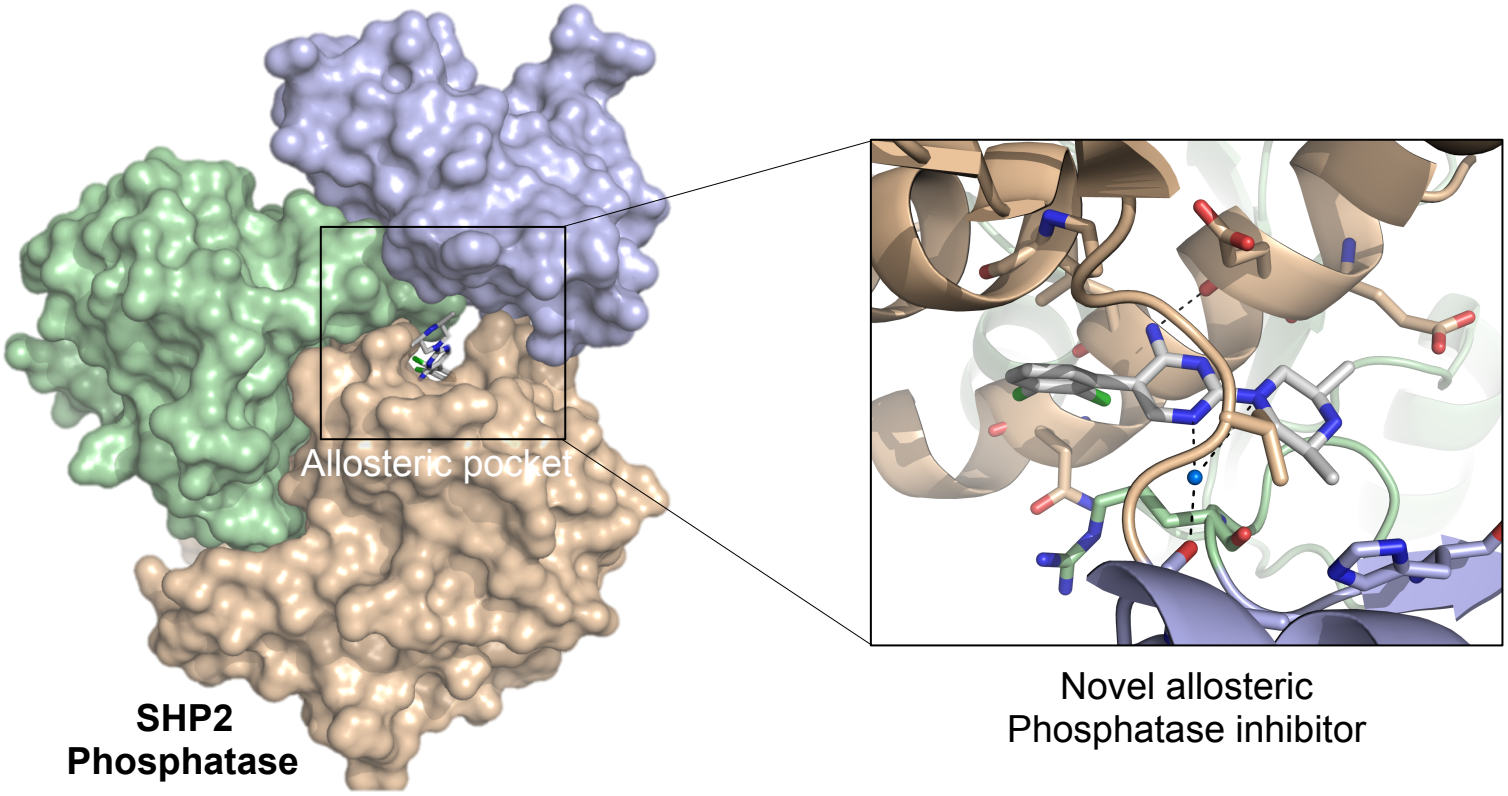
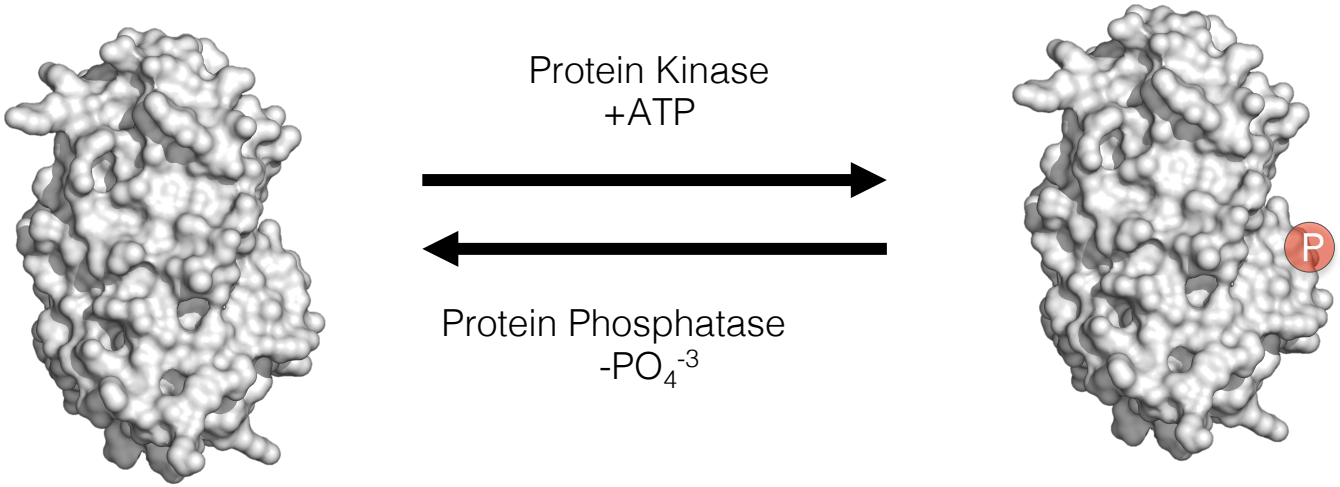
Allosteric Inhibition of SHP2: Identification of a Potent, Selective, and Orally Efficacious Phosphatase Inhibitor



