SAR and structural biology of NEK kinase inhibitors

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16th SCI/RSC Medicinal Chemistry Symposium

CANCER RESEARCH UK

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The Royal Marsden NHS

NHS Foundation Trust

ICR The Institute of Cancer Research

ICR The Institute of Cancer Research

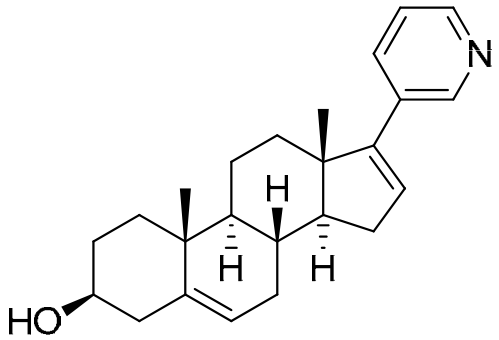
CRUK Cancer Therapeutics Unit

CRUK Cancer Therapeutics Unit

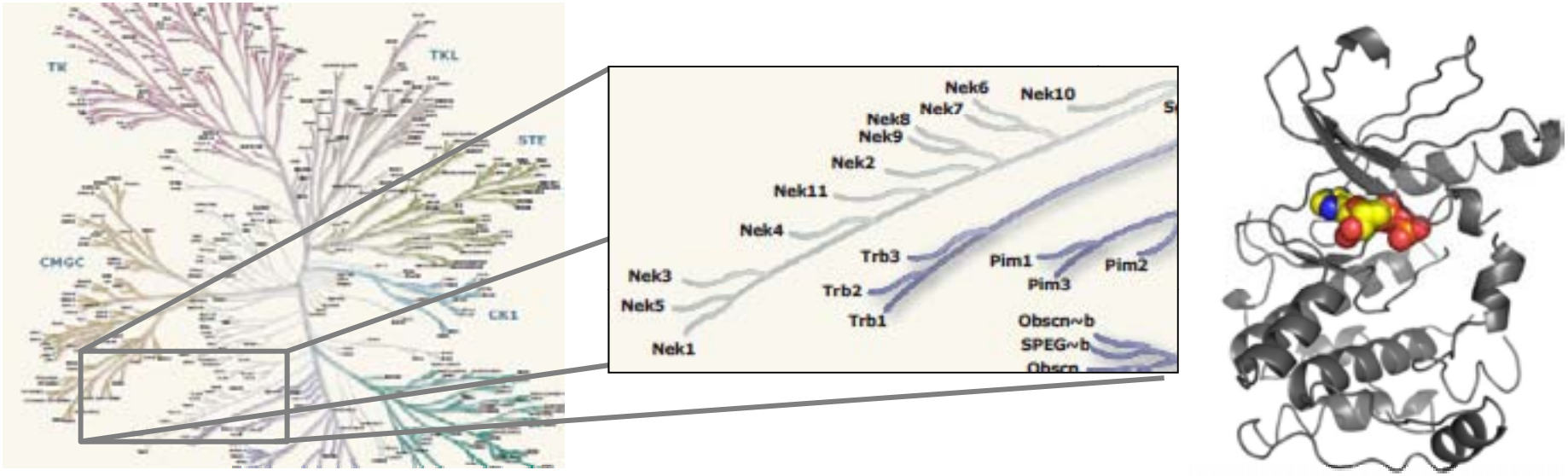
- 160 staff
- Focused on small molecule drug discovery
- Includes essential expertise and technologies (e.g. HTS, structural biology, pharmacology..)
- 40 chemists at the bench
- Currently 6 compounds in clinical trials

Abiraterone:

Approved by the FDA in April 2011 and by the EMEA in September 2011

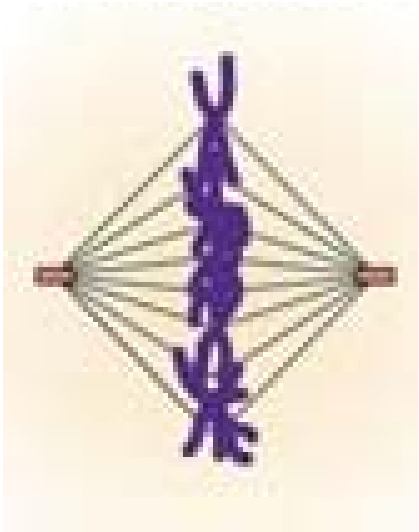


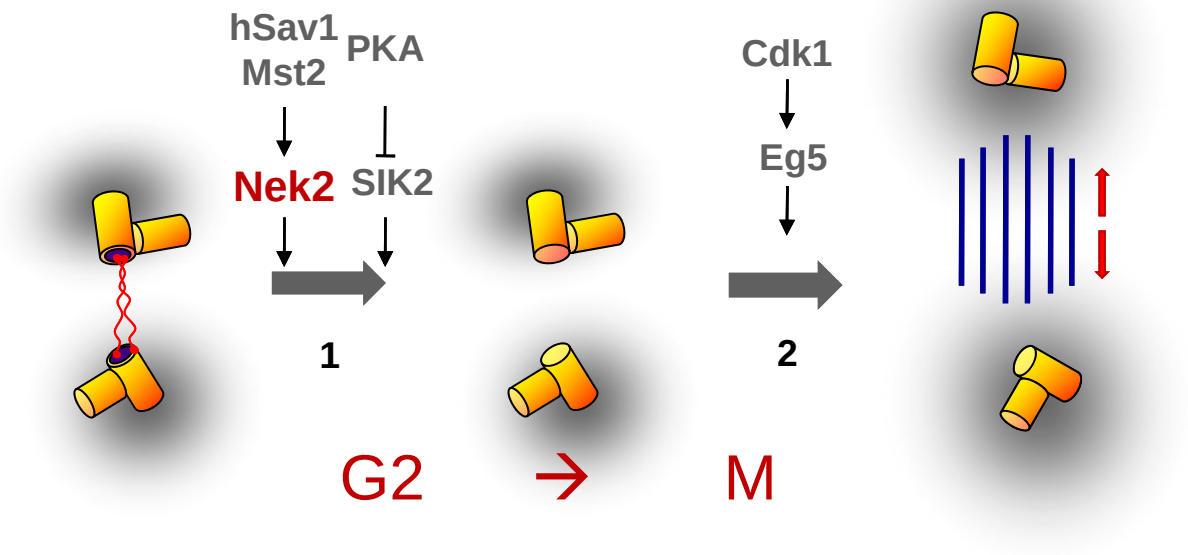
Nek2: Introduction



- Ser/Thr kinase, member of the Nek family
- Very few inhibitors of Nek kinases are known to date
- Nek2 is a cell cycle kinase that facilitates centrosome separation
- Numerous other Nek2 substrates have been reported but remain to be confirmed.

