

2nd L&L session / 1st MedChemXChange: Protein Kinases as Drug Targets

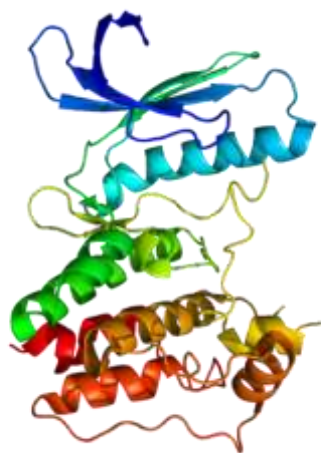
Protein Kinases and the Development of Chemical Probes for the Unexplored Kinome

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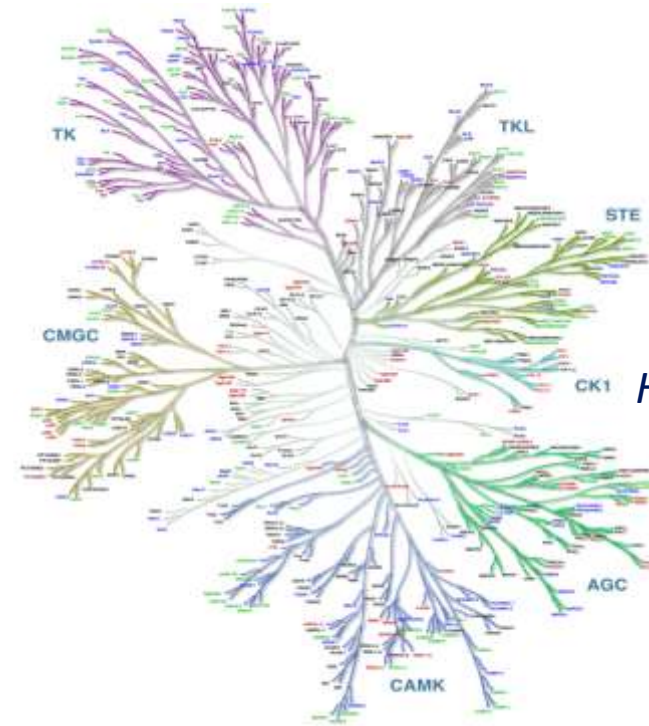
Institut Químic de Sarrià (IQS), Universitat Ramon Llull (URL)
School of Engineering, Department of Organic and Pharmaceutical Chemistry

September 11th 2025, Barcelona, Spain

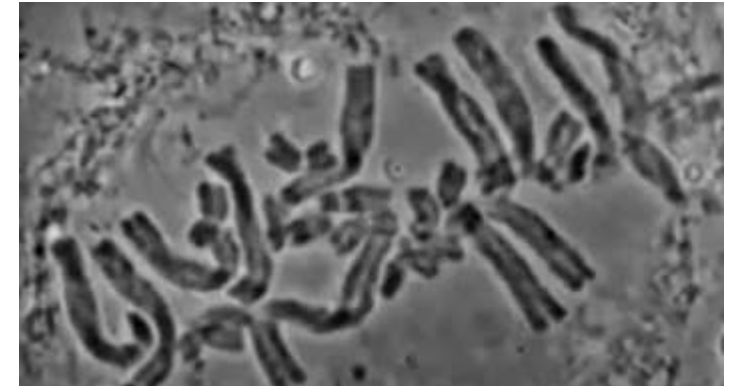


...**2% of eukaryotic genes** and **more than 500 protein kinases** are predicted based on the human genome sequencing...

...**Up to 30% of all human proteins may be modified by kinase activity**, and kinases are known to **regulate the majority of cellular pathways**, especially those involved in signal transduction...



Human Kinome



https://www.youtube.com/watch?v=L61Gp_d7evo

Regulation of cell signaling by Protein Kinases through phosphorylation



Edmond H. Fischer



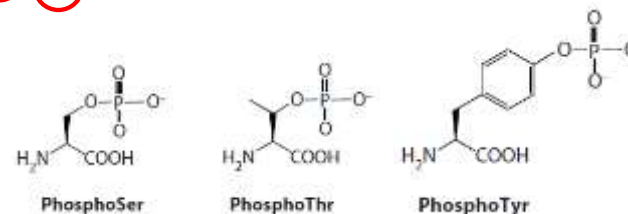
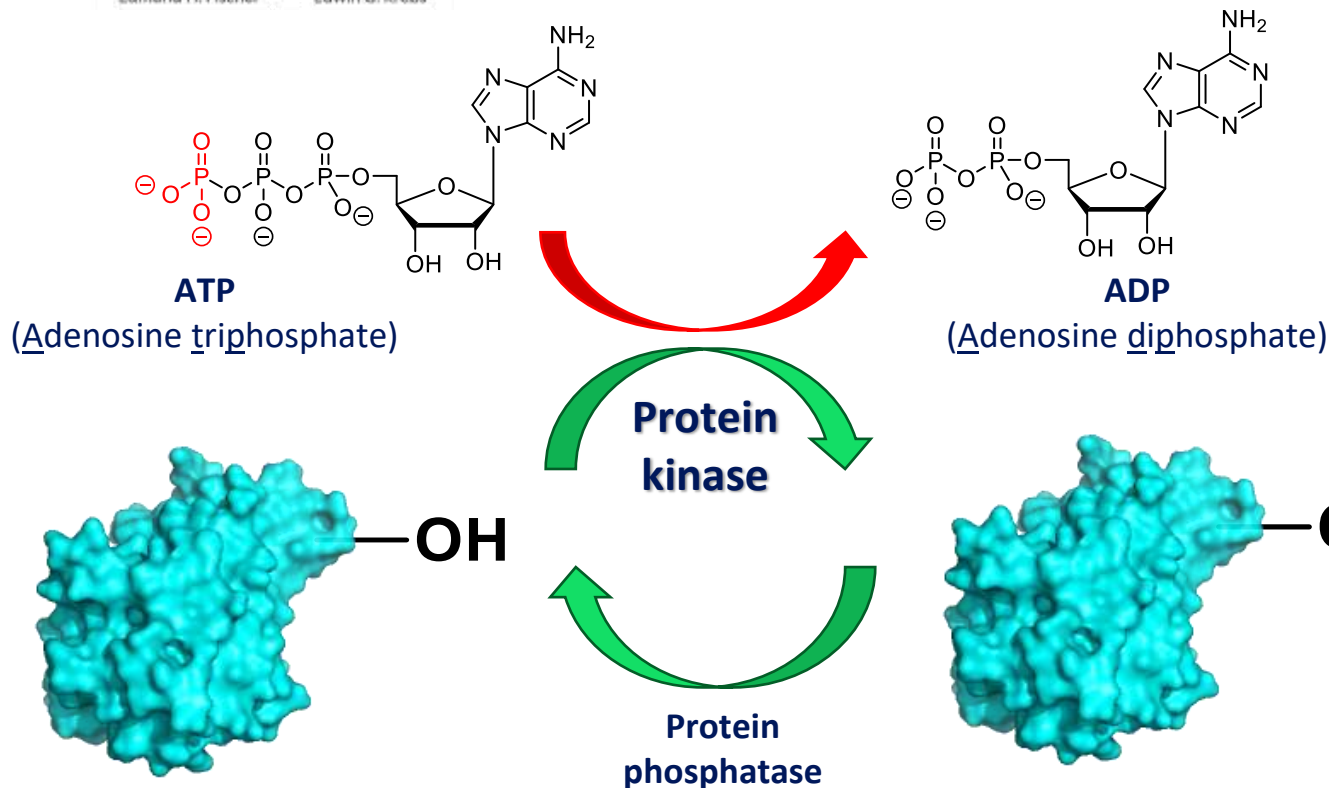
Edwin G. Krebs

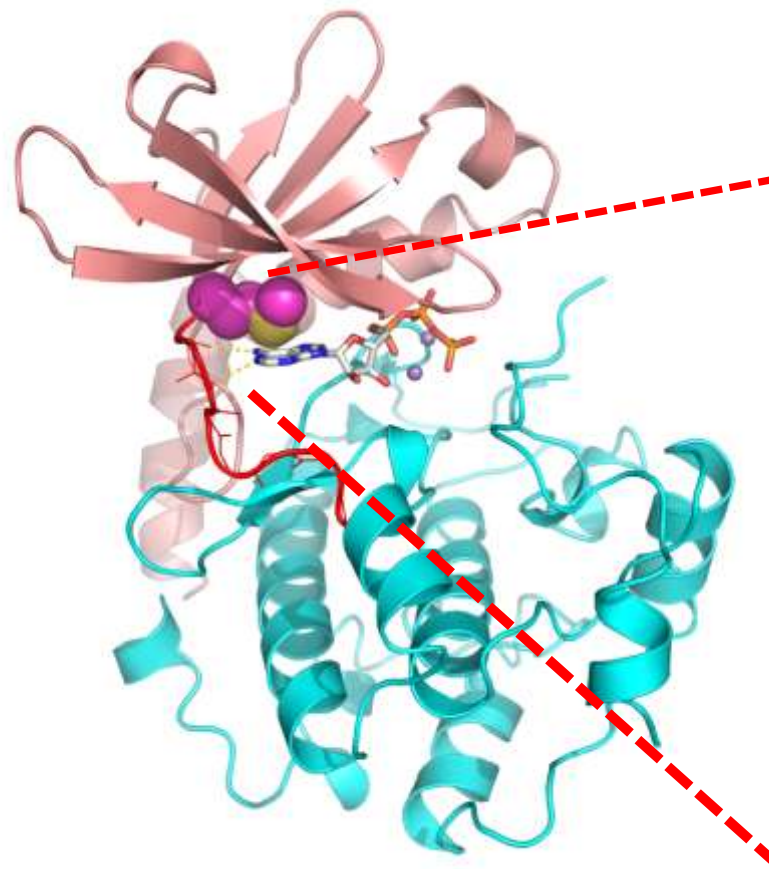
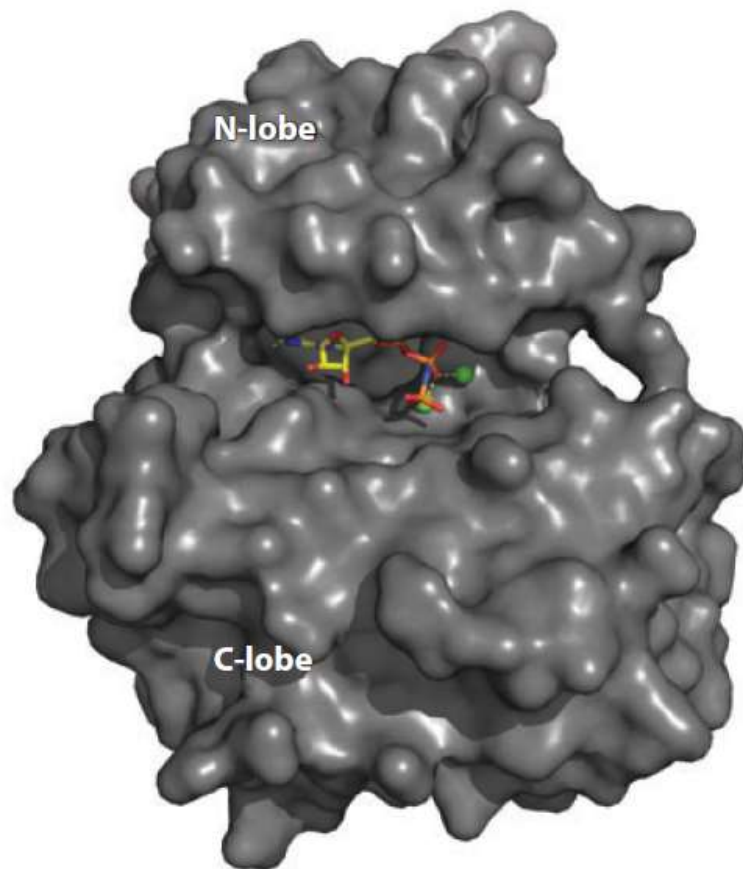


Nobel Prize in Physiology or Medicine (1992)

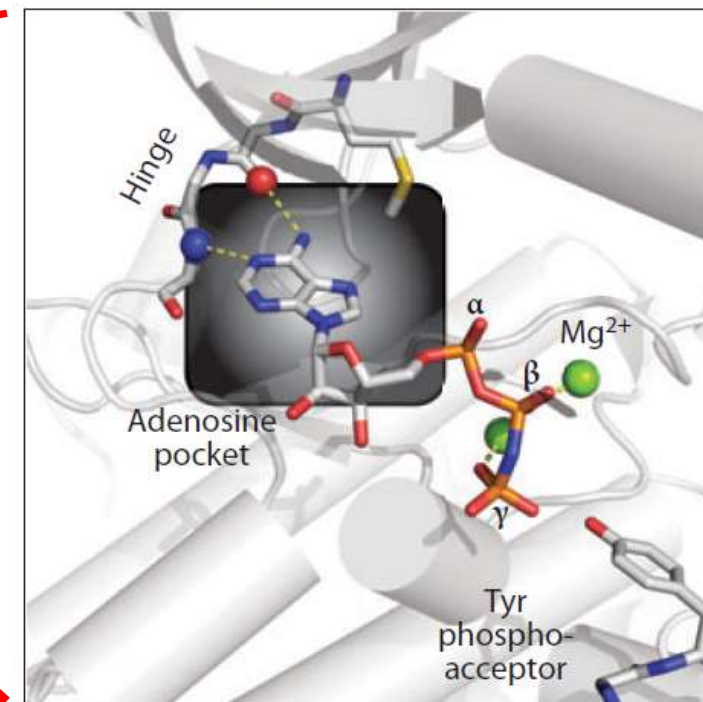
"for their discoveries concerning reversible protein phosphorylation as a biological regulatory mechanism"

...resulting in a **functional change of the substrate** by changing enzyme activity, cellular location, or association with other proteins.





