Better Health, Brighter Future

Structure Guided Design and Optimization of Selective Kinase Inhibitors from Fragment Starting Points

Steve Woodhead

Streamlining Drug Discovery and Development : April 14th 2016

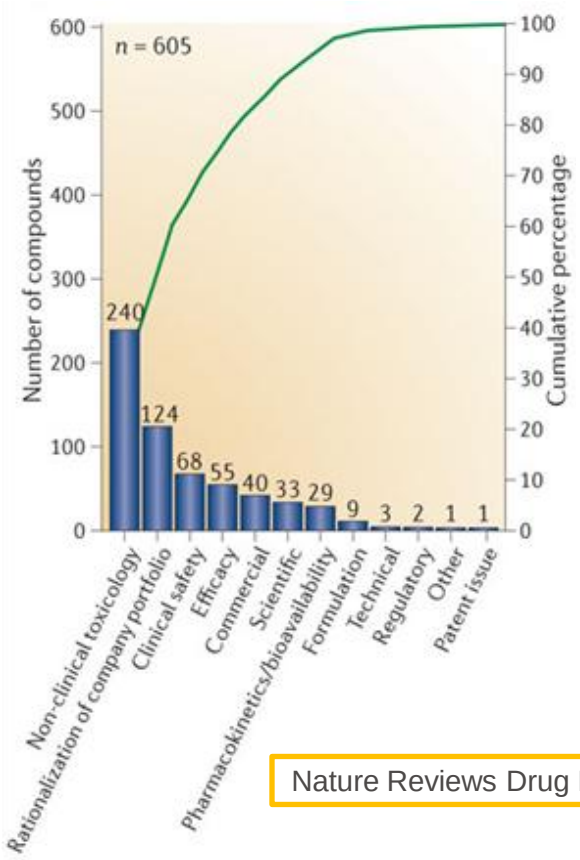
Takeda California

Outline

- Challenges in Kinase Drug Discovery
- Takeda's FBDD platform
  - Why start with fragments?
- Design of selective BTK inhibitors
- Same fragment, different kinase…

Challenges in Kinase Drug Discovery

- Attrition rates in small molecule R&D are high.
  - The most common reason for failure is non-clinical toxicology or clinical safety.



Nature Reviews Drug Discovery **2015**, 14, 475–486

Challenges in Kinase Drug Discovery

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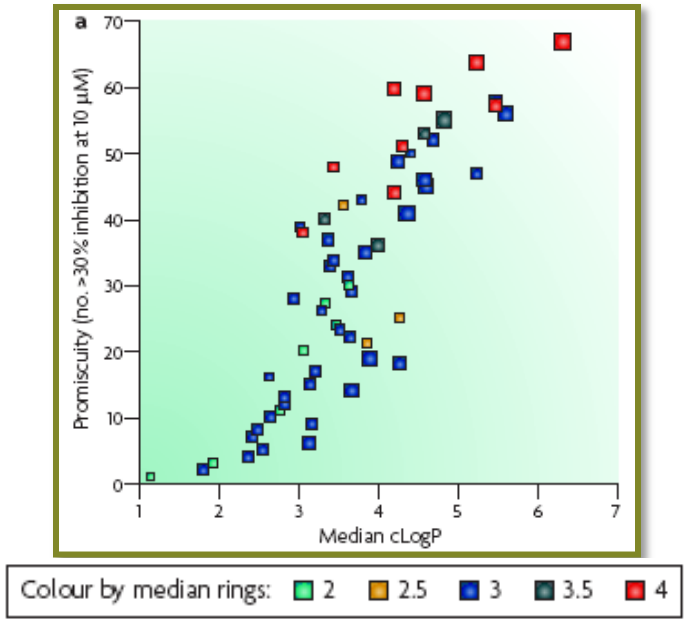
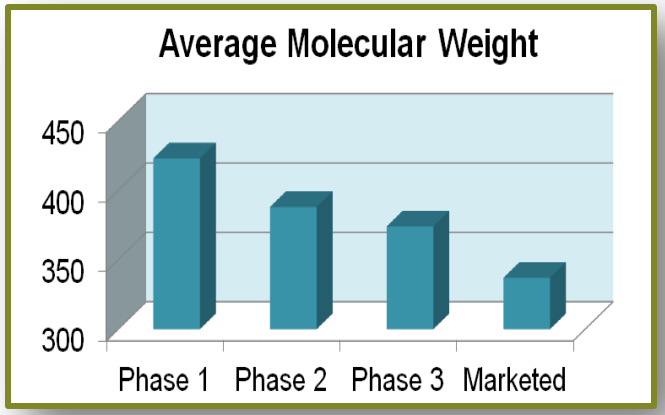
- Unwanted ‘off-target’ activity is often the source.
  - Undesirable Physicochemical properties



Taken from Leeson and Springthorpe
Nature Rev./Drug Disc. 2007, 6, 881-890.



A Comparison of Physiochemical Property Profiles of Development and Marketed Oral Drugs

Mark C. Wenlock,* Rupert P. Austin, Patrick Barton, Andrew M. Davis, and Paul D. Leeson

Departments of Physical & Metabolic Science and Medicinal Chemistry, AstraZeneca R&D Charnwood, Bakewell Road, Loughborough, Leicestershire, LE11 5RH, United Kingdom

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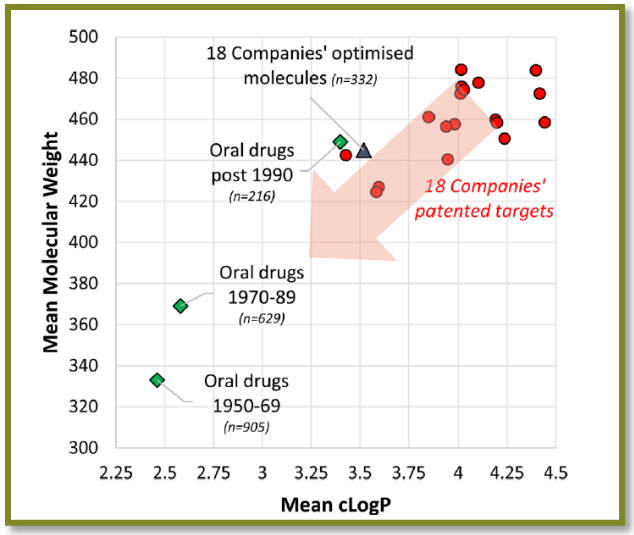
  - Undesirable Physicochemical properties

Molecular Property Design: Does Everyone Get It?

Paul D. Leeson*,† and Robert J. Young‡

†Paul Leeson Consulting Ltd., The Malt House, Main Street, Congerstone, Nuneaton, Warwickshire CV13 6LZ, U.K.
‡Medicines Research Centre, GlaxoSmithKline, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, U.K.



Challenges in Kinase Drug Discovery

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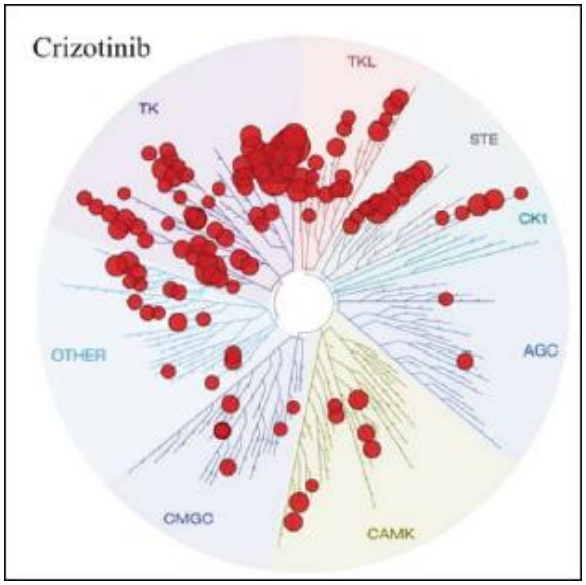


Taken from Nature Biotechnology 2011, 29,1046–1051

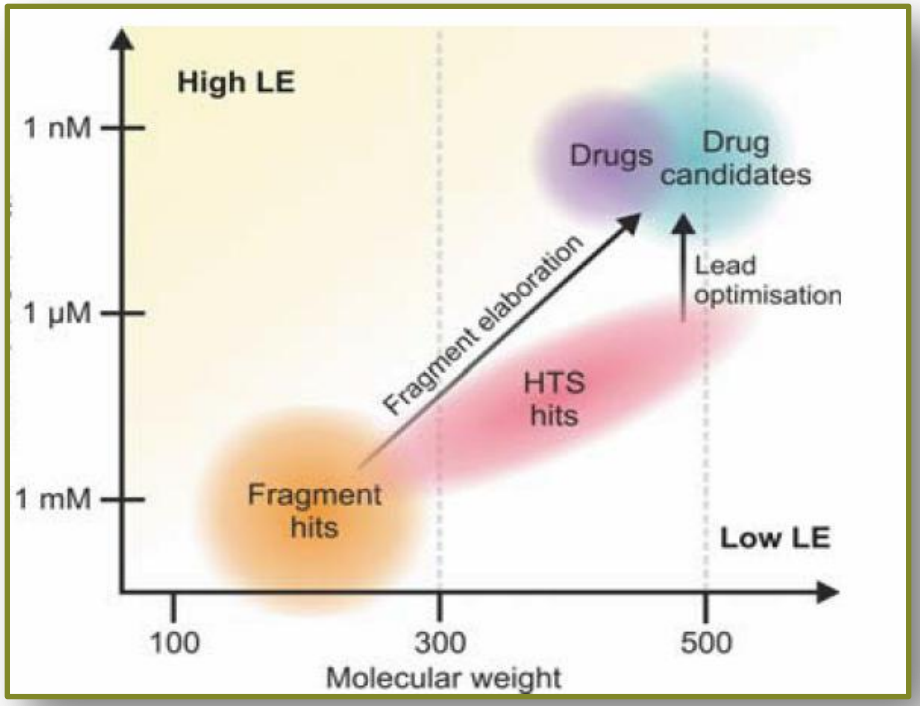
Challenges in Kinase Drug Discovery

- Attrition rates in small molecule R&D are high.
  - The most common reason for failure is non clinical toxicology or clinical safety.
- Unwanted ‘off-target’ activity is often the source.
  - Undesirable Physicochemical properties
  - All kinases share the same substrate…
- The majority of kinase inhibitors have low therapeutic margins.
  - Only one kinase drug is on the market for a non-oncology indication.