Evan Carder
Wipf Group Current Literature
August 06, 2016

[1]. J. Med. Chem., Article ASAP
[2] *Nature* **2016**, 535, 148.

Regulation of Cell Signaling



Protein Kinase

- *ca.* 518 protein kinases
- Catalyze the phosphorylation
- Control the amplitude of the response
- Receptor and Non-receptor kinases
- Serine/Threonine and Tyrosine

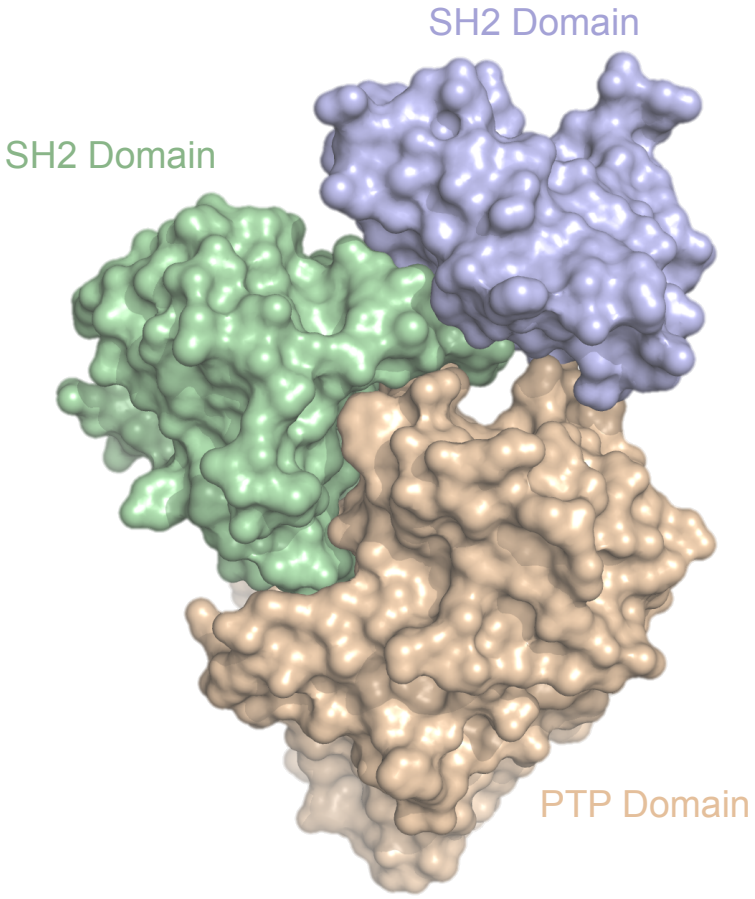
Protein Phosphatase

- *ca.* 107 protein phosphatases
- Catalyze de-phosphorylation
- Control the rate and duration of the response
- Receptor and Non-receptor kinases
- Classical tyrosine-specific PTPs (37 genes)
- Dual specificity phosphatases (65 genes)

[1]. *Drug Discovery Today* **2016**, 21, 1..
[2]. *Nat. Rev.* **2006**, 7, 833.