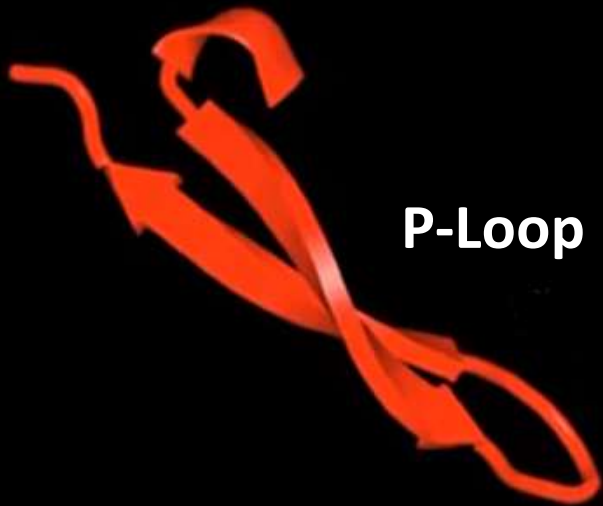
Protein kinase active site



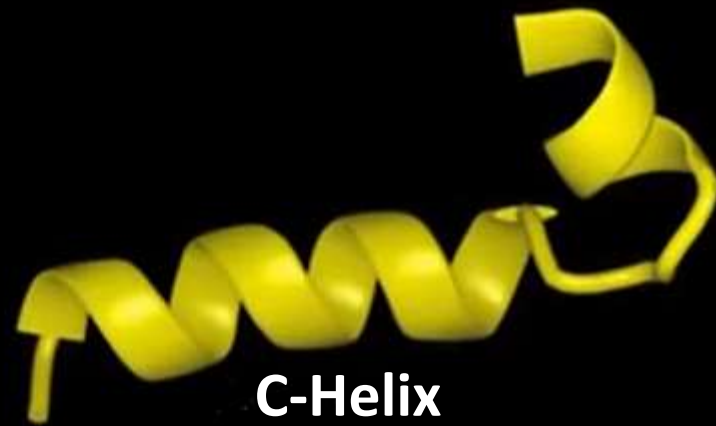
Structure of a typical protein kinase domain displaying ATP binding site and conserved elements around it



P-Loop



C-Helix



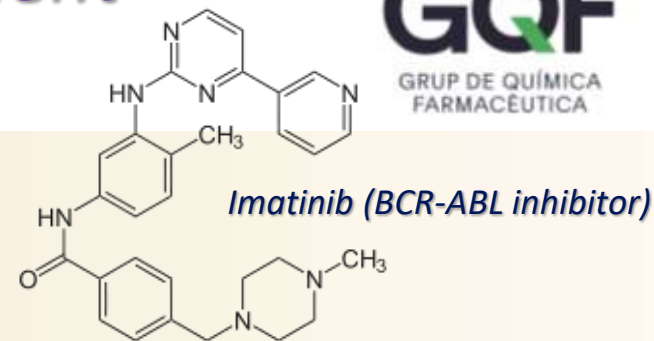
Hinge



Activation-Loop



Timeline: Key events in the development of protein kinase inhibitors



Src kinase

The first oncogene is shown to be a protein kinase.

Isoquinoline sulphonamides are shown to inhibit protein kinases.

Pharmaceutical companies start to develop inhibitors of protein tyrosine kinases.

PD98059 is shown to reverse the phenotype of RAS-transformed cell lines by preventing the activation of MKK1 by RAF.

The first nanomolar inhibitors of receptor protein tyrosine kinases are developed.

Cytokine-synthesis anti-inflammatory drugs are shown to inhibit p38 MAPK.

Gleevec (CGP57148, STI-571) enters human clinical trials for the treatment of CML.

Gleevec is approved for clinical use in the United States.

1978

1981

1984

1986

Late 1980s

1991

1994

1995

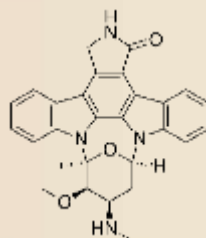
1996

1999

2001

Tumour-promoting phorbol esters are shown to activate PKC.

Staurosporine is shown to be a nanomolar inhibitor of PKC.



Three-dimensional structure of the first protein kinase is determined (PKA).

The immunosuppressant cyclosporin is shown to inhibit a protein phosphatase (PP2B).

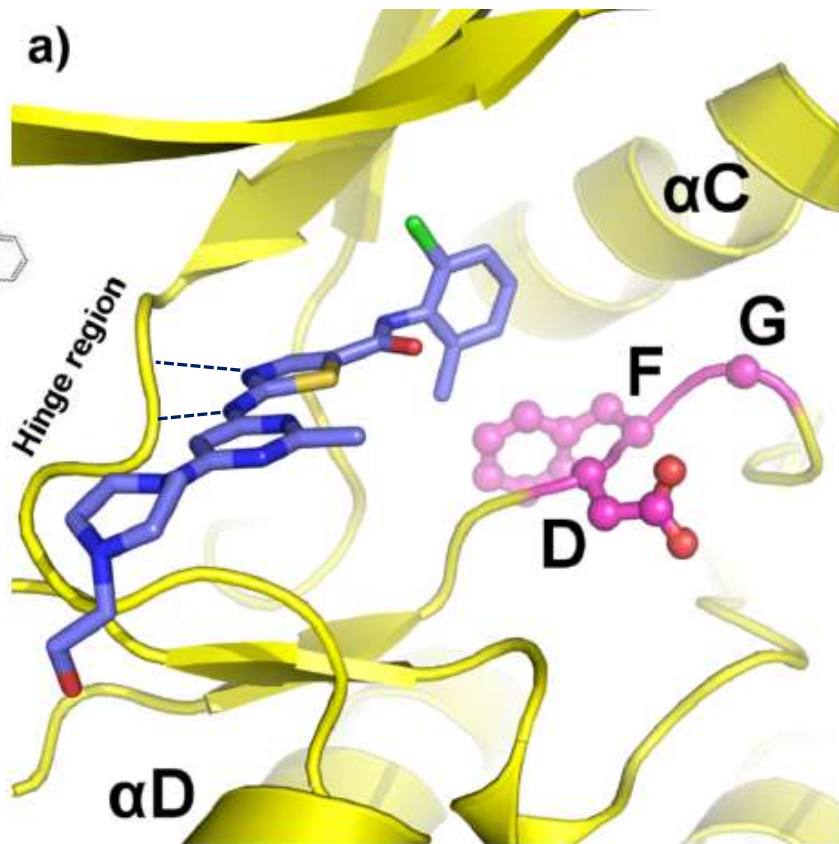
The immunosuppressant rapamycin is shown to inhibit a protein kinase (Tor).

The first protein-kinase inhibitor (fasudil, HA1077) is approved in Japan for the treatment of cerebral vasospasm.

Rapamycin is approved for clinical use as an immunosuppressant in the United States.

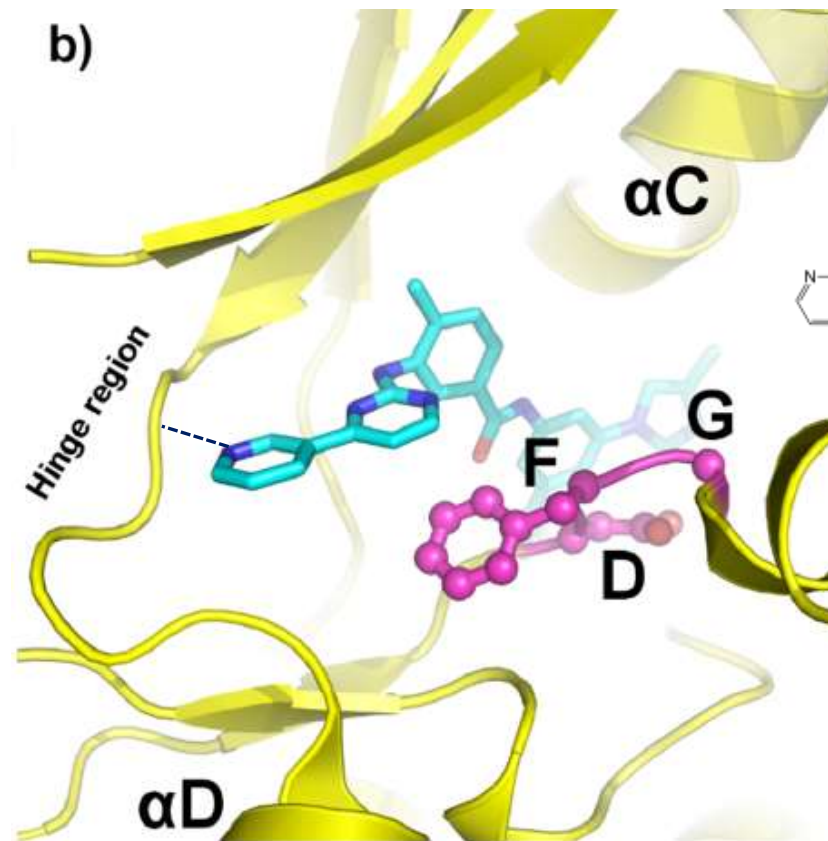
CML, chronic myelogenous leukaemia; EGF, epidermal growth factor; MAPK, mitogen-activated protein kinase; MKK1, mitogen-activated protein kinase kinase 1; PKA, protein kinase A; PKC, protein kinase C; Tor, target of rapamycin.

