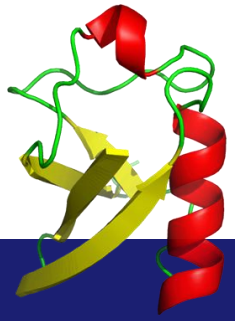
PROTEIN DYNAMICS



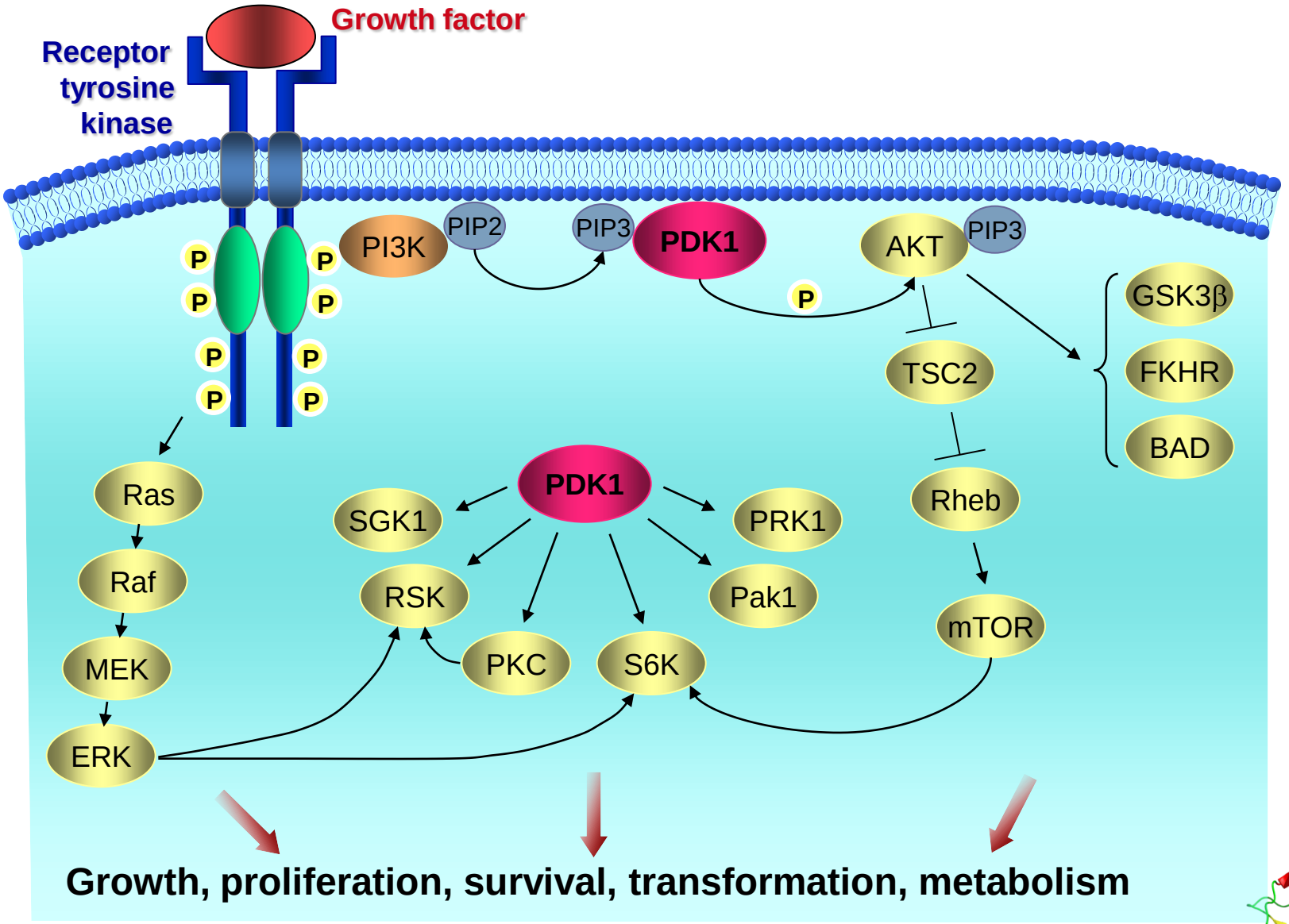
GlaxoSmithKline Oncology

FBLD: A PDK1 Case Study

Jeff Axten

GlaxoSmithKline
Oncology Research
Protein Dynamics DPU
Collegeville, PA 19426

PDK1 Signaling



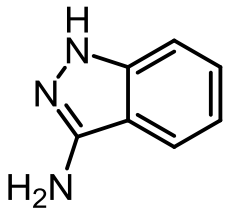
PROTEIN DYNAMICS

PDK1 Fragment Screen

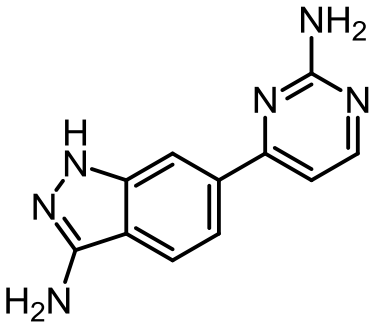
Kinase ATP-site Directed Fragment Set