The Challenge, particularly outside of oncology, is to deliver selective inhibitors within desirable druglike property space.

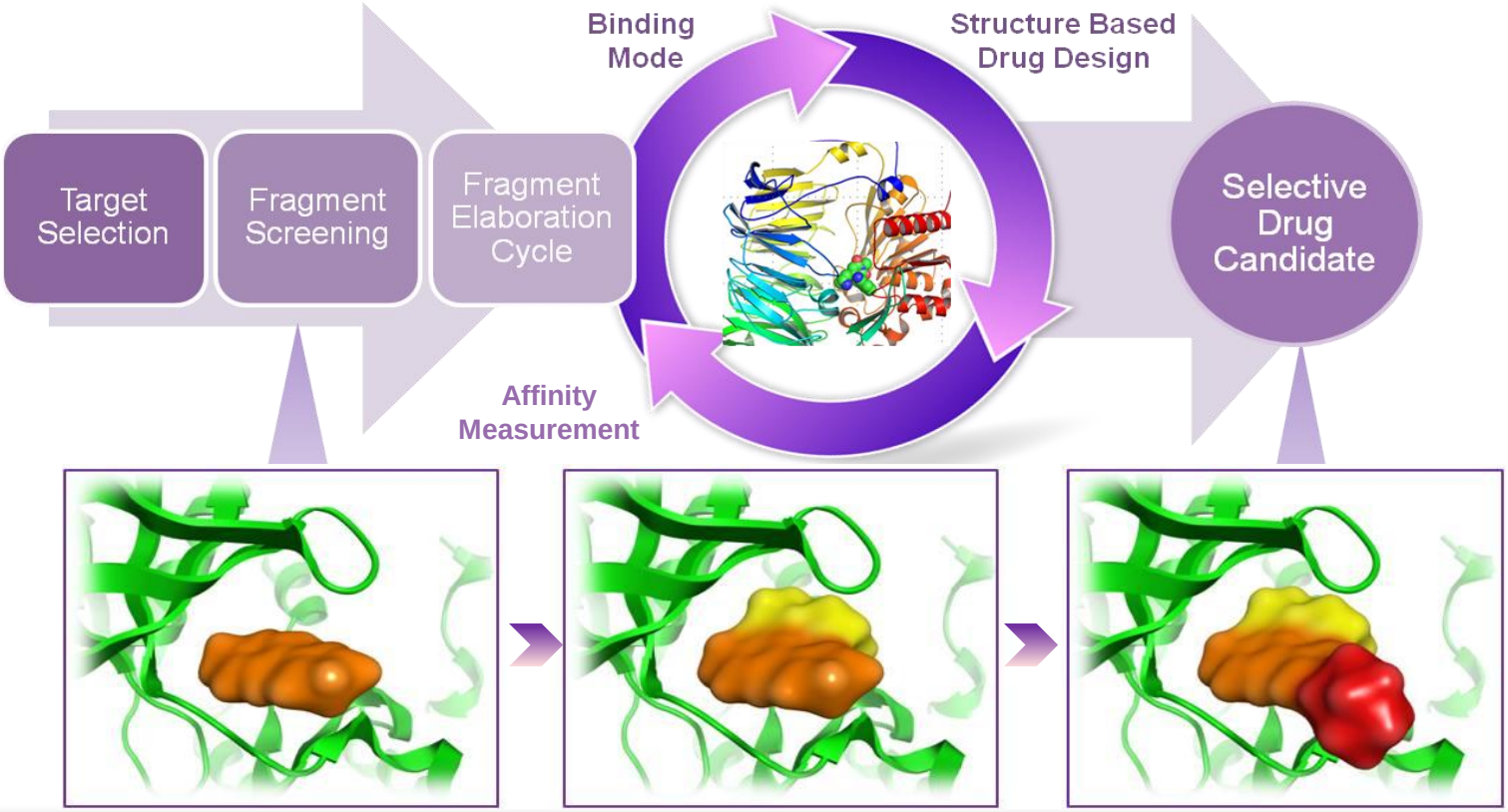
Why FBDD?

- Fragments are efficient binders.
  - Judicious fragment elaboration allows the optimization of molecules with desirable physical properties.



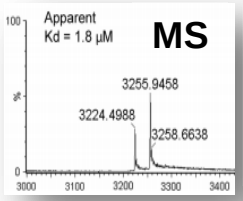
Why FBDD?

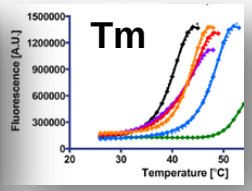
- Efficient optimization of fragments to drug candidates is enabled by crystallography and SBDD.
  - Opportunity to design for specificity.

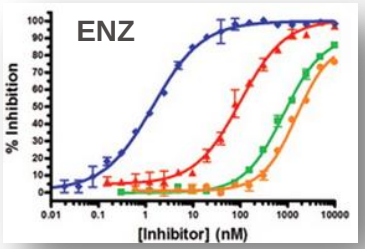


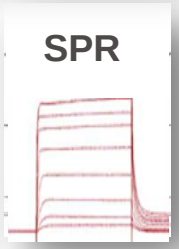
Takeda FBDD Platform and Capabilities

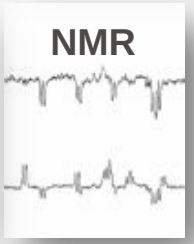
Biochemical & Biophysical Screening, Binding Kinetics and hit Characterization



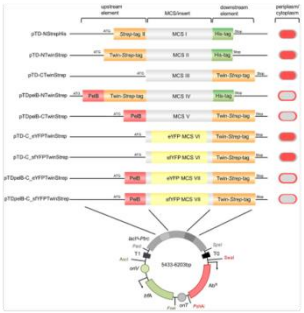




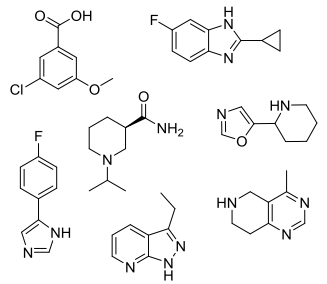




Protein production & Protein Construct Design



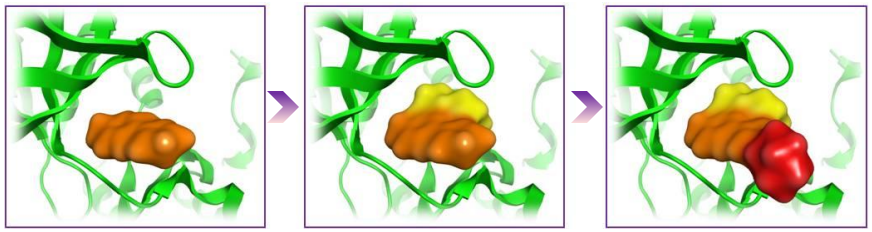
Fragment Libraries



BPL; 3D; 19F

Hits

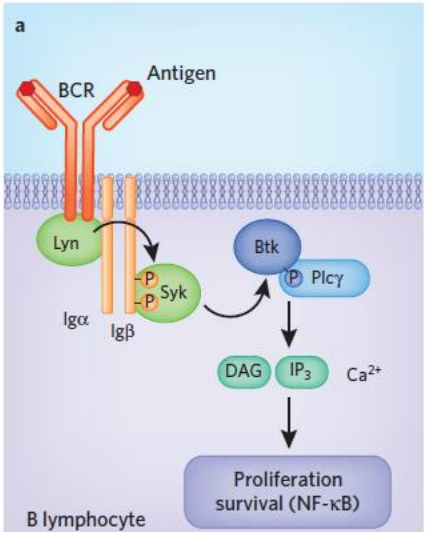
Crystallography & SBDD

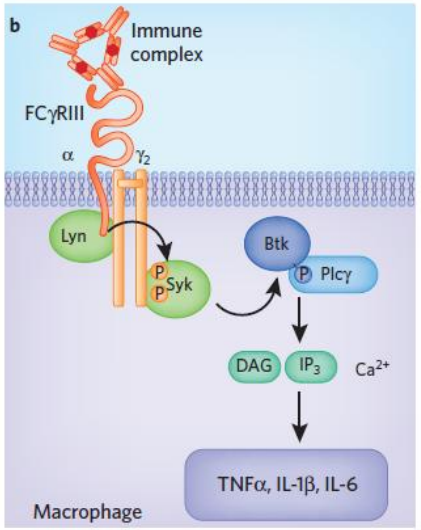


Leads

Tool Molecules: i.e. HTS, Literature

Bruton's Tyrosine Kinase - Btk



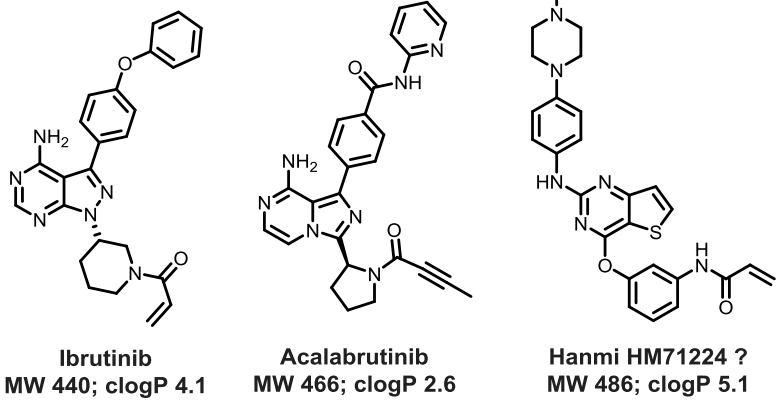


- Tec family kinase required for B-cell receptor signaling in B cells and FCγ receptor signaling in myeloid cells
- Aberrant signaling through Btk implicated in the pathogenesis of diffuse large B-cell lymphoma, mantle cell lymphoma and chronic B-cell leukemia
- Ibrutinib (PCI32765) approved for the treatment of mantle cell lymphoma
- Btk has high structural similarity with LCK (SRC family kinase anti-target)
- Btk is not expressed in T cells. LCK is expressed in T cells. Selectivity over LCK was sought to avoid T cell driven pharmacology.