Types of Kinase Inhibitors: *Type I and II*



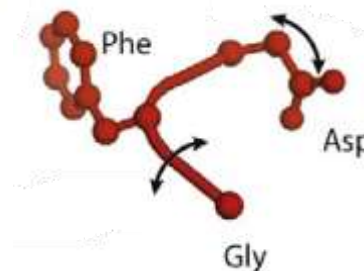
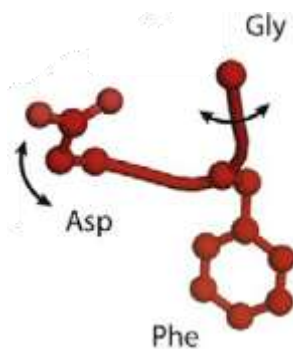
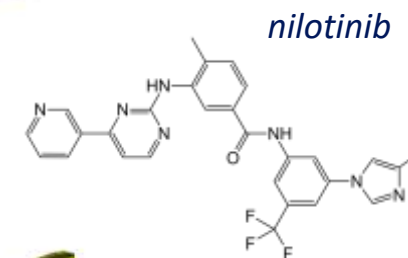
"DFG-in"

Type I kinase inhibitor
(active conformation of the kinase)

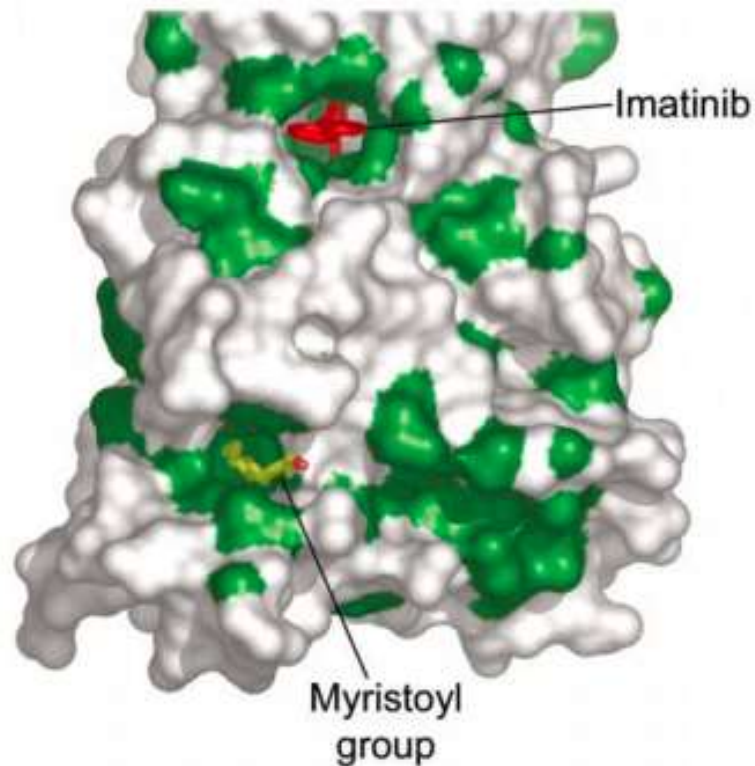


"DFG-out"

Type II kinase inhibitor
(inactive conformation of the kinase)

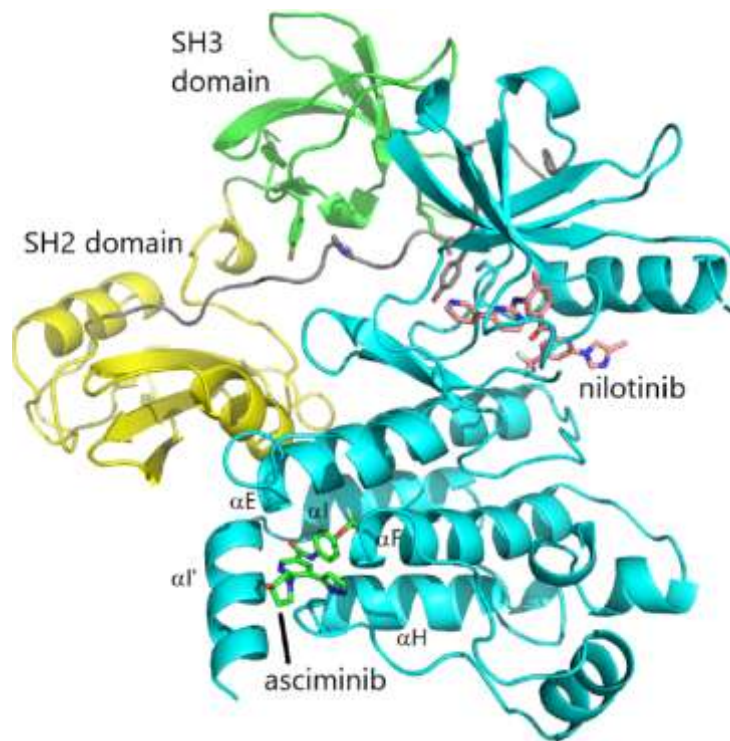


2003



Abelson leukemia virus tyrosine kinase (c-Abl)

2021

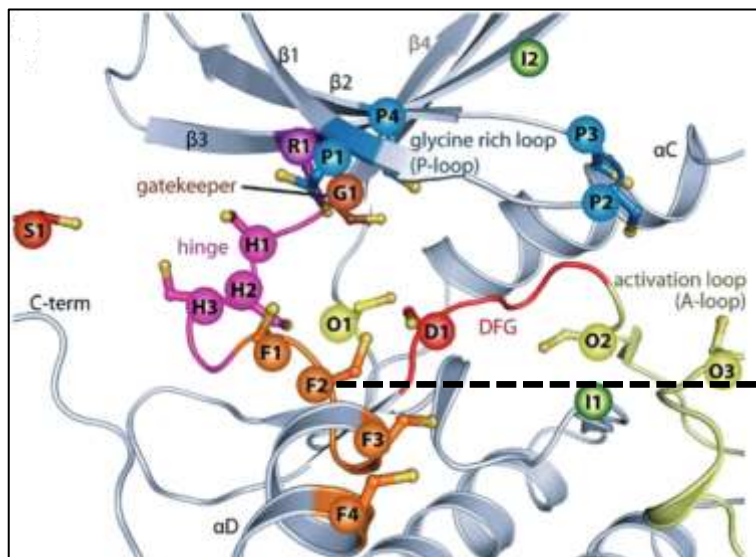
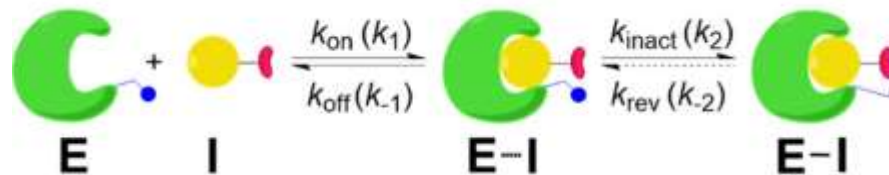


Novartis receives FDA Breakthrough Therapy designations for investigational STAMP inhibitor asciminib (ABL001) in chronic myeloid leukemia

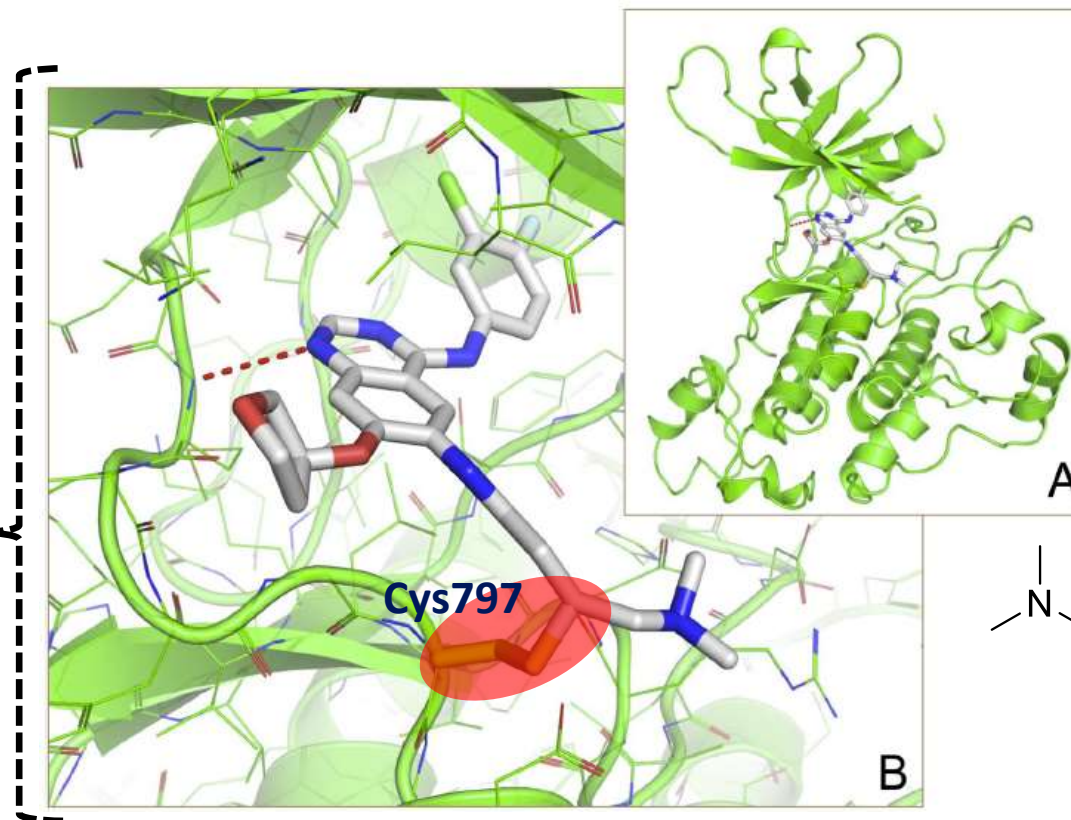


Types of Kinase Inhibitors: *Covalent*

General two-step mechanism of irreversible- and reversible-covalent protein binding

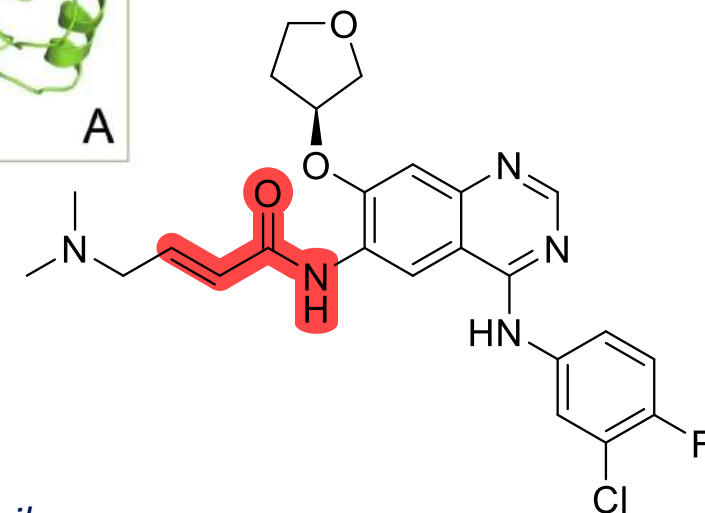


Location of cysteine residues in close proximity to the kinase active site.



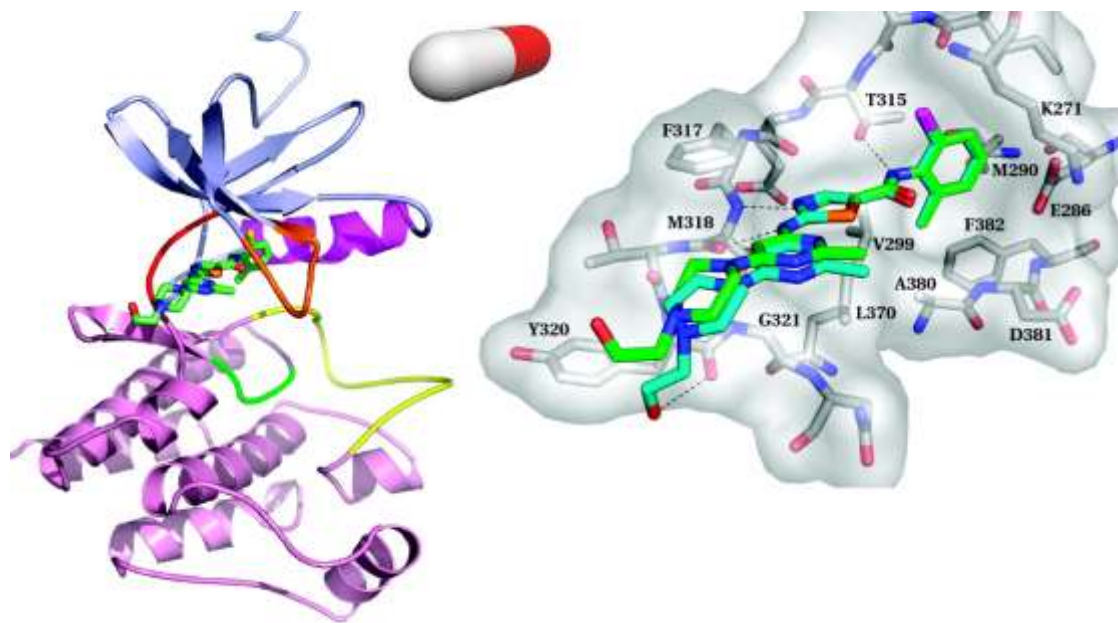
Crystal structure of wild-type EGFR in complex with afatinib