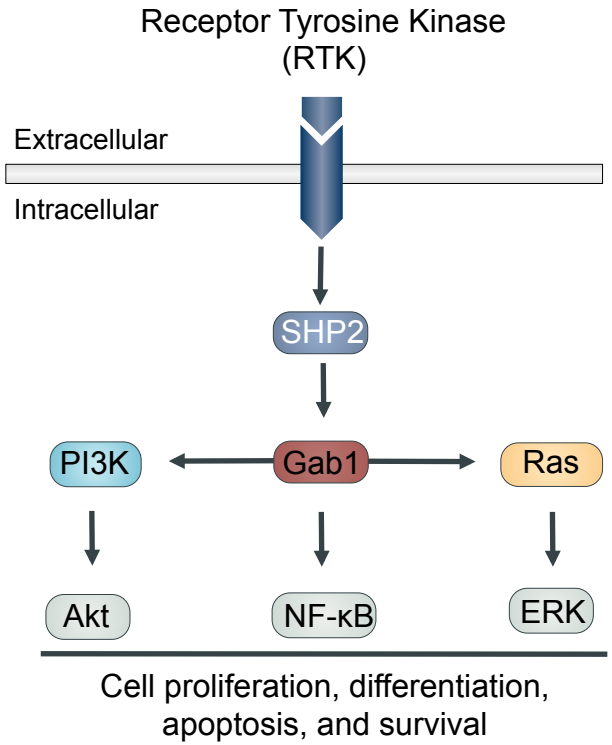
SHP2 Structure

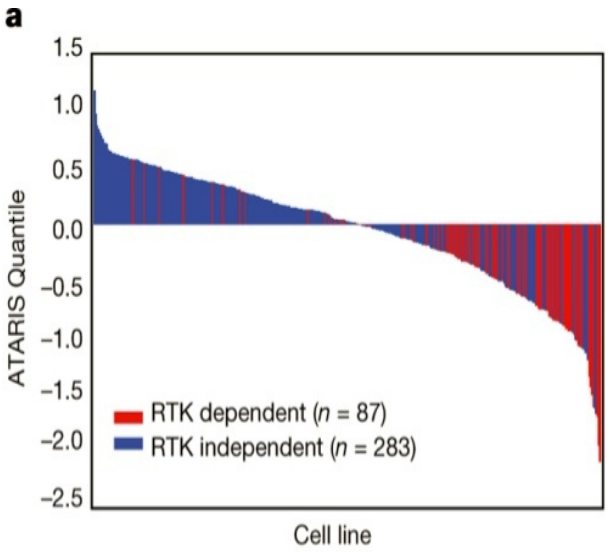
- Non-transmembrane, cytoplasmic protein tyrosine phosphatase (PTP)
- PTP domain
  - Enzymatic region responsible for catalyzing de-phosphorylation
- Src-homology-2 domains (SH2)
  - Tandem SH2 domains
  - Bind to phosphorylated tyrosine residues
  - Regulatory domains
  - Flank the catalytic domain (PTP domain)
  - Regulate enzymatic activity

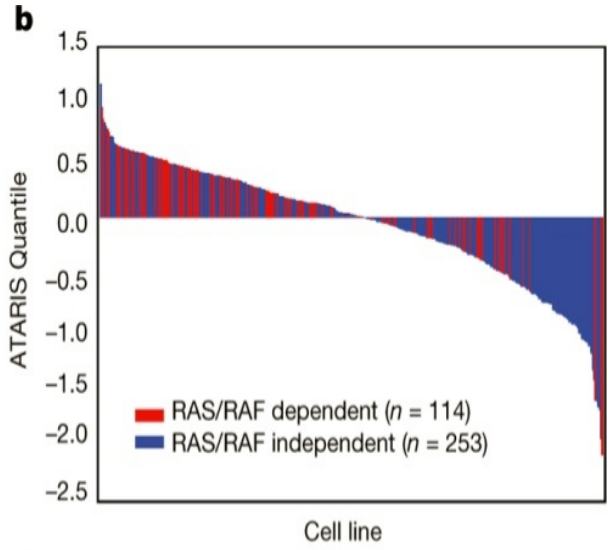


[1].*Histol. Histopathol.* **2007**, 22, 1251.

SHP2 Cell Signaling

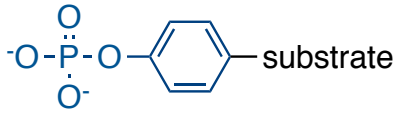






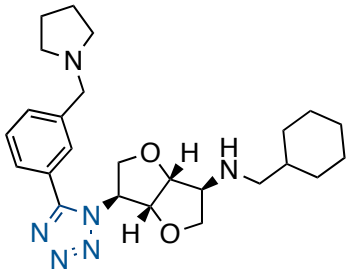
[1]. *Nature* **2016**, 535, 148.
[2]. *Histol. Histopathol.* **2007**, 22, 1251.
[3]. *Nat. Rev.* **2006**, 7, 833.

