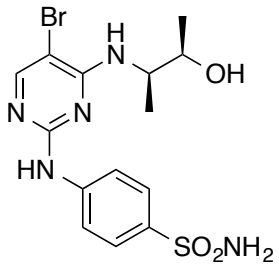
Optimization of a HTS hit led to the identification of BAY 1000394



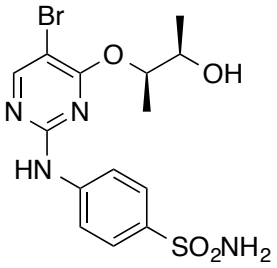
A (HIT)
CDK2 IC_{50} = 100 nM
CDK1 IC_{50} = 100 nM
MCF7 IC_{50} = 4 μM



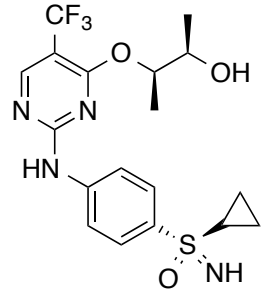
ZK-304709
CDK2 IC_{50} = 4 nM
CDK1 IC_{50} = 50 nM
VEGF-R2 IC_{50} = 34 nM
CA-II IC_{50} = 514 nM
MCF7 IC_{50} = 266 nM



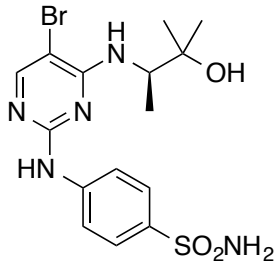
B
CDK2 IC_{50} = 10 nM
CDK1 IC_{50} = 10 nM
VEGF-R2 IC_{50} = 59 nM
CA-II IC_{50} = 403 nM
MCF7 IC_{50} = 30 nM



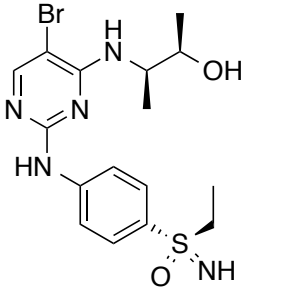
D
CDK2 IC_{50} = 9 nM
CDK1 IC_{50} = 4 nM
VEGF-R2 IC_{50} = 122 nM
CA-II IC_{50} = ND
MCF7 IC_{50} = 48 nM



BAY-1000394 (Roniciclib)
CDK2 IC_{50} = 9 nM
CDK1 IC_{50} = 7 nM
VEGF-R2 IC_{50} = 163 nM
CA-II IC_{50} > 10 μM
MCF7 IC_{50} = 15 nM



C
CDK2 IC_{50} = 13 nM
CDK1 IC_{50} = 14 nM
VEGF-R2 IC_{50} = 158 nM
CA-II IC_{50} = 529 nM
MCF7 IC_{50} = 182 nM



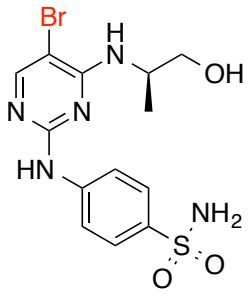
E
CDK2 IC_{50} = 2 nM
CDK1 IC_{50} = 3 nM
VEGF-R2 IC_{50} = 32 nM
CA-II IC_{50} > 10 μM
MCF7 IC_{50} = 44 nM

- *Promising preclinical profile: developed as a multitarget CDK and VEGF-R inhibitor*
- *Failed in phase I due to dose-limited absorption and high interpatient variability*

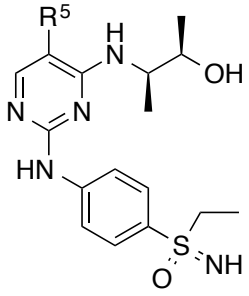
ChemMedChem **2013**, *8*, 1067.
Bioorg. Med. Chem. Lett. **2015**, *25*, 3420.

In this study

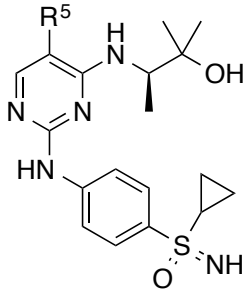
- Investigation of the binding kinetics of roniciclib and related compounds, as well as other previously identified potent CDK inhibitors.
- Roniciclib displays prolonged residence times on CDK2 and CDK9.
- Variation of the substituent at the 5-position of the pyrimidine scaffold results in changes of up to 3 orders magnitude of the drug-target residence time.
- X-ray cocrystal structures of apo-CDK2 with **6**, **5**, and roniciclib were solved.