Hendrix, *Nat Chem Biol*. **2011**
Kuppers, *Nature Rev. Cancer,* **2005**, 5, 251-62

Published Lead Series and Team Objective

- Published Btk inhibitor series were either covalent or high molecular weight >500 Da and with low ligand efficiency ≤ 0.3

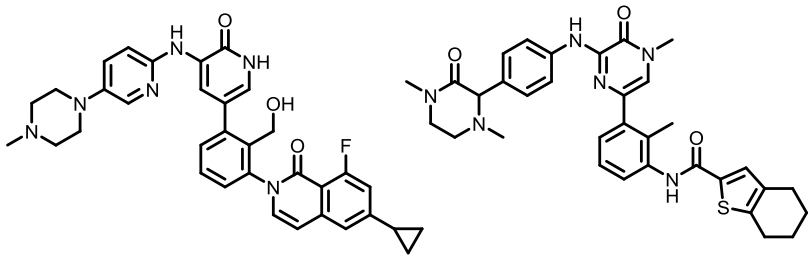


Ibrutinib
MW 440; clogP 4.1

Acalabrutinib
MW 466; clogP 2.6

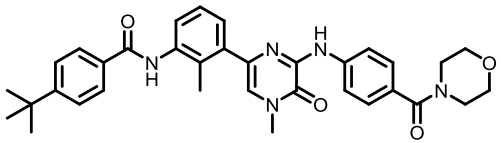
Hanmi HM71224 ?
MW 486; clogP 5.1

Irreversible inhibitors



RN486 Roche
Mwt 592 Da; clogP 3.9
Btk Kd 0.3 nM
LE 0.30 LLE 5.6

GDC0834 Gilead
Mwt 596 Da; clogP 5.0
Btk IC_{50} 6 nM
LE 0.27 LLE 4.1



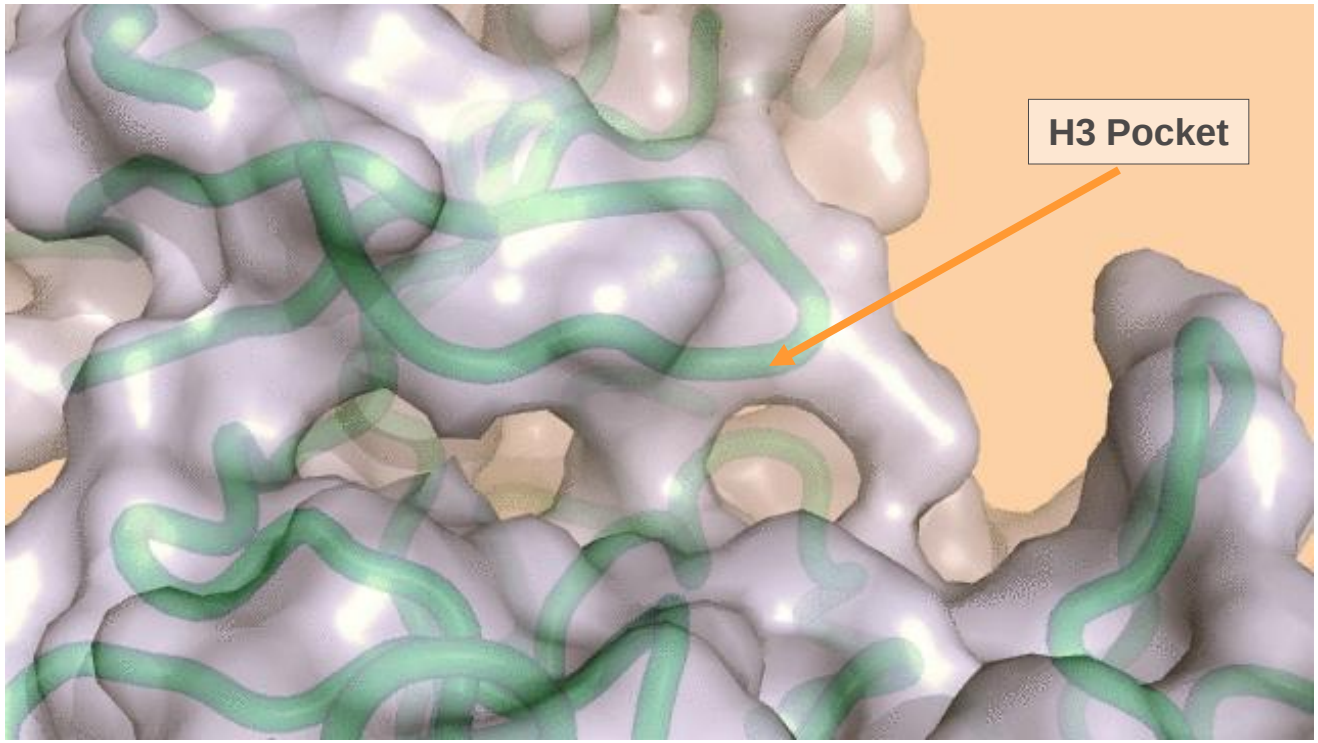
CGI1746
Mwt 579 Da; clogP 4.1
Btk IC_{50} 6 nM
Lck IC_{50} inactive
LE 0.26 LLE 4.1

Reversible inhibitors

Could we deliver a reversible Btk inhibitor with high selectivity against LCK with a molecular weight ≤ 400 Da? (Ro4 compliant)

Btk Conformational Changes & LCK Specificity

- H3 pocket is formed by the A-loop in Btk but not in Lck. Occupying H3 pocket provides specificity against Lck



- Other kinases cannot adopt this conformation which allows CGI1746 molecule to bind both the hinge and occupy the H3 pocket putatively resulting in the observed specificity.

Screening Strategy and Results

Flow Scheme

Single point screen of SURF library at 200μM Caliper assay

↓

IC_{50} on hits ≥ 40% inhibition

↓

Confirmation STD ^{1}H NMR

↓

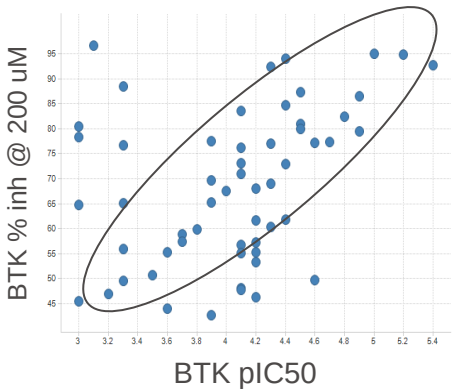
X-ray crystallography

Results

506 Hits 4.6% hit rate

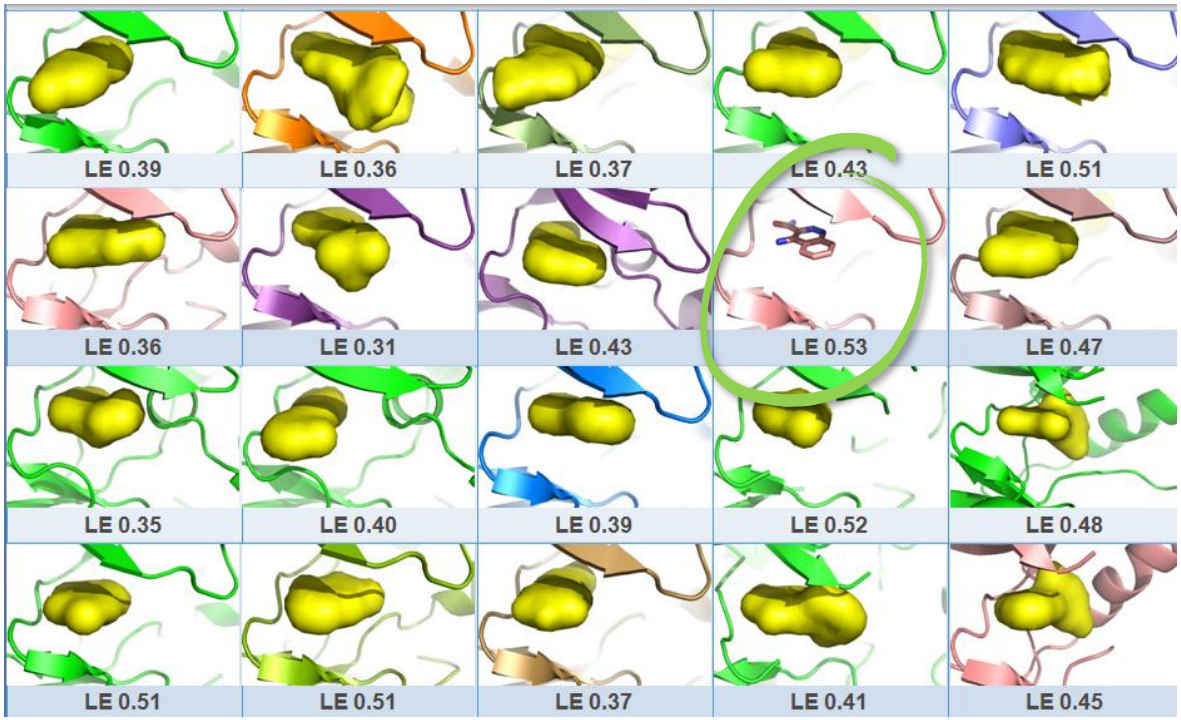
↓

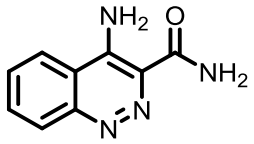
IC_{50} on 50 compounds



20 fragment crystal structures

20 Diverse Fragment Structures





Cinnoline fragment 1 had the highest LE and largest -ΔH component

Hit Prioritization

X-ray crystallography

↓

Vector analysis filter

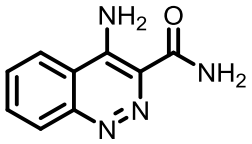
↓

ITC Experiments

↓

Rank by -ΔH and LE

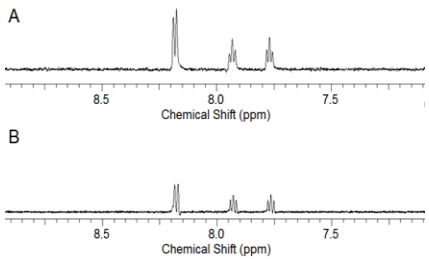
Cinnoline Hit Characterization and Growth vector