Afatinib
(EGFR inhibitor)
-non-small cell lung cancer

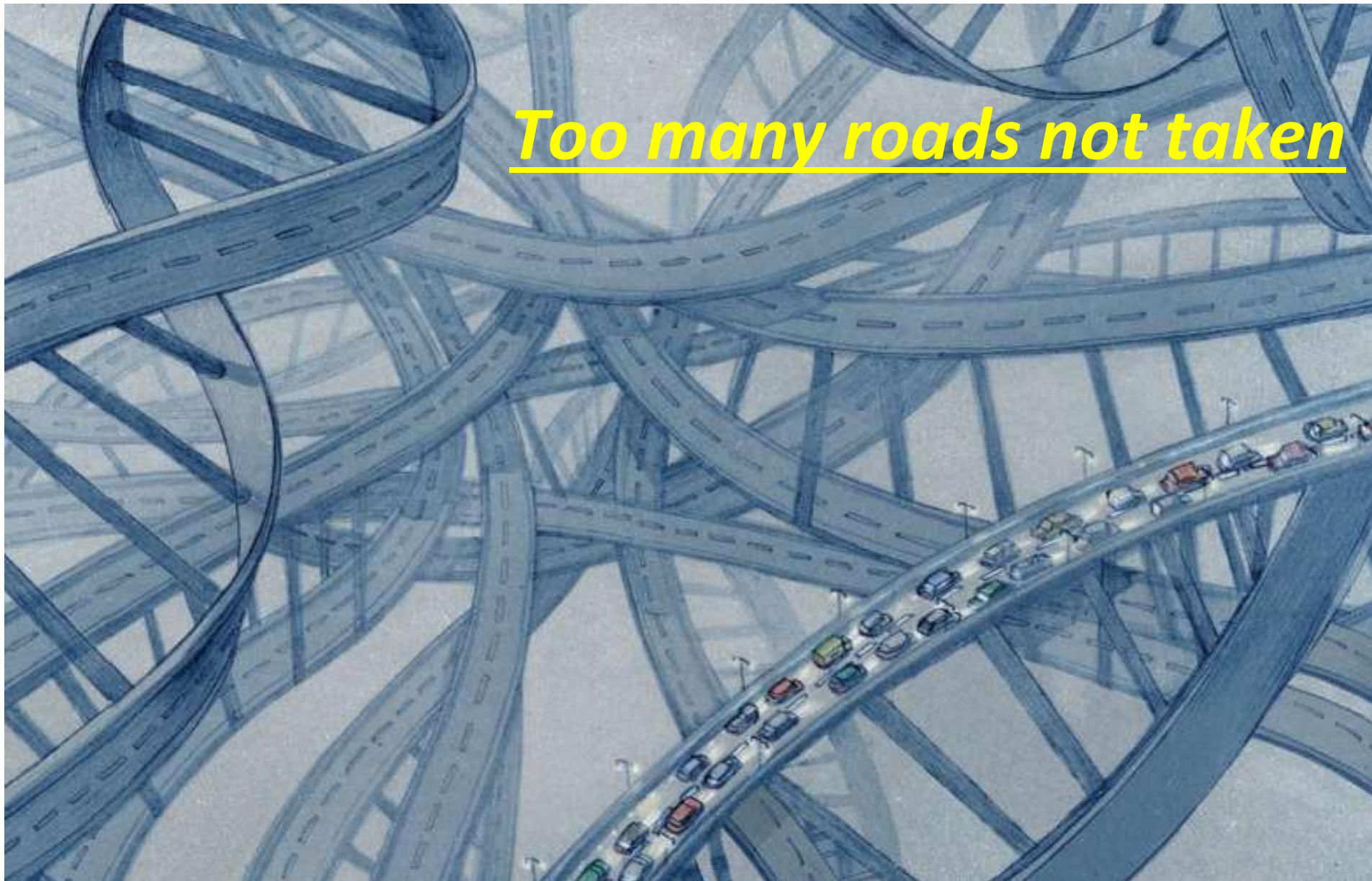


Protein kinases are important therapeutic targets of drugs

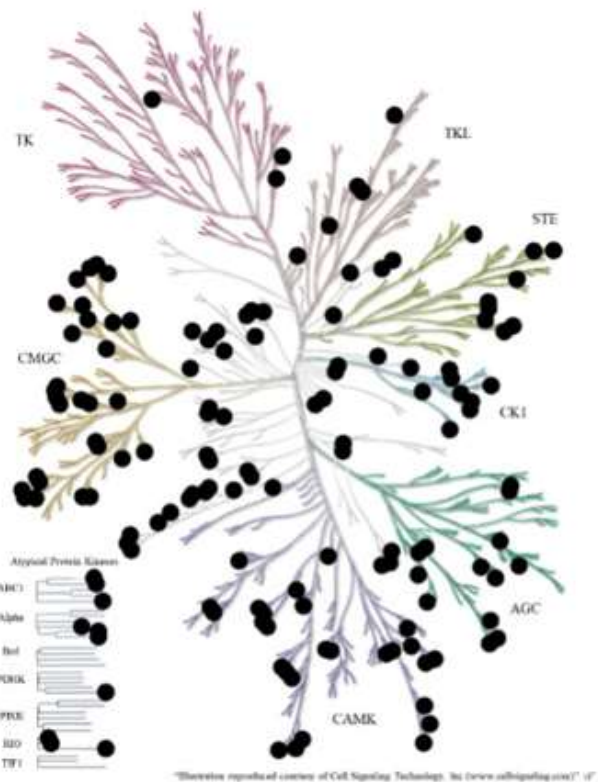
There are **88** FDA-approved small molecule protein kinase inhibitors (May 2025)



- **Oncology**
- Immune system
- Inflammation
- Degenerative disease



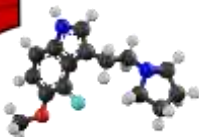
"Dark Side of the Kinome"



- **~50 % of the kinome is still considered as chemically under or unexplored**, meaning a lack of elaborate drug discovery activity or having only very promiscuous and poorly characterized ligands.
- **Only 25% of the kinome is covered** by the currently approved PKIs.
- Literature shows a strong genetic links of many other kinases to diseases.
- Wide avenue for medicinal chemistry and chemical biology exploration in the kinase field (potential new drug targets).

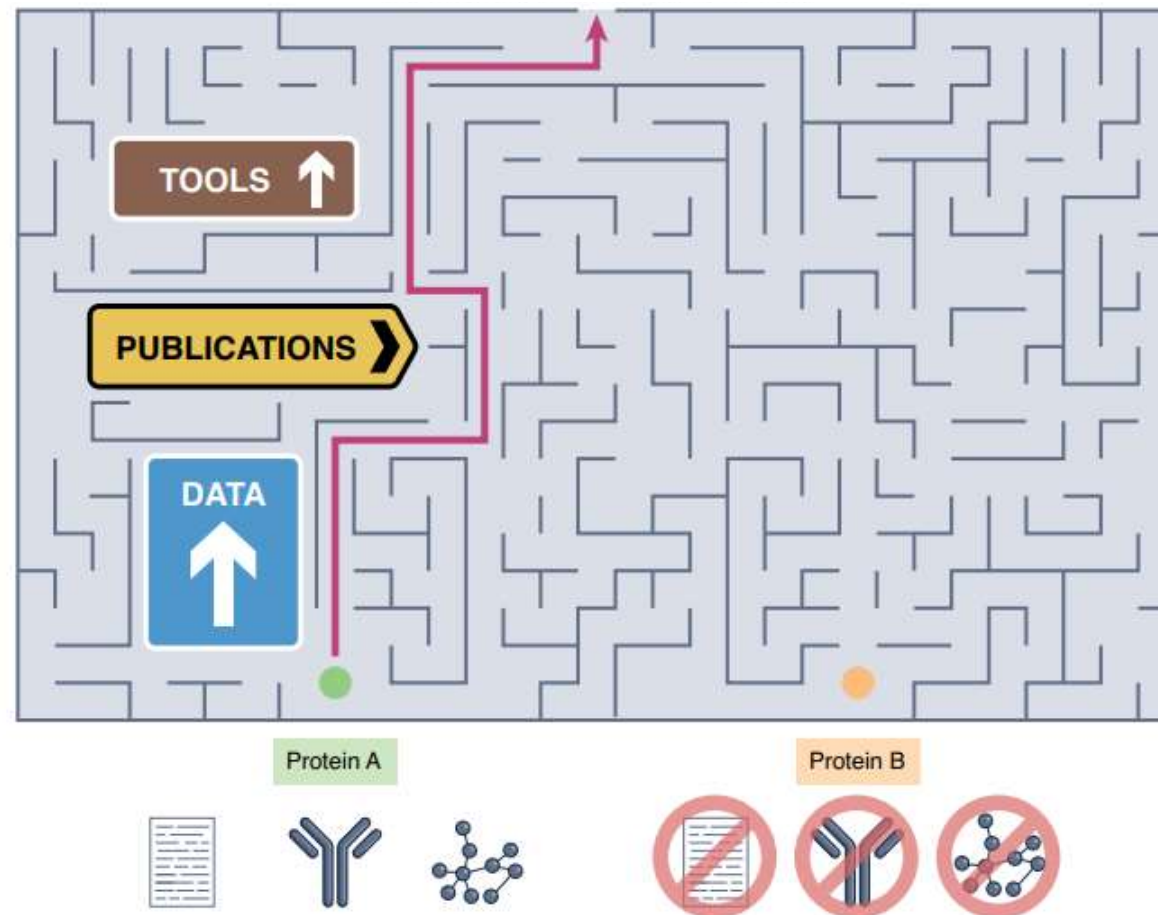
Voßen et al. *J. Med. Chem.* **2024**, 67, 19, 17919–17928; Koch, S.; Bajorath, J. *RSC Med. Chem.* **2025**, 10.1039.D5MD00306G

...Chemical probes for target (in)validation of protein kinases are needed!!!

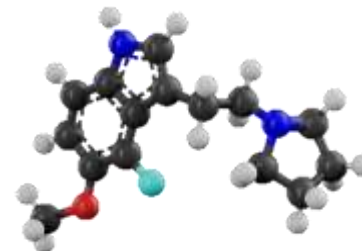




Function and role in disease understood



Protein annotation inequality impedes biomedical progress



What is a Chemical Probe???

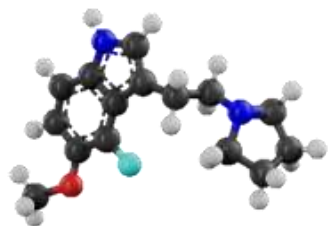


Why is it so useful???



...is defined as a **highly selective small-molecule modulator** of a protein's function that allows the user to answers mechanistic and phenotypic questions about its molecular target...

It is used to interrogate and establish **possible correlations between specific chemical target modulation and the resulting phenotype** (*biological tractability*) in a relevant biological system.



A High-Quality Chemical Probe must meet the following criteria:

- Biochemical Potency: <100 nM (at the K_m of ATP)
- Cellular target engagement: <1 μ M
- Selectivity: >30-fold versus a broad panel of kinases (with exception of closely related paralogues)
- Negative control: >100-fold weaker on kinase target
- Freely available

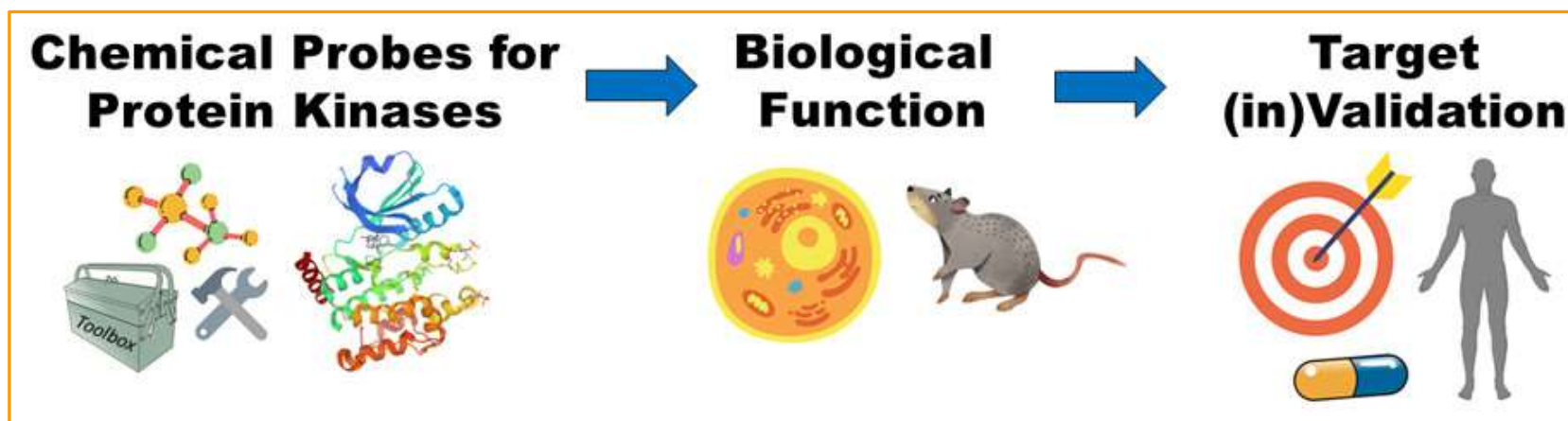
Chemical Probes as powerful and valuable tools in drug discovery

Chemical probes to inform target selection

...chemical probes comprise the most impactful of all classes of biomedical reagents, as judged by citations and usage.