Nek2 and Centrosome Separation



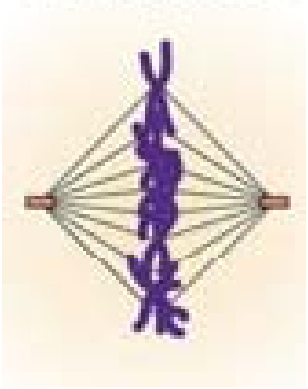


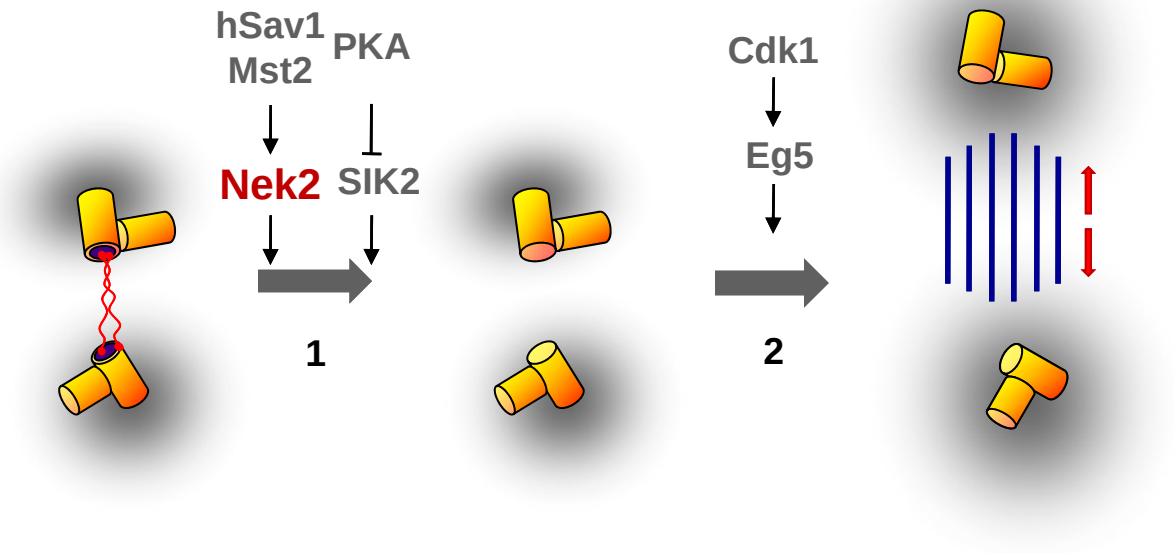
Nek2 facilitates centrosome seperation by phosphorylating C-Nap1, one component of the linker connecting the centrosomes

Nek2 and Cancer

- Shown to be over-expressed in cancer cell lines and tumour tissue
- Nek2 over-expression induces mitotic errors promoting aneuploidy
- RNAi knock down leads to growth inhibition and induction of apoptosis in cell lines *in vitro* and *in vivo*
- Somatic mutations have been reported for a few cell lines

Hypothesis: Inhibitors of Nek2 will be anti-mitotic with particular efficacy against cell lines / tumours with high Nek2 expression/activity.





Nek2 Project

Collaborative project:

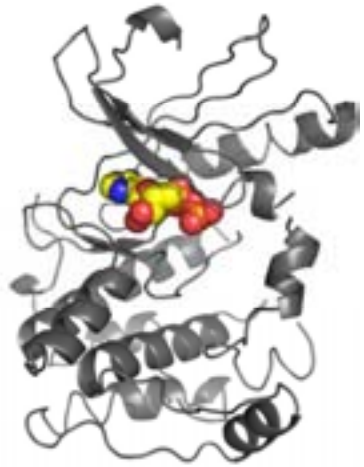
Roger Griffin, **Herbie Newell**, Newcastle University

Andrew Fry, University of Leicester

Richard Bayliss, University of Leicester

Wynne Aherne, **Swen Hoelder**,

ICR, CRUK Cancer Therapeutics Unit

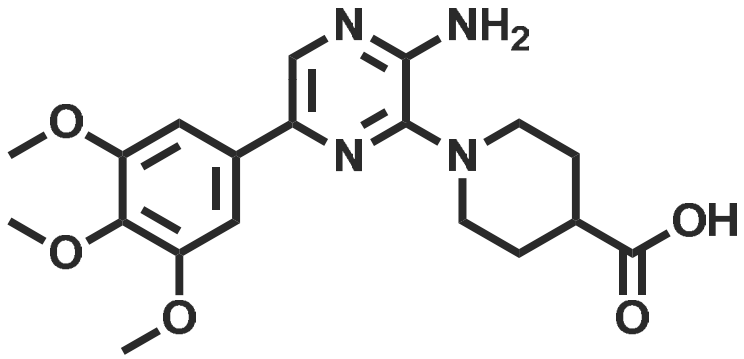


Aims:

- To discover potent and selective small molecule inhibitors of NEK2 as chemical probes.
- To investigate the role of Nek2 in tumour biology / mitosis and to identify patient populations that are likely to respond to Nek2 inhibitors.

Nek2 Screening

Screening of our in-house library gave a low hit rate but identified aminopyrazines as modestly potent inhibitors:

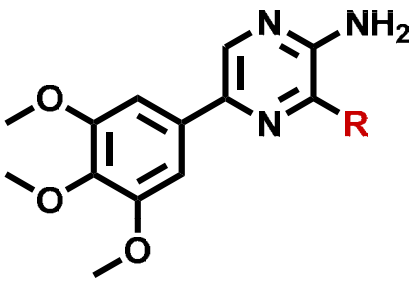


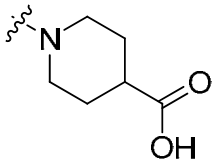
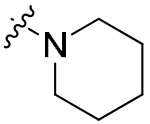
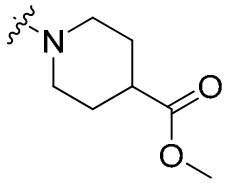
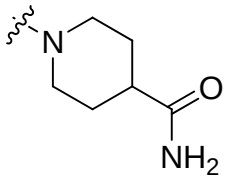
- **IC_{50}(Nek2):** 1µM
- **Ligand eff.:** 0.3 ($\Delta G/N(_{heavy\ atoms})$)
- **Stable in human and mouse microsomes**
- **Low permeability in PAMPA / CaCo-2**
- **PSA**:120Å^2
- **cLogP:** ~3

Aims for hit follow up

- Increase activity
- Lower polarity (PSA)

Pyrazine SAR

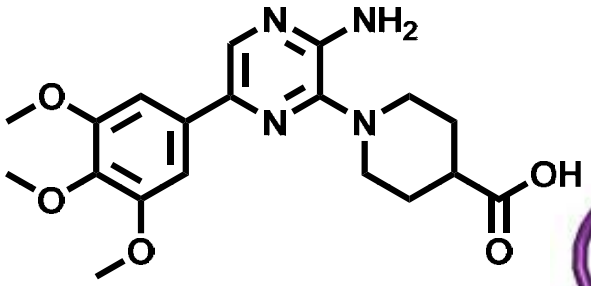


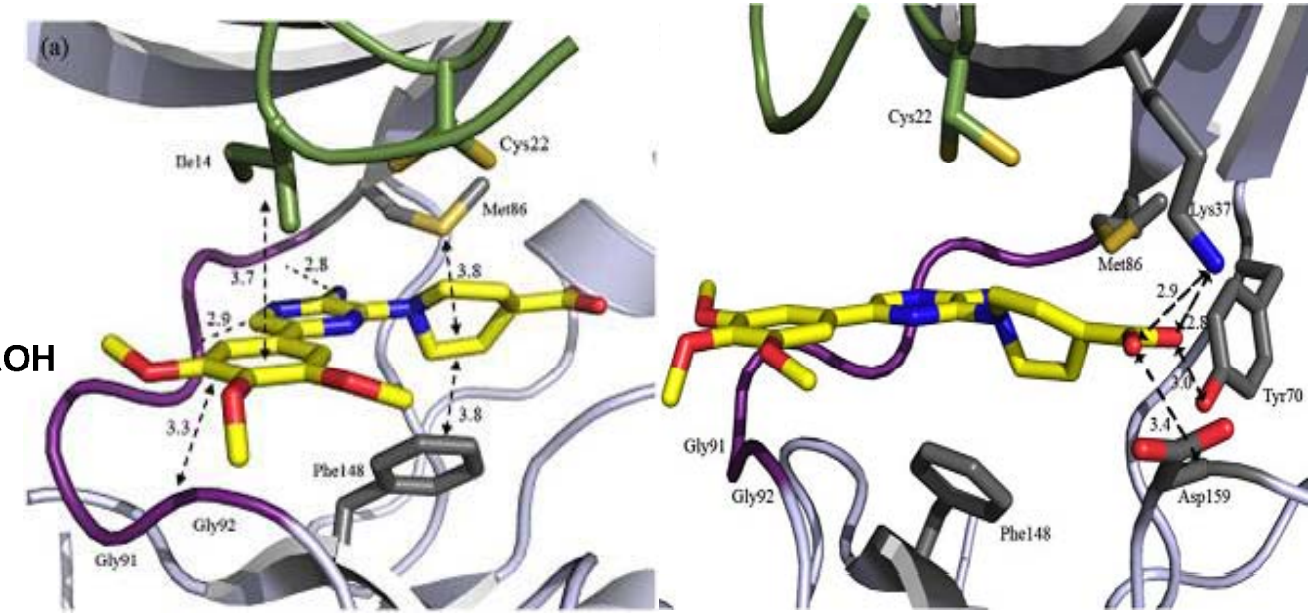
				
IC50 (μM)	**1**	**>50**	**18**	**6**
LE	**0.3**	**-**	**0.22**	**0.26**
tPSA	**120**	**83**	**110**	**126**