Cmpd 1

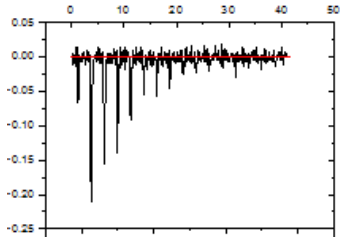
Fragment Hit

Btk IC_{50} 3.5 μM
LE 0.53



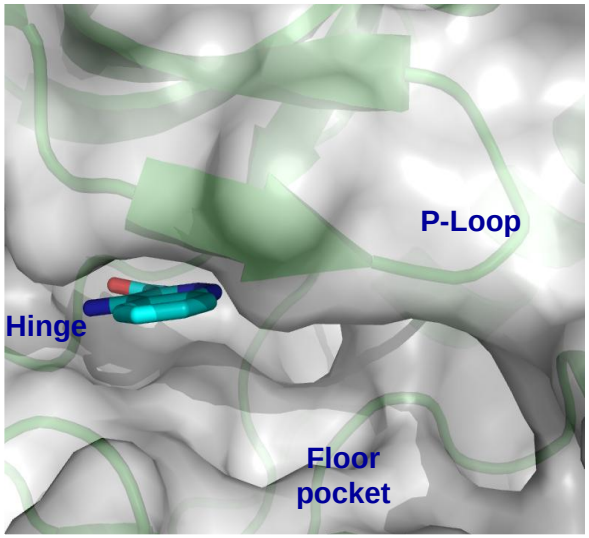
STD ^{1}H NMR

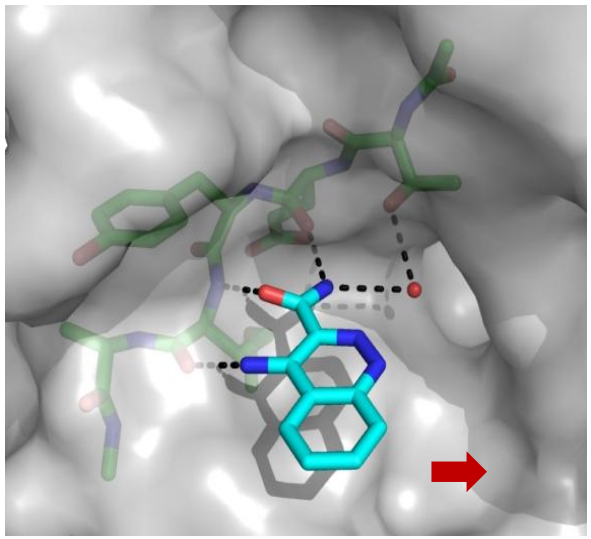
25% saturation of signal



Isothermal Calorimetry

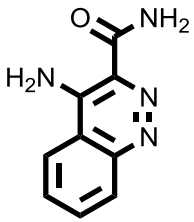
Btk Kd 2.7 μM
ΔH -11.4 Kcal/mol



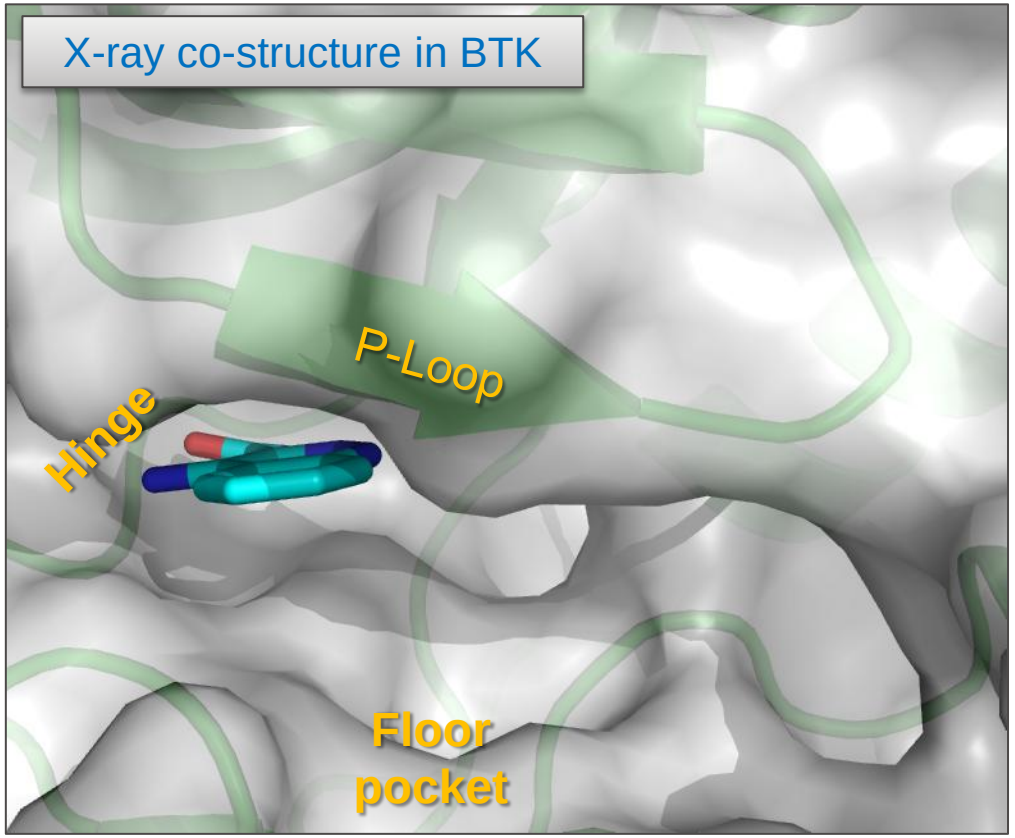


X-ray Co-crystal Structure – Btk kinase domain

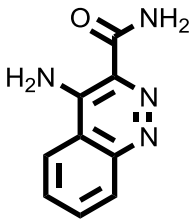
Btk Cinnoline Series



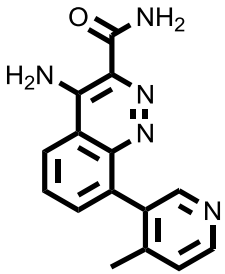
Fragment Hit
Cmpd 1
Btk IC_{50} 4 μM
LE .53



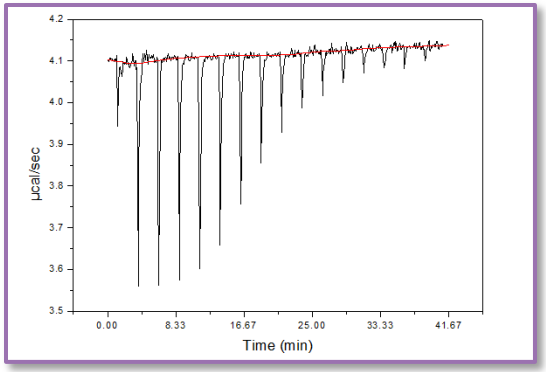
Btk Cinnoline Series

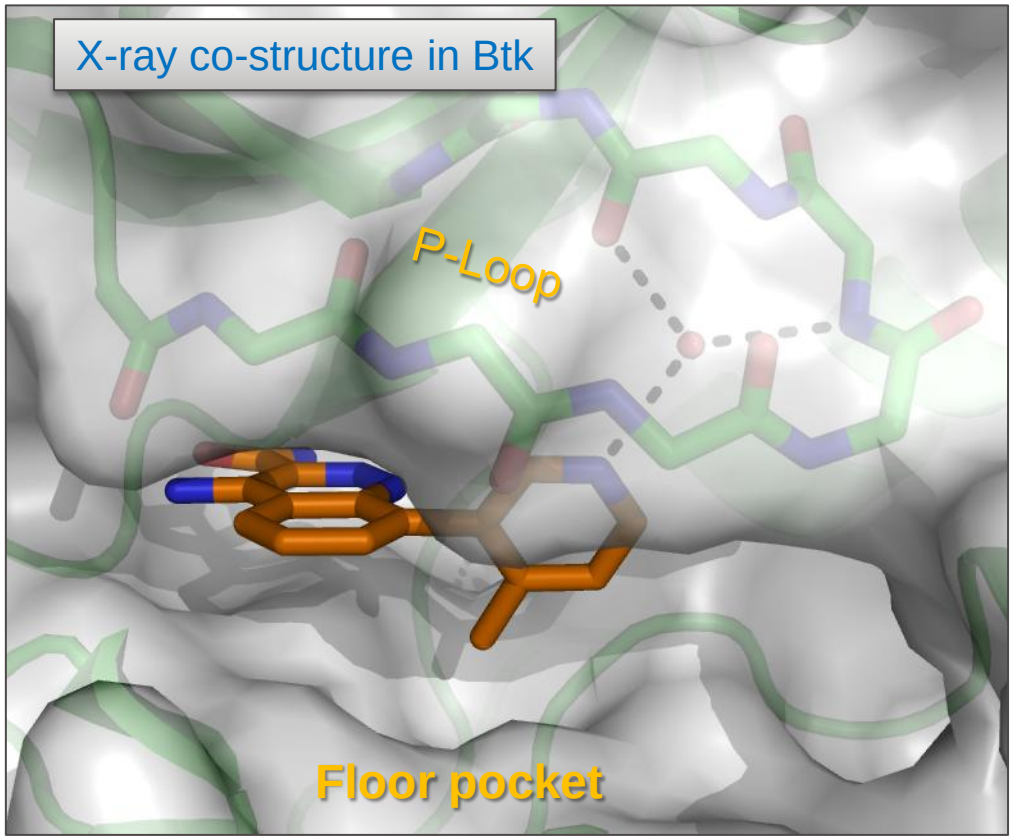


Fragment Hit
Cmpd 1
BTK IC_{50} 4 μM
LE .53



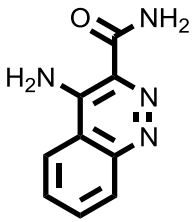
Cmpd 2
Btk IC_{50} 100nM
Lck IC_{50} 6300 nM
LE 0.45
ITC
ΔH -16.8 Kcal/mol
ΔS -22.1 Kcal/mol



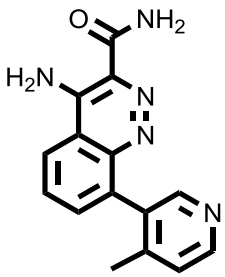


The binding of Compound 2 to BTK is driven by enthalpy, but not entropy.