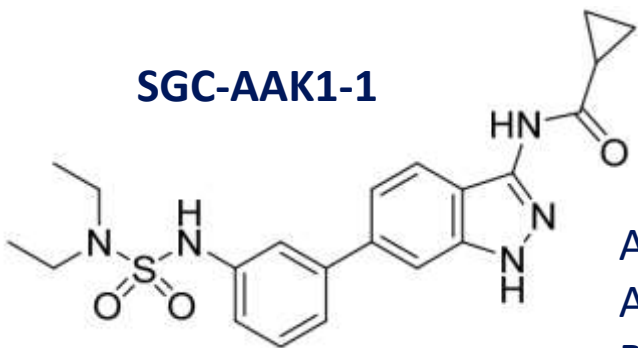
...open access to high-quality chemical probes can inform initial target selection.



High-Quality Chemical Probes used for Target Validation of Protein Kinases

AAK1 and BIKE/BMP2K

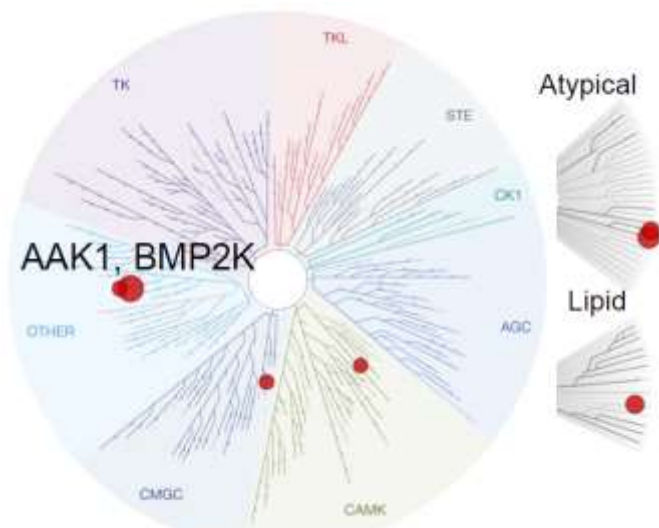
SGC-AAK1-1



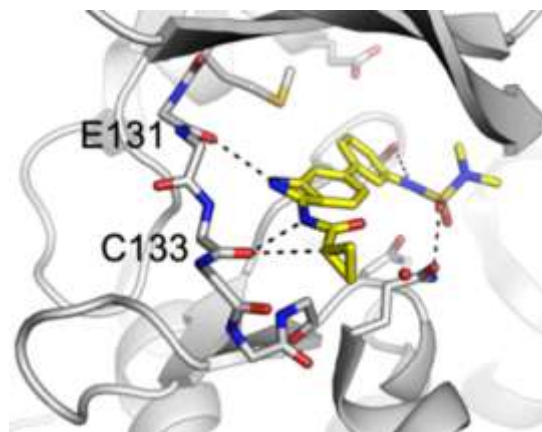
AAK1: $K_i = 9.1$ nM

AAK1: $IC_{50} = 240$ nM (nanoBRET)

BIKE: $K_i = 17$ nM



at **1 μ M** concentration and tested against over **400** wild-type human kinases



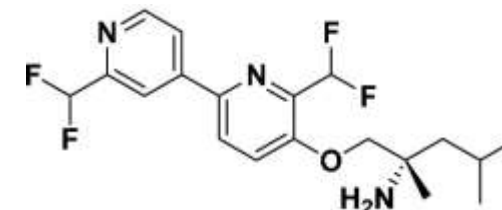
PDB code: 5I3O



Lexicon Pharmaceuticals Announces Topline Results from Phase 2b PROGRESS Study Evaluating Pilavapadin (LX9211) in Adults with Diabetic Peripheral Neuropathic Pain

March 3, 2025

Pilavapadin achieved meaningful pain reduction versus placebo and was well-tolerated in the 10 mg dose, meeting the Company's objectives for the study

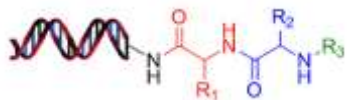


Pilavapadin/LX9211 (Lexicon)

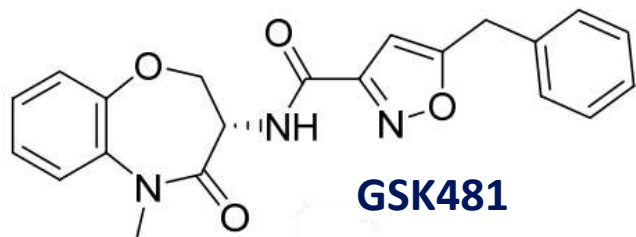
Clinical phase 2b

Diabetic Peripheral Neuropathic Pain

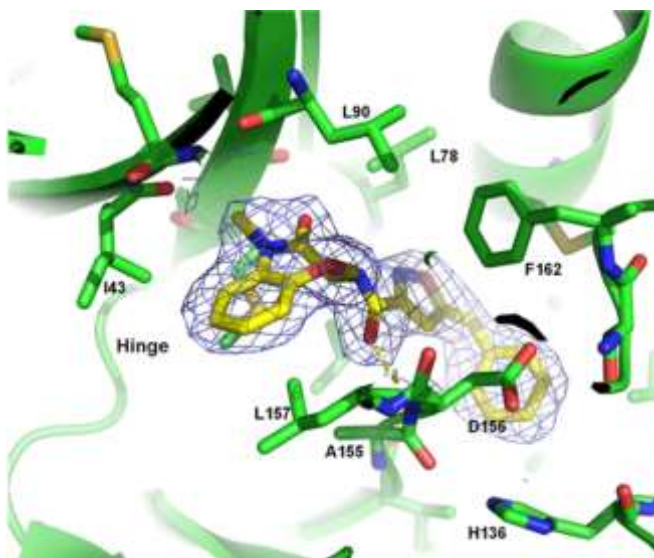
High-Quality Chemical Probes used for Target Validation of Protein Kinases



Identified by DEL screening (~7.7 billion cmpds)

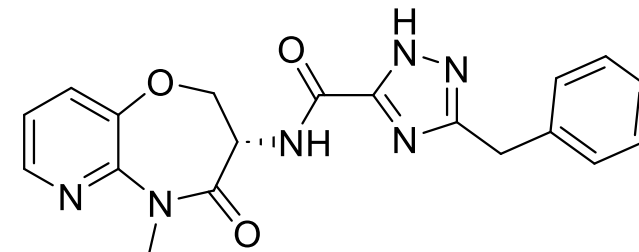


RIPK1: IC₅₀ = 1.3 nM
U937 cell: IC₅₀ = 10 nM



PDB code: 5HX6

RIPK1

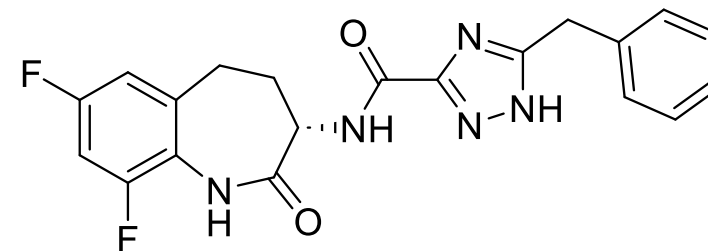


Eclitasertib/SAR443122 (Sanofi)

Clinical phase 2

Moderate to Severe Ulcerative Colitis

<https://clinicaltrials.gov/study/NCT05588843>

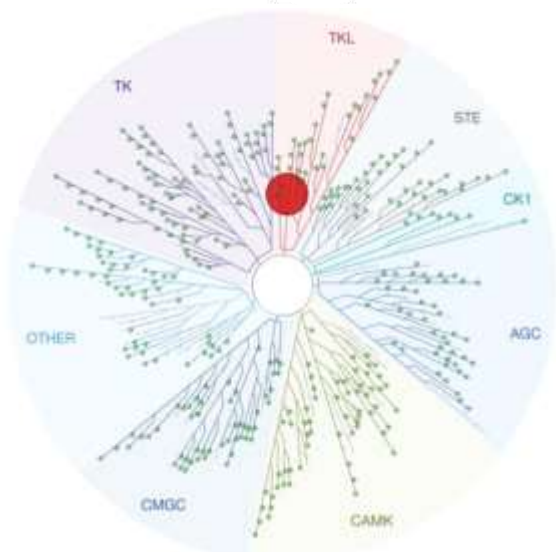


GSK095 (GlaxoSmithKline)

Clinical phase 1/2 – discontinued

Pancreatic ductal adenocarcinoma

<https://www.clinicaltrials.gov/study/NCT03681951>



Remarkable selectivity: no off-targets
at **10 μM** in two large kinase assay panels
(**318** kinases and **456** kinases)

High-Quality Chemical Probes used for Target **(In)**validation of Protein Kinases



SGC-CK2-1

new probe

*...is exquisitely selective
for CK2 α and CK2 α' at 1 μ M*

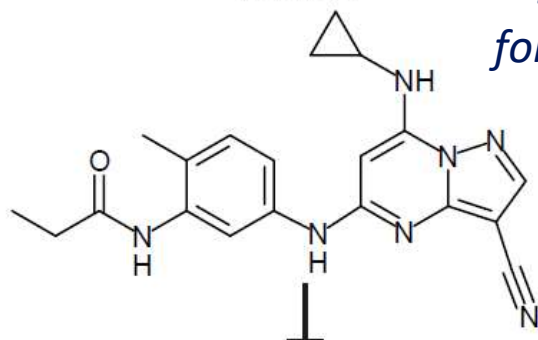
CK2

...unlike many previous studies demonstrated, **selective CK2 inhibition was not able to generate broadly antiproliferative effect...**

...Of the **140 cancer cell lines** tested, **SGC-CK2-1** only inhibited cell proliferation below 500 nM in **one cell line** (U-937)...

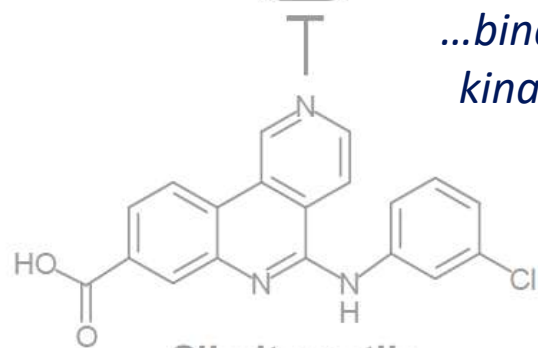
*...binds 28 human
kinases at 1 μ M*

Maybe CK2 is not really important in oncology, but now, using this chemical probe, this kinase could be better investigated in other fields!!!