- Several compounds in this series were prepared but most proved to be significantly less active
- Very few compounds showed comparable activity

→ **Steep SAR**

Aminopyrazine crystal structure

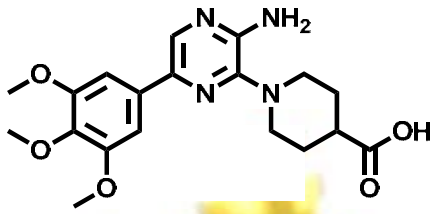


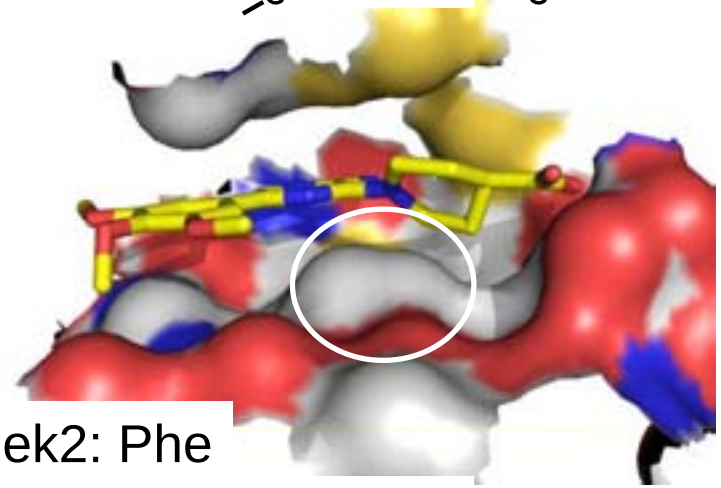


- Aminopyrazine engages in 2 hydrogen bonds with the hinge region.
- CO_2H group involved in hydrogen bond network in the back pocket.

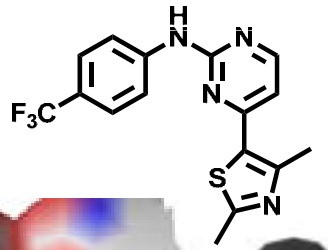
Whelligan DK, Solanki S, Taylor D, Thomson DW, Cheung KM, Boxall K, Mas-Droux C, Barillari C, Burns S, Grummitt CG, et al. (2010). J Med Chem 53, 7682-7698.

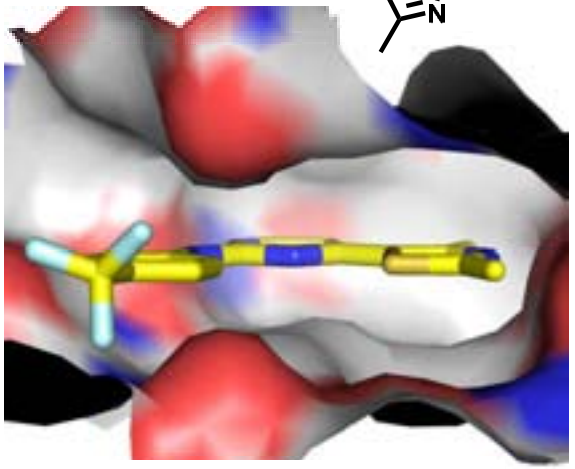
The effect of Phe148 on inhibitor design





Nek2: Phe





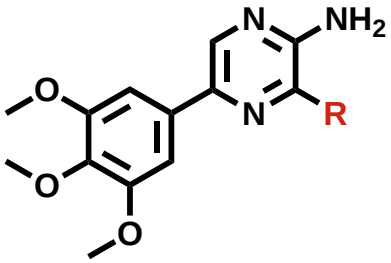
CDK2: Leu (1PXL)

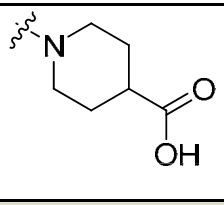
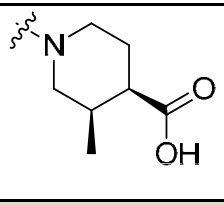
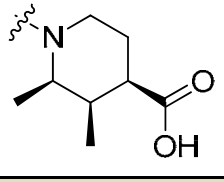
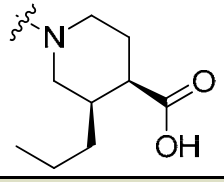
Phe148:

- Rare AA at this position (usually leucine)
- Most likely interferes with binding of many typical kinase scaffolds and made optimisation of aminopyrazine series challenging.

Whelligan DK, Solanki S, Taylor D, Thomson DW, Cheung KM, Boxall K, Mas-Droux C, Barillari C, Burns S, Grummitt CG, et al. (2010). J Med Chem 53, 7682-7698.

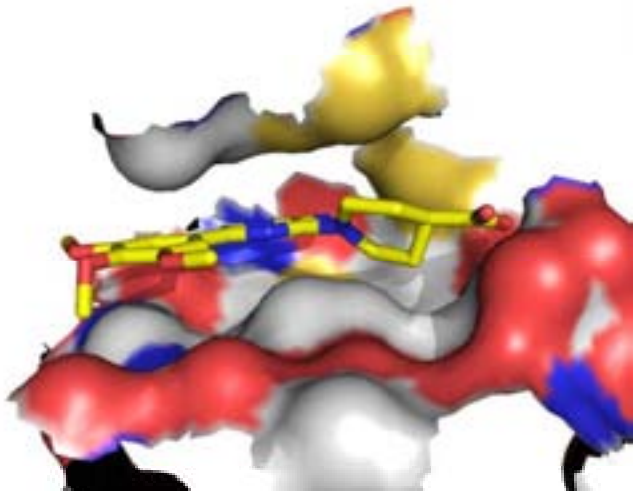
Substituted piperidine derivatives



				
IC50 (μM)	**1**	**0.4**	**0.2**	**11**
LE	**0.3**			
PAMPA	**low**	**low**	**low**	**low**

- Optimisation of hydrophobic contacts led to increase in activity
- Still no signs of cellular activity most likely due to poor permeability
- Larger substituents not tolerated, steep SAR

→ Series abandoned



Phe148

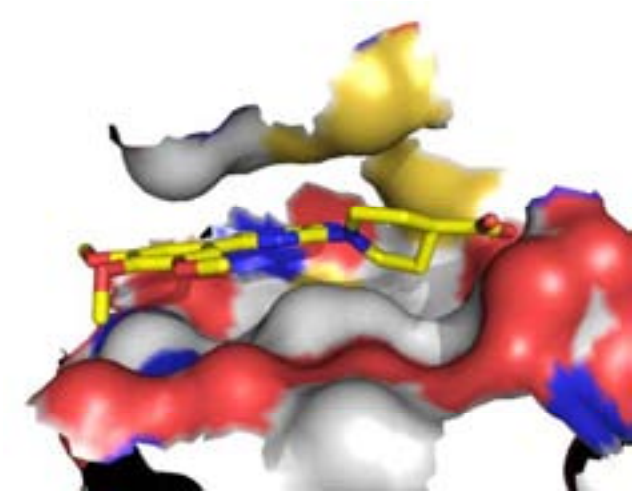
Trends in Kinase Selectivity: Insights for Target Class-Focused Library Screening

Shana L. Posy,[†] Mark A. Hermsmeier,[‡] Wayne Vaccaro,[§] Karl-Heinz Ott,[‖] Gordon Todderud,[⊥] Jonathan S. Lippy,[⊥] George L. Trainor,[§] Deborah A. Loughney,[†] and Stephen R. Johnson*[,†]