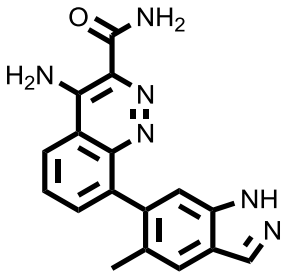
Btk Cinnoline Series



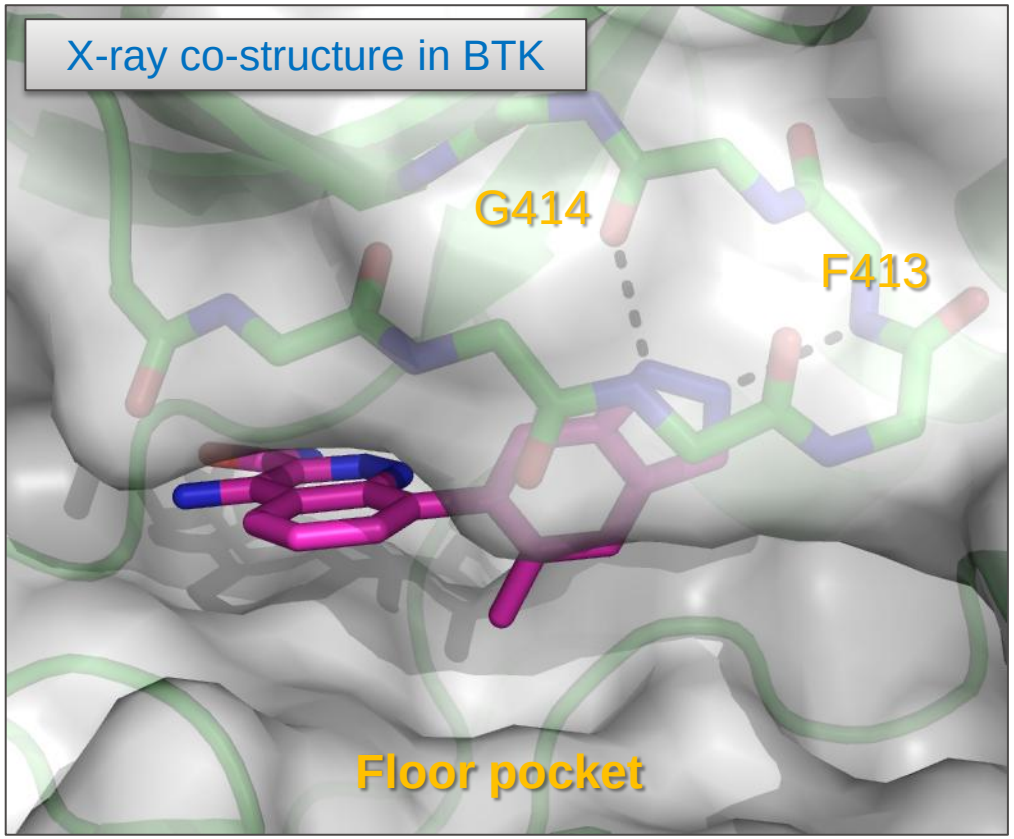
Fragment Hit
Cmpd 1
BTK IC_{50} 4 μM
LE .53



Cmpd 2
BTK IC_{50} 100nM
LCK IC_{50} 6300 nM
LE 0.45

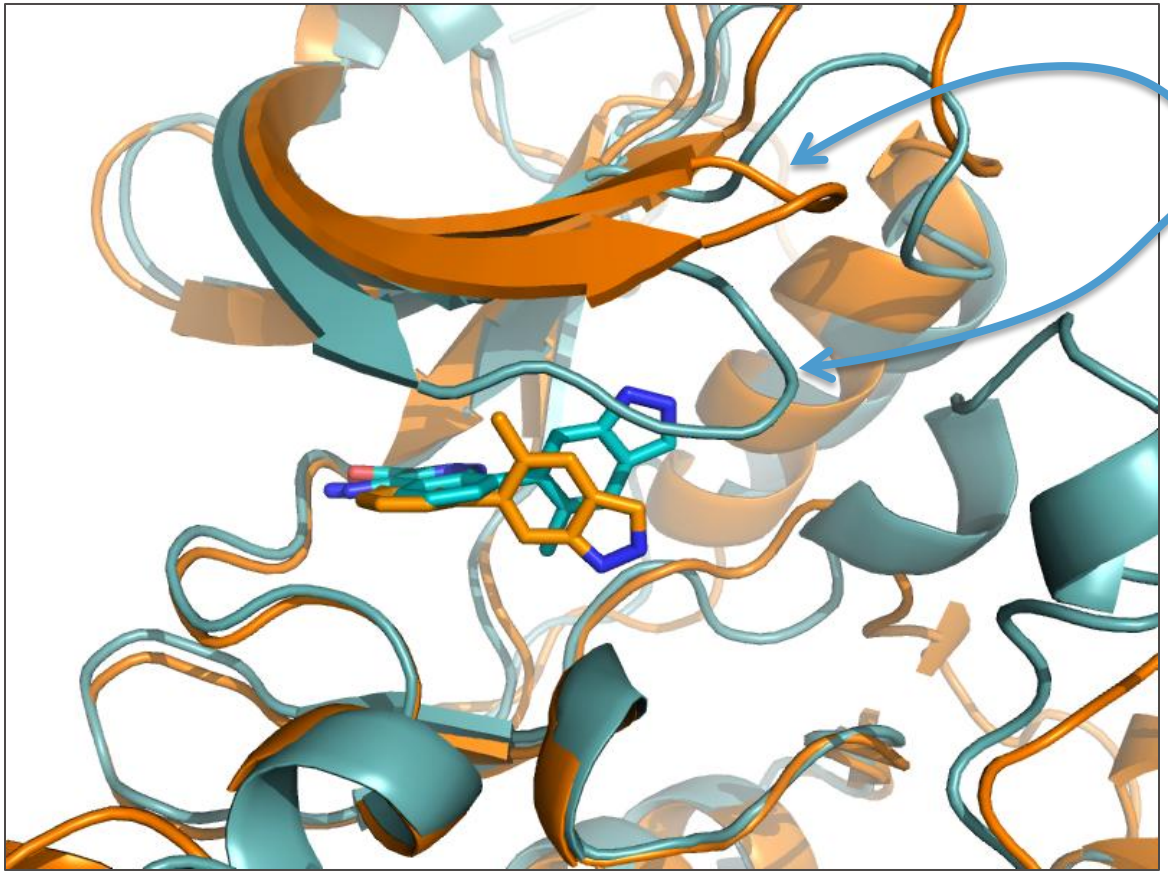


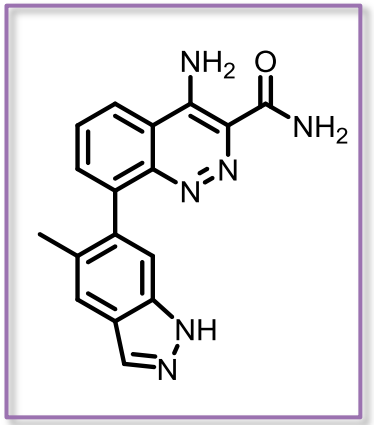
Cmpd 3
Btk IC_{50} 4nM
LCK IC_{50} 412 nM
LE .48



~103X selective over Lck
MW 318

Compound 3 in Btk and Lck



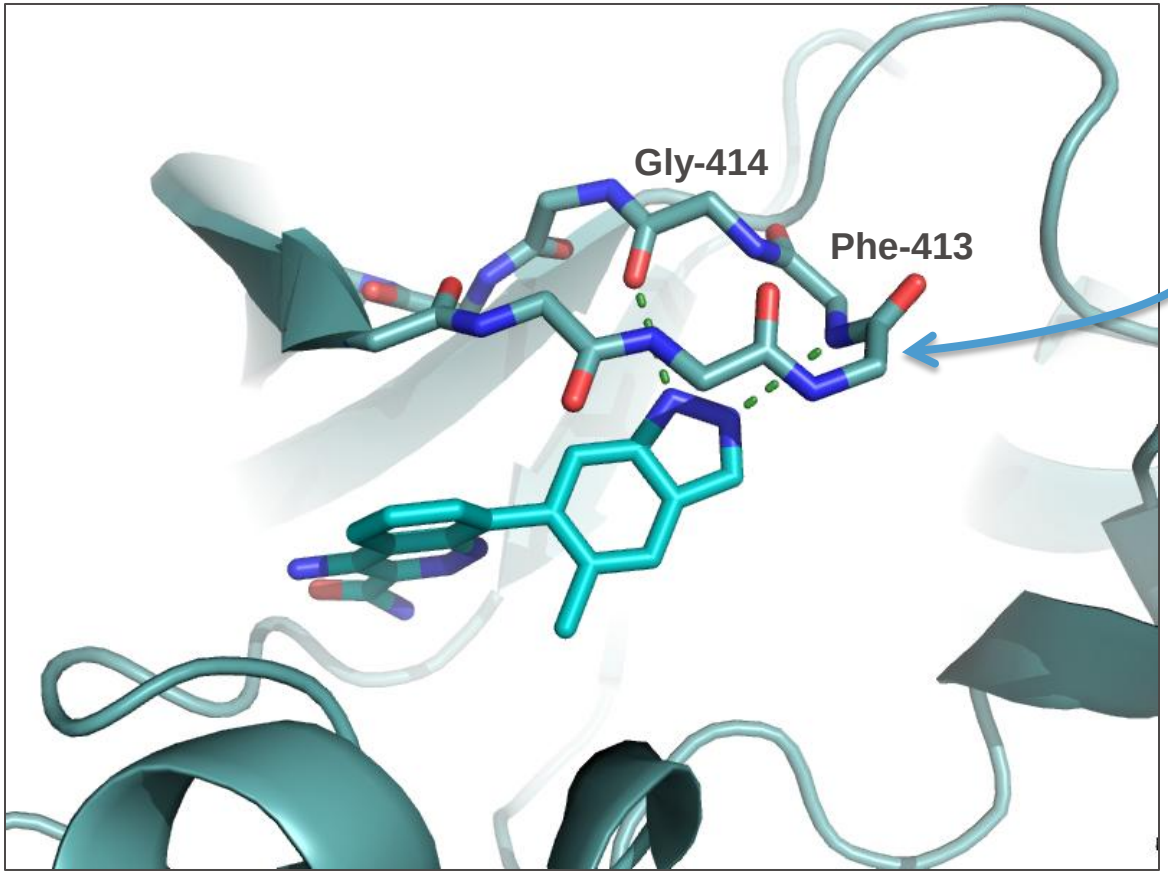


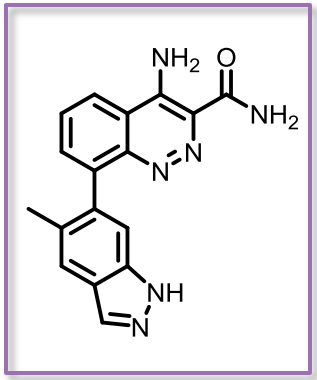
Selectivity is driven by ability of Btk P-loop to clamp down onto the ligand. The Lck P-loop cannot adopt this conformation

