PSDI 2012

Fragment-Based Drug Design With Limited Resources

Michael Kothe
Senior Scientist
Genzyme
Sanofi Boston R&D Hub

genzyme
A SANOFI COMPANY

11/12/2012

Small Molecule Drug Discovery at Genzyme

Relied mostly on high-throughput screening

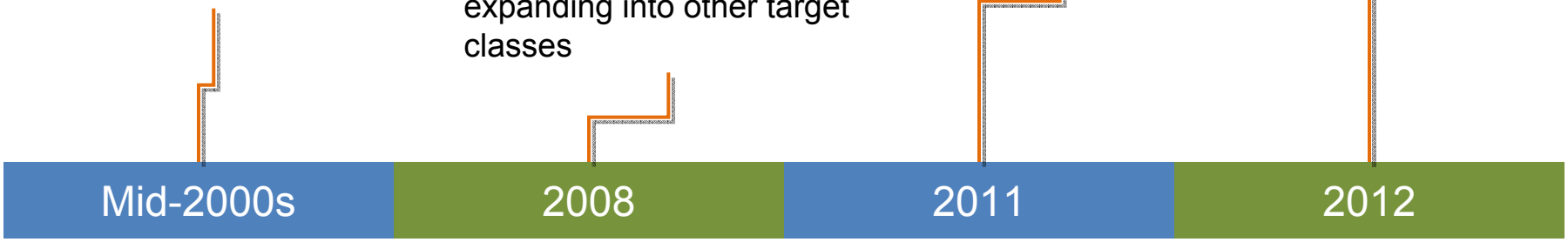
- Largely commercial, non-proprietary compounds in library
- Capabilities not optimal for high priority targets

Added fragment-based drug design (FBDD) capability

- Seek to efficiently access priority targets with modest resources
- Initial focus on kinases, expanding into other target classes

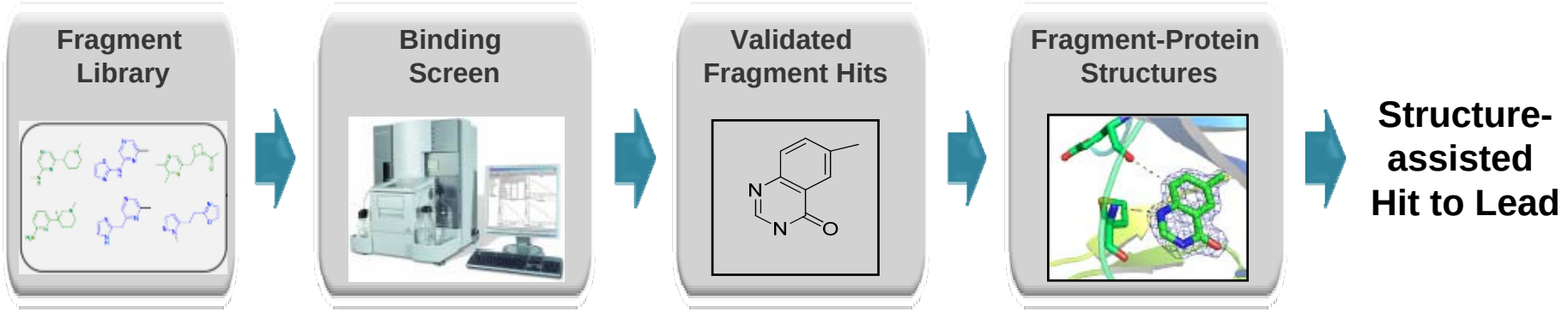
Sanofi acquisition of Genzyme

Genzyme R&D unit within Sanofi Boston R&D Hub



Goal for this presentation is to share our experience using the FBDD approach in the past 4 years

Considerations For The Genzyme FBDD Effort



- **Sizable, high quality fragment library**
  - ~4000 fragments from multiple vendors, filtered by rule of 3 and functional groups
  - No strong curation, purpose was to get started quickly
- **Effective screening system to identify true binders**
  - Relying primarily on SPR for screen
  - SPR, TR-FRET and DSF to confirm hits
- **Co-localized and closely integrated gene to structure effort**
  - Established second Genzyme SB group on site
  - Considerable investment in automation improved throughput
- **Decision to pursue an opportunistic blend of FBDD and SBDD**

Resourcing The Genzyme FBDD Capability

Pre-existing Genzyme expertise 2008

- Protein expression /purification ***(not on site)***
- Structural biology (Biologics) ***(not on site)***
- Enzyme/cell assays
- Compound screening
- Pharmacology
- Chemistry
- ADME

New hires/training

- Computational chemistry
- Kinase drug design
- Structural biology/FBDD
- SPR methods