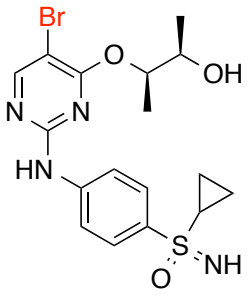
ZK-304709



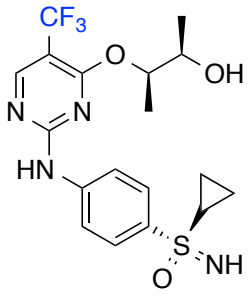
1 R^5 = Br
2 R^5 = CF_3



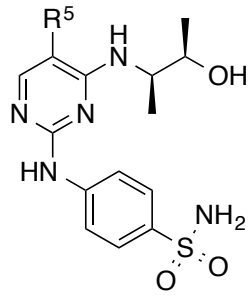
3 R^5 = Br
4 R^5 = CF_3



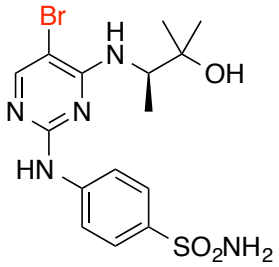
5



BAY-1000394 (Roniciclib)



6 R^5 = H
7 R^5 = F
8 R^5 = Cl
9 R^5 = Br
10 R^5 = CF_3

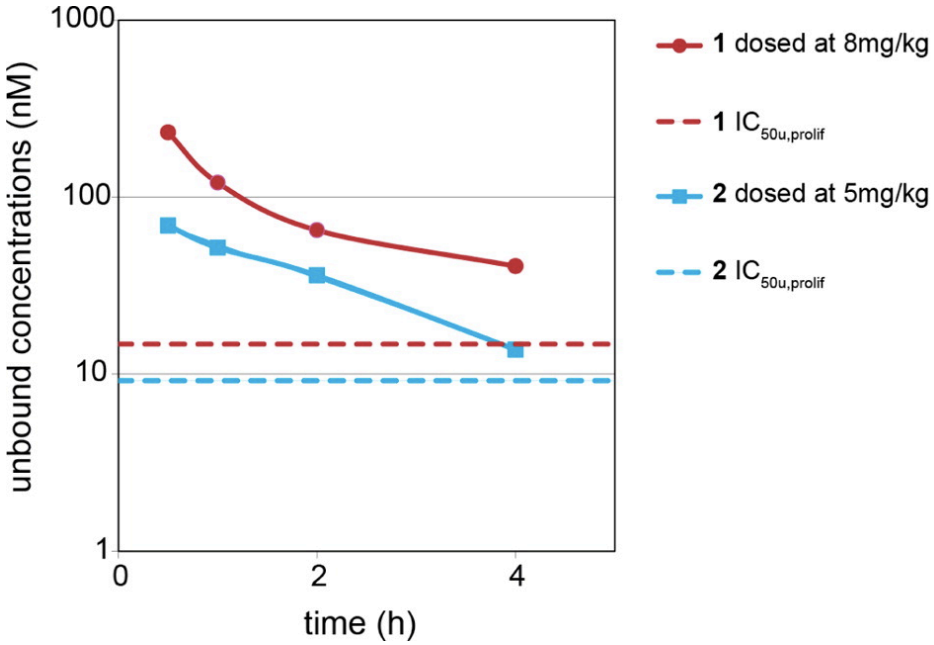


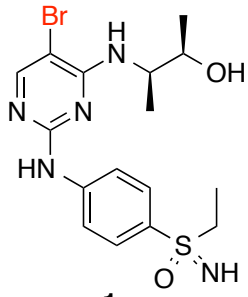
11

ACS Chem. Biol. **2016**, 11, 1710-1719

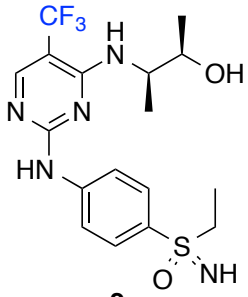
Exposure levels in mouse serum after oral administration of 1 and 2

- In HeLa-MaTu xenograft models, 5-CF_3 analogs show superior efficacy in tumor growth inhibition compared to their corresponding 5-Br analogs
- 5-Br derivative **1** has a superior exposure profile (both total (AUC) and unbound exposure (AUC_u)) than 5-CF_3 analog **2** in mouse serum.
- The pharmacological efficacy of 5-CF_3 derivative **2** over 5-Br analog **1** cannot be explained by an increased exposure in this species.