Cmpd 3
Btk co-structure

Cmpd 3
Lck co-structure

Compound 3 in Btk

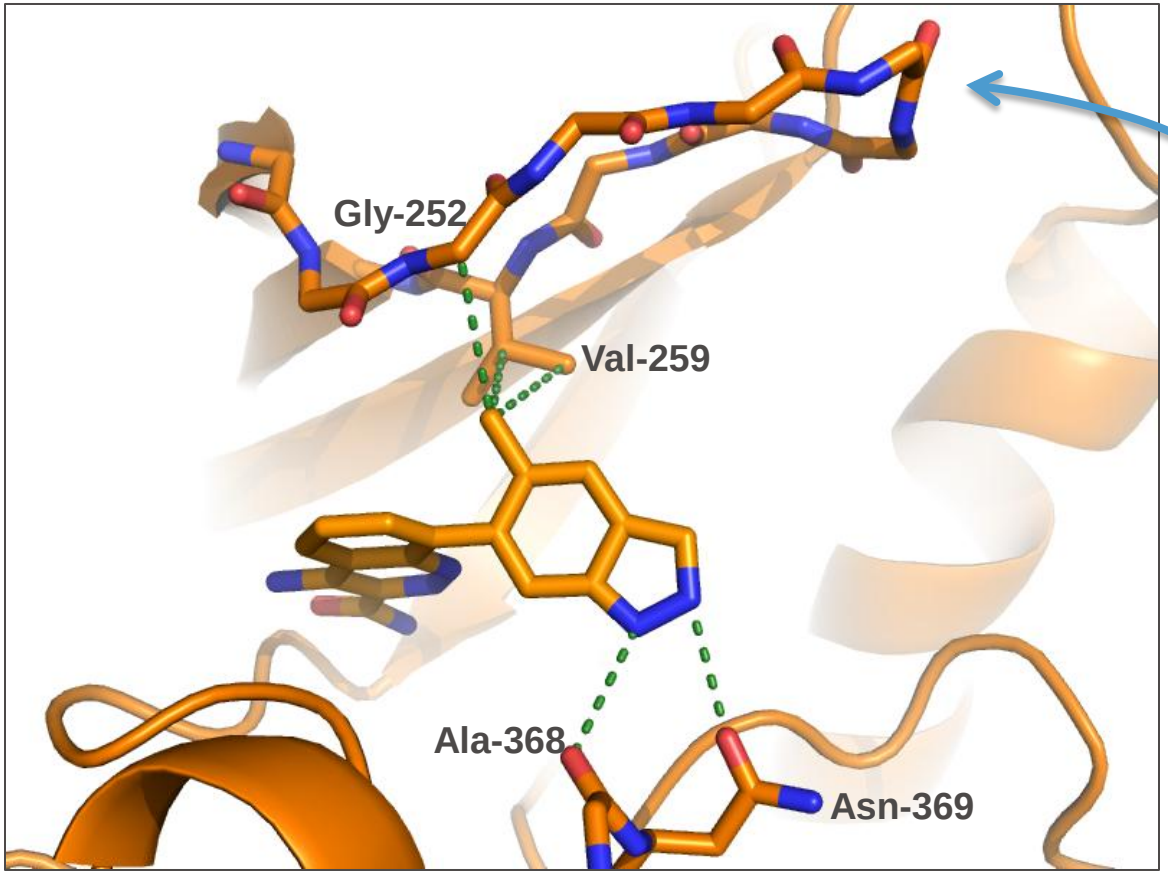


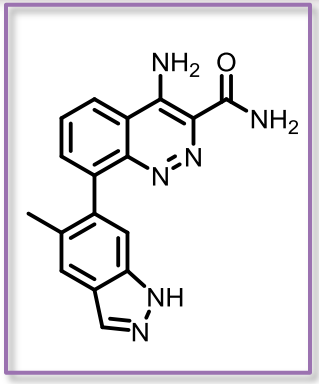


Indazole of compound 3 makes high quality H-bonds to the backbone atoms of the P-loop. Additional potency may be derived from induced fit shape-complimentarity.

Cmpd 3
Btkco-structure

Compound 3 in Lck





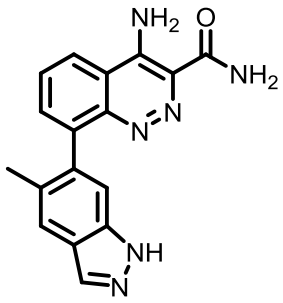
Cmpd 3
Lck co-structure

Compound 3 makes two entirely different interactions with Lck:

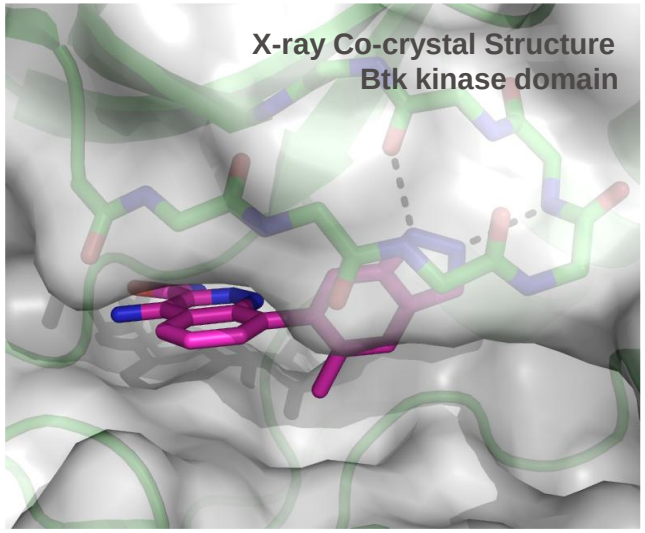
- Methyl group of Compound 3 is proximal to Gly-252 and Val-259 in the P-loop

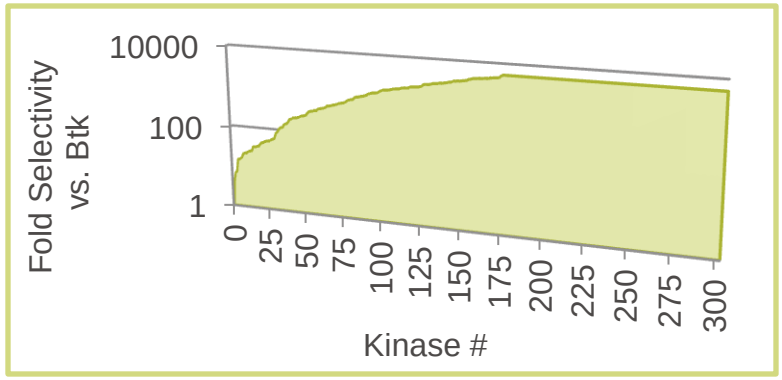
- Indazole makes an interaction with Ala-368 and Asn-369 in the ribose-binding region of the pocket.

Compound 3 Selectivity: kinome, Tec & Src families



Mwt 318 Da
LogD 1.8
tPSA 123
Btk IC_{50} 4nM
pBtk EC_{50} 28 nM
RWB EC_{50} 160 nM



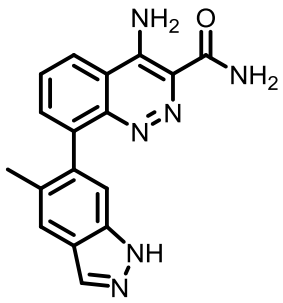


Tec/Src Selectivity

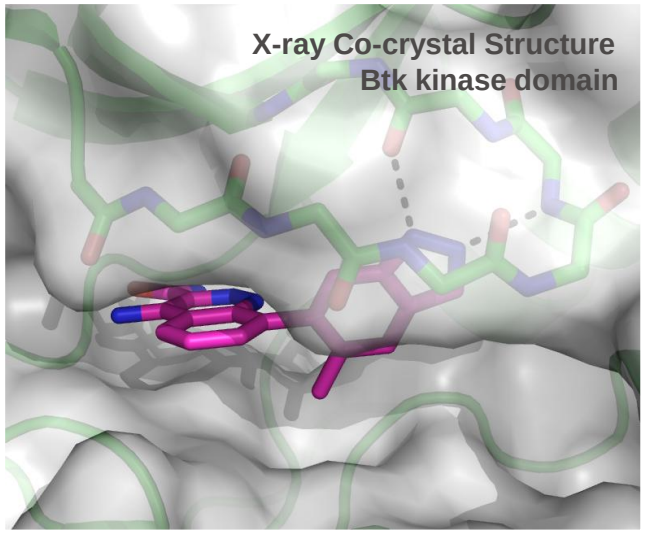
Kinase	Kinase Family	Selectivity ratio[c]
Blk	**Tec**	**18**
Bmx	**Tec**	**18**
Itk	**Tec**	**160**
Tec	**Tec**	**29**
Lck	**Src**	**103**
Fgr	**Src**	**21**
Frk	**Src**	**21**
Hck	**Src**	**50**
LynA	**Src**	**41**
LynB	**Src**	**27**
Src	**Src**	**34**

For additional discussion see: Smith C.R. et al, *J. Med. Chem*., **2015**, 58 (14), pp 5437–5444

Compound 3 Physicochemical Properties and PK



Mwt 318 Da
LogD 1.8
tPSA 123
Btk IC_{50} 4nM
pBtk EC_{50} 28 nM

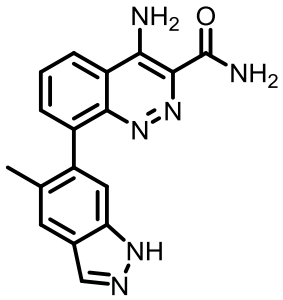


Membrane Permeability		
MDR AB: nm/s; Efflux ratio	13; 6.5	
MOCK AB: nm/s; Efflux ratio	61; 0.3	
Thermodynamic solubility: μg/mL	Free base	Phosphate salt
JP1 pH 1.2	110	95
JP2 pH 6.8	0.6	31
GCDC pH 6.5	59	14
Water	0.3	224
Melting Point	>350°C	305°C