- 3 hires (Q3 2008)

Total FTE devoted to FBDD

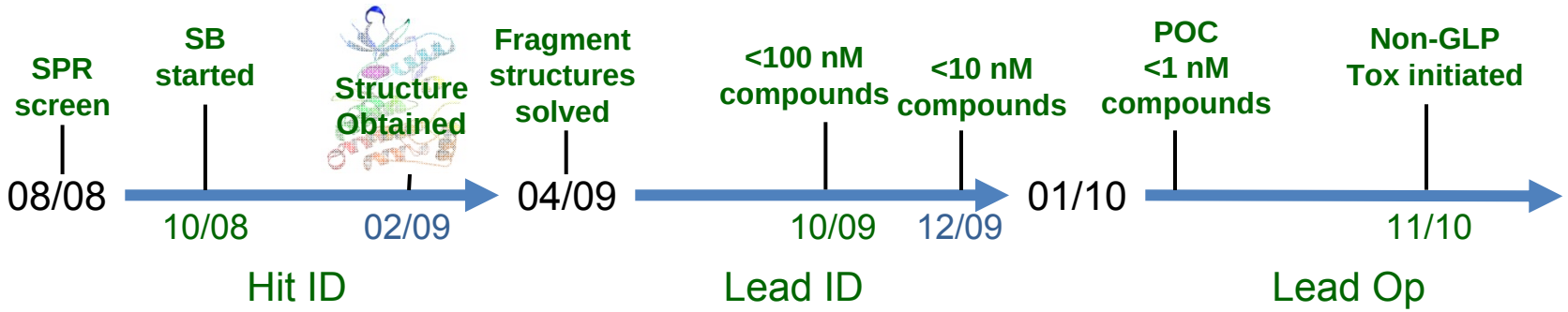
- 2 expression/purification, 2 SB, 1 screening, 2 comp chem, ~10 med chem (+ 5-10 CRO)
- Plus enzyme assays, ADME, etc.
- Some SB and expression/purification help from other groups at Genzyme

Identification of Active Fragment Compounds

Target	Method	Fragments Screened	Confirmed Hits	Fragment Structures
A (TK)	SPR	1500	21	17 (out of 21 attempted)
B (TK)	SPR	1200	TBD	5 (out of 9 attempted)
C (STK)	SPR	1500	32+	24 (out of 32 attempted)
D (TK)	TR-FRET	13000	~200	32 (out of 40 attempted)
E (STK)	SPR	4000	~60	14 (out of 16 attempted)
F (NK)	SPR	4000	11	1 (out of 11 attempted)
G (NK)	NMR/SPR	900+	TBD	4 (out of 54 attempted)

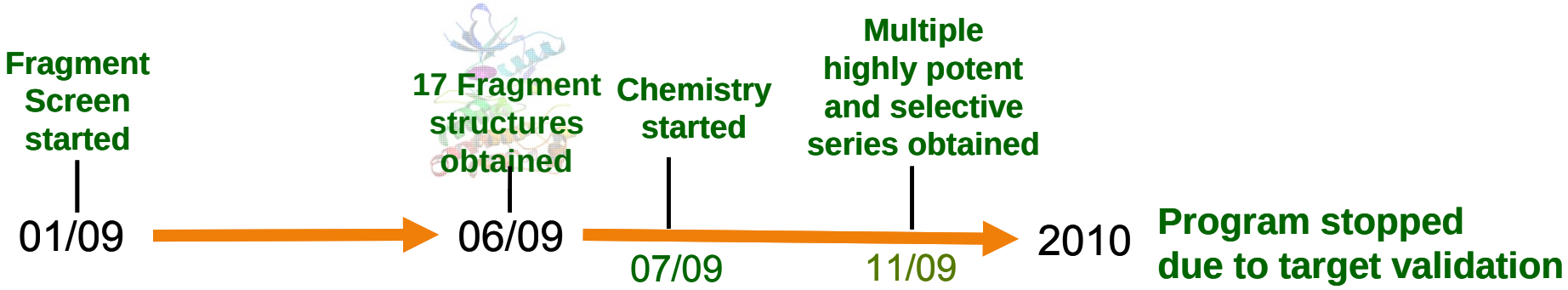
- **Screen hit rates ~1.5 % for kinases**
- **~75 % of confirmed hits yield co-crystal structures for kinases**
  - Significantly lower success rate for non-kinases (n=2)
- **96 fragment co-crystal structures determined for 7 targets**

Resources and Productivity: Example 1



- **Med. chem. optimization of 4 fragment hit series**
  - 2 series progressed to lead op
  - Additional SBDD molecular redesign chemotype also developed
    - Leveraging novel FBDD SAR with existing competitor inhibitor
- **Start of lead ID to late lead optimization in ~18 months**
  - 2 distinct chemotypes progressed to non glp tox studies
    - Pk / kinase selectivity / potency meeting Target Product Profile
    - One FBDD series plus SBDD molecular redesign series
- **Chemistry resourcing: 5 in-house med chemists plus 9 CRO**

Resources and Productivity: Example 2



- **4 sub-100nM series generated (2 sub 10nM)**
- **~50 structures solved during the course of the project**
- **Chemistry resourcing:**
  - 3 in-house med chemists plus 3 CRO
  - 5-6 months.