[†]*Computer-Assisted Drug Design, Applied Biotechnology, Bristol-Myers Squibb Research and Development, P.O. Box 4000, Princeton, New Jersey 08543, United States,* [‡]*Chemistry Informatics, Bristol-Myers Squibb Research and Development, P.O. Box 4000, Princeton, New Jersey 08543, United States,* [§]*Discovery Chemistry, Bristol-Myers Squibb Research and Development, P.O. Box 4000, Princeton, New Jersey 08543, United States,* [‖]*Bioinformatics, Applied Biotechnology, Bristol-Myers Squibb Research and Development, P.O. Box 4000, Princeton, New Jersey 08543, United States, and* [⊥]*Lead Evaluation, Applied Biotechnology, Bristol-Myers Squibb Research and Development, P.O. Box 4000, Princeton, New Jersey 08543, United States*

J.Med. Chem 2011, p. 54:

→ Screening of **21851** kinase focused compounds against **317** kinases



	Hit rate	Neighbour hit rate
Average all kinases	3.3 %	22%
Nek2	0.25 %	3.7%
Average Phe kinases	1%	13%

- **Nek2 shows a (neighbour) hit rate much lower than average**
- **Kinases with a Phe in the equivalent position show lower than average hit rate**

Benzimidazoles

Design of potent thiophene inhibitors of polo-like kinase 1 with improved solubility and reduced protein binding

Kyle A. Emmitte[a,*], George M. Adjebang[a], C. Webb Andrews[a], Jennifer G. Badiang Alberti[a], Ramesh Bambal[b], Stanley D. Chamberlain[a], Ronda G. Davis-Ward[a], Hamilton D. Dickson[a], Daniel F. Hassler[a], Keith R. Hornberger[a], Jeffrey R. Jackson[b], Kevin W. Kuntz[a], Timothy J. Lansing[a], Robert A. Mook Jr.[a], Kristen E. Nailor[a], Mark A. Pobanz[a], Stephon C. Smith[a], Chiu-Mei Sung[b], Mui Cheung[c]

[a] Five Moore Drive, Research Triangle Park, NC 27709, GlaxoSmithKline, USA
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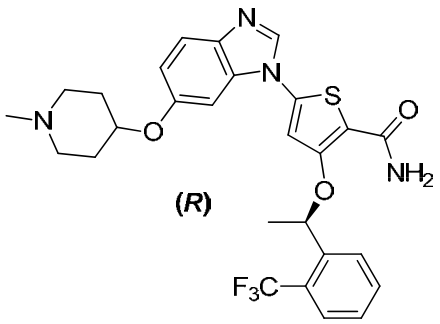
[c] 709 Swedeland Road, King of Prussia, PA 19406, GlaxoSmithKline, USA



Properties of published compound:

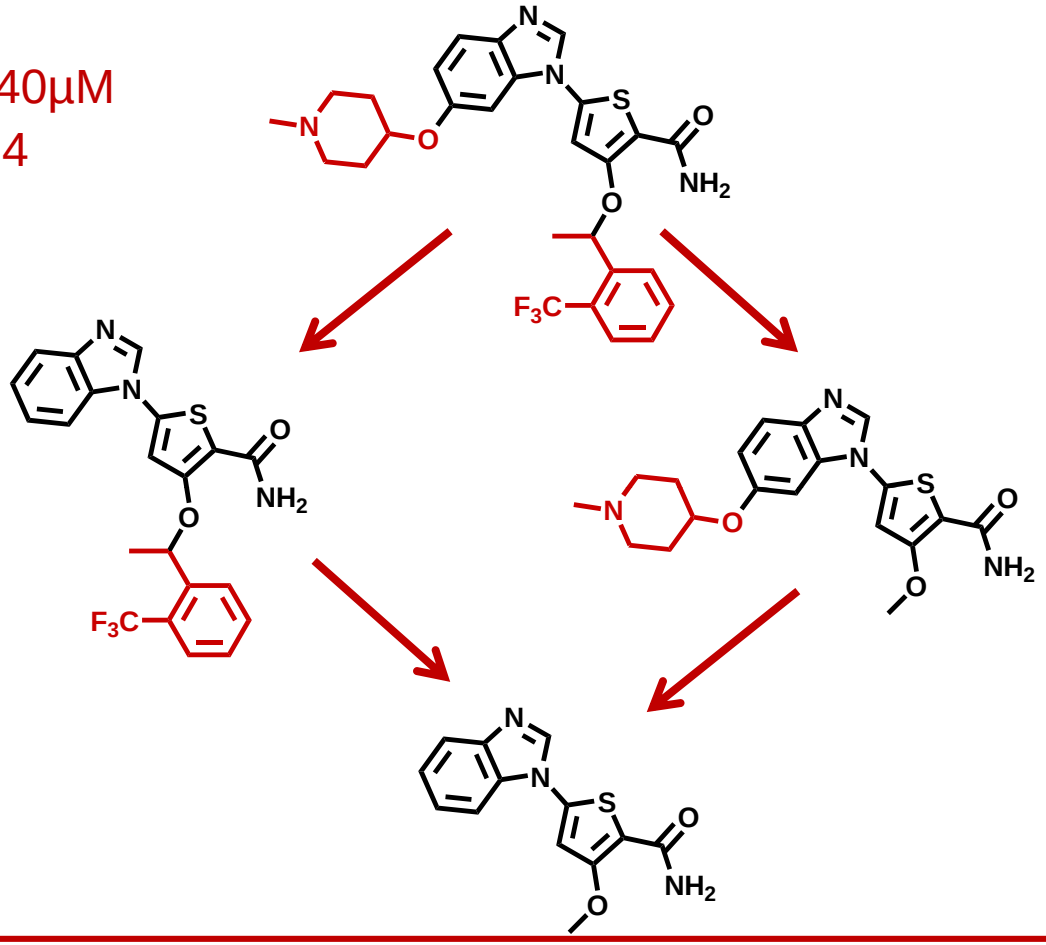
- Measured IC_{50} (rac) **Nek2:** 140 nM, **Plk1**: < 8 nM
- Ligand efficiency: 0.24
- MW 545 / cLogP 5

Aims for initial exploration:

- Selectivity vs. Plk1
- Improve activity without increasing lipophilicity and MW

Truncated benzimidazoles

Nek2: 0.140µM
LE: 0.24

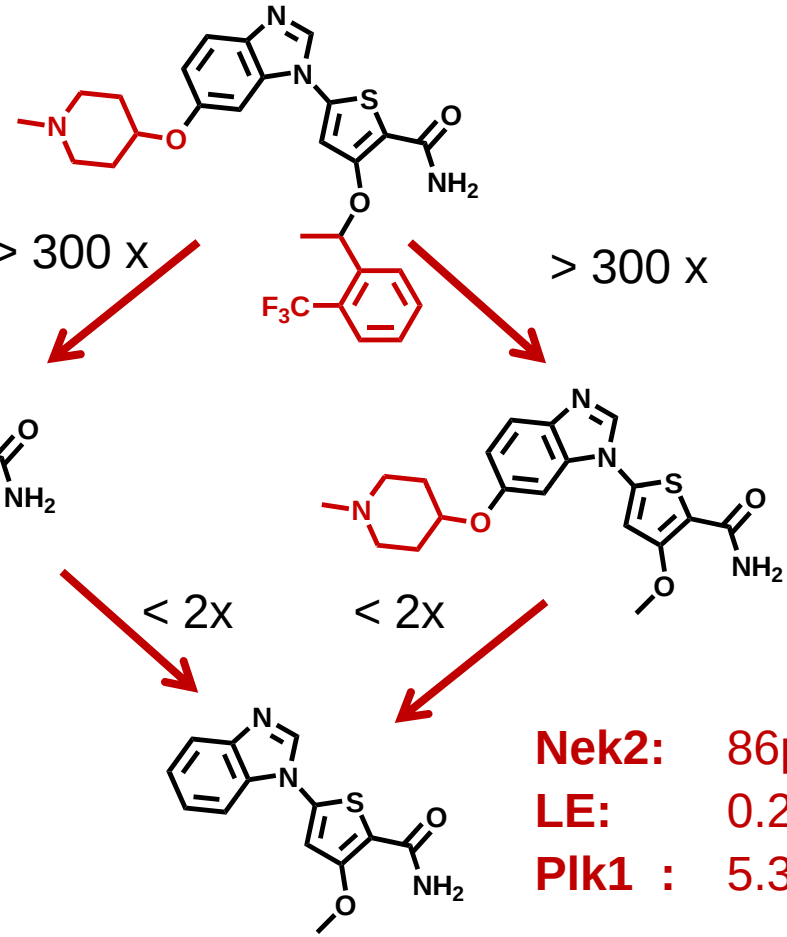


Hypothesis:

- Poor LE driven by substituents optimised for Plk1.
- Truncation will deliver a more ligand efficient core template.