CK2 α CK2 α'

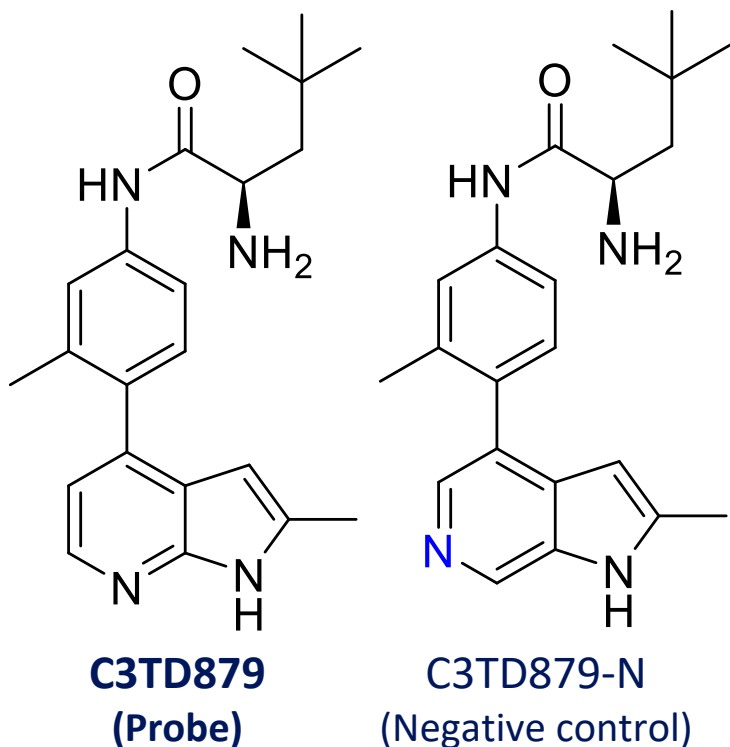
DYRKs CK2 α CK2 α' HIPKs
DAPKs GRKs PIMs CLKs
others



Silmitasertib

old probe

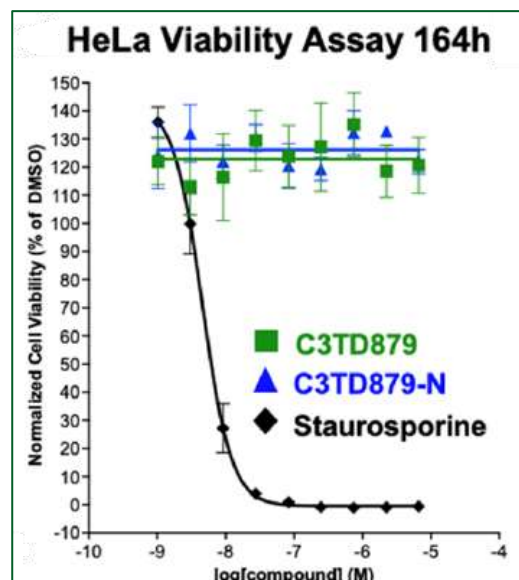
High-Quality Chemical Probes used for Target **(In)**validation of Protein Kinases



In a panel of 373 human kinases and follow-up biochemical dose-response assays, only 2 off-targets with $IC_{50} < 1 \mu M$.

CITK

...There are many in vivo and in vitro genetic studies suggesting CITK inhibition as a promising strategy for the treatment of different kinds of cancers...



- HeLa cells did not demonstrate antiproliferative effect
- No effect below $1 \mu M$ in a panel of 60 different tumor cell lines.

Structural features of CITK other than its catalytic function may play a more important role in cancer cell growth!!!

<https://www.chemicalprobes.org/>

Chemical Probes.org

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September 25

How sharing an inhibitor targeting the SOS1-RAS axis generated new insights in oncology and diabetes

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1 2

Q Search targets Select from list

Search targets (example: PARP1, PIK3CA)

*required field

EVALUATE PROBES FOR MY TARGET

ICR The Institute of
Cancer Research

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TECNIOspring

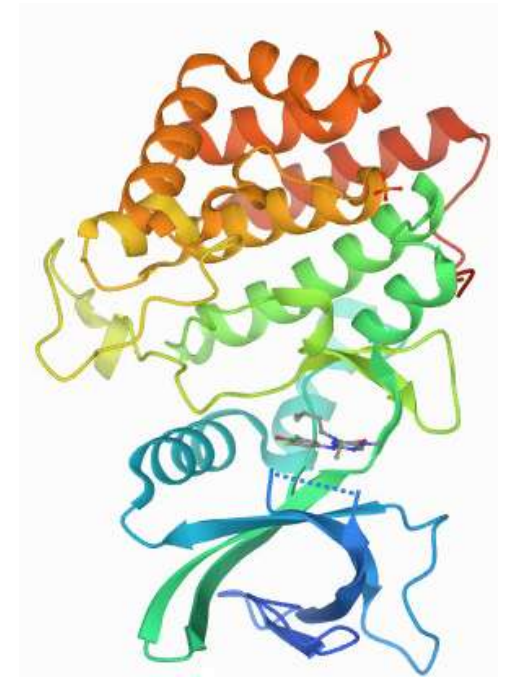
ACCIO Generalitat de Catalunya
Government of Catalonia

Vaccinia-related kinases 1 and 2 (VRK1 and VRK2)

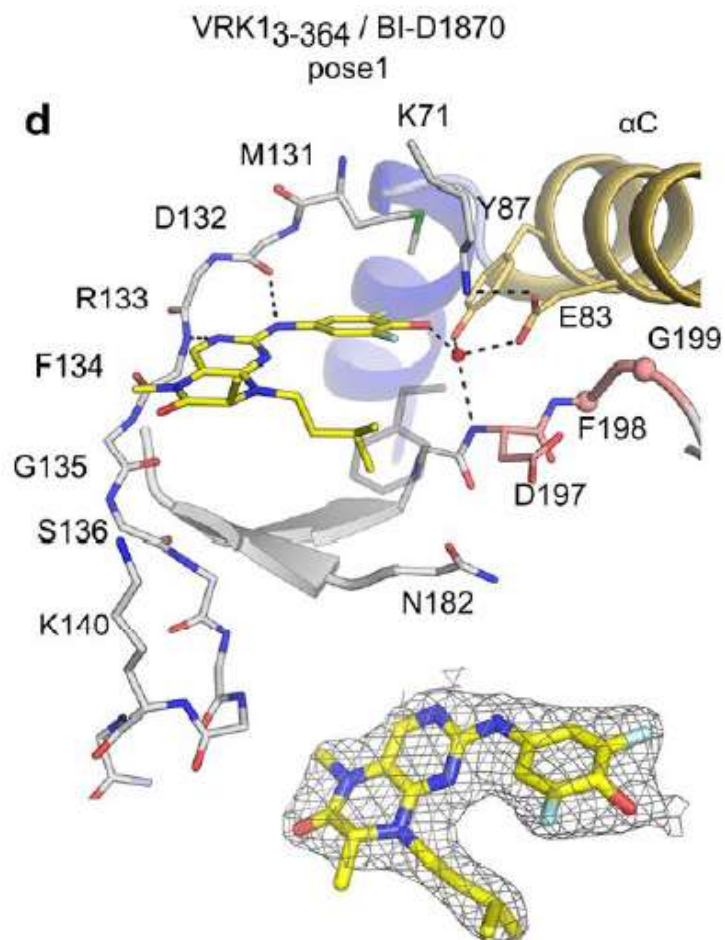
- **VRK1** is overexpressed in various solid tumors and has been linked to a poor prognosis in patients with breast cancer and glioma.

• *implicated in the DNA damage response and genome instability
(phosphorylation of p53 and H2AX)*

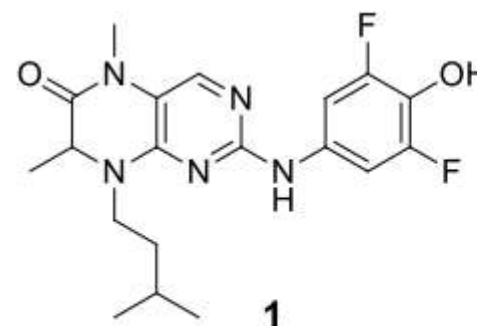
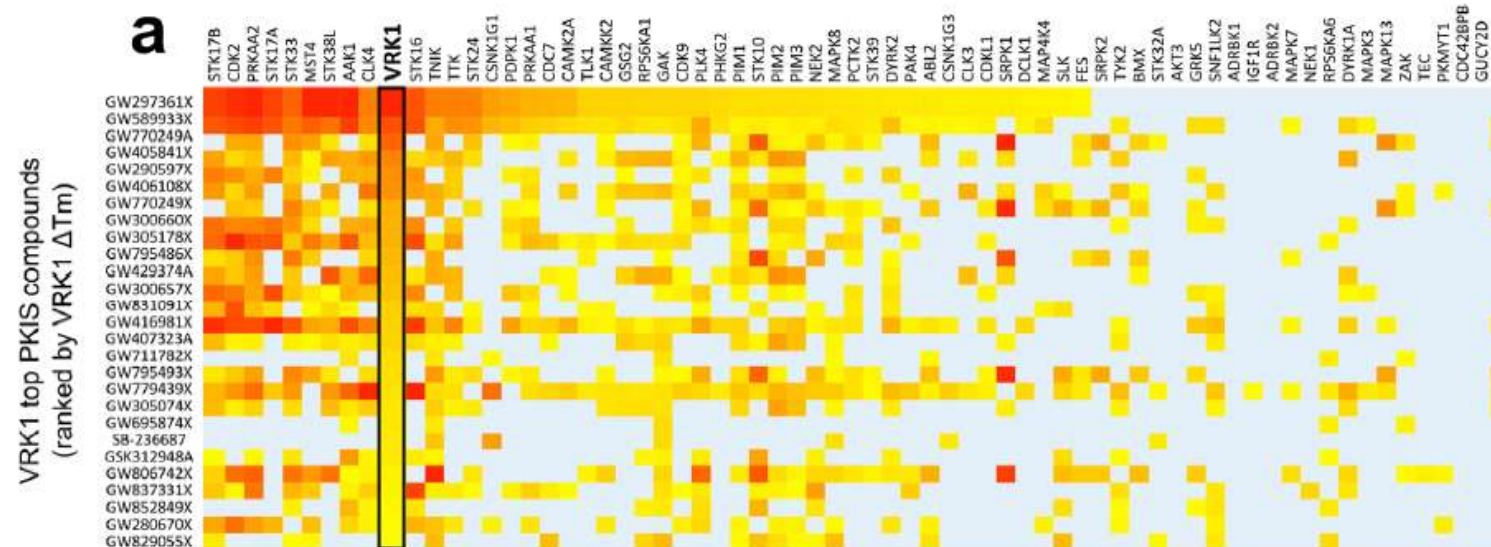
- **VRK2** biological role is less clear, but studies has also been demonstrated to phosphorylate p53 and its apoptosis downregulation effect.



Kinase Target: **VRK1**

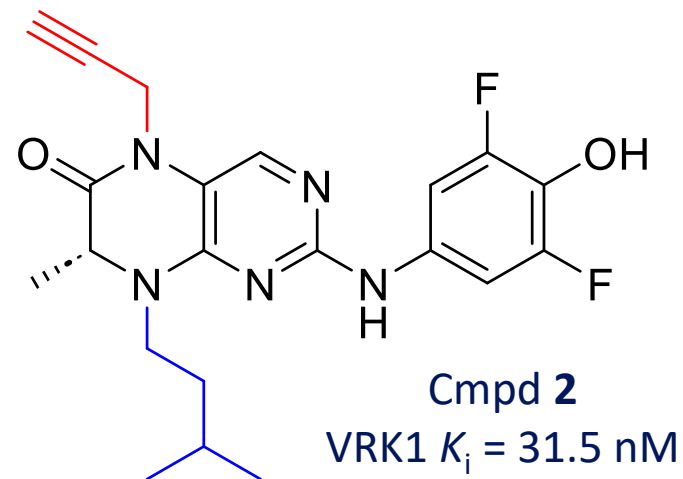
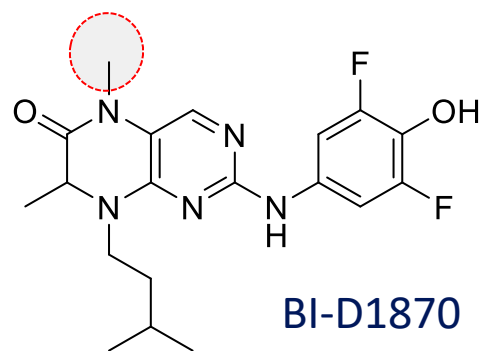
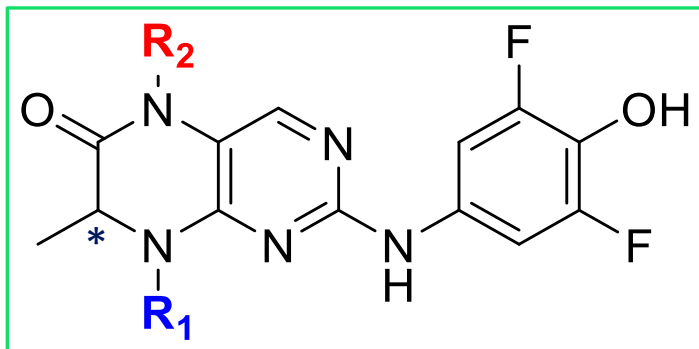