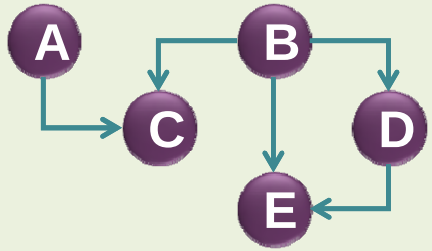
Some General Observations: Strategy

Med Chem buy in and team co-localization was critical to success

- Strong commitment to FBDD across groups
- Screening, SB, chemistry closely coordinated
- Flexibility to react rapidly to results and changing priorities

Early focus on kinases created synergies

- Assay platforms and reagents
- Chemotypes propagated to new targets



Pragmatic blend of FBDD and SBDD

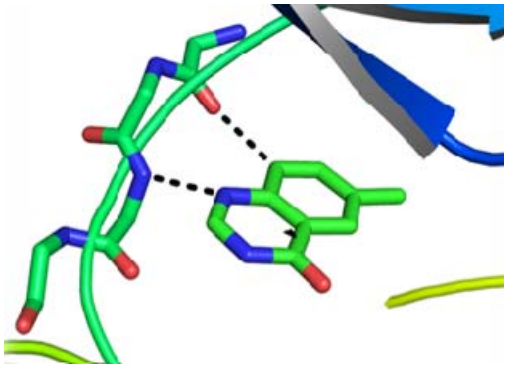
- Not relying on traditional fragment expansion alone
- Scaffold-hopping
- Deconstruction of reference compounds
- Traditional SBDD

FBDD enabled access to desirable and previously inaccessible targets

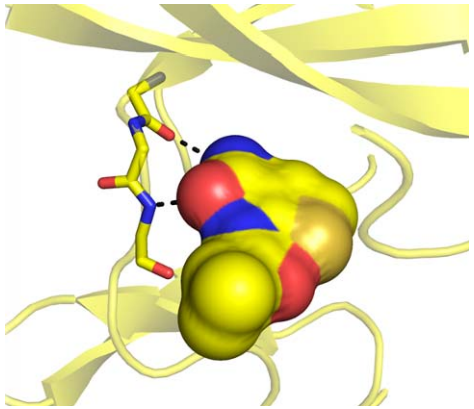
- Not constrained by existing screening deck
- IP designed in and not required up front
- Fast progress through lead ID yielding potent, selective compounds
- Generated low- and sub-nM compounds for 5 targets

Some General Observations: Structures

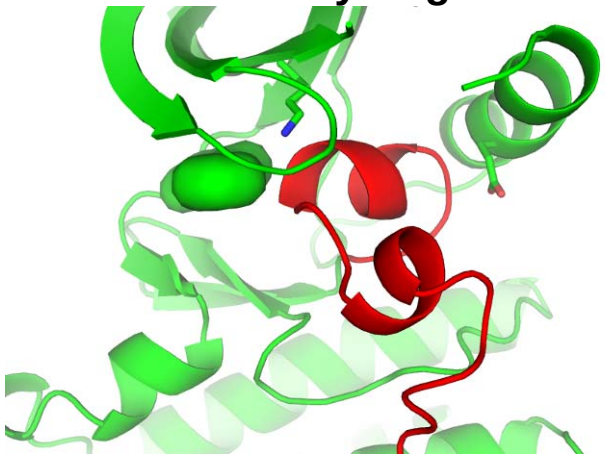
Kinase fragments are predominantly hinge binders



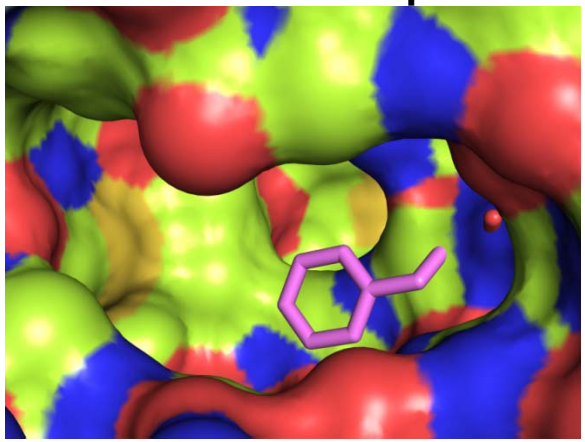
Ubiquitous fragment observed binding to 4 kinase targets