1
CDK2 IC_{50} = 2 nM
CDK1 IC_{50} = 3 nM
VEGF-R2 IC_{50} = 32 nM
CA-II IC_{50} > 10 μM
MCF7 IC_{50} = 44 nM



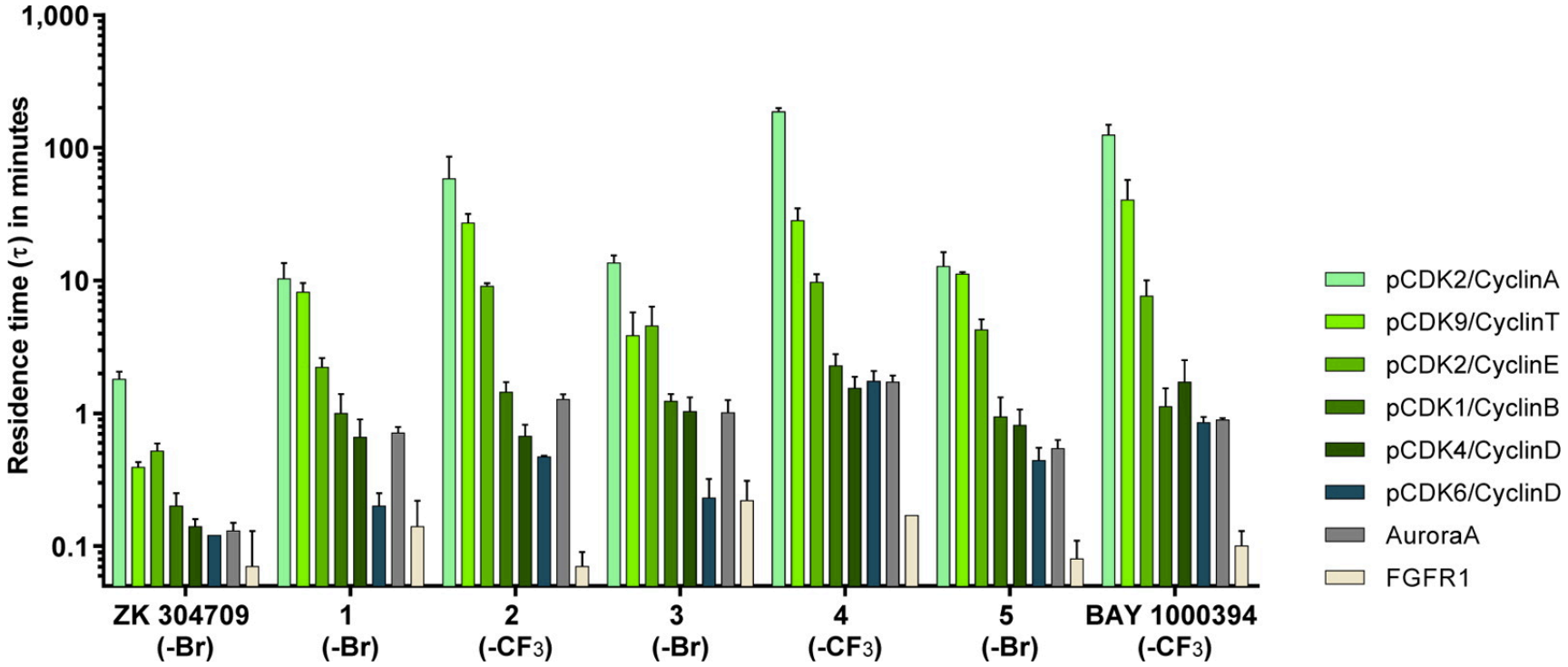
2
CDK2 IC_{50} = 5 nM
CDK1 IC_{50} = 5 nM
VEGF-R2 IC_{50} = 71 nM
CA-II IC_{50} > 10 μM
MCF7 IC_{50} = 26 nM

Compound	1	2
$AUC_{0\text{-tlast}}$ (mg·h·l^{-1})	0.654	0.219
$AUC_{0\text{-tlast,norm}}$ (kg·h·l^{-1})	0.082	0.044
AUC_u (mg·h·l^{-1})	0.144	0.057
$AUC_{norm,u}$ (kg·h·l^{-1})	0.018	0.011
$C_{max,u}$ (nM)	232	69
IC_{50u} (nM)	14.7	9.2
$C_{max,u}$ / IC_{50u}	15.7	7.5

Unbound plasma levels at therapeutic doses vs antiproliferative IC_{50u} of **1** and **2** in mice.

Structure-kinetic relationship study

- Binding kinetics of ZK 304709, compounds **1–5**, and roniciclib (BAY 1000394) on cyclin-activated CDKs in surface plasmon resonance (SPR) spectroscopy experiments

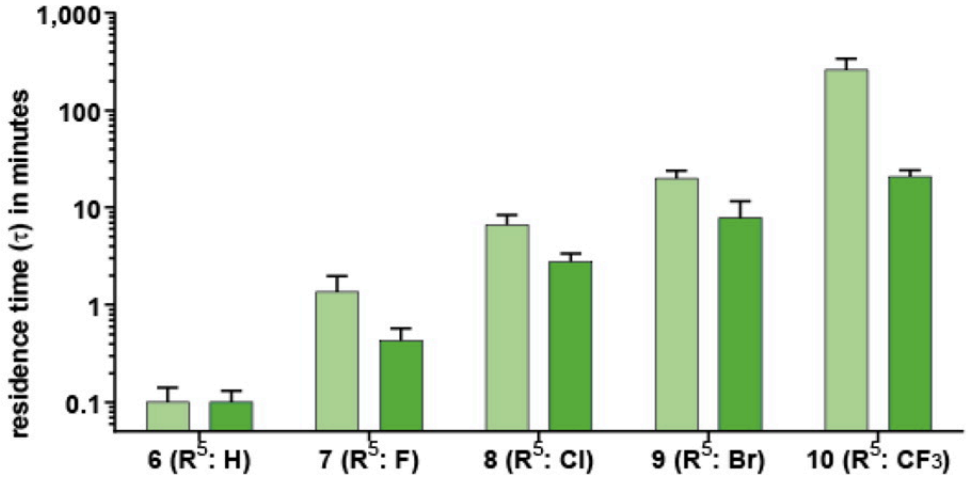


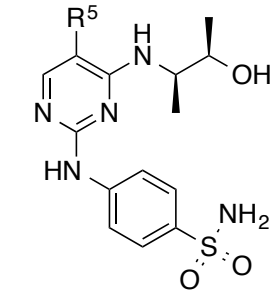
- Comparison of the residence times (τ) of ZK 304709, compounds **1–5**, and roniciclib (BAY 1000394). The 5-CF_3 derivatives have longer residence times than their 5-Br analogues. Residence times were calculated from SPR experiments at 25 °C.