Design of Phosphatase Inhibitors

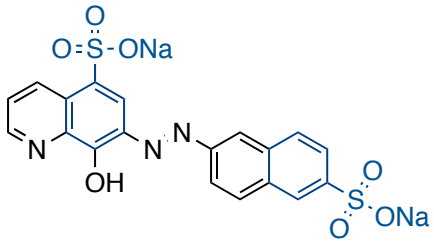


Phosphorylated Tyrosine

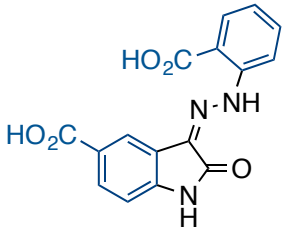
SHP2 Inhibitors possessing ionizable functional groups



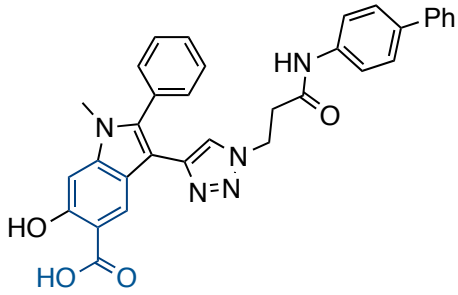
NAT6-297775
IC_{50} (SHP2): 2.5 uM



NSC-87877
IC_{50} (SHP2): 0.3 uM



IC_{50} (SHP2): 0.8 uM

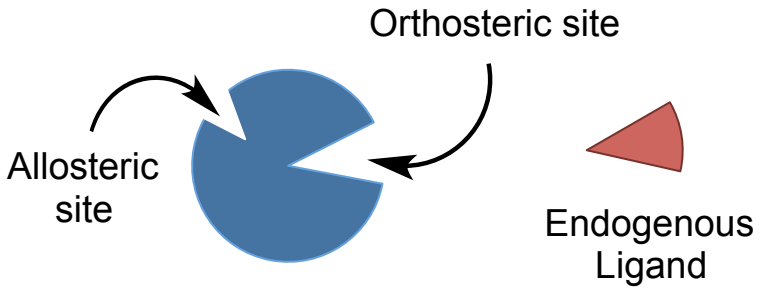


IIB08
IC_{50} (SHP2): 5.5 uM

Allosteric Modulators

Orthosteric binding site – a distinct binding site for a protein's endogenous ligand

Allosteric binding site – a site that is topologically and functionally distinct to the orthosteric binding site.



- Allosteric modulators: regulation of protein activity by binding of an effector molecule at a site other than the protein's orthosteric site.
  - Mechanism: Allosteric modulators traditional impact protein activity by inducing conformational change that can either enhance or reduce protein activity
  - Significance: Greater selectivity may be obtained by targeting allosteric sites – including subtype selectivity within receptor families. Also, allosteric modulators can have improved physiochemical and drug metabolism/pharmacokinetic properties

[1]. *Annu. Rev. Pharmacol. Toxicol.* **2014**, 54, 165. [2]. *Med. Princ. Pract.* **2013**, 22, 418. [3] *Nat. Biotech.* **2014**, 22, 1113.

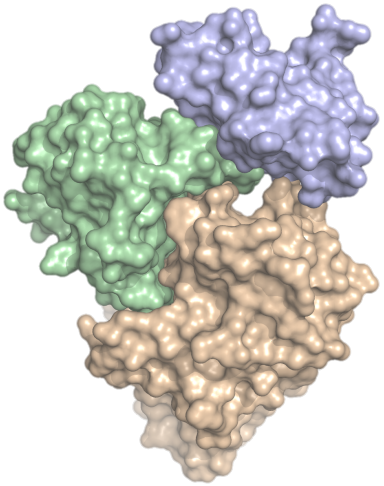
Central hypothesis. Targeted inhibition of SHP2 leads to RTK-dependent cancer reduction.

Aim 1. Development of an allosteric SHP2 small molecule inhibitor

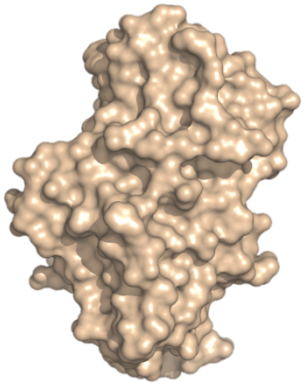
- Subaim 1.1. Identify allosteric small molecule inhibitor
- Subaim 1.2. Optimize Hit by SAR and SBDD
- Subaim 1.3. Biologically evaluate SHP2 inhibitor