Large conformational changes in target protein induced by fragment binding

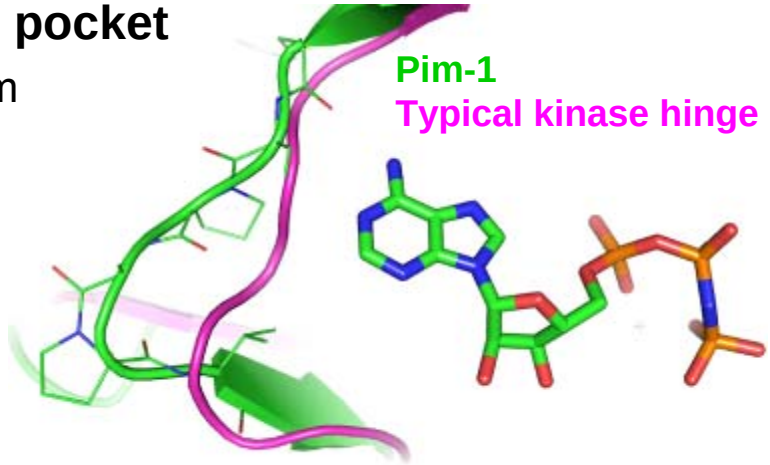


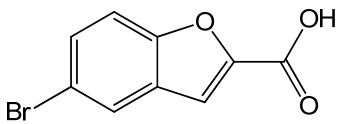
Fragment-driven Phe switch allows access to back pocket



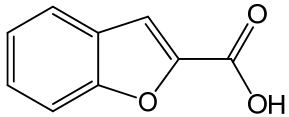
FBDD Example: Pim-1 Kinase

- **Ser/Thr protein kinase with role in proliferation**
- **Activated in FLT3 mutated leukemias**
- **Unusual hinge region results in unique ATP pocket**
  - Proline insertion limits H-bonding and creates room
- **Fragment screen:**
  - ~1500 fragments screened by SPR (75uM)
  - >32 hits confirmed in Pim-1 biochemical assay
  - Benzofuran carboxylic acids among the hits

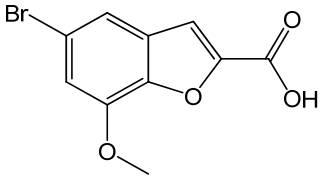




IC50 = 8.5 uM
LE = 0.54



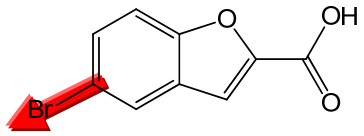
IC50 = 119 uM
LE = 0.45



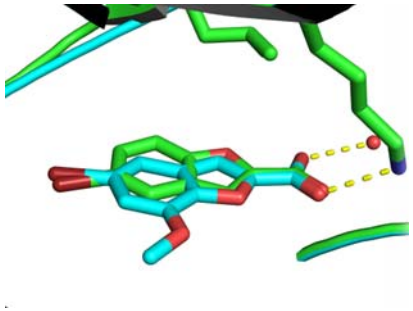
IC50 = 5.8 uM
LE = 0.48

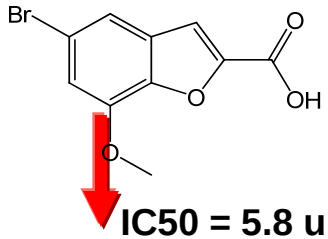
Xiang et al. *BMC Lett.* (2011)

Pim-1 Kinase Fragment Binding Modes
(Benzofuran Carboxylic Acids)