8

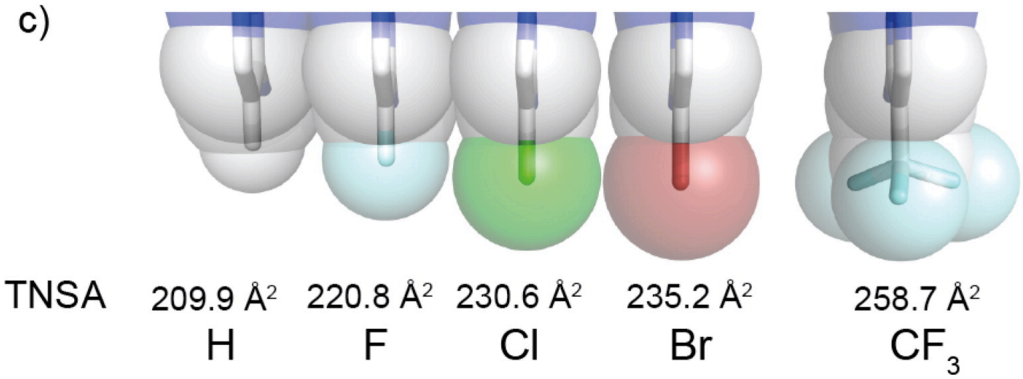
Effect of 5-halogen substituents

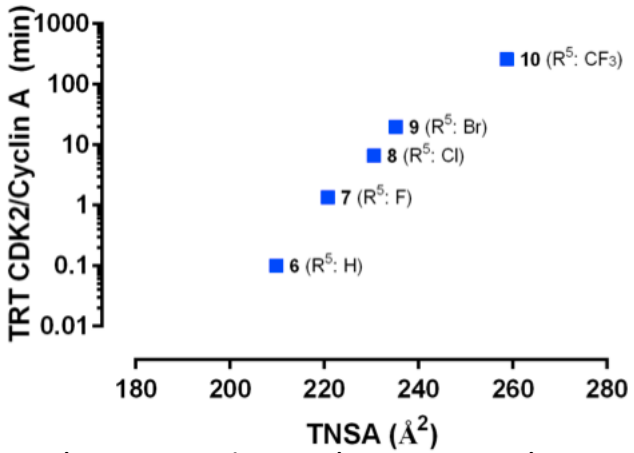
- Upon variation of 5-H to 5-Br and to 5-CF_3 substitution, an increase in the residence time of 3 orders of magnitude was observed.
- Stepwise increase in the residence time within the halogen series of F, Cl, Br modifications at this position.