*The first X-ray crystal of a
VRK1/ligand complex!!! (PDB: 5UVF)*

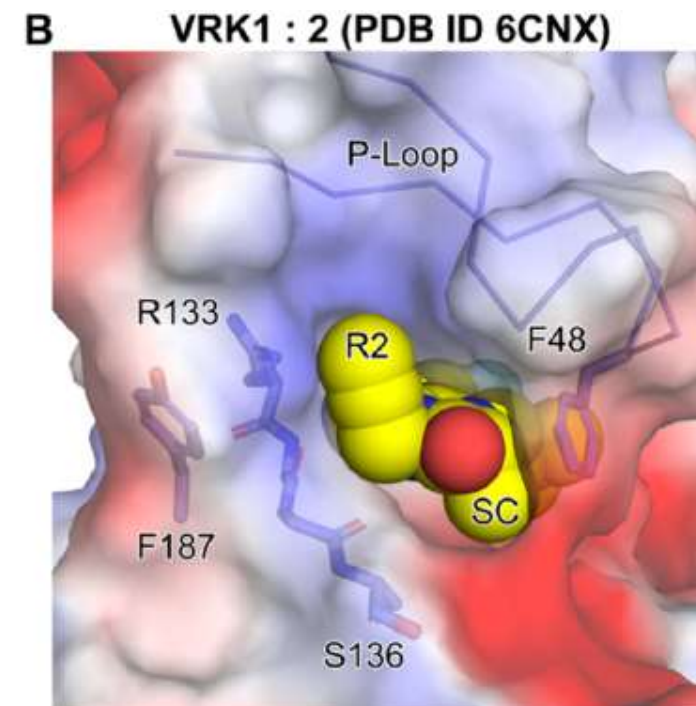
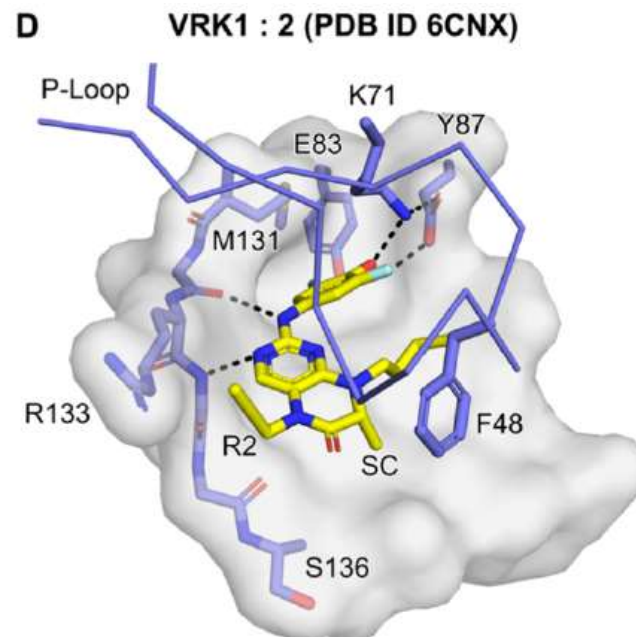


BI-D1870

Promiscuous p90 RSK inhibitor

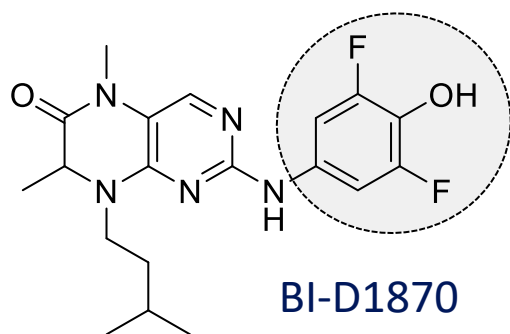
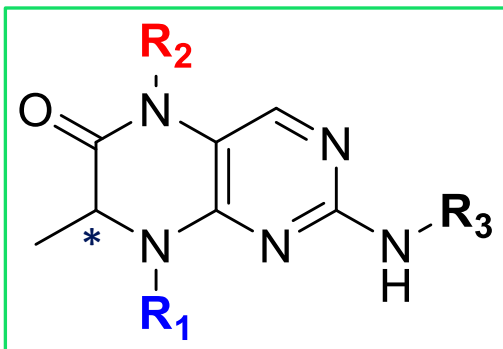


R2 propargyl group directed into the space between the hinge region and the kinase P-Loop.

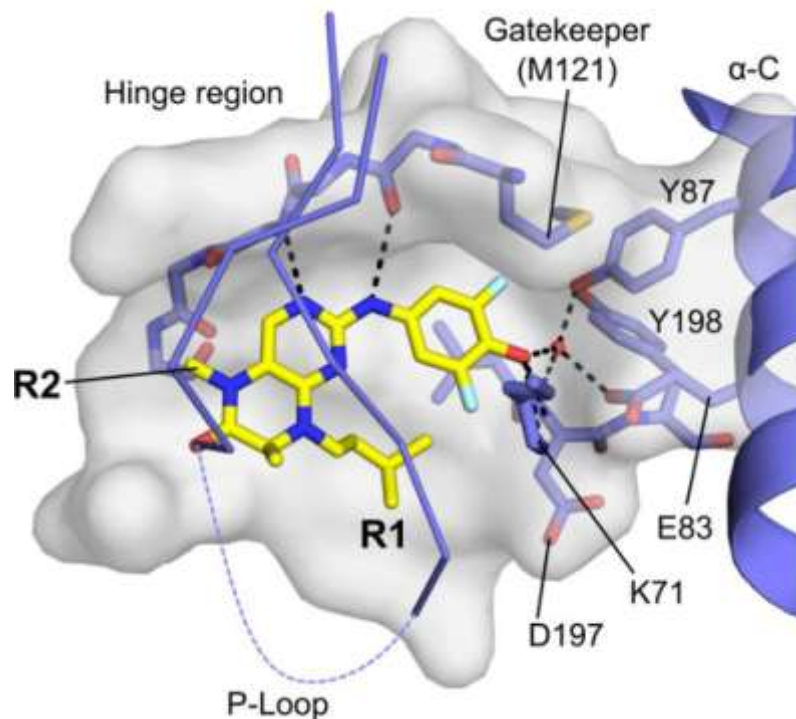


*>40 cmpds synthesized
and*

9 X-ray co-crystal structures



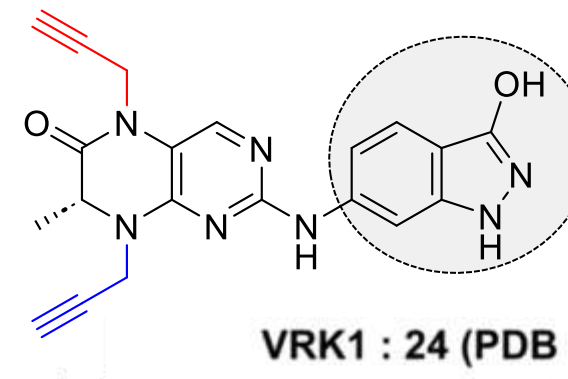
Canonical binding mode of BI-D1873
and VRK1 (PDB: 5UVF)



...presence of a structural water mediating
extensive hydrogen bond...

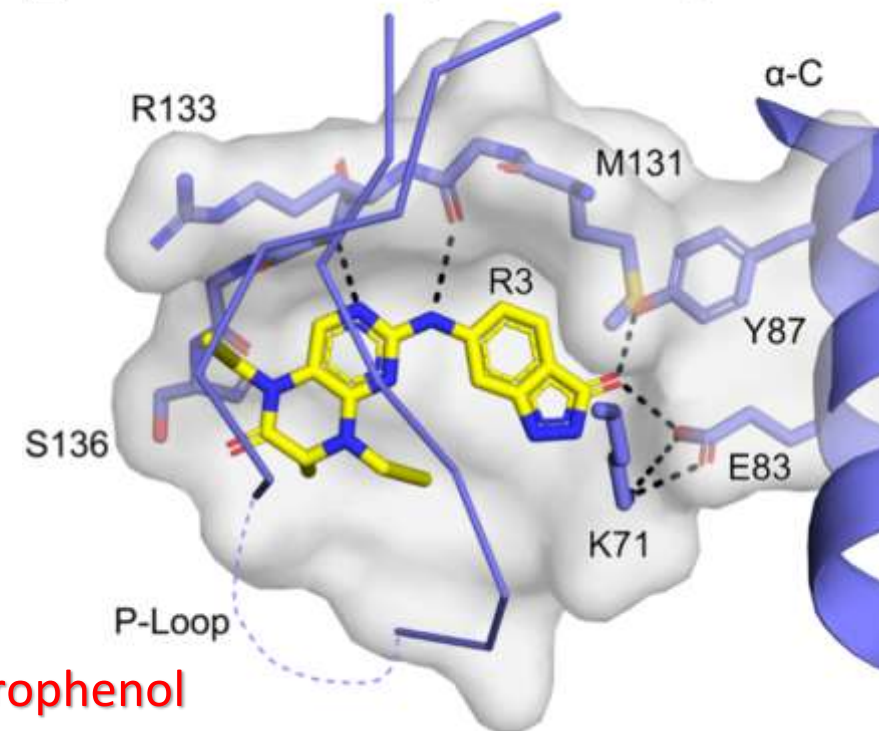


Aided by an *in silico* approach we replaced the difluorophenol
moiety 5,000 different commercially available R3 moieties...

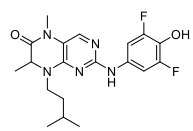
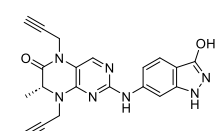


Cmpd **24**
VRK1 K_i = 17.9 nM

VRK1 : 24 (PDB ID 6NPN)

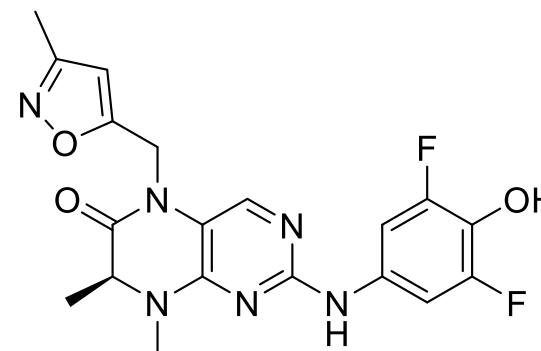


Kinome-wide Selectivity Profile for Selected VRK1 Inhibitors

	Entry	S_{50}^a
BI-D1870 	1	0.114
	2	0.163
	3	0.028
	7	0.031
	8	0.044
	11	0.075
	23	0.125
	24	0.119
	35	0.025
	36	0.024
	38	0.032
	39	0.038

^aDefined as the fraction of kinases tested having <50% residual activity.

1.0 μ M across 320 human kinases (ProQinase)