Solanki S, Innocenti P, Mas-Droux C, Boxall K, Barillari C, van Montfort RLM, Aherne GW, Bayliss R, Hoelder S (2011). Journal of Medicinal Chemistry, 54 (6), pp 1626–1639

Truncated benzimidazoles



Nek2: 0.140µM
LE: 0.24

Nek2: >50µM
LE: <0.21

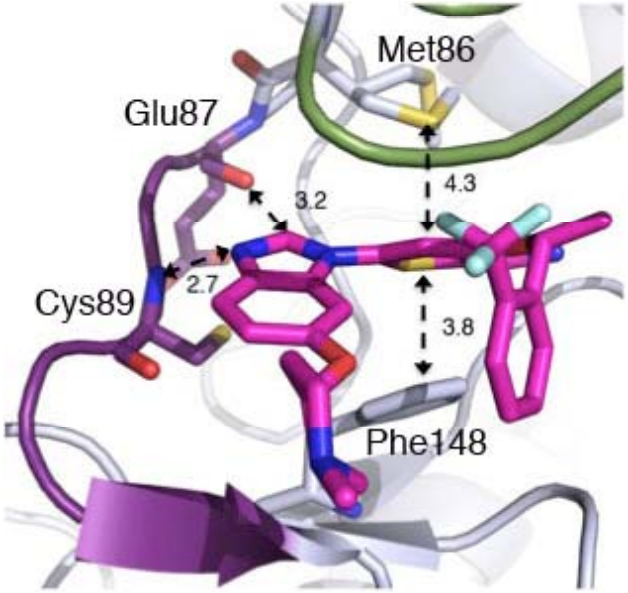
Nek2: 64µM
LE: 0.21

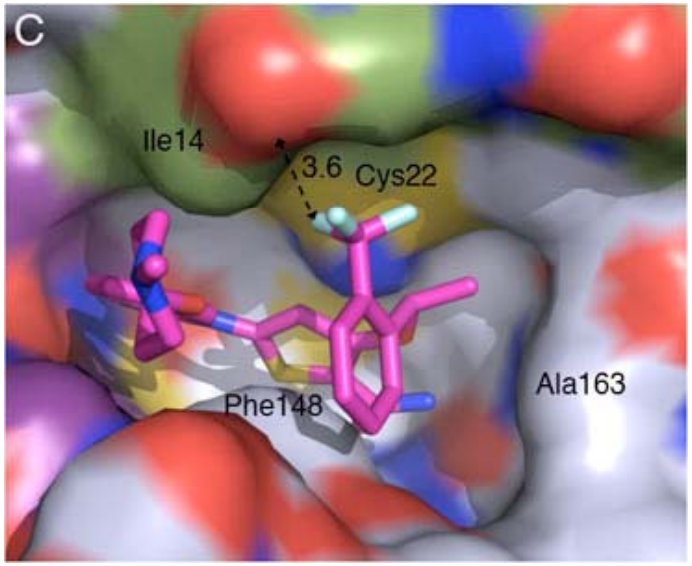
Nek2: 86µM
LE: 0.29
Plk1 : 5.3 µM

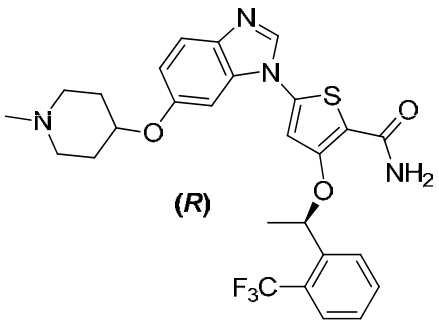
- Pronounced non linear (and steep) SAR for Nek2
- Truncated scaffold significantly more efficient on Plk1

Solanki S, Innocenti P, Mas-Droux C, Boxall K, Barillari C, van Montfort RLM, Aherne GW, Bayliss R, Hoelder S (2011). Journal of Medicinal Chemistry, 54 (6), pp 1626–1639

Benzimidazole Crystal Structure







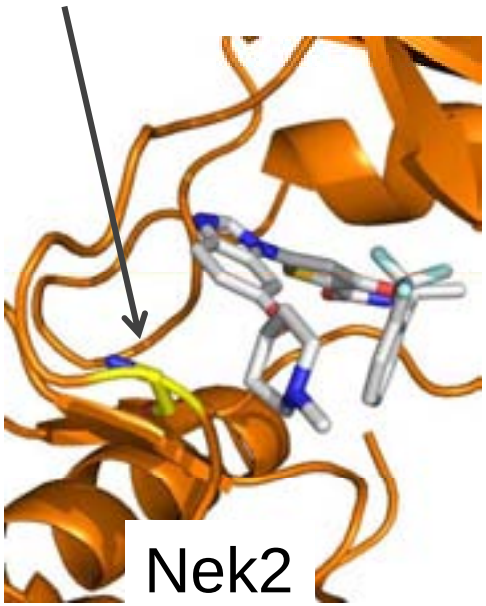
- Thiophene sandwiches between Phe148 and Met86
- Piperidine ring does not engage in H-bonding
- Phenyl group does not engage in hydrophobic contacts.

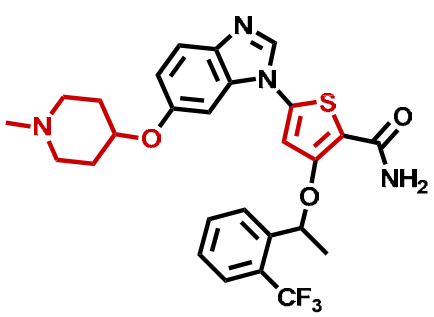
→ It would have been very challenging to identify this compound through structure-based design or FBDD

Solanki S, Innocenti P, Mas-Droux C, Boxall K, Barillari C, van Montfort RLM, Aherne GW, Bayliss R, Hoelder S (2011). Journal of Medicinal Chemistry, 54 (6), pp 1626–1639

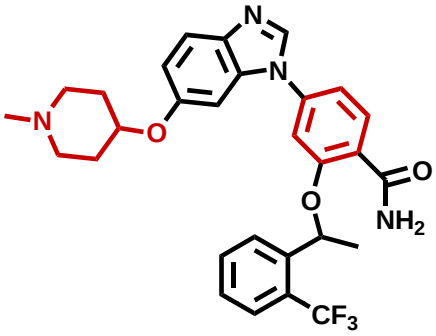
Selectivity vs. Plk1

Nek2: Gly
PLK1: Arg

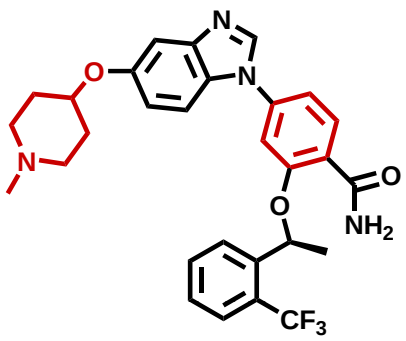




Nek2: **0.**140µM
LE: 0.24
Plk1 : <0.008µM



Nek2: **0.66 µM**
LE: **0.22**
Plk1 : **0.2 µM**

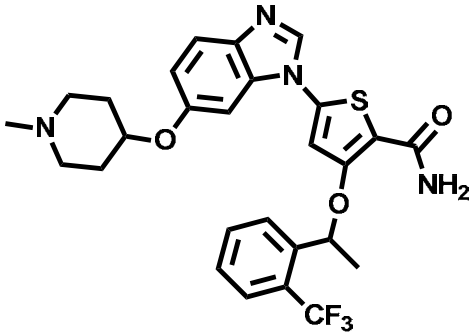


Nek2: **0.36 µM**
LE: **0.23**
Plk1 : **50 µM**

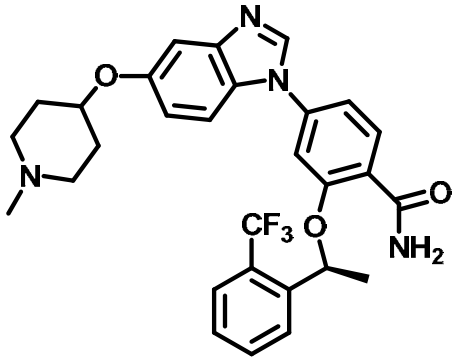
2 modifications designed based on a Gly → Arg sequence difference and published SAR data led to 10000 fold change in selectivity