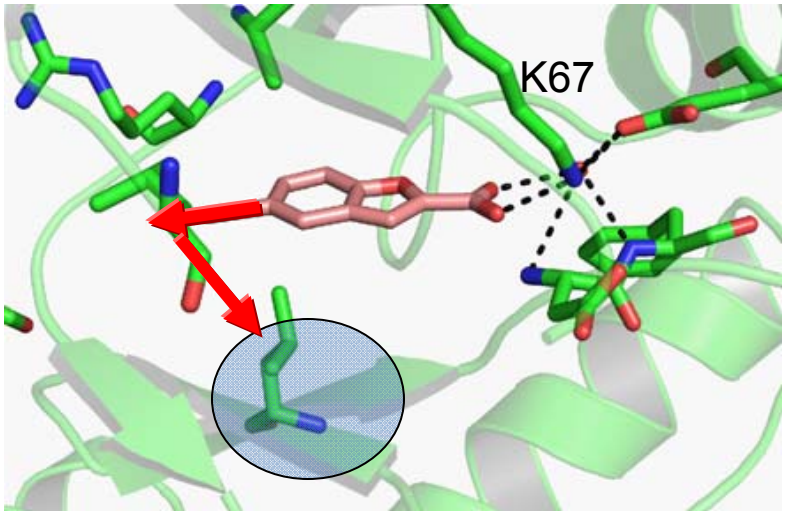


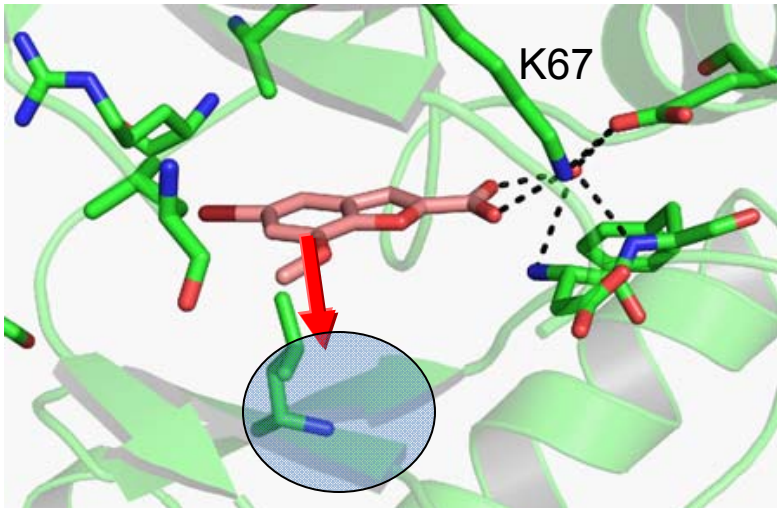
IC50 = 8.5 uM
LE = 0.54





IC50 = 5.8 uM
LE = 0.48



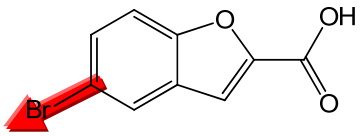


- Carboxylic acid salt bridge with lysine 67
- Bromo interaction with hydrophobic hinge pocket
- Methoxy group inverts binding orientation
- Both fragments expanded in same direction with different vectors

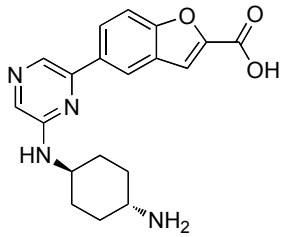
Xiang et al. *BMC Lett.* (2011)