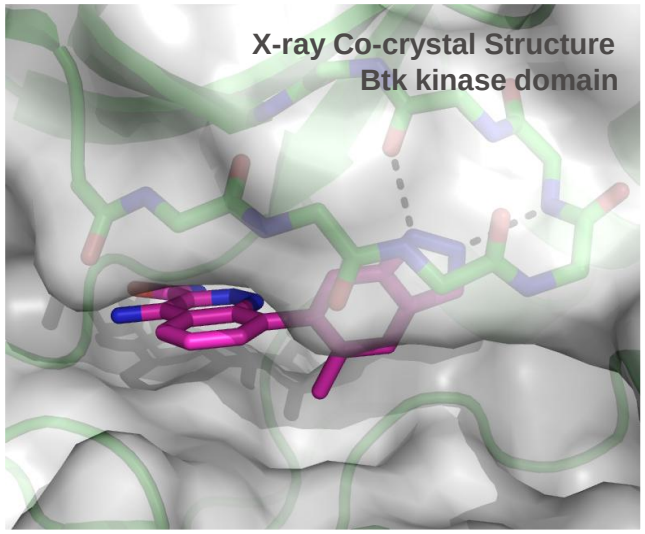
Pharmacokinetics					
Species	IV Cl mL/min/kg	Eh	Vd (L/Kg)	$T_{1/2}$ (IV, h)	% F
Mouse	18	0.2	0.8	1.6	93
Rat	12	0.2	1.5	1.6	70

Low membrane permeability with Pgp and poor JP2 solubility with high MP concerned team

Compound 3 Physicochemical Properties and PK

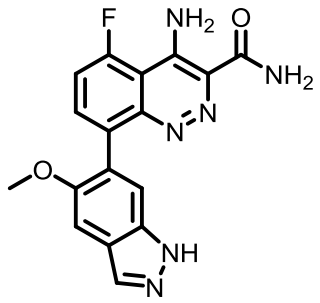


Mwt 318 Da
LogD 1.8
tPSA 123
Btk IC_{50} 4nM
pBtk EC_{50} 28 nM



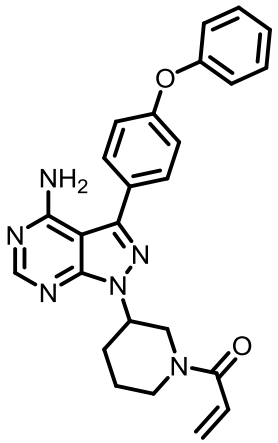
Further Optimization

Compound 4 is more potent, permeable and Lck selective

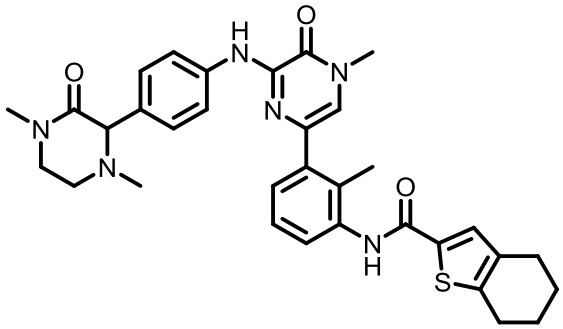


Compound 4
Mwt 352
tPSA 133
Btk IC_{50} .8 nM
Lck IC_{50} 192 nM (240X)
pBtk EC_{50} 8 nM
RWBC EC_{50} 20 nM
P_{A-B} 36 nm/sec

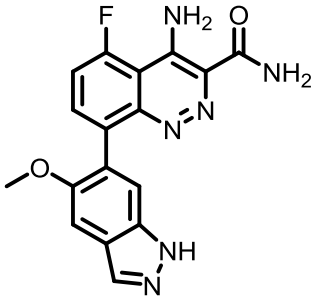
Btk Summary



Ibrutinib
MW 440
Pharmacyclics
Irreversible



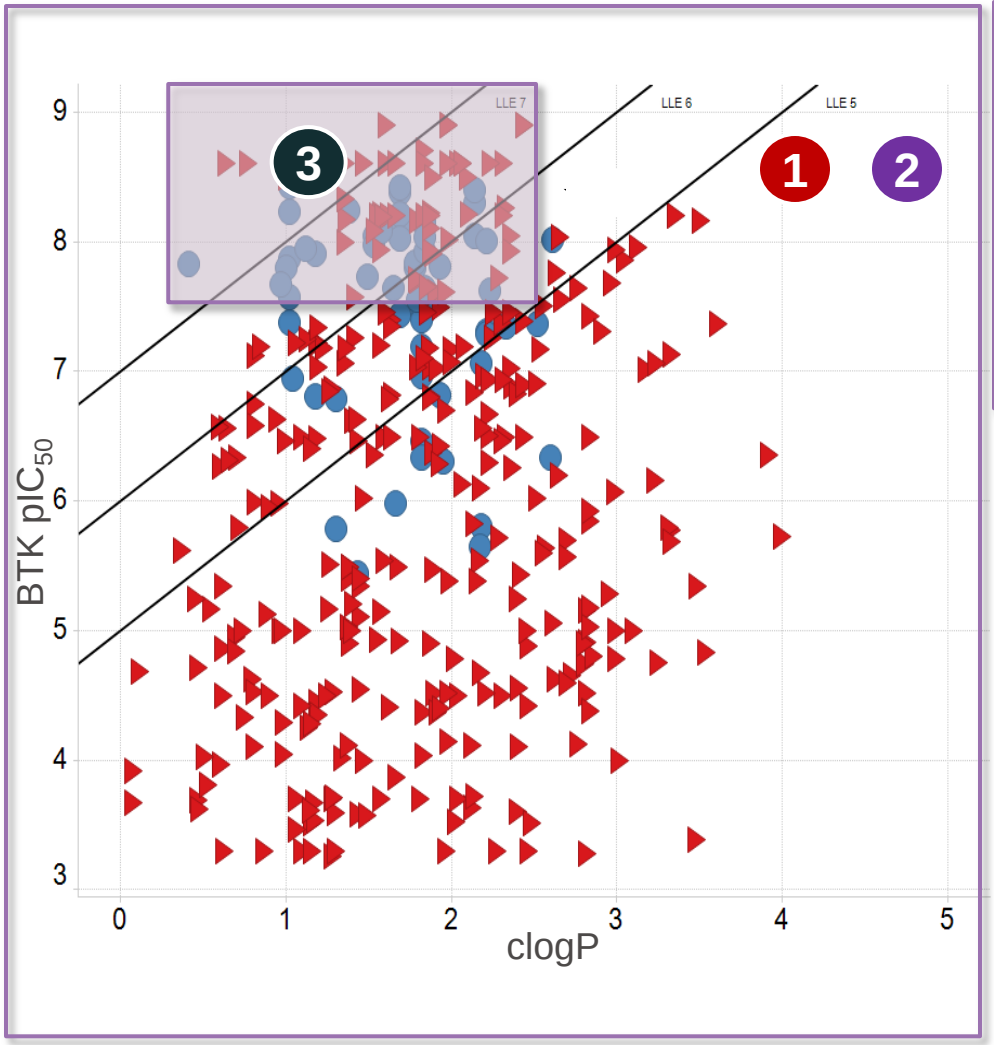
GDC0834 Gilead
Mwt 596 Da
Btk IC_{50} 6 nM
LE 0.27 LLE 4.6



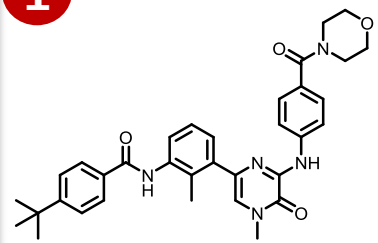
Takeda
Mwt 352 Da
Btk IC_{50} .8 nM
LE 0.45 LLE 7.5

A FBDD approach was successful in discovering a novel reversible Btk inhibitor with molecular weight ≤ 400 Da with good LCK selectivity which was achieved without H3 pocket occupancy through optimal P-loop interaction