Identification of an allosteric SHP2 small molecule inhibitor

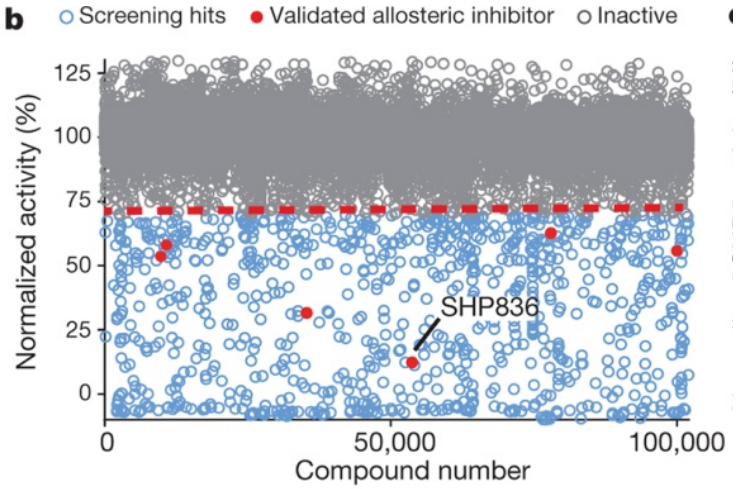
100,000 Compounds
Library Screen



Full Length SHP2

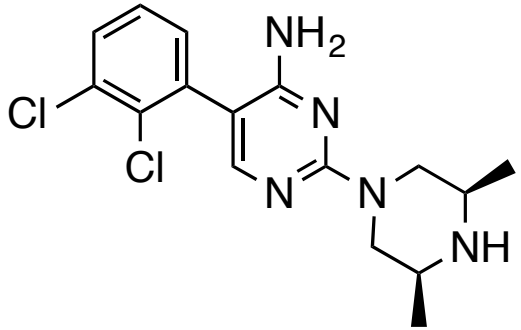


PTP Domain



Nature **2016**, 535, 148.