Pim-1 Kinase Fragment Expansion

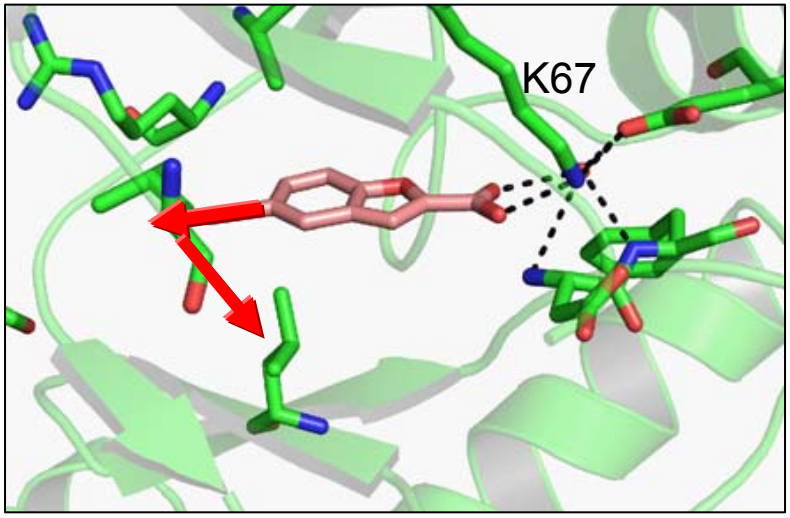
Example 1

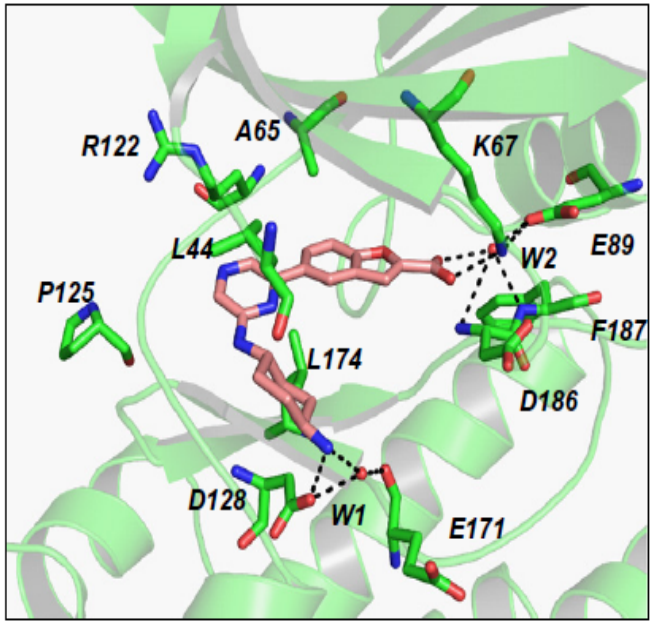


IC50 = 8.5 uM
LE = 0.54



IC50 = 120 nM
LE= 0.37



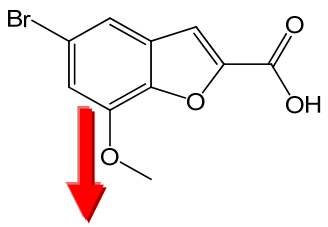


- Aromatic ring replacements essentially equipotent
- Further functionalization of ring produced limited additional potency
- Most potent compounds form H-bonds with D128 and E171

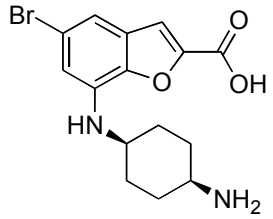
Xiang et al. *BMC Lett.* (2011)

Pim-1 Kinase Fragment Expansion

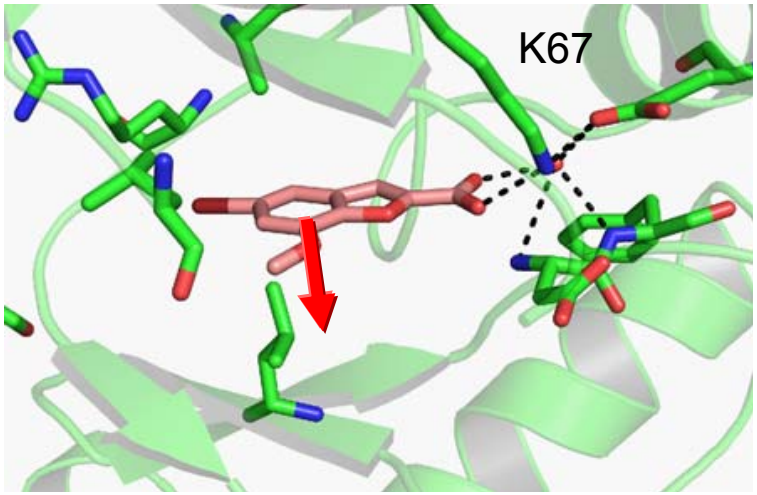
Example 2

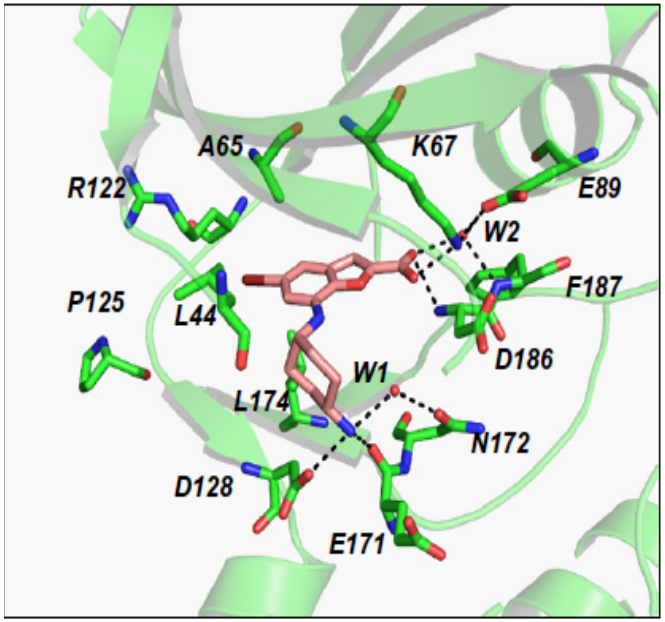


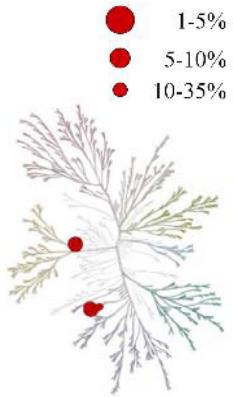
IC50 = 5.8 uM
LE = 0.48



IC50 = 1 nM
LE= 0.59







Ambit panel
at 100 nM

- Maintain Bromo hinge pocket interaction
- R groups again added targeting D128 and E171, leading to highly potent cpds
- Stereochemistry of benzofuran addition important for potency
- Most potent compound is highly selective