Selectivity can be achieved in drug-like space

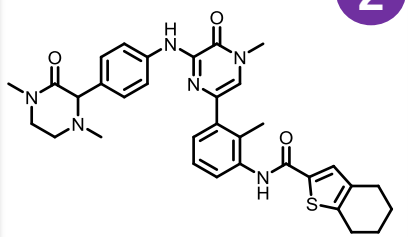


1



CGI1746

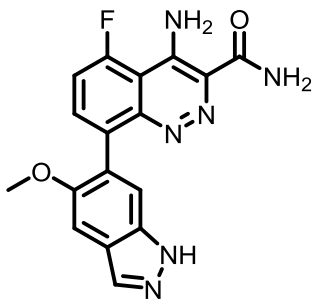
2



Gilead

From Competitors

3

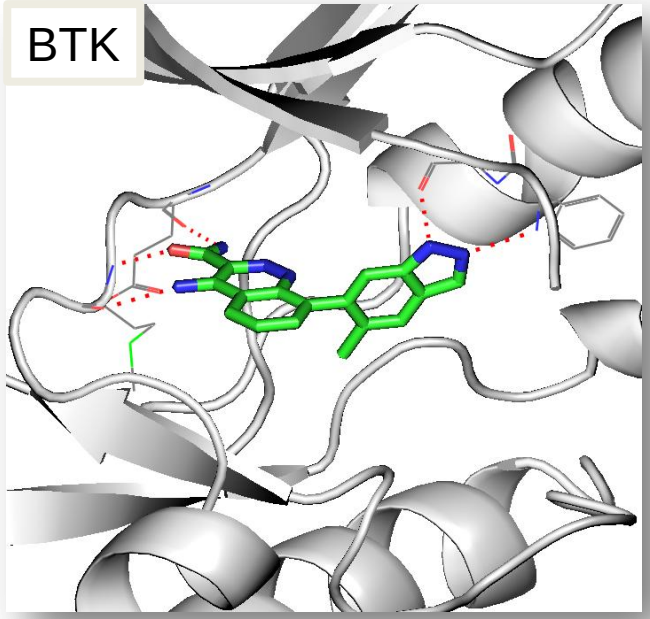


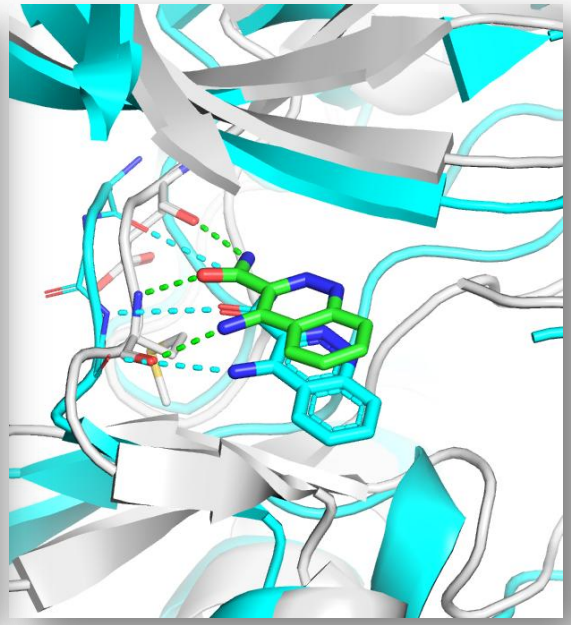
LCK IC_{50} 240 nM
BTK IC_{50} 2nM

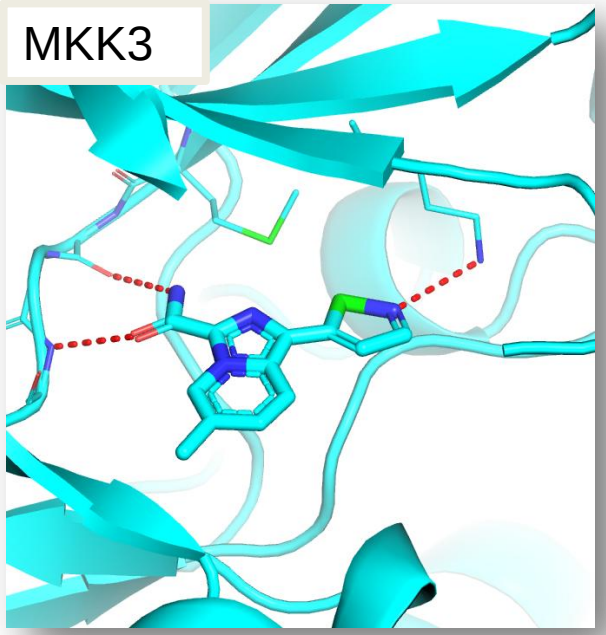
MW 352

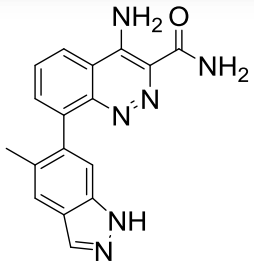
From FBDD

Same Fragment, Different Kinase

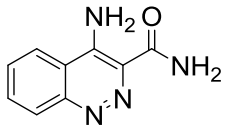




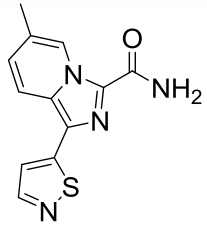




BTK pIC_{50} 8.7



BTK pIC_{50} 5.4
MKK3 pIC_{50} 4.4



MKK3 pIC_{50} 8.5

Same high efficiency fragment led to different series in BTK and MKK3

Summary

- FBDD is a proven and powerful approach to lead identification and is a cornerstone of Takeda California's lead generation strategy.
- Fragments are optimal starting points for delivering selective and drug-like kinase inhibitors.
  - SBDD is a crucial component of fragment optimization
- The same fragment hit can be optimized to distinct selective leads for different kinase targets

Acknowledgements

BTK Team

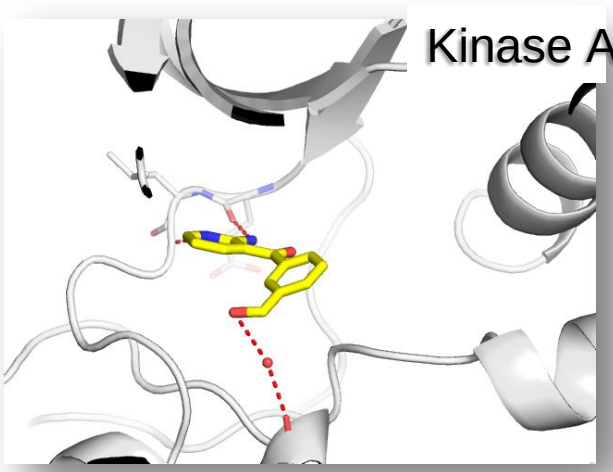
MKK3 Team

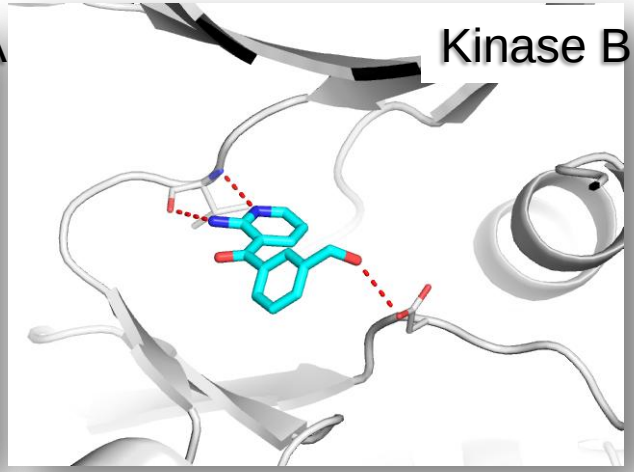
TCAL Scientists

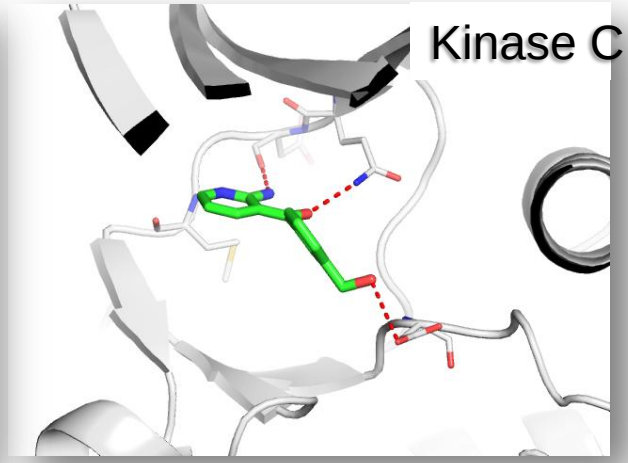
Appendix

But aren't fragments promiscuous?

This same high efficiency fragment was identified and crystallized in 3 different kinases

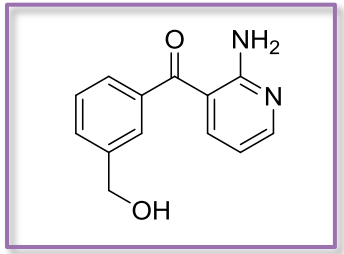




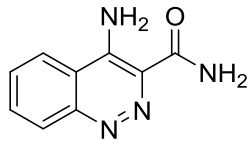


Different binding modes in each protein allow different vectors for optimization

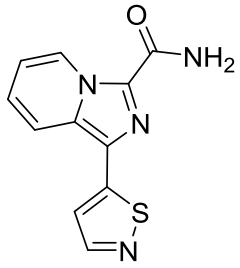
Opportunities for different IP, selectivity, etc



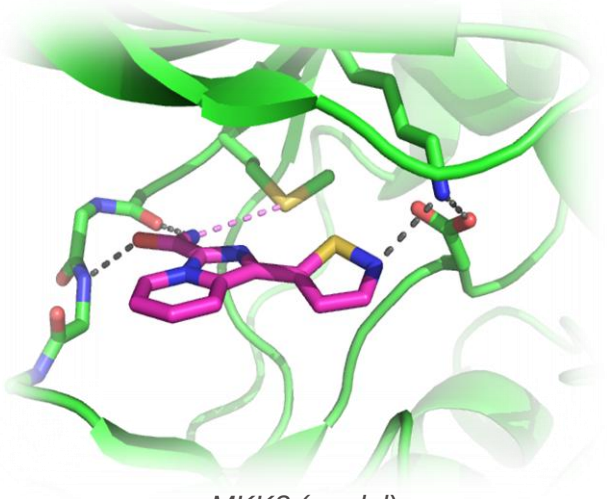
Same Fragment, Different Kinase



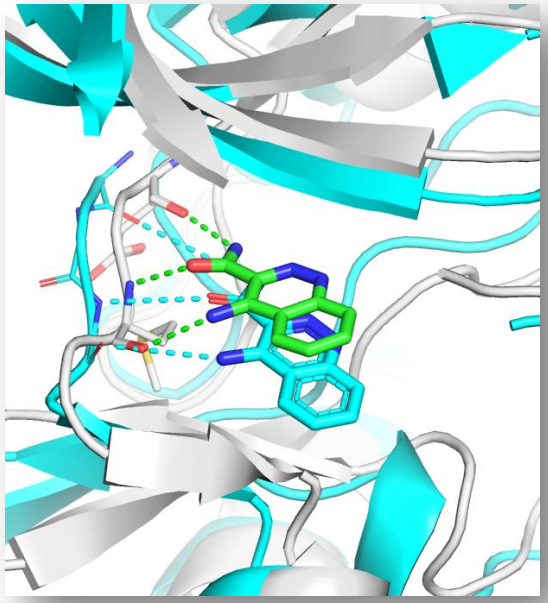
BTK pIC_{50} 5.4
MKK3 pIC_{50} 4.4



MKK3 pIC_{50} 8.5
LE 0.67; LLE 7.6



MKK3 (model)



Cyan – MKK3 (model)
Grey - BTK

MKK6	p38a	p38g	JNK1	JNK2	ERK1
12	>100	>100	>100	>100	>100
ERK2	MEK1	MEK2	TAK1	MLK3	ASK1
>100	86	>100	23	>100	>100

Fold selectivity against a panel of MAPK signaling kinases (invitrogen)

See Adams *et al*. Bioorganic & Medicinal Chemistry Letters, 2015 (corrected proof available online).