Hit

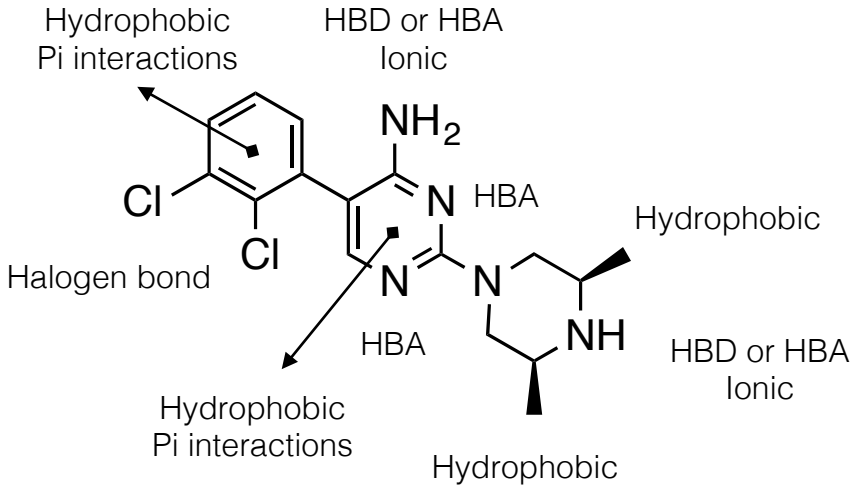


SHP836
Allosteric Modulator
SHP2 IC_{50} = 12 uM

SHP836 – Chemical Analysis

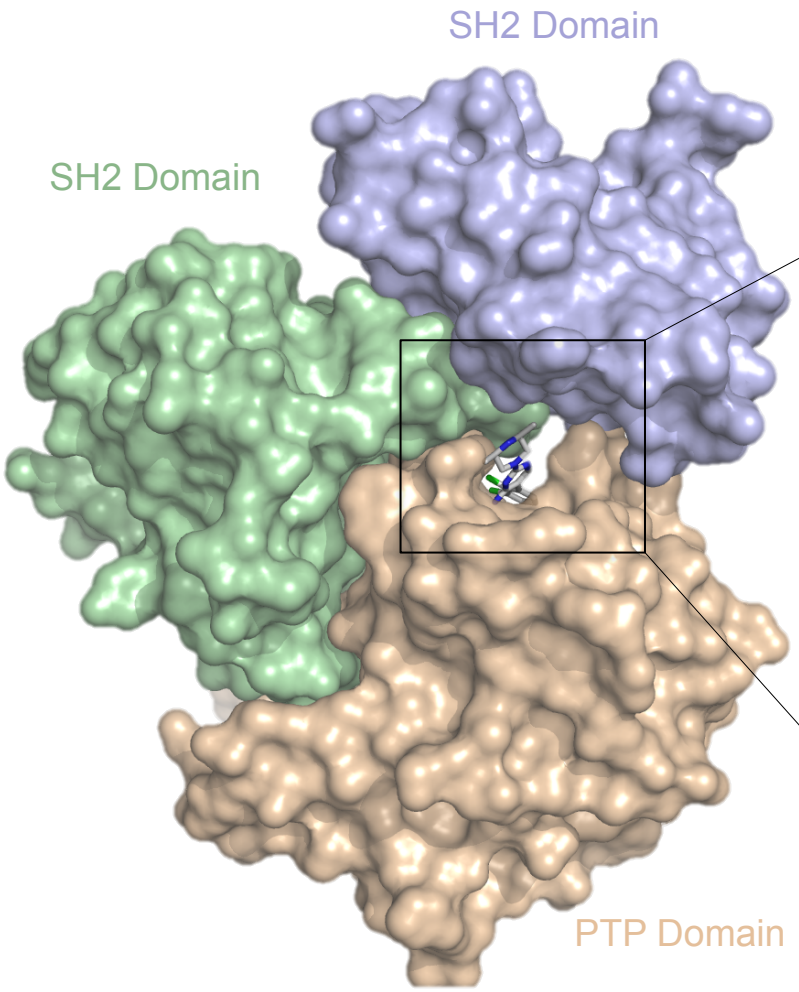
Intermolecular Interactions

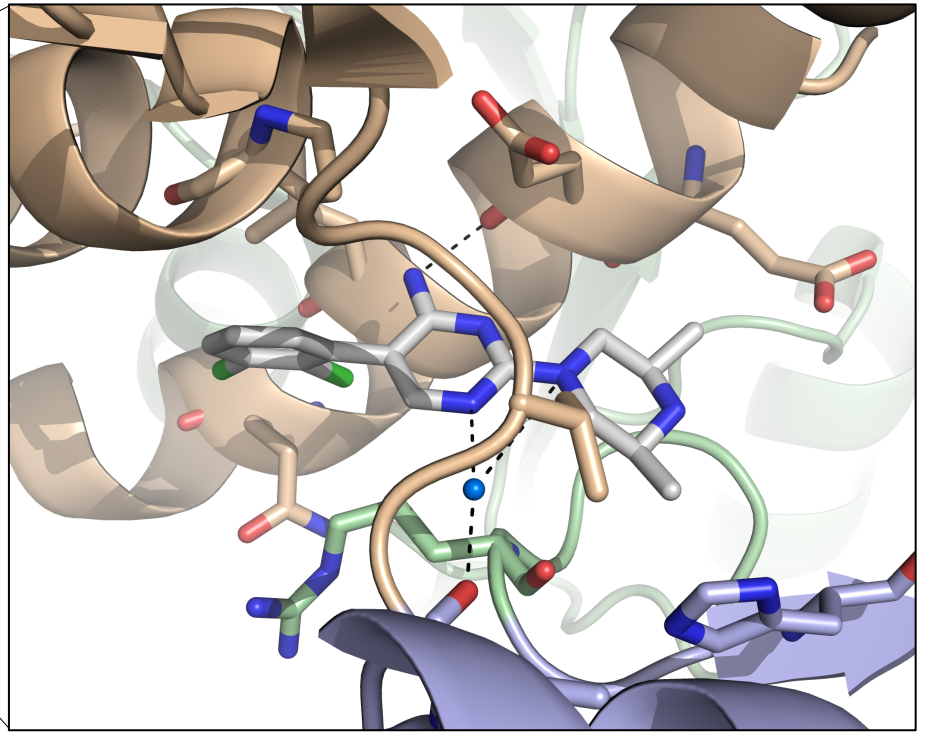
- Ion-dipole and dipole-dipole interactions
- Ionic or electrostatic bonds
- Pi interactions
- Hydrophobic interactions
- Hydrogen bonds
- Halogen bonding



[1]. *J. Med. Chem.* **2010**, 53, 5061.