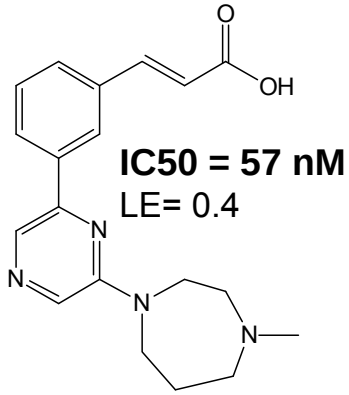
Xiang et al. *BMC Lett.* (2011)

Pim-1 Kinase

Molecular redesign with fragment information obtained by literature search

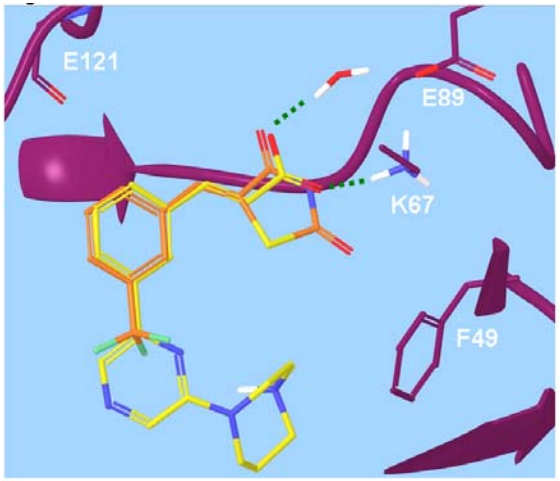
- **Potent thiazolidinedione (THZ) screening hit published**
  - Docked protonated with h bonding to hinge
- **THZ is a known acid isotere**
  - Acid binding to gatekeeper preferred
  - Molecule redocked deprotonated
  - Docking mode superimposes well with BI carboxylate screening hit xtal structure

IC50 = 24 nM
LE= 0.58



IC50 = 57 nM
LE= 0.4

Xia et al *J. Med. Chem.* **2009,** *52,* 74
Qian et al J. Med. Chem. **2009,** *52,* 1814



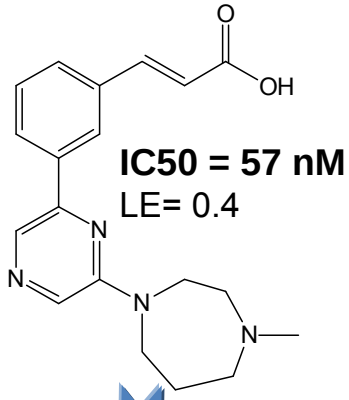
Good et al J. Med. Chem. (2012)

Pim-1 Kinase

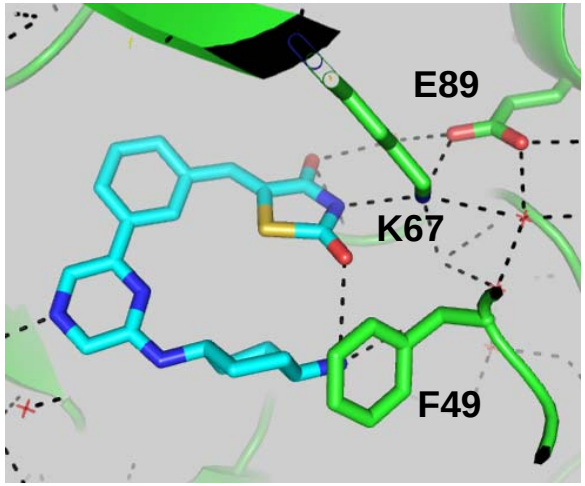
Molecular redesign with fragment information obtained by literature search

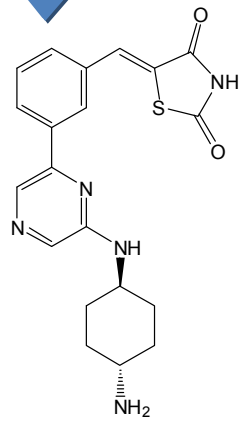
- **Potent thiazolidinedione (THZ) screening hit published**
  - Docked protonated with h bonding to hinge
- **THZ is a known acid isotere**
  - Acid binding to gatekeeper preferred
  - Molecule redocked deprotonated
  - Docking mode superimposes well with BI carboxylate screening hit xtal structure
- **Created hybrid molecule with improved potency and good LE**
  - Binding mode confirmed by Xtal structure
  - THZ makes predicted interactions