Solanki S, Innocenti P, Mas-Droux C, Boxall K, Barillari C, van Montfort RLM, Aherne GW, Bayliss R, Hoelder S (2011). Journal of Medicinal Chemistry, 54 (6), pp 1626–1639

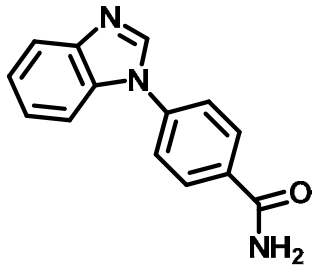
Summary Benzimidazoles



Nek2: 0.140µM
LE: 0.24
Plk1 : <0.008µM



Nek2: 0.36 µM
Plk1: > 50µM
LE: 0.23
cLogP 5.1



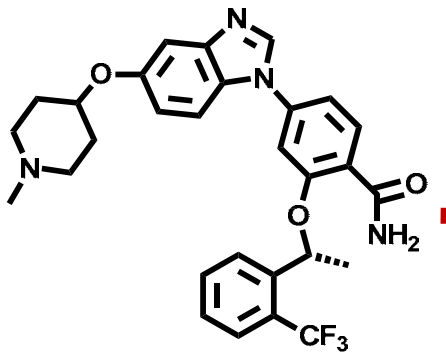
Nek2: 60 µM
LE: 0.29

- Potency and LE still modest, partially due modest efficiency of core scaffold
- No cellular activity

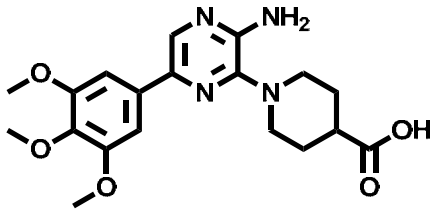
Hypothesis: Pharmacophore can be explored to give potent inhibitors if a more efficient hinge binding scaffold can be found

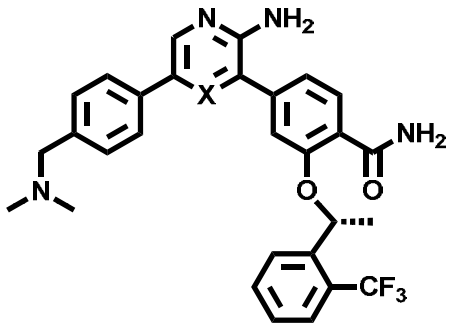
Hybridising both series

Nek2: 0.340µM
Plk1: > 50µM
LE: 0.24
cLogP 5.1



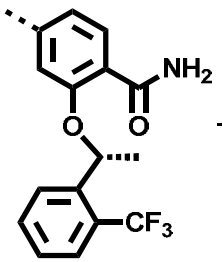
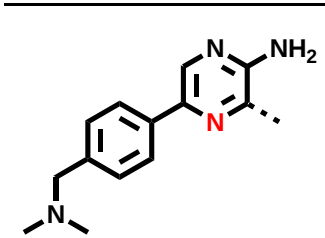
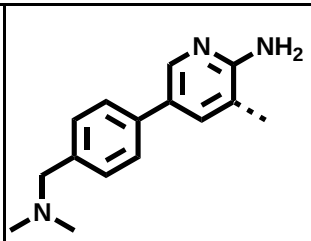
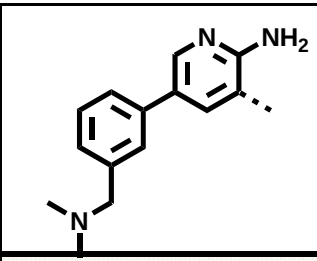
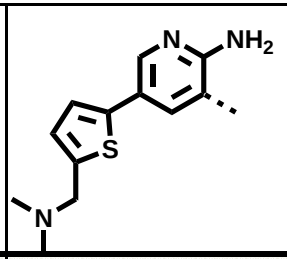
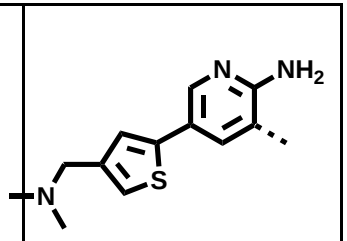
Nek2: 1.0 µM
LE: 0.3





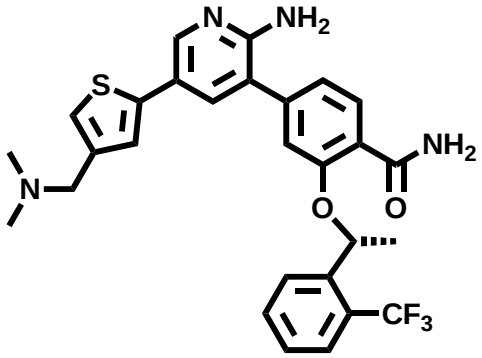
Superposition of crystal structures suggested that the benzimidazole pharmacophore can be grafted onto hinge binder from first series

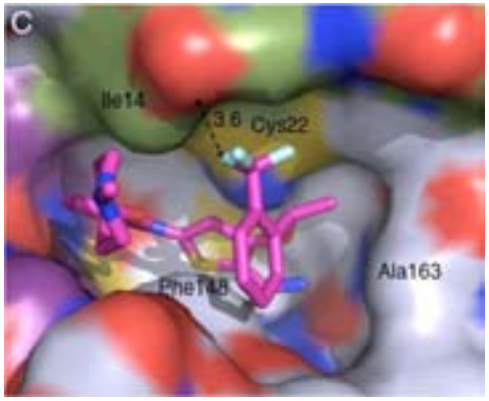
Hybrid compounds

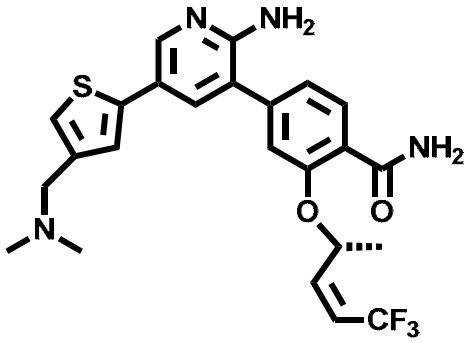
						
NEK2	0.8 µM	0.12 µM	0.21 µM	0.04 µM	0.05 µM
PLK1	1.5 µM	1.6 µM	3.3 µM	0.5 µM	1.3 µM
Ratio	~2	~13	~15	~13	~25
LE	0.21	0.23	0.23	0.27	0.27

- Hybridisation of both series led to compounds with improved potency but limited PLK1 selectivity
- Weak signs of cellular activity

Truncated hybrid compound







R-Isomer

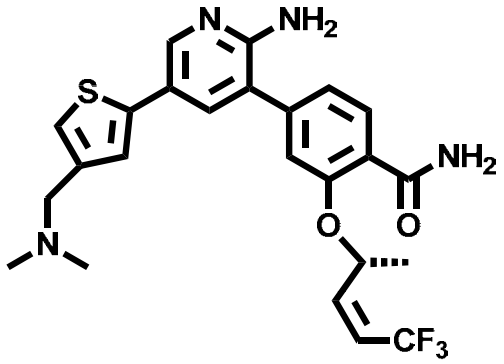
Nek2: 0.035 µM
Plk1: 0.82 µM
Ratio: 24
LE: 0.27 / cLogP: 5.4