6 R^5 = H
7 R^5 = F
8 R^5 = Cl
9 R^5 = Br
10 R^5 = CF_3



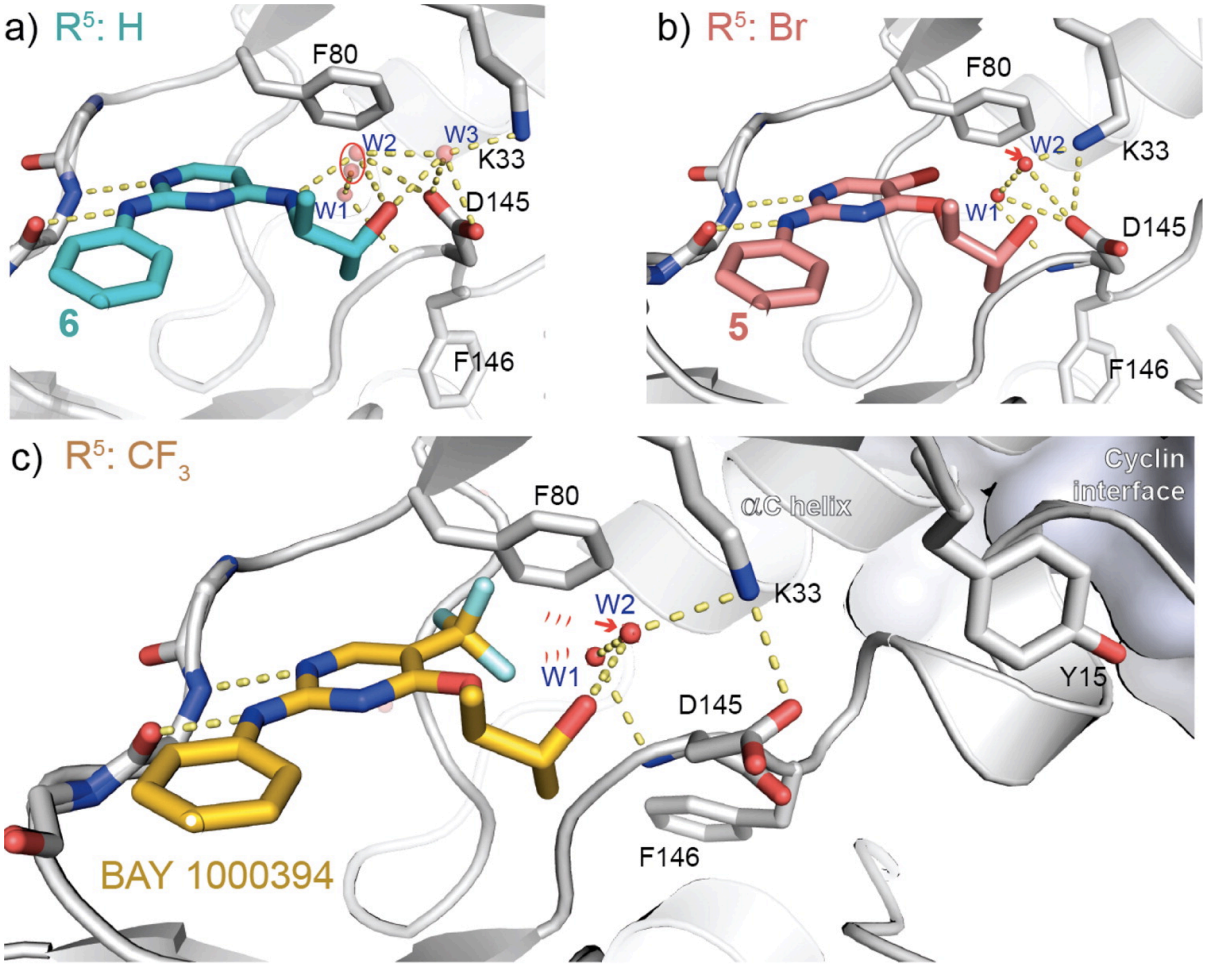


Display of van der Waals (vdW) volumes of the substituents at the aminopyridine 5-position with corresponding topological non-polar surface areas (TNSA)

9

Structure and thermodynamic characterization

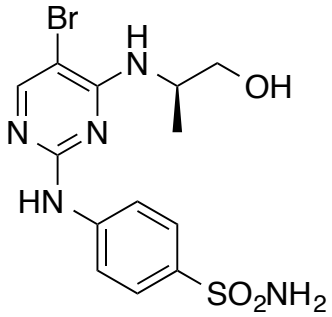
- X-ray co-crystal structures of apo-CDK2 with **6** (5-H), **5** (5-Br), and roniciclib (5-CF_3) were solved.



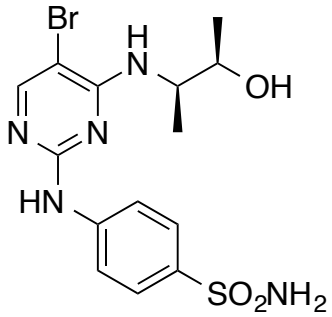
- halogen-water bridge interaction
- Interacts with water molecules
: coordinated by the phenyl ring of the gatekeeper F80 residues and side chain of the catalytic lysine residue K33.

- The increased residence time of 5-CF_3 analogs cannot be attributed to an increased halogen bond energy
- Large vdW volume of the CF_3 group cause a repulsive effect on the W1 and W2 water molecules, resulting in a distal relocation of these toward K33 -> flipping the ε-amino group of K33
- K33 maintains its salt bridge to D145 and thereby induces a flip of D145 that in parallel relocates F146.
- A displacement of Y15 of the β1−β2 loop in the vicinity of the αC helix and cyclin interface.

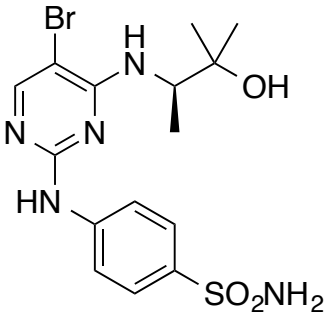
4-position side chain derivatives: different binding kinetics on CDK2