IC50 = 24 nM
LE= 0.58



IC50 = 57 nM
LE= 0.4





IC50 = 0.47 nM
LE= 0.46

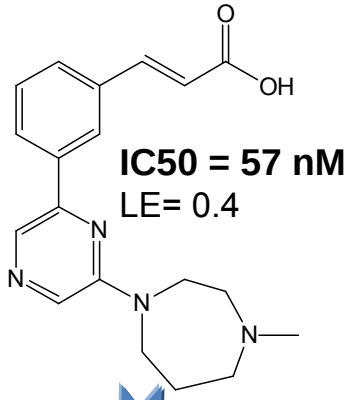
Good et al J. Med. Chem. (2012)

Pim-1 Kinase

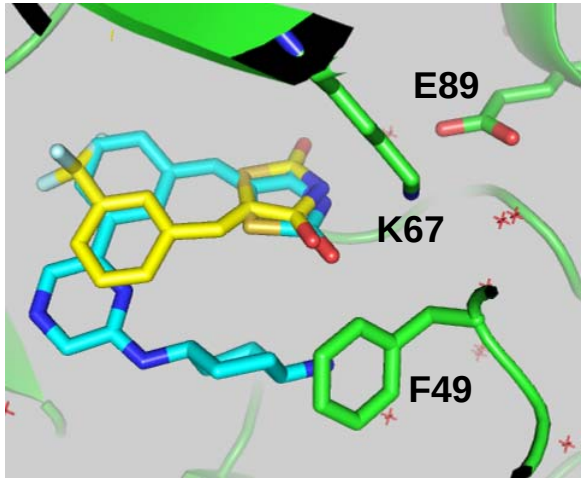
Molecular redesign with fragment information obtained by literature search

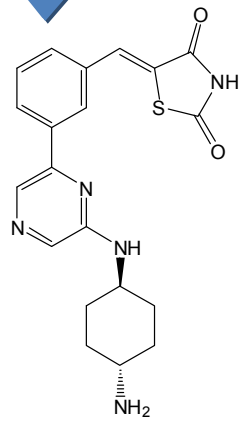
- **Potent thiazolidinedione (THZ) screening hit published**
  - Docked protonated with h bonding to hinge
- **THZ is a known acid isotere**
  - Acid binding to gatekeeper preferred
  - Molecule redocked deprotonated
  - Docking mode superimposes well with BI carboxylate screening hit xtal structure
- **Created hybrid molecule with improved potency and good LE**
  - Binding mode confirmed by Xtal structure
  - THZ makes predicted interactions
- **THZ fragment structure solved after hybrid synthesis**
  - THZ binding mode matches, hydrophobic portion is flipped compared to docking result

IC50 = 24 nM
LE= 0.58



IC50 = 57 nM
LE= 0.4





IC50 = 0.47 nM
LE= 0.46

Good et al J. Med. Chem. (2012)

Genzyme FBDD: Major Achievements & Lessons

- **Pragmatic FBDD/SBDD blend had significant impact on several projects**
  - Ran seven fragment screens, generated nanomolar compounds for 5 targets
  - Created novel scaffolds from fragment hits
  - Moved projects quickly through Hit ID to Lead Op
- **We were able to have an impact quickly despite limited prior FBDD experience and moderate resources**
  - Synergy from early focus on kinases
  - Flexibility to address IP issues early on
  - No need for diligent curation of fragment library
- **Close team integration and buy in was important to success**
  - Strong commitment to the approach gave FBDD a “fair shot”
  - Co-localization of activities created opportunities for frequent and informal communication

Acknowledgements

- ***In Vitro* Biology**
- Jinyu Liu
- David Stepp
- Tanya Gladysheva
- Gary Asmussen
- Stephanie Davis
- Trisha Fremgen
- Becky Lam
- Leah Kennedy
- Jill Gregory
- Meghan Harris
- Angela Quinn
- Hans Biemann
- Jim Lillie

Medicinal Chemistry

- Andy Good
- Markus Metz
- John Kane
- Andrew Scholte
- Yibin Xiang
- Bradford Hirth
- Katherine Jancsics
- Andrew Papoulis
- Elyse Bourque
- Erika Johnston
- Renato Skerlj

Analytical Sciences

- Kim Alving

Therapeutic Protein Discovery (Framingham)

- Annu Jain
- Meta Foster
- Jason Pinckney
- Kim Bishop
- Bill Brondyk

Therapeutic Protein Research (Framingham)

- Ronnie Wei
- Tracey Reihle