R-Isomer

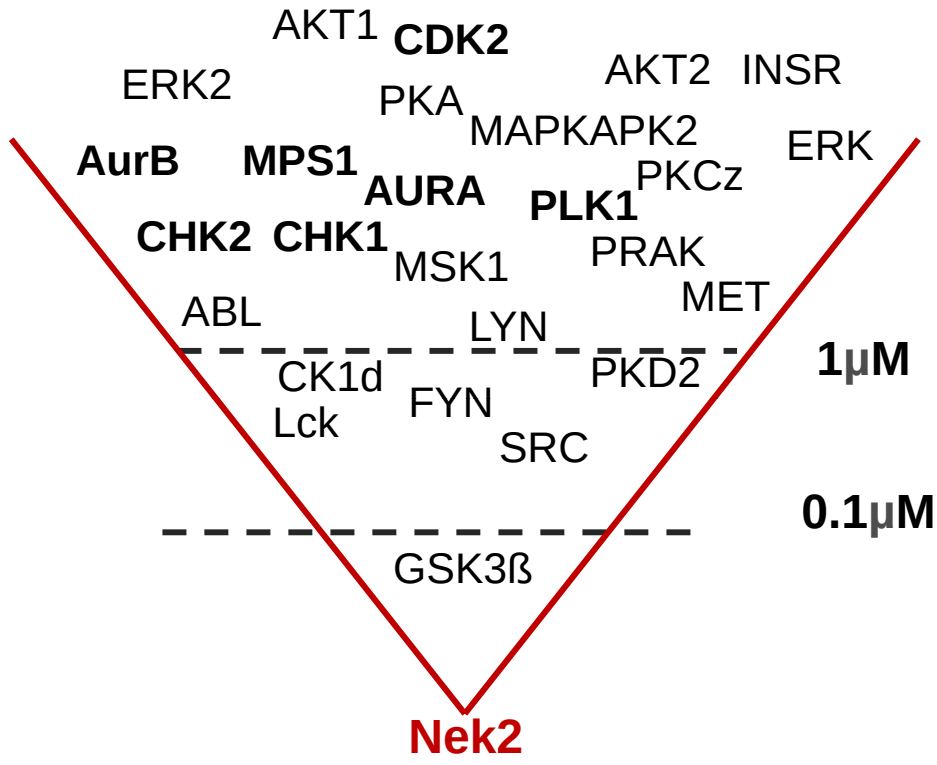
CCT250863
Nek2: 0.022 µM
Plk1: 5.8
Ratio: **260**
LE: 0.31 / cLogP: 4.3

- Structure-based trimming of the phenyl group lead to a compound with comparable activity and much improved PLK1 selectivity, ligand efficiency and lipophilicity

Kinase selectivity

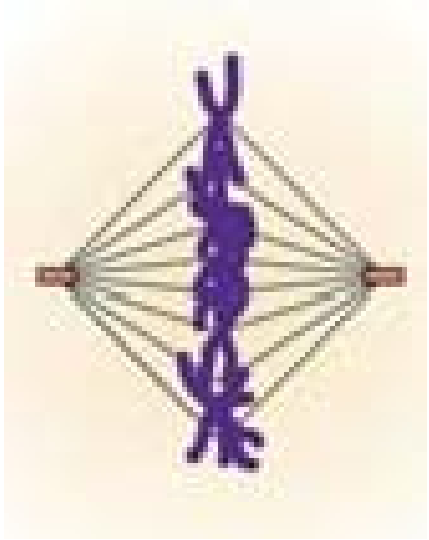


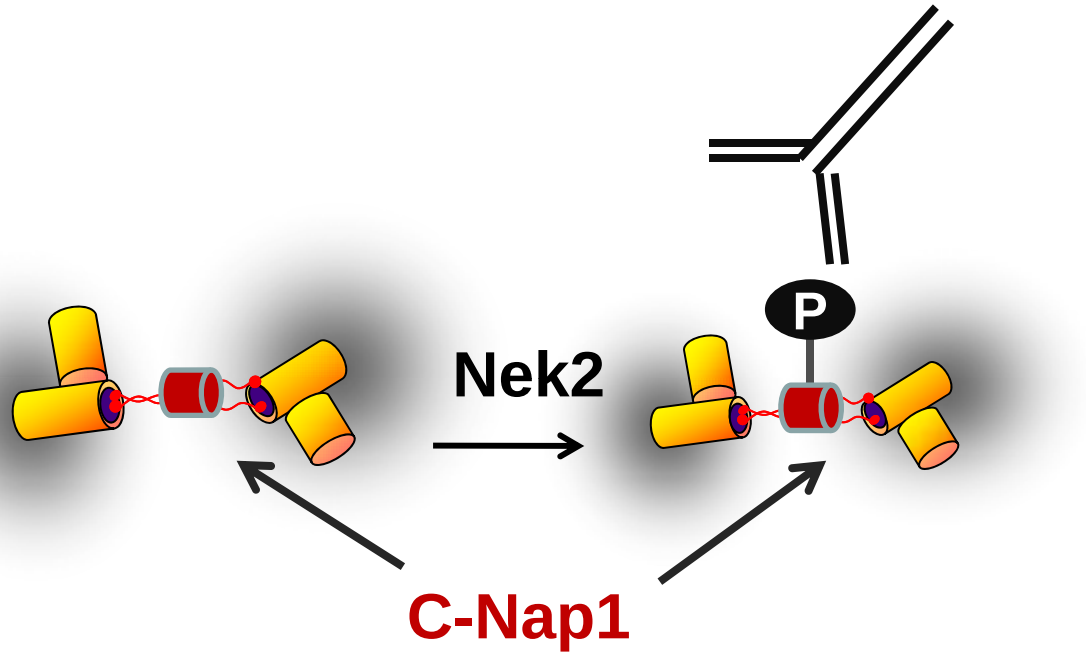
CCT250863
(R-Isomer)
Nek2: 0.022 µM
Plk1: 5.8



- >100x selective against most kinases
- no significant inhibition of cell cycle kinases
- only 10-20x for SRC family and 5x for GSK3ß

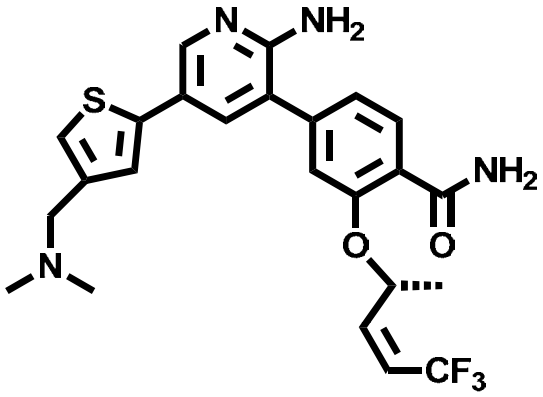
NEK2 mechanism based cell assay





Quantification of C-Nap1 phosphorylation was achieved using high content imaging in Aphidicolin synchronised U2OS cells.

Cellular Activity



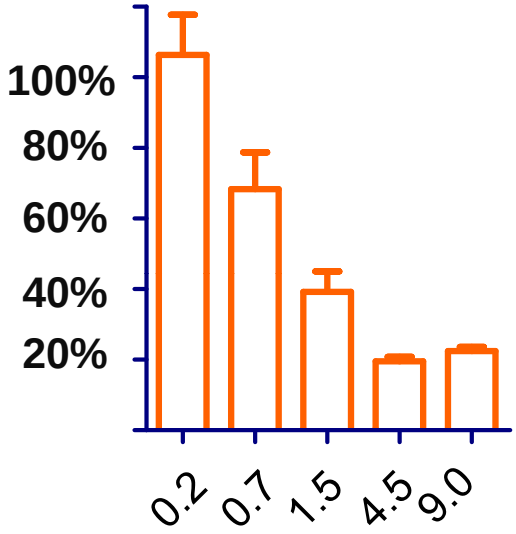
CCT250863

Nek2: 0.022 µM

Plk1: 5.8

GI_{50} U2OS 1.8uM (96h)

C-Nap1 phosphorylation (% of control)