ZK 304709
CDK2 IC_{50} = 4 nM



9
CDK2 IC_{50} = 10 nM



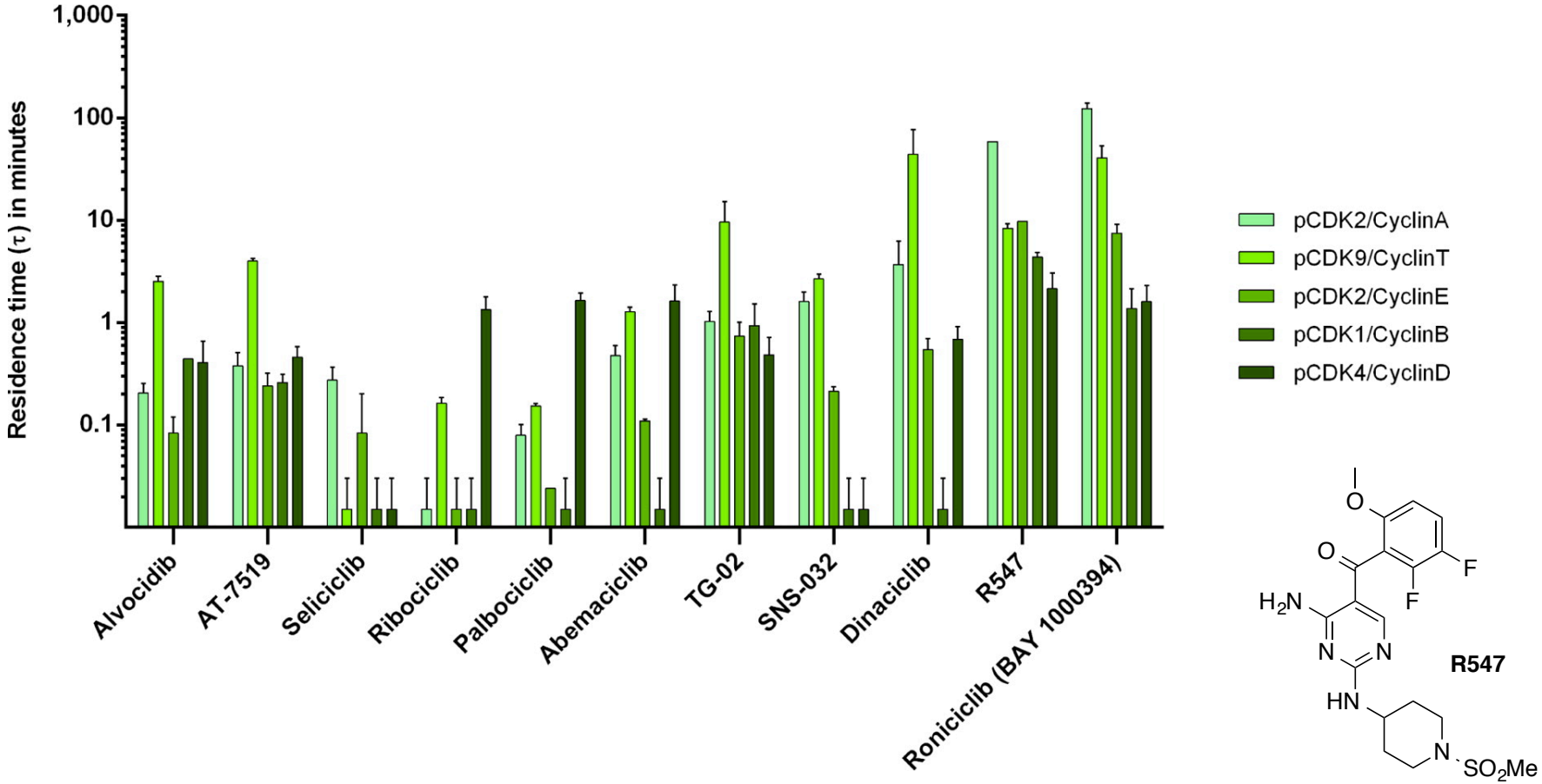
11
CDK2 IC_{50} = 13 nM

Binding kinetics of ZK 304709 and compounds 9 and 11

: *sec*- and *tert*-alcohol derivatives **9** and **11** exhibit approximately one order of magnitude lower association and dissociation rates than primary alcohol ZK 304709.

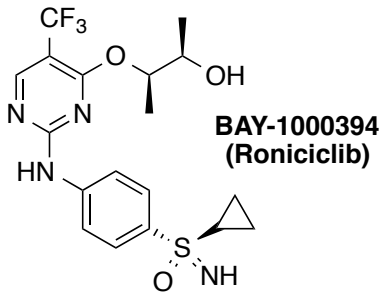
Compound	pCDK2/CyclinA		pCDK2/CyclinE		HeLa-MaTu
	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	Cell prolif. IC_{50} (M)
ZK 304709	1.47E+07	9.23E-03	1.89E+07	3.19E-02	4.75E-08
9	3.40E+06	8.41E-04	2.18E+06	2.13E-03	5.00E-09
11	4.91E+06	8.90E-04	3.78E+06	1.27E-03	5.00E-09