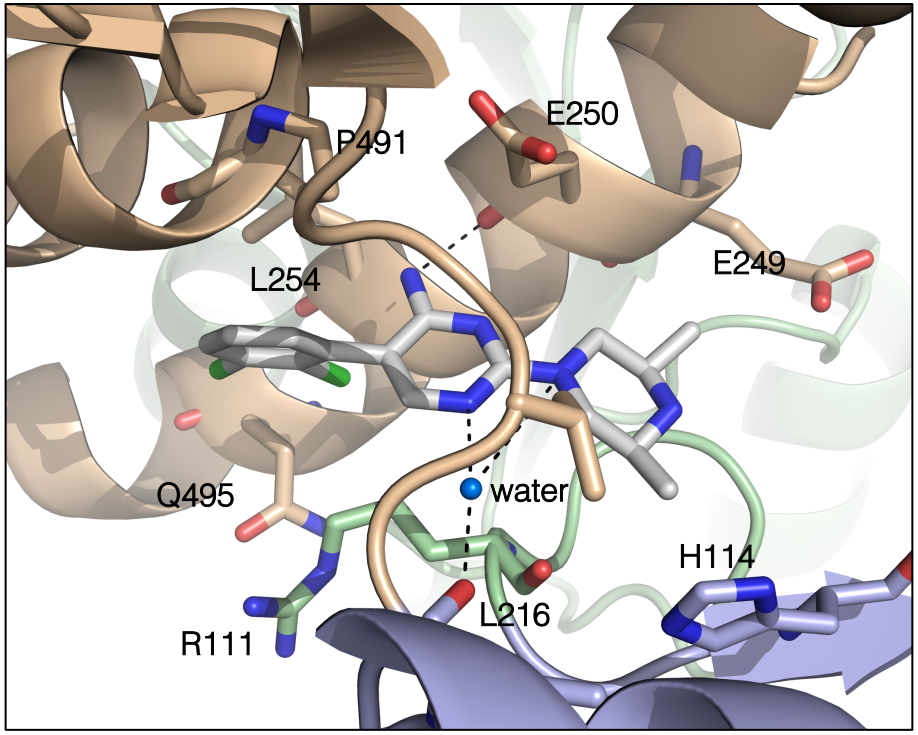
SHP836 & SHP2 Co-crystal Structure





PDB: 2SHP

SHP836 & SHP2 Co-crystal Structure: Key Intermolecular Interactions

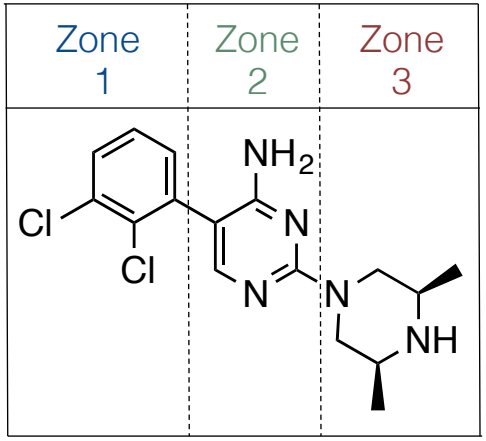


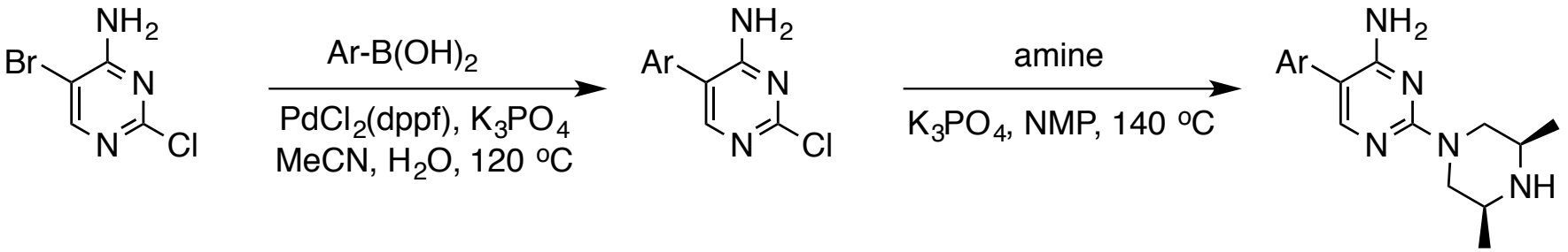
PDB: 2SHP

Protein-Ligand Interactions

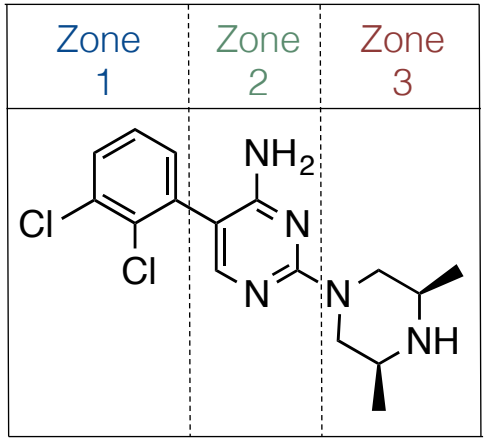
- -NH2 form H-bonding with Glu250 backbone
- Methyl groups form van der Waals interactions with His114 and Glu249
- The dichlorophenyl ring resides in a hydrophobic pocket (Leu254, Gln257, Pro491)
- Cationic-Pi interaction with dihlorophenyl ring and Arg111
- H-bonding network with water molecules

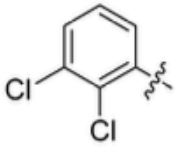
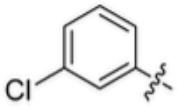
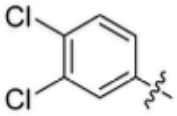
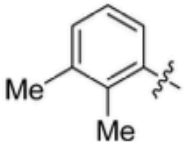
Optimization by SAR and SBDD



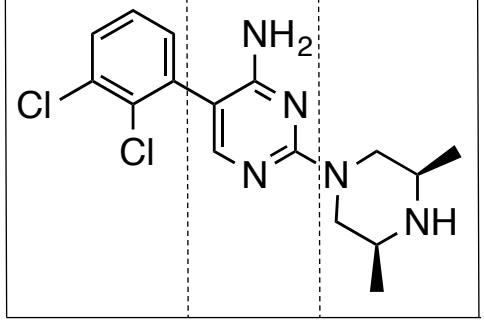


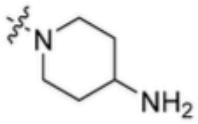
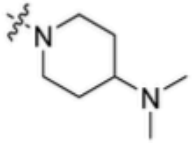
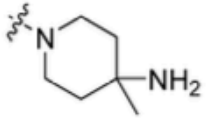
Zone 1



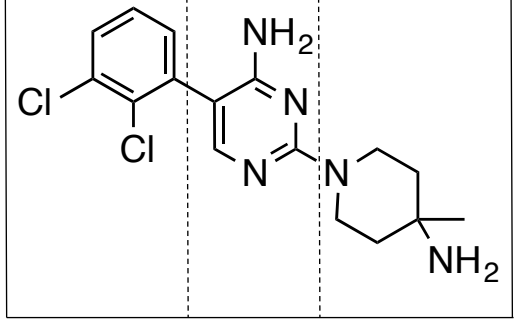
Compound	Ar	SHP2 IC_{50} (μM) (*p-ERK* (μM))
SHP836 (2)		12
3		99
4		54
5		>100