PDK1 biochemical assay screen

NMR filter

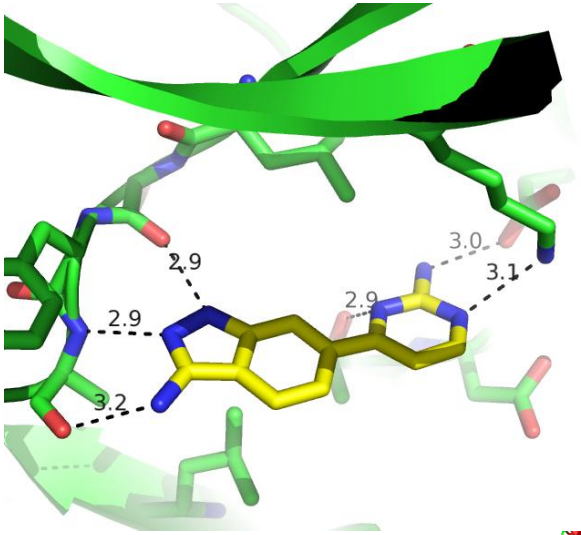


Follow-up screening



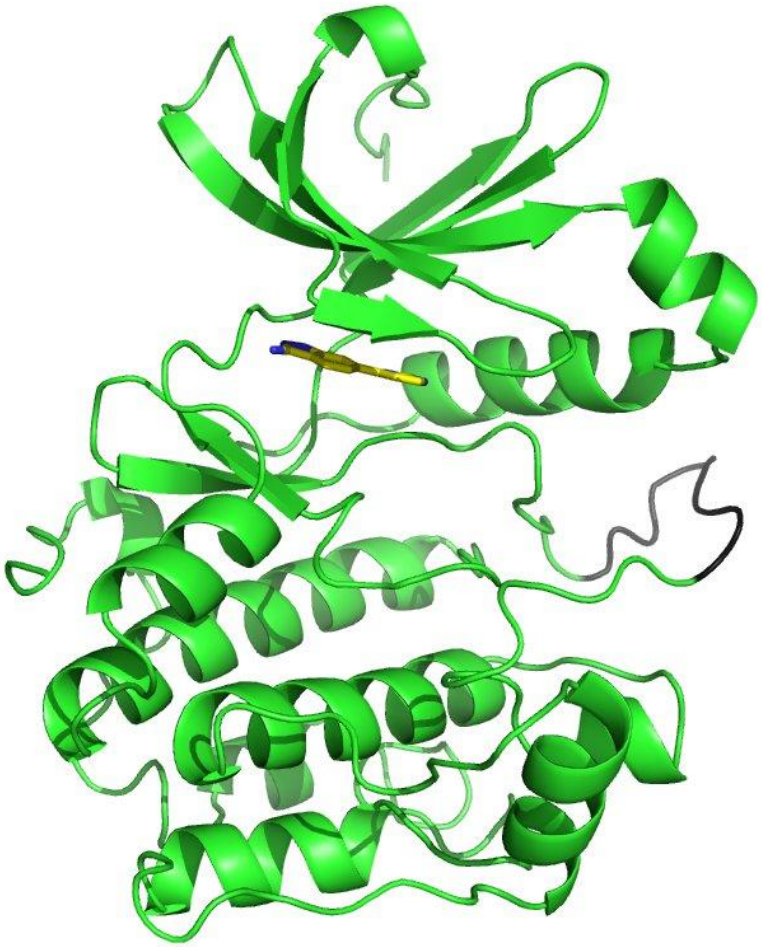
PDK1 pIC_{50} = 3.51 (311 μM)
LE = 0.48

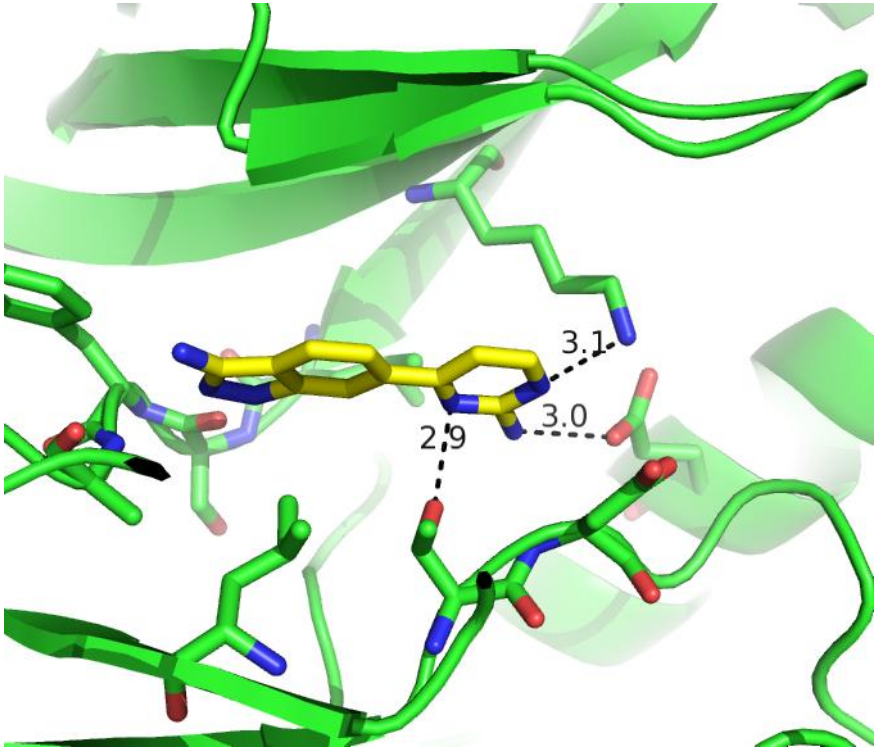
PDK1 pIC_{50} = 6.43 (0.37 uM)
LE = 0.52



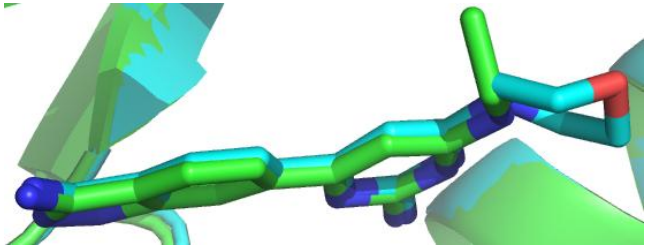
Medina, J.R. *et. al*. *ACS Med. Chem. Letters* **2010**, *July 22nd ASAP*