Comparison of the residence times (τ) of CDK inhibitors

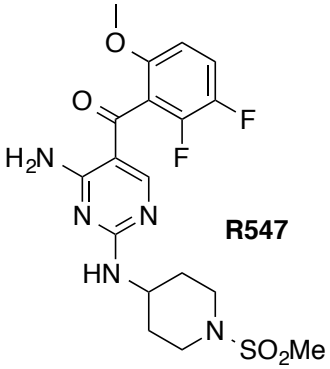


*Residence times were calculated from SPR experiments at 25 °C

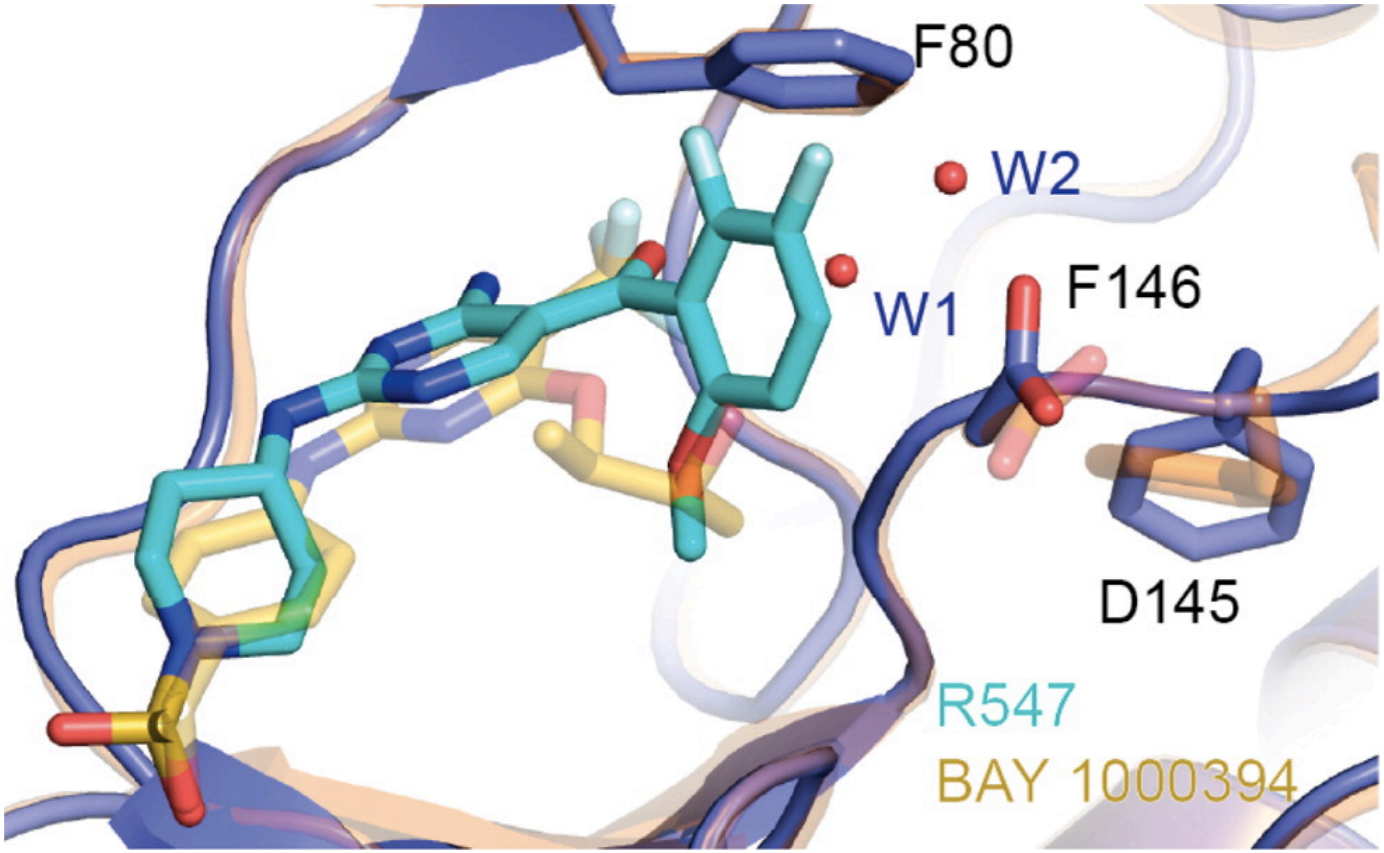
Comparison of the X-ray cocrystal structures: R547 vs Roniciclib



BAY-1000394 (Roniciclib)