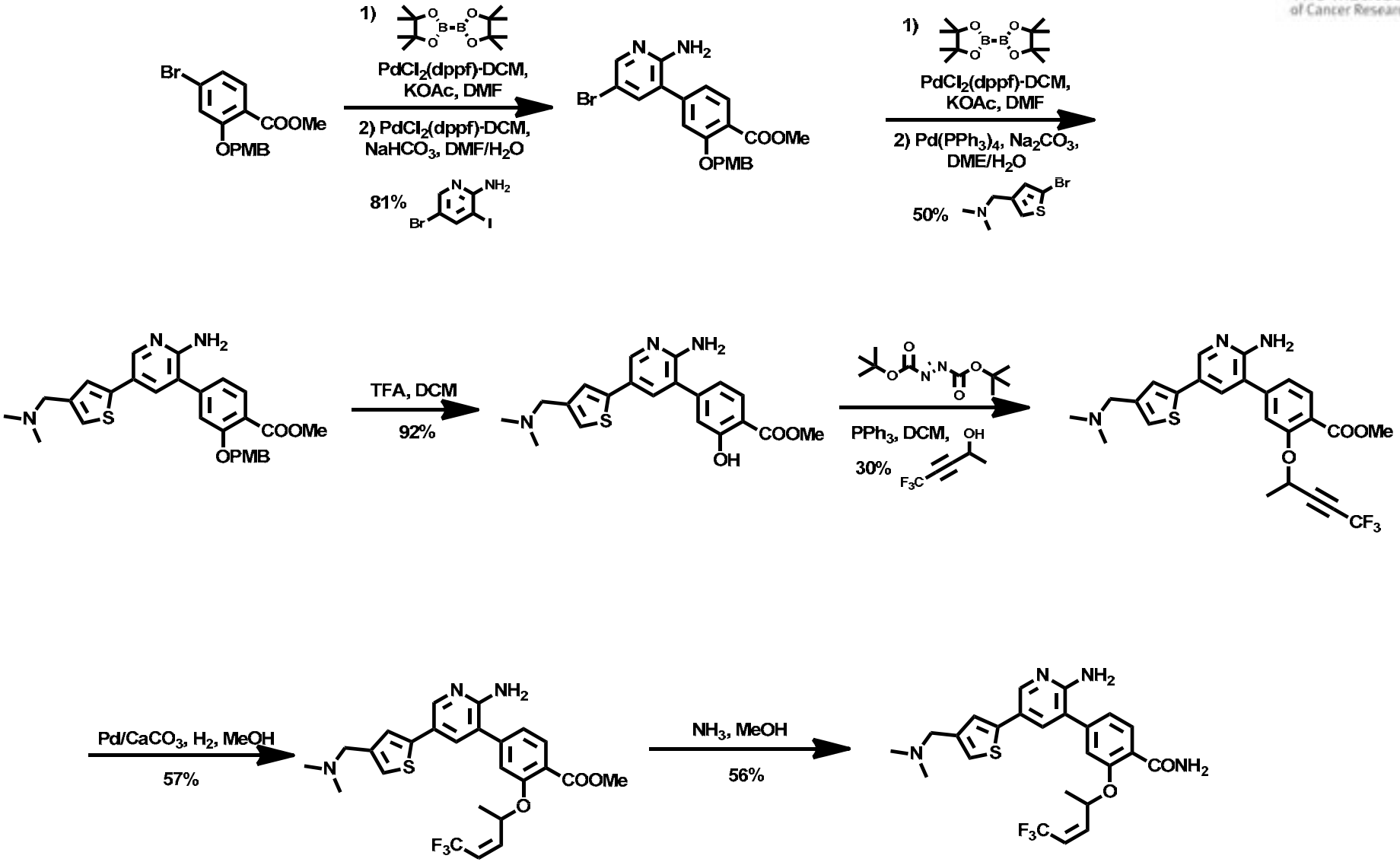


IC_{50} = 1µM

- Dose dependent PD marker modulation (IC_{50} = 1 µM)
- Modest cytotoxicity @ ≥ 2µM, potentially due to off target effects

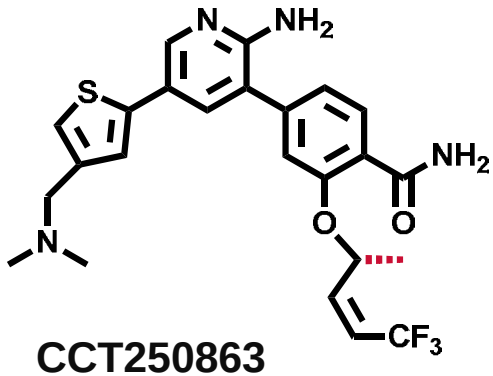
→ **CCT250863 selected as a chemical probe to investigate the role of Nek2 in mitosis and tumour biology.**

CCT250863 Synthesis



Summary

- Ligand and structure-based data confirm our hypothesis that Nek2 is challenging to target at least partially due to Phe148.
- We explored two different series, hybridising both led to potent and selective chemical probe which modulated cellular NEK2 biomarkers
- A published serendipitous finding was critical to define the pharmacophore for potent Nek2 inhibitors
- Structural biology enabled the design of selective and potent inhibitors with improved ligand efficiency and lipophilicity
- Use of this and another chemical probe is ongoing to investigate the role of Nek2 in mitosis and tumour biology and to identify tumours that are sensitive to Nek2 inhibition.



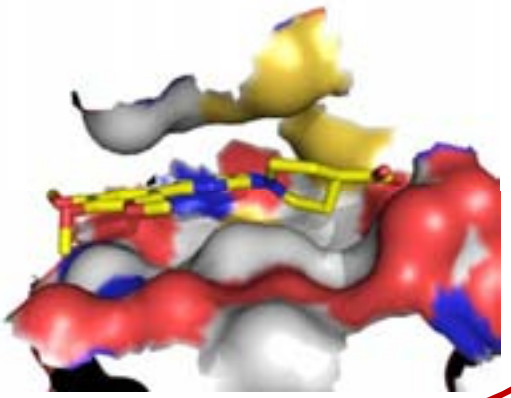
CCT250863

Nek2: 0.022 µM

Plk1: 5.8

LE: 0.31

cLogP: 4.3



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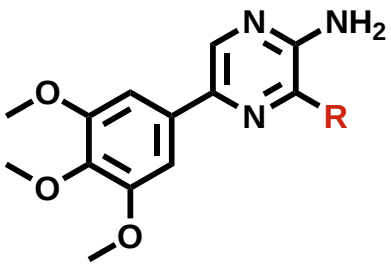
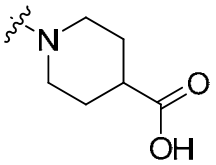
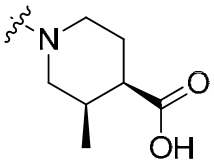
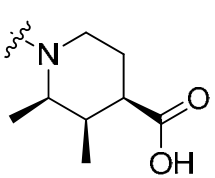
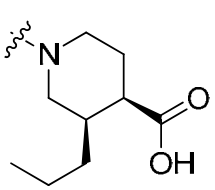
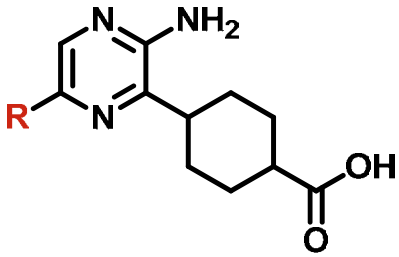
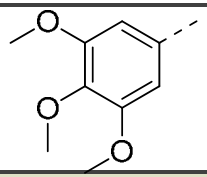
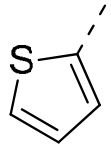
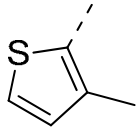
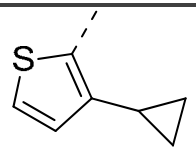


				
IC50 (µM)	**1**	**0.4**	**0.2**	**11**
LE	**0.3**			
PAMPA	**low**	**low**	**low**	**low**



				
IC50 (µM)	**1.0**	**4.0**	**3.0**	**1.0**
LE	**0.3**	**0.35**	**0.35**	**0.34**
PAMPA	**low**	**low**	**low**	**low**

- Optimisation of hydrophobic contacts only led to minor increase activity
- Low permeability persisted

→ Series abandoned

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