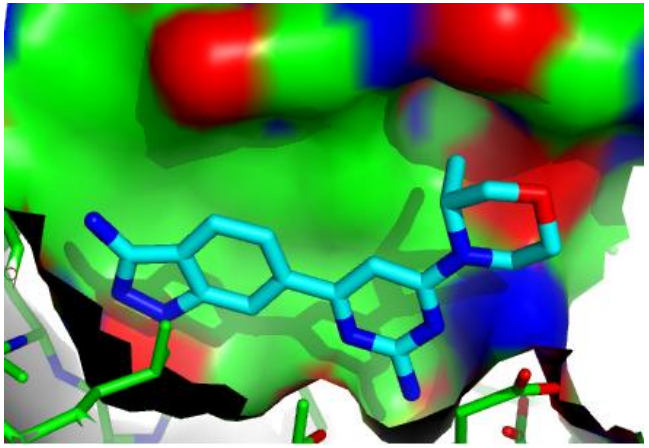
PROTEIN DYNAMICS

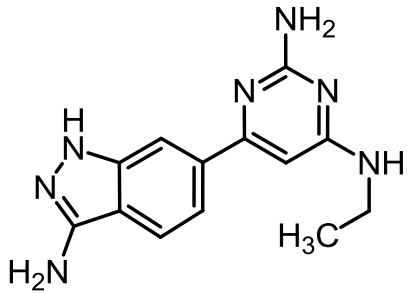


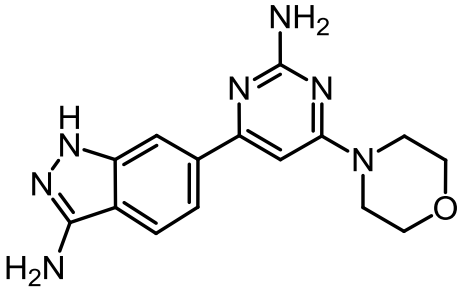


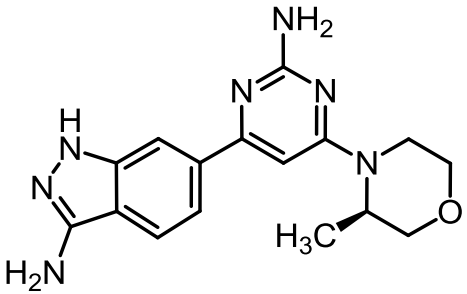
PROTEIN DYNAMICS



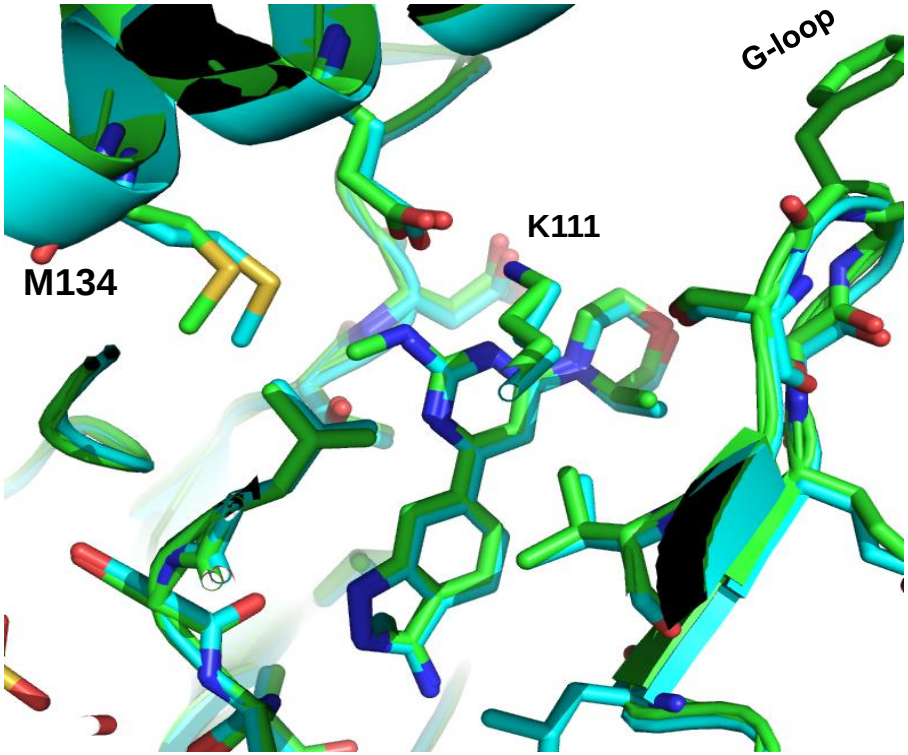


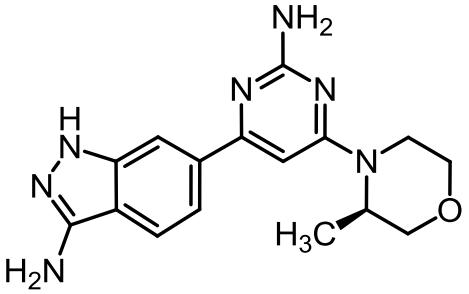




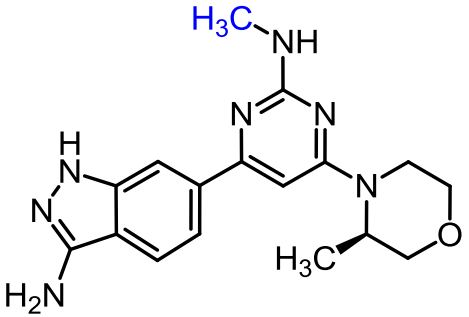


pIC_{50}			
PDK1 (LE)	**6.81 (0.47)**	**6.45 (0.38)**	**7.21 (0.41)**
ALK5	6.3	<5	<5
AurA/B	5.6/5.0	<5/4.7	<5/4.7
IKK1	5.3	<4.6	<4.6
ROCK	<5	<5	<5





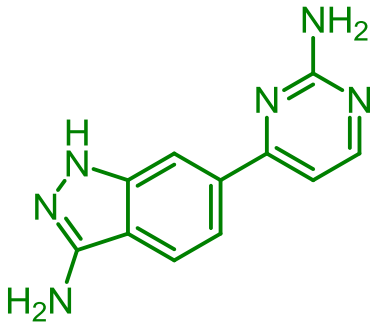
PDK1 pIC_{50} = 7.21 (LE = 0.40)

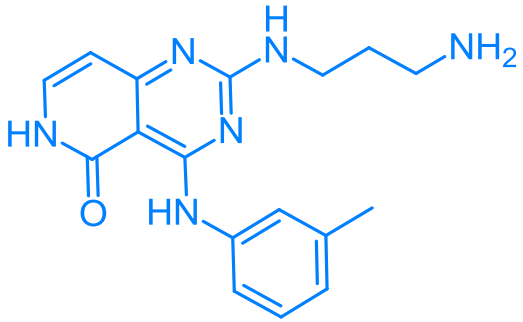


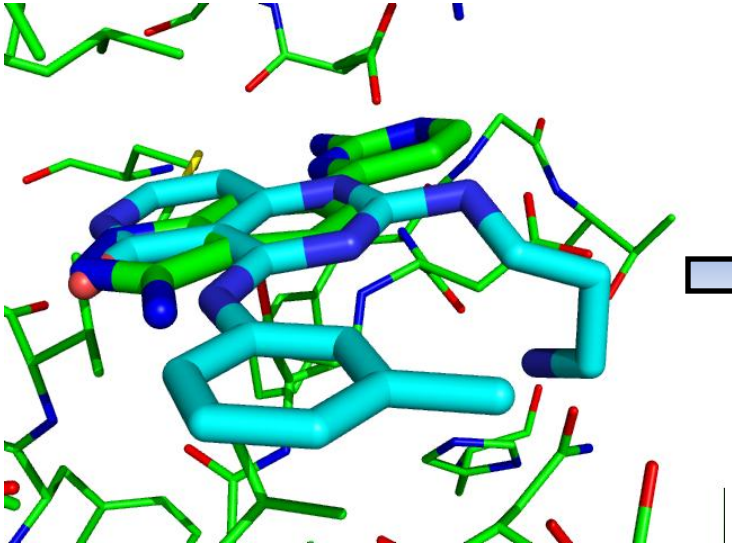
PDK1 pIC_{50} = 6.97 (LE = 0.38)

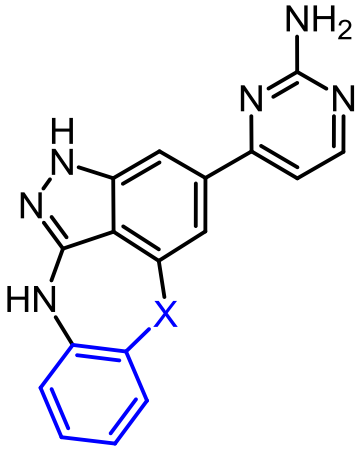
- M134 shows significant shift with added methyl group
- Larger groups not tolerated
- All other significant residues similar

PROTEIN DYNAMICS

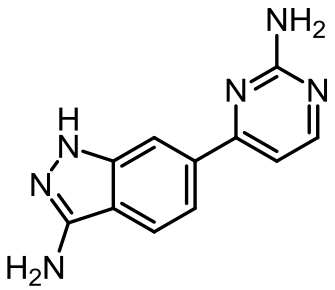


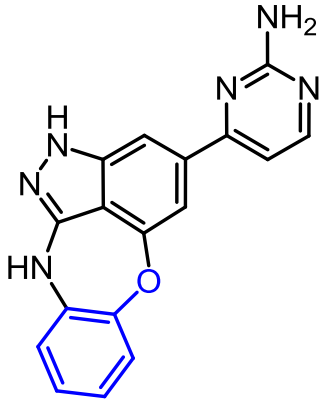


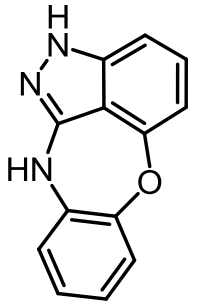




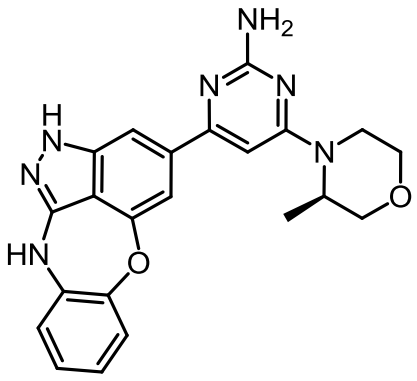
- Designed for potency
- Novel ring system

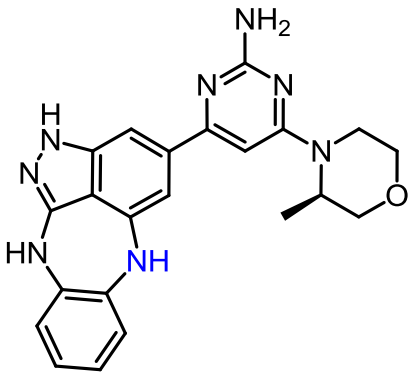


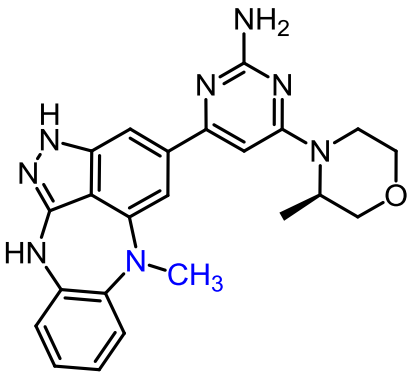




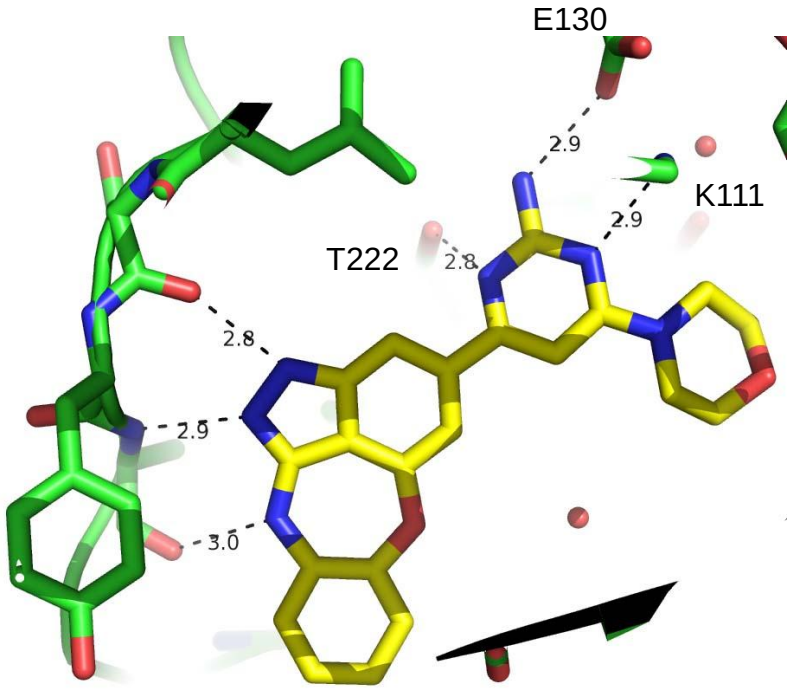
PDK1 pIC_{50} (LE)	**6.43 (0.52)**	**8.54 (0.49)**	**5.81 (0.48)**
ALK5 pIC_{50}	5.7	7.6	6.7
AurA/B pIC_{50}	6.3/5.4	8.3/7.6	7.3/7.7
ROCKI pIC_{50}	5.9	6.7	< 5

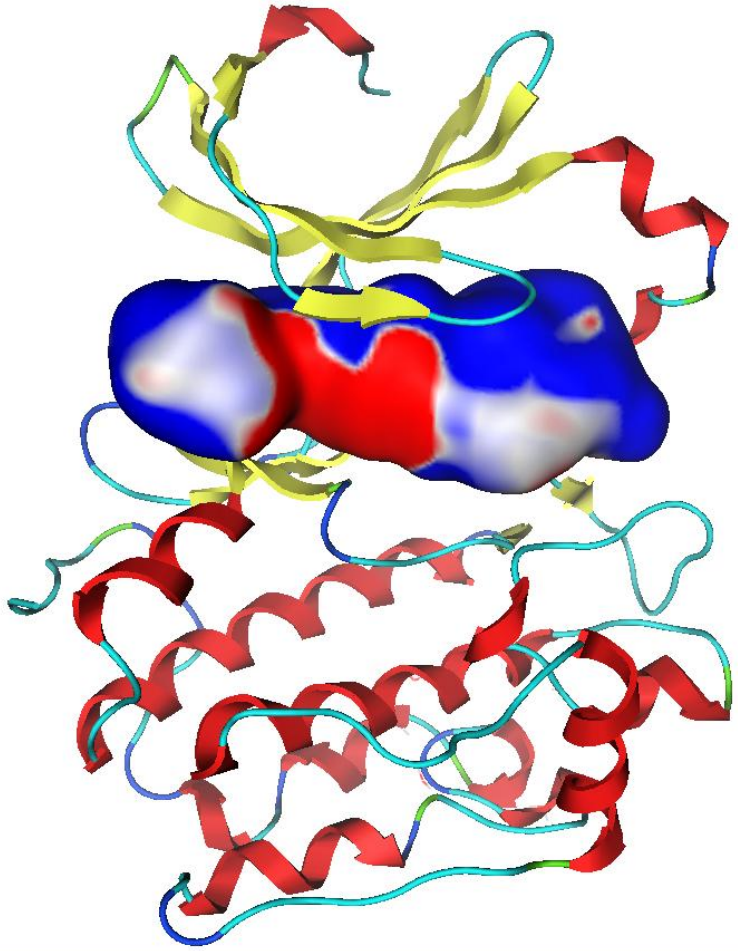




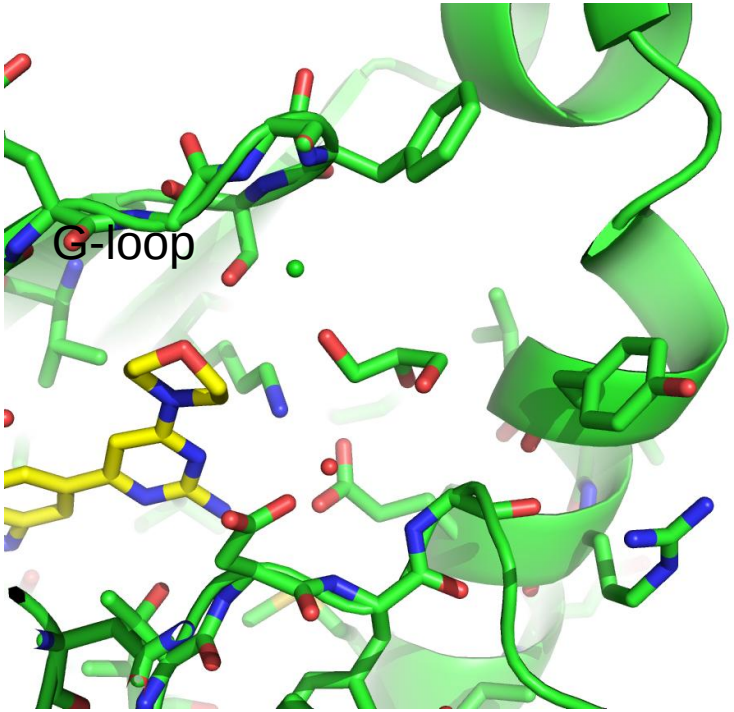


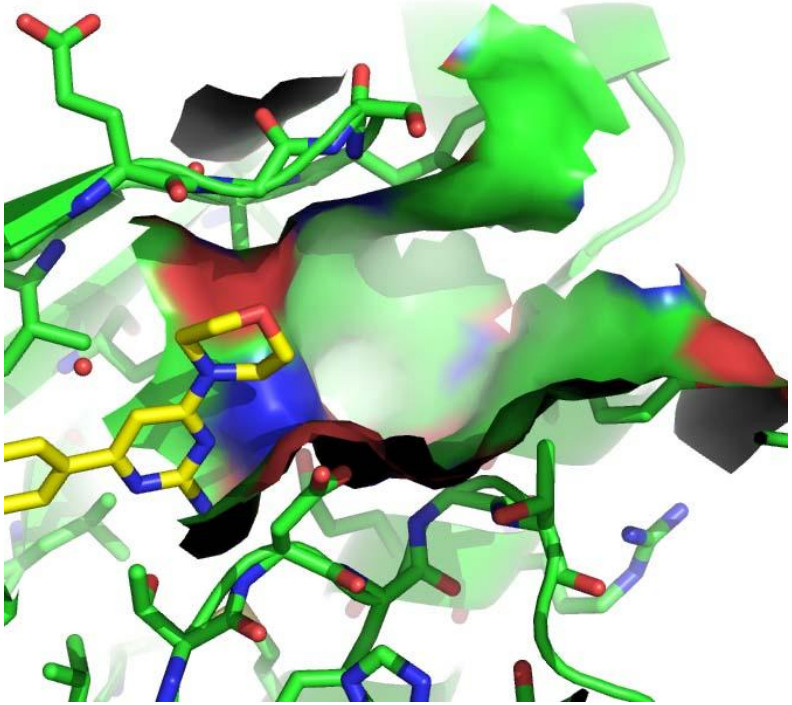
PDK1 pIC_{50} (LE)	**8.93 (0.39)**	**9.33 (0.41)**	**9.18 (0.39)**
ALK5 pIC_{50}	6.0	6.4	6.2
AurA/B pIC_{50}	7.3/6.8	8.4/7.7	7.4/7.0
ROCKI pIC_{50}	5.2	6.0	5.6
Cell data (PC-3)			
pAKT(T308) IC_{50}	263 nM	23 nM	182 nM
pAKT(S473) IC_{50}	>29,300 nM	>7,331 nM	>29,300 nM
pRSK(S221) IC_{50}	1,022 nM	25 nM	132 nM



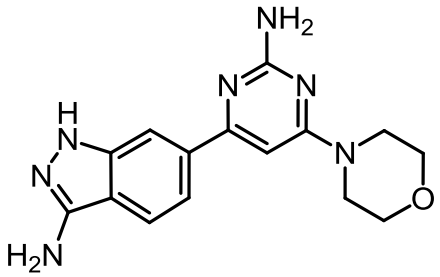


PROTEIN DYNAMICS

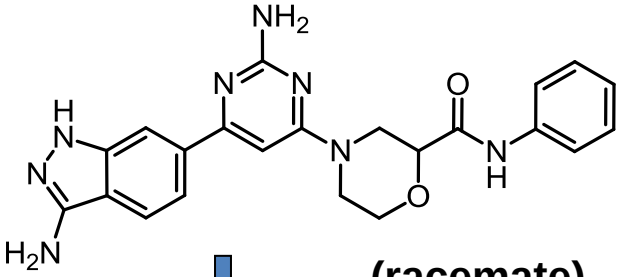




- Glycerol is added after the crystals have grown to allow freezing crystals for data collection
- Removing the glycerol/Chlorine and generating a surface shows a very deep pocket.



PDK1 pIC_{50} = 6.45 (LE = 0.38)



(racemate)

PDK1 pIC_{50} = 7.40 (LE = 0.32)

Cell Data (PC-3)

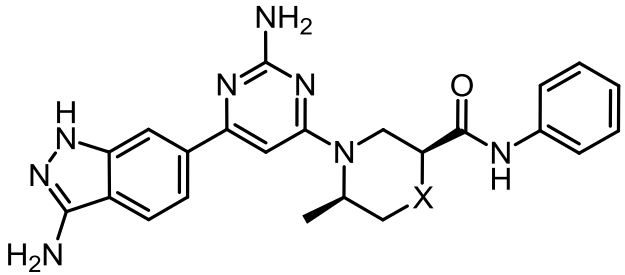
pAKT(T308) IC_{50}	1,617 nM
pRSK(S221) IC_{50}	2,684 nM

X=C

PDK1 pIC_{50} = 8.54 (LE = 0.35)

Cell Data (PC-3)

pAKT(T308) IC_{50}	327 nM
pRSK(S221) IC_{50}	333 nM

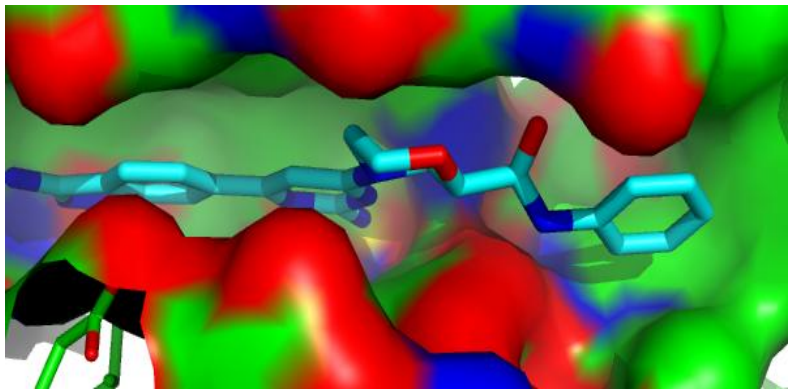


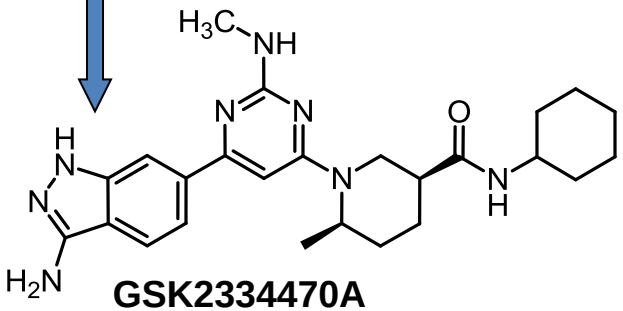
X=O

PDK1 pIC_{50} = 8.29 (LE = 0.34)

Cell Data (PC-3)

pAKT(T308) IC_{50}	264 nM
pRSK(S221) IC_{50}	594 nM



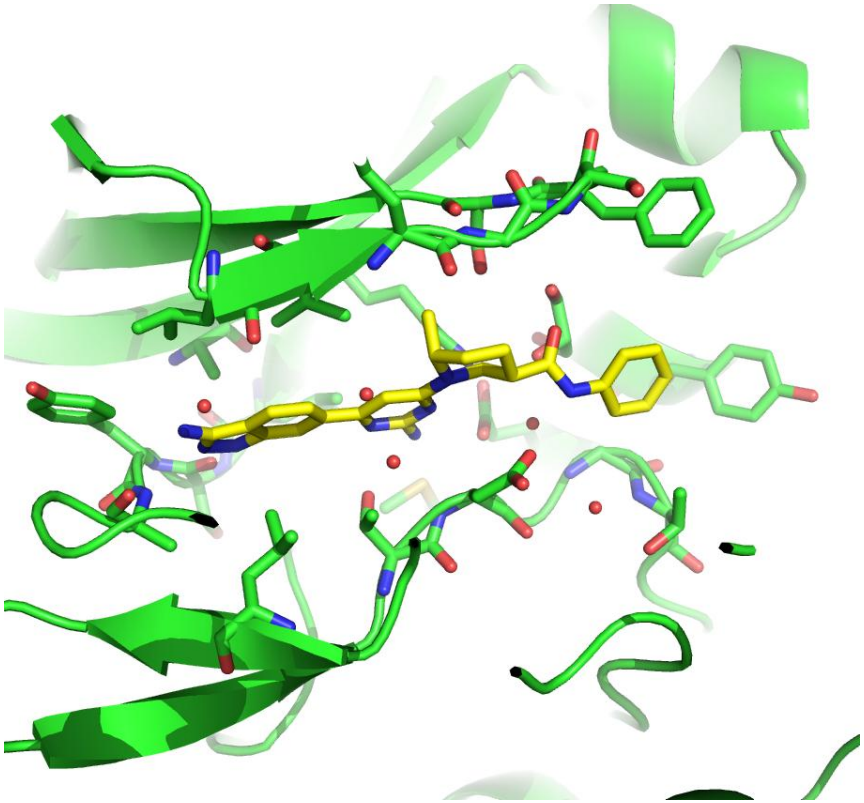


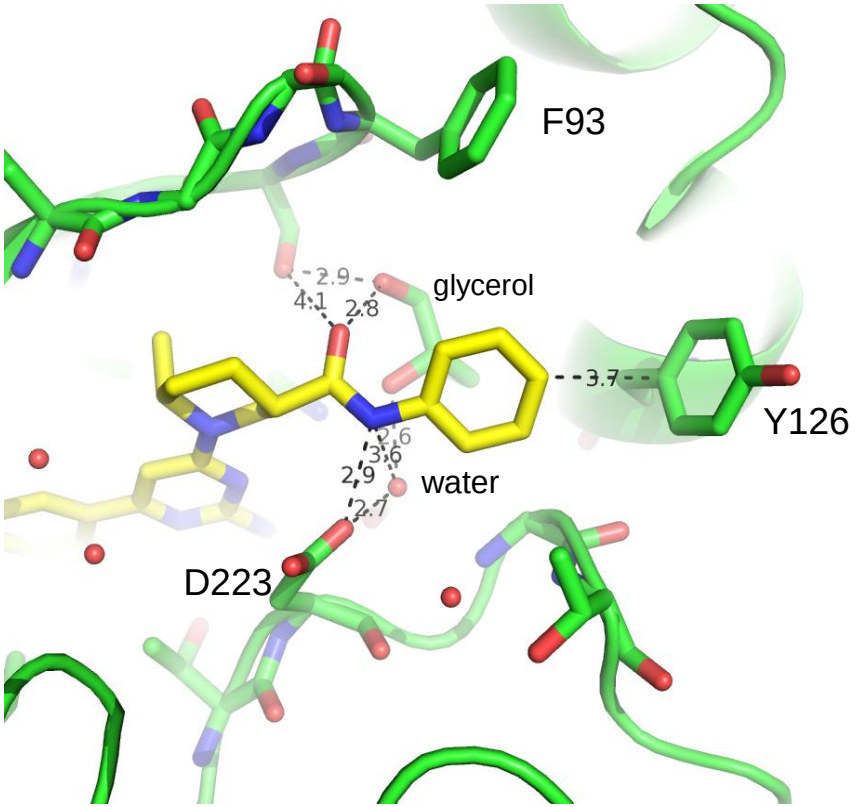
GSK2334470A

PDK1 PIC_{50} = 8.66 (LE = 0.35)

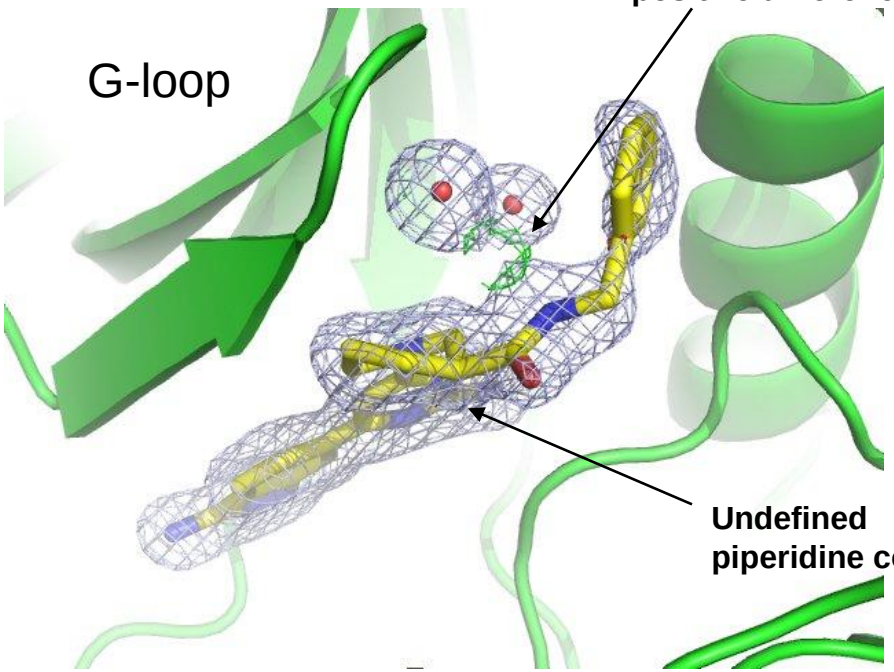
Cell Data (PC-3)

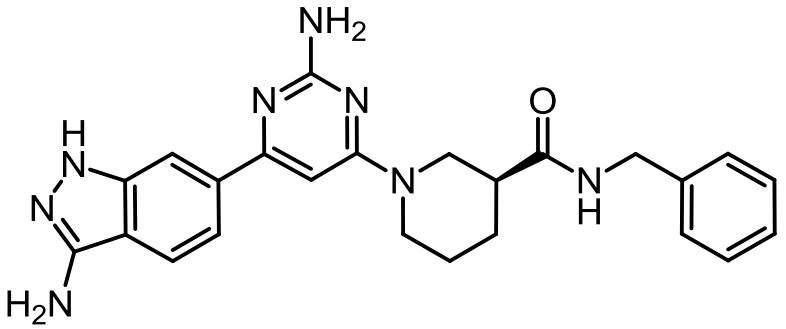
pAKT(T308) IC_{50}	113 nM
pRSK(S221) IC_{50}	293 nM





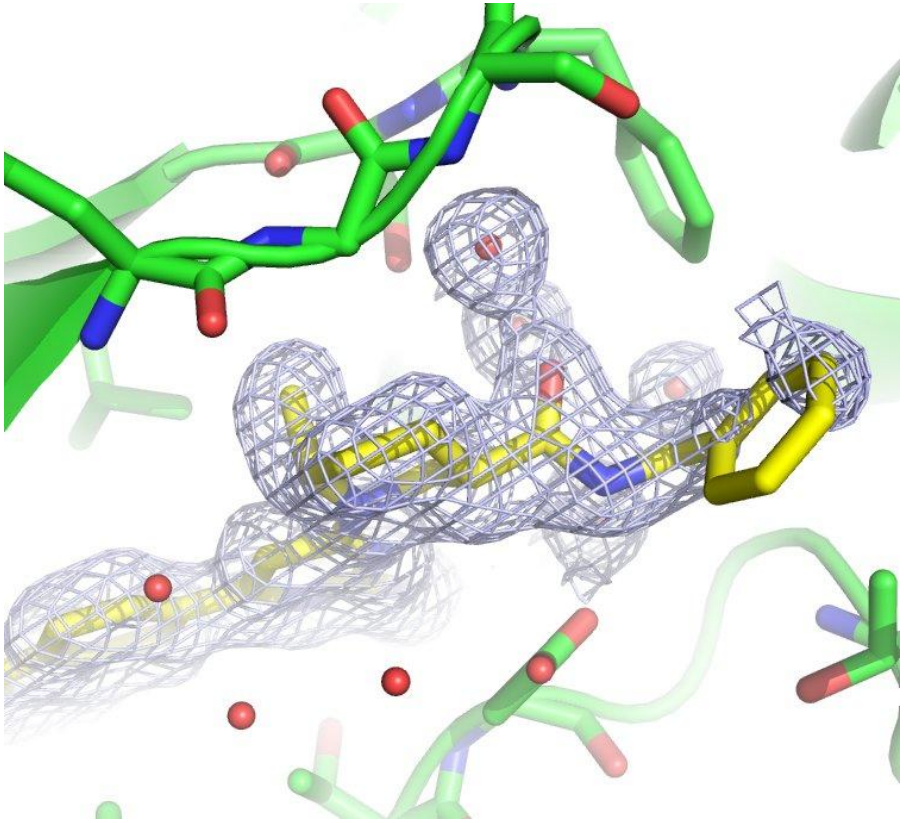
PROTEIN DYNAMICS

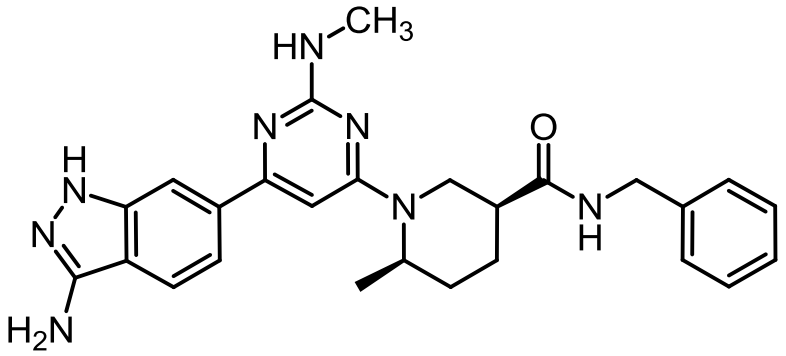




PDK1 pIC_{50} = 7.09 (LE = 0.29)

PROTEIN DYNAMICS





PDK1 PIC50 = 8.75 (LE = 0.34)

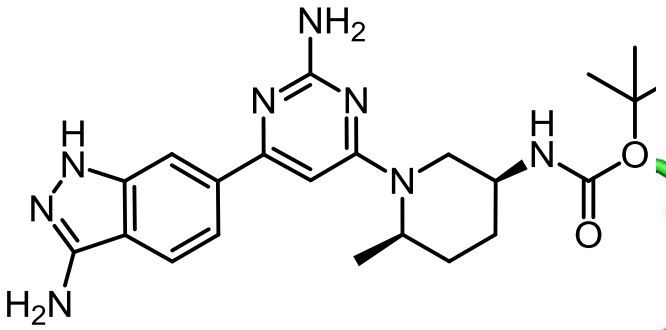
Cell Data (PC-3)

pAKT(T308) IC_{50} 92 nM

pRSK(S221) IC_{50} 107 nM

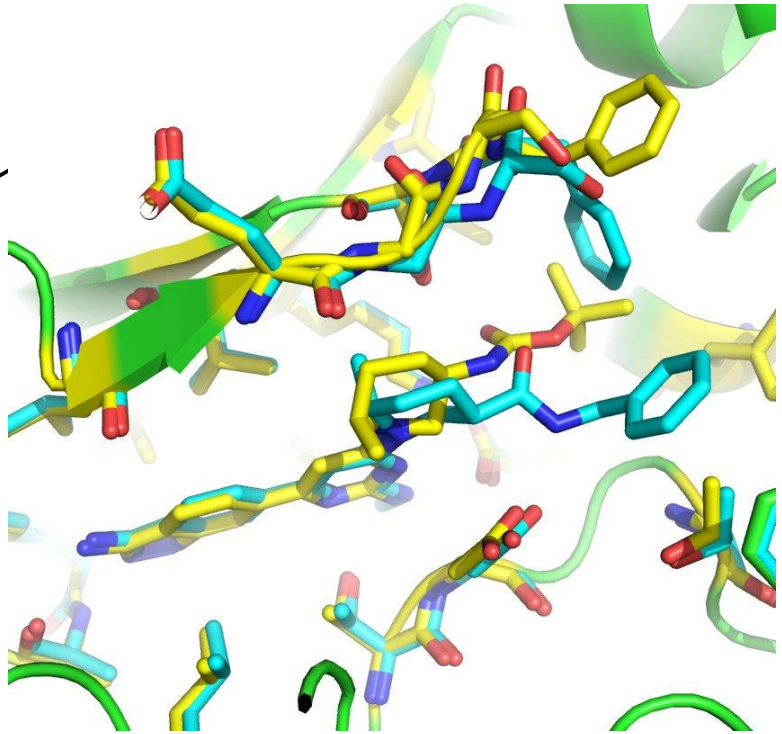
PROTEIN DYNAMICS

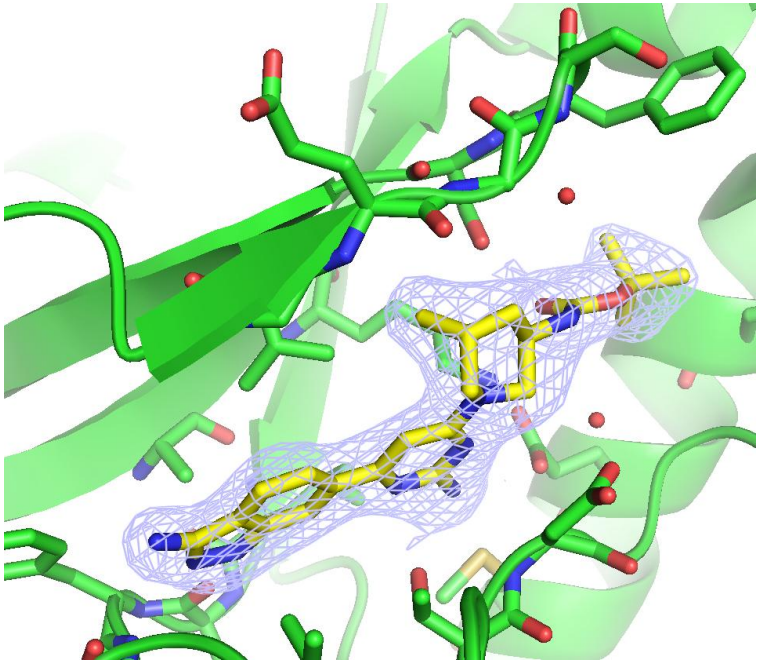
- Peculiar activity of racemate vs. “preferred” stereochemistry with carbamate linker

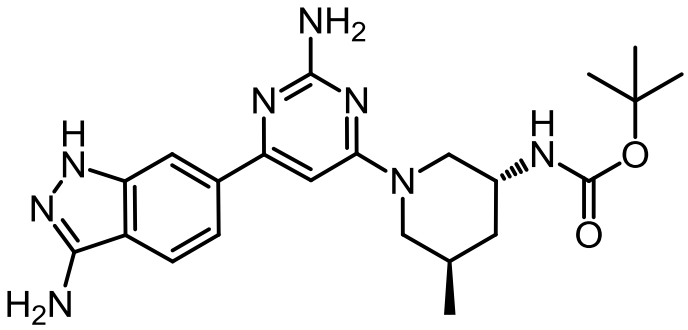


(cis-racemate)
PDK1 pIC_{50} = 7.75 (LE = 0.33)

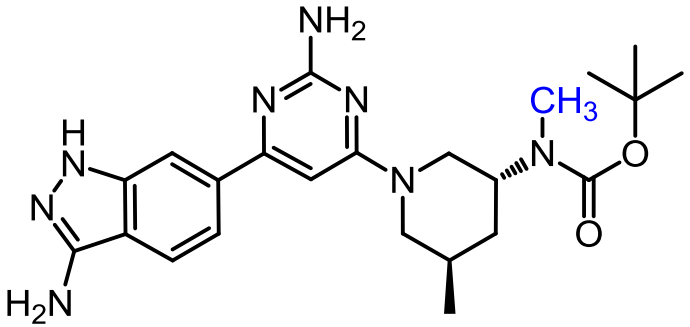
(3S, 6R)
PDK1 pIC_{50} = 6.24 (LE = 0.27)



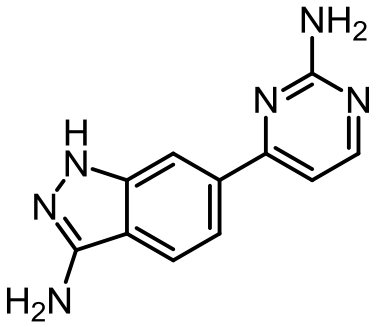




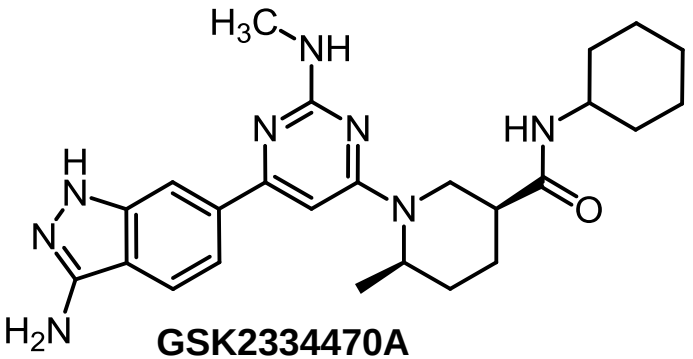
PDK1 pIC_{50} = 8.15 (LE = 0.35)
Cell Data (PC-3)
pAKT(T308) IC_{50} 74 nM
pRSK(S221) IC_{50} 151 nM



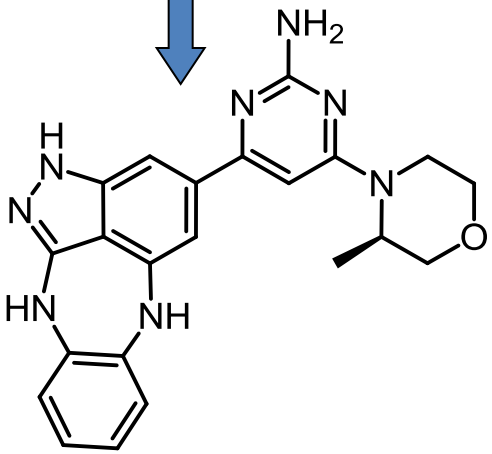
PDK1 pIC_{50} = 9.13 (LE = 0.38)
Cell Data (PC-3)
pAKT(T308) IC_{50} 9 nM
pRSK(S221) IC_{50} 19 nM



PDK1 pIC_{50} = 6.43 (LE = 0.52)
Aurora A/B pIC_{50} 6.4/5.5

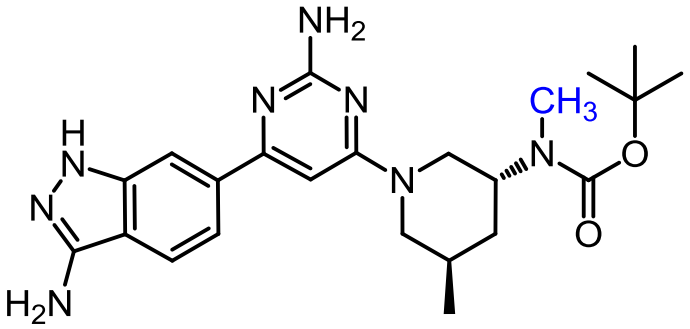


GSK2334470A
PDK1 PIC_{50} = 8.66 (LE = 0.35)
Aurora A/B pIC_{50} 4.4/5.5



PDK1 pIC_{50} 9.33 (LE = 0.41)
Aurora A/B pIC_{50} 8.4/7.7

Cell Data (PC-3)
pAKT(T308) IC_{50} 23 nM
pRSK(S221) IC_{50} 25 nM



PDK1 pIC_{50} = 9.13 (LE = 0.38)
Aurora A/B pIC_{50} 6.9/6.6

Cell Data (PC-3)
pAKT(T308) IC_{50} 9 nM
pRSK(S221) IC_{50} 19 nM

Acknowledgements

Seth Grant
Jesús Medina
Celine Duquenne
Mark Bobko
Bill Li
Art Shu
Dirk Heerding
Chris Becker
Chuck Blackledge
William Miller
Karen Evans
Leanna Shuster
Dai-Shi Su
Stuart Romeril
Daryl Scherzer
Yanhong Feng
Brian Dombroski
Phil DeYoung

Antony Chadderton
Lois Wright
Bruce Wisely
Derek Parks
Gary Smith
Edgar Wood
Tony Leesnitzer
Mike Schaber
Jeff Gross
Tony Choudhry
Nino Campobasso
Paris Ward
Pat Brady
Jacques Briand
Donald Huddler
Sridhar Rabindran
Christine Gardiner
Hong Xiang