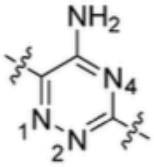
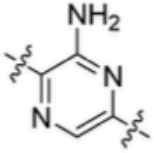
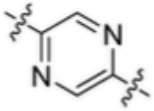
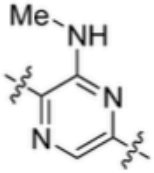
Zone 3

Zone 1	Zone 2	Zone 3
		

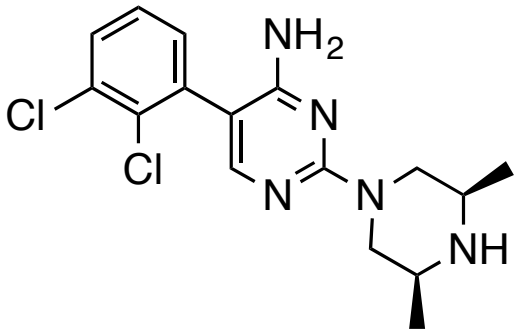
Compound	NR_1R_2	SHP2 IC_{50} (μM) (*p-ERK* (μM))
6		1.3
7		6.5
8		0.26 *(1.98)*

Zone 2

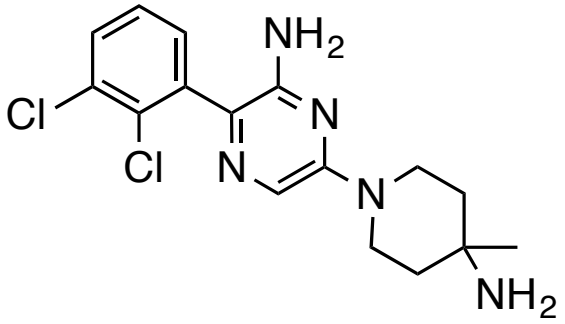
Zone 1	Zone 2	Zone 3
		

Compound	Core	SHP2 IC_{50} (μM) (*p-ERK* (μM))
9		0.30 *(0.62)*
1		0.07 *(0.25)*
10		5.7
11		22

Improvement

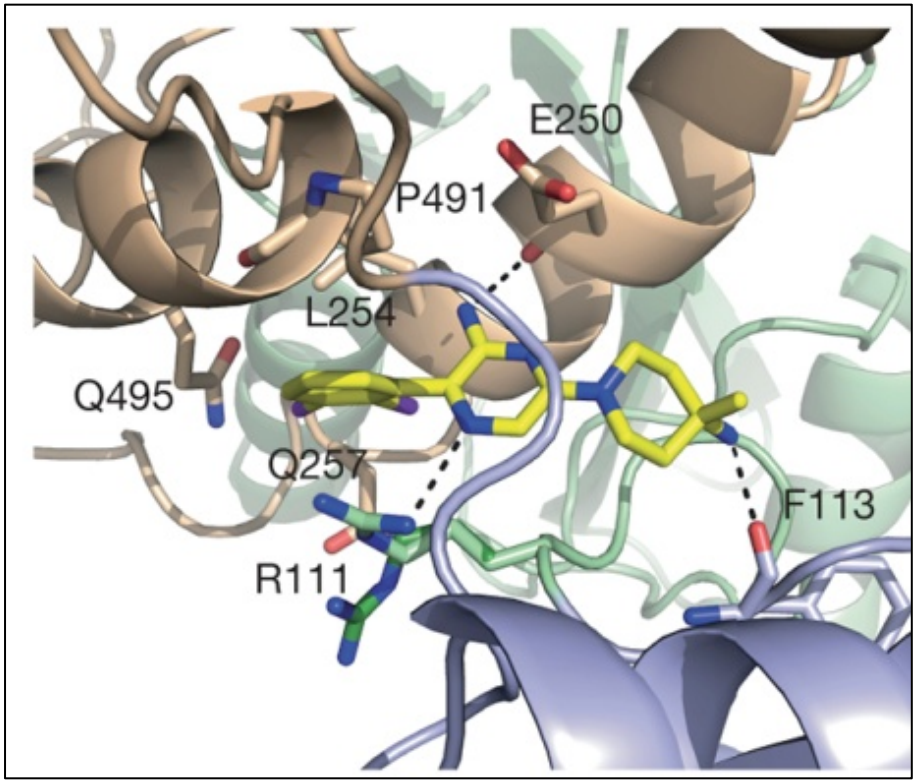


SHP836
SHP2 Allosteric Modulator
SHP2 IC_{50} = 12 uM



SHP099
SHP2 Allosteric Modulator
SHP2 IC_{50} = 0.07 uM

SHP099 & SHP2 Co-crystal Structure: Key Intermolecular Interactions