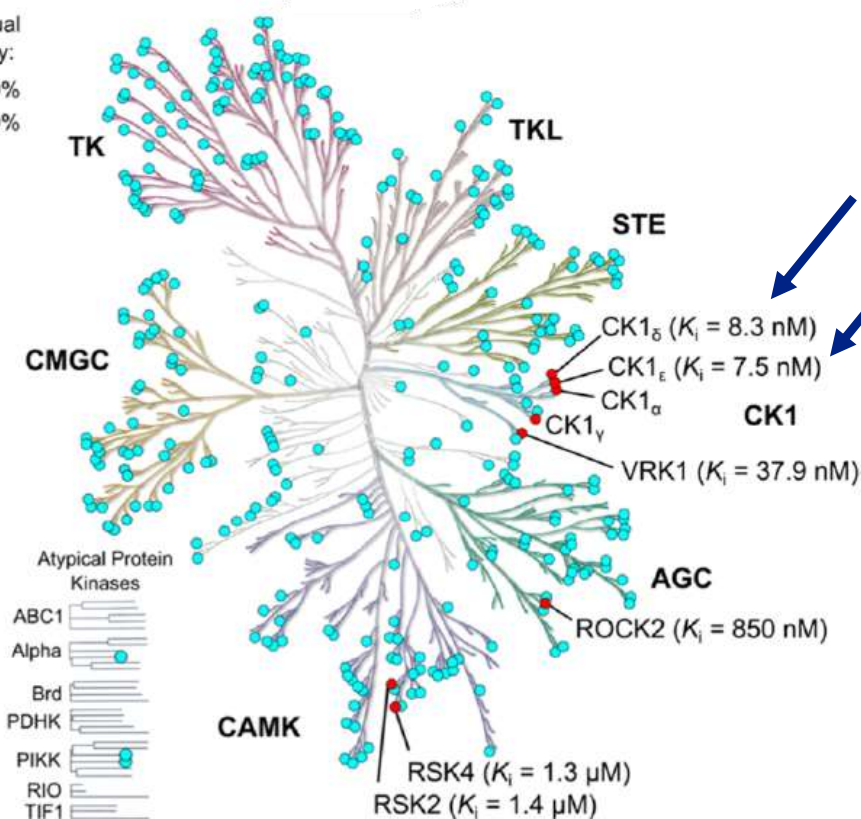
Cmpd 36

VRK1 K_i = 37.9 nM

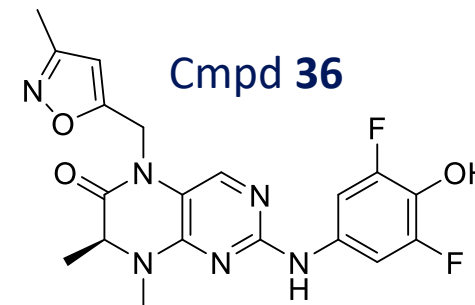
VRK2 IC_{50} = >3.5 μ M*

*accurate K_i determination not possible

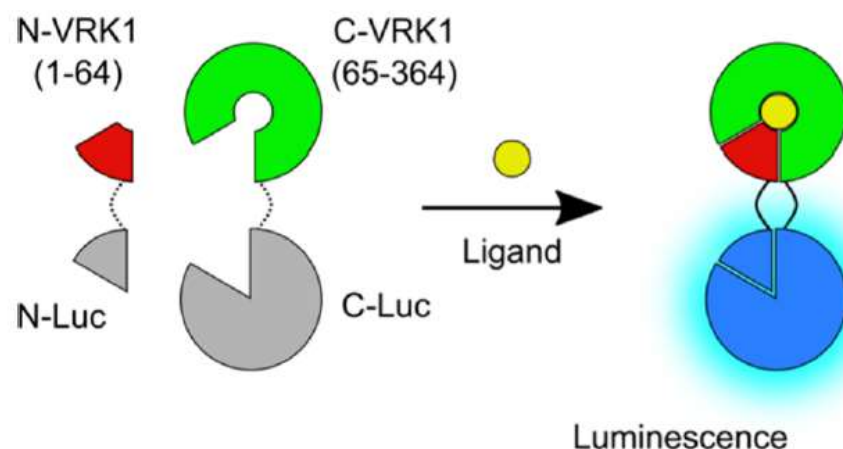
Residual activity:
● > 50%
● < 50%



Cmpd 36 engages with VRK1 in live, intact human cells

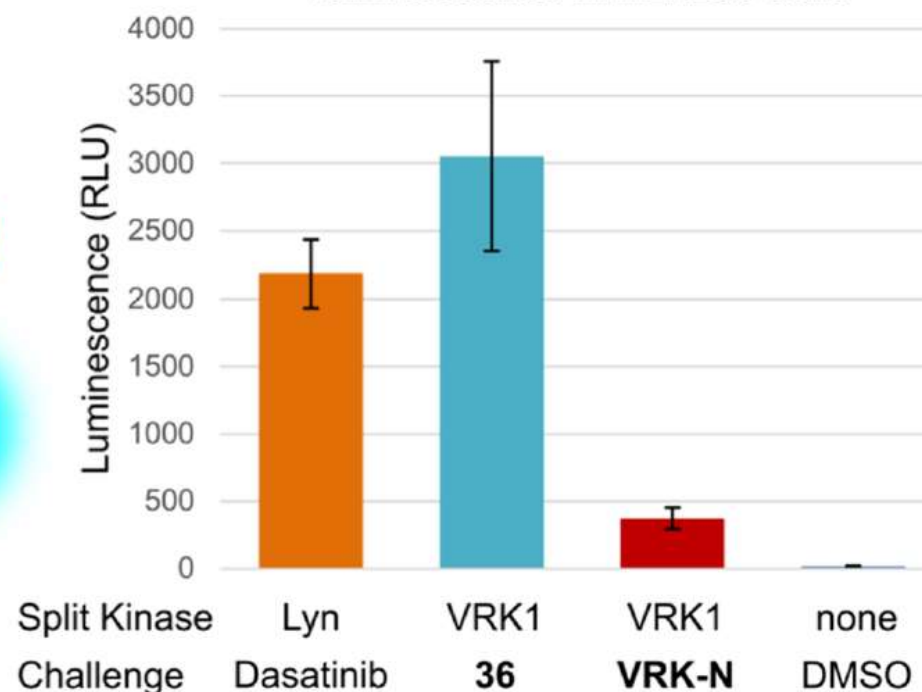


A Split VRK1 strategy



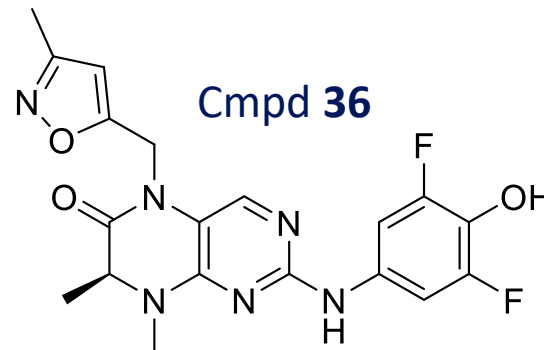
*Split kinase/split luciferase
complementation strategy for VRK1
cellular target engagement in live cell*

B Ligand binding-induced luminescence in HEK293 cells



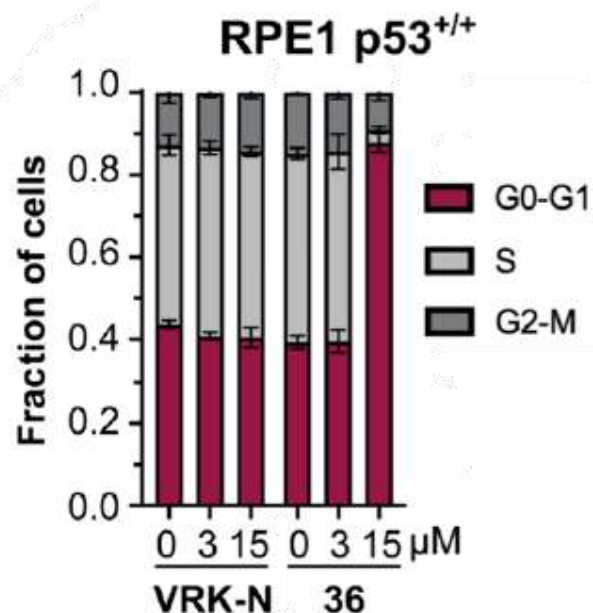
*Light emission by HEK293 cells cotransfected with plasmids encoding N and C-terminal VRK1/Luciferase fragments and treated with **36**, VRK-N (negative control) or **dasatinib** (positive control). Compounds were added at 30 μ M final concentration.*

	BI-D1870	Cmpd 36
Aqueous kinetic solubility (μM)	<1.6	31.0 ± 19.1
Metabolic stability in liver human microsomes ($t_{1/2}$ min)	32.2 ± 6.1	> 145

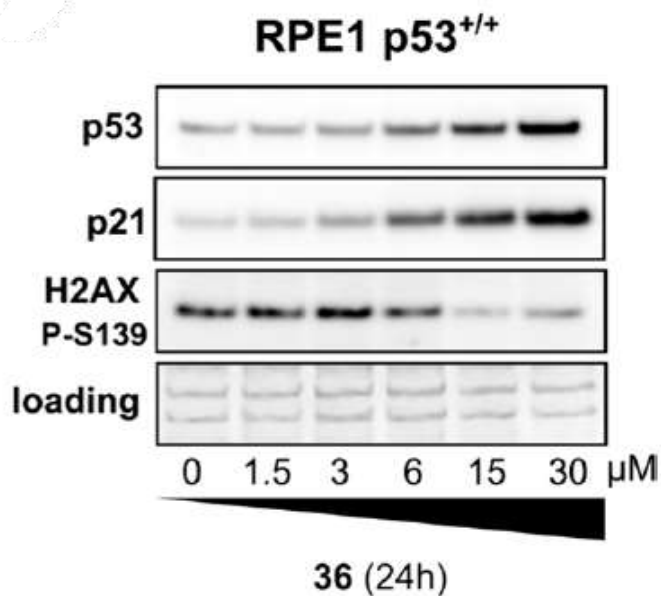


no significant inhibition in cytochrome P450 isoforms (1A2, 2C9, 2C19, 2D6, and 3A) at 10 μM .

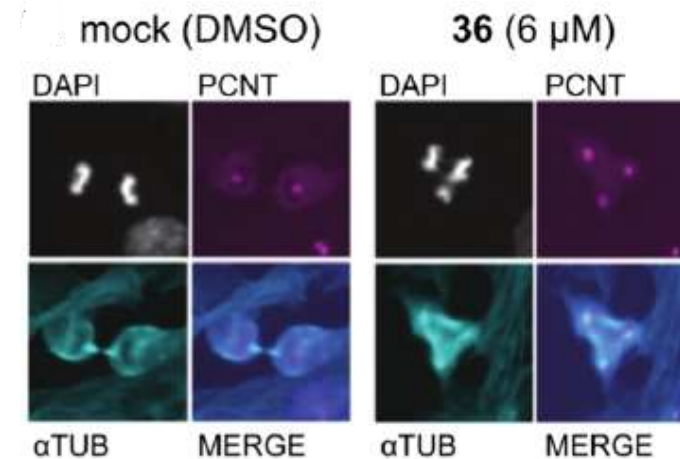
Cmpd 36 triggered cell cycle arrest and genome instability



36 triggered p53-proficient cells arrested in the G0/G1 phase after 24 h.

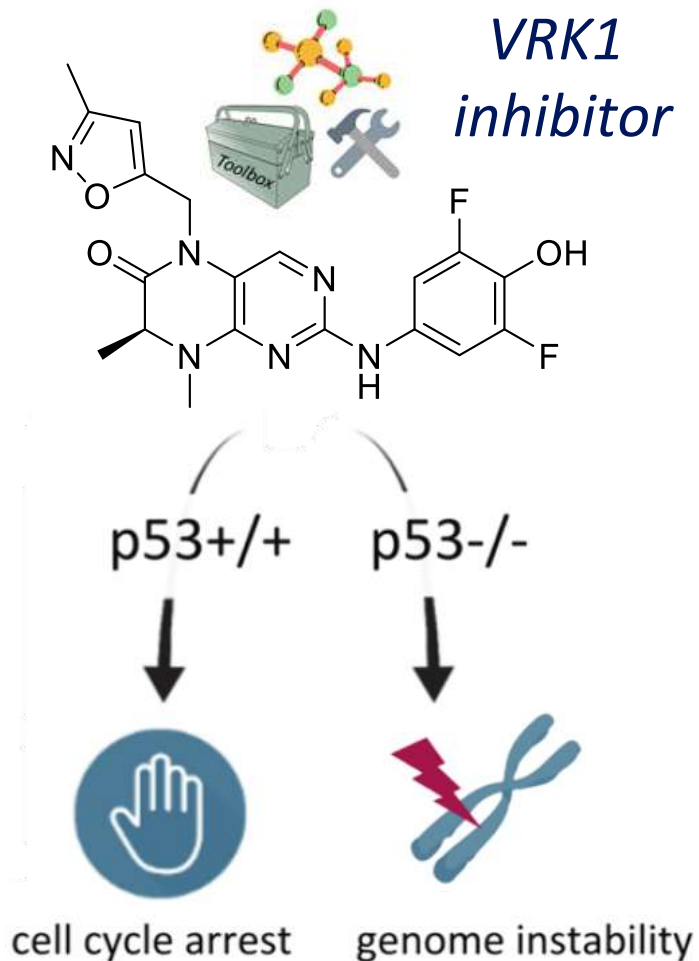


36 reduced the levels of phosphorylated histone H2AX in a dose-dependent manner



Microscopy images showing **36**-induced multipolar spindles and ensuing chromosome segregation defects in mitotic cells

Take home message!!!



Acknowledgements

We Never Walk Alone...



Fernando H. S. Gama
Hatylas Azevedo
Alessandra Mascarello
Cristiano R. W. Guimarães
Marcos A. Ferreira Jr.



Jonathan M. Elkins (*Oxford*)
Opher Gileadi (*Karolinska*)
Carina Gileadi (*Karolinska*)
Michael Hawgood (*Karolinska*)
Ann-Sofie Nilsson (*Karolinska*)
Bruno Urien (*Karolinska*)
Bennie B. L. G. Lemmens (*Karolinska*)
Dimitrios-Ilias Balourdas (*Frankfurt*)
Susanne Mueller (*Frankfurt*)
Stefan Knapp (*Frankfurt*)



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Thank You!!!
Moltes Gràcies!!!
iii Muchas Gracias!!!
Muito Obrigado!!!



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