R547

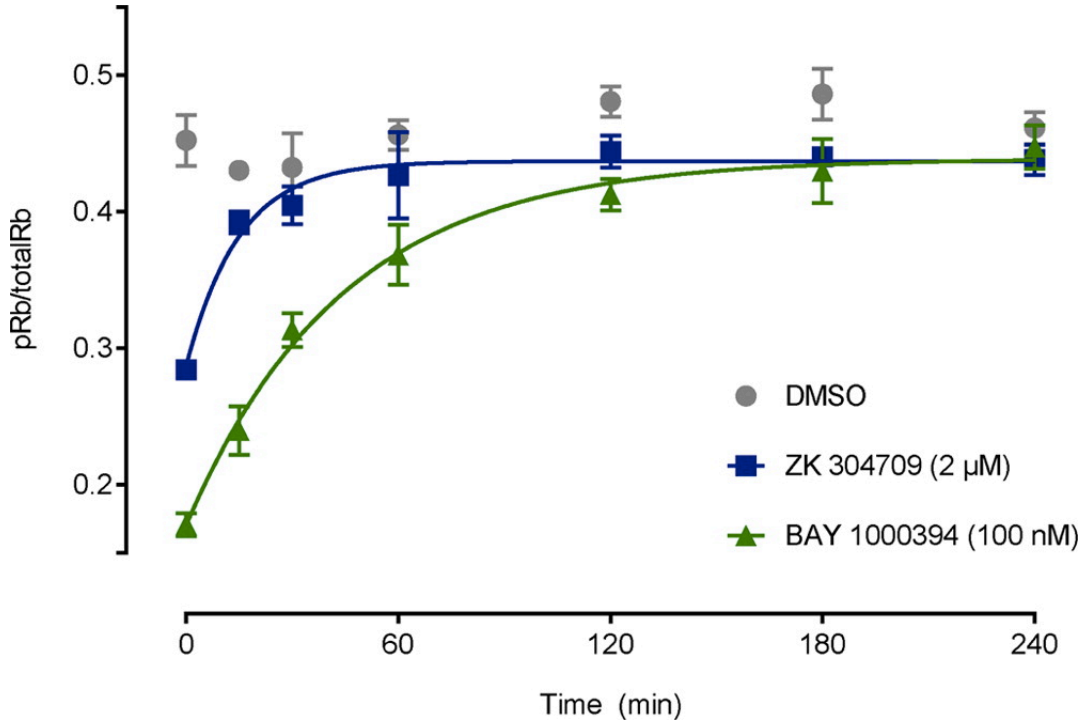


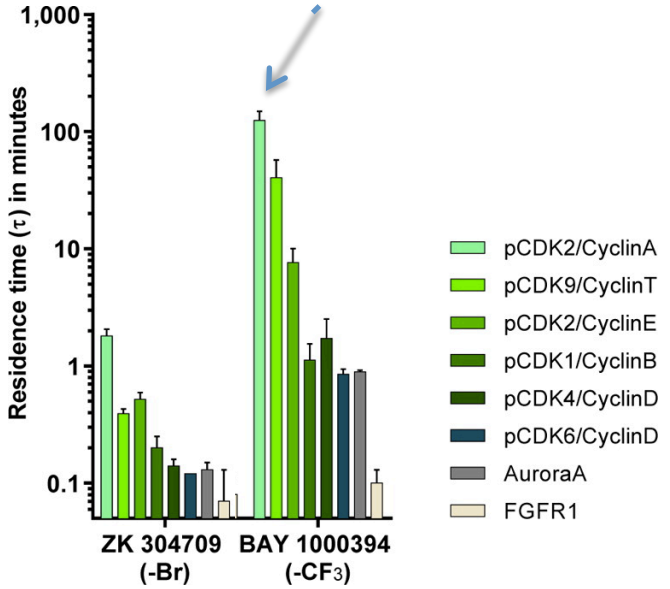
R547-CDK2 X-ray cocrystal structure (cyan, PDB ID: 2FVD) superimposed on the roniciclib (BAY 1000394)-CDK2 cocrystal structure (gold, present work, PDB ID: 5IEV). In both structures, the DFG motive adopts a similar position.

Cell-based phosphorylation recovery assay in HeLa-MaTu cells

- At the first data point after compound washout, retinoblastoma protein (RB) phosphorylation levels for ZK 304709 and roniciclib were 28% and 17%, respectively.
- Recovery to 80% pRB within the first 15 min for ZK 304709 & 60-120 min for roniciclib.

→ The prolonged pRB recovery half-time of roniciclib-treated cells of ~45 min is consistent with the in vitro residence time of roniciclib on pCDK2/cyclin A (123 min, arrow).





RB phosphorylation recovery in HeLa-MaTu cells after compound wash-out. HeLa-MaTu cells were treated with CDK inhibitors ZK 304709 and roniciclib (BAY 1000394) at at least 10-fold HeLa-MaTu antiproliferation IC_{50} concentrations. x axis, time after the removal of the inhibitor; y axis, ratio of pRB to total RB levels measured via ELISA. Data were fitted to a single exponential yielding rRB recovery time constants of t = 14.9 min for ZK 304709 and t = 44.3 min for BAY 1000394.

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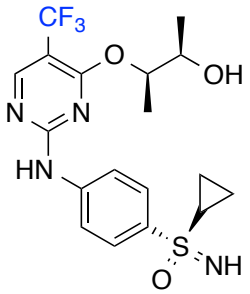
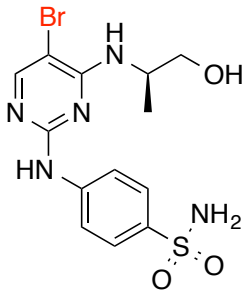
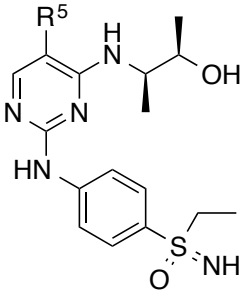
Summary



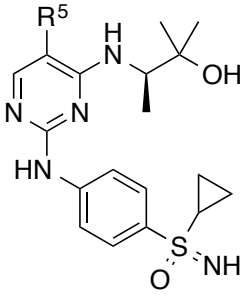
BAY-1000394 (Roniciclib)



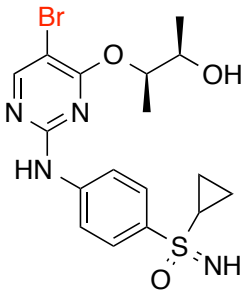
ZK-304709



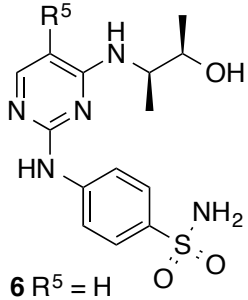
1 R^5 = Br
2 R^5 = CF_3



3 R^5 = Br
4 R^5 = CF_3



5



6 R^5 = H
7 R^5 = F
8 R^5 = Cl
9 R^5 = Br
10 R^5 = CF_3

- Roniciclib (BAY 1000394) is a type I pan-CDK (cyclin-dependent kinase) inhibitor which has revealed potent efficacy in xenograft cancer models.
- Introduction of the apolar CF_3 group to the aminopyrimidine scaffold induces a rearrangement of the hydration network, accompanied by a conformational adaption of the DFG motif based on the X-ray cocrystal structure.
- The link between DFG-loop adaption and increased residence time is further corroborated by the fact that an unrelated CDK inhibitor, R547, induces a similar DFG-loop adaption while displaying a comparable residence time on pCDK2/cyclin A.
- The in vitro measured target residence times of roniciclib are reflected in a cell-based mechanistic assay (the sustained inhibibition of RB phosphorylation in HeLa-MaTu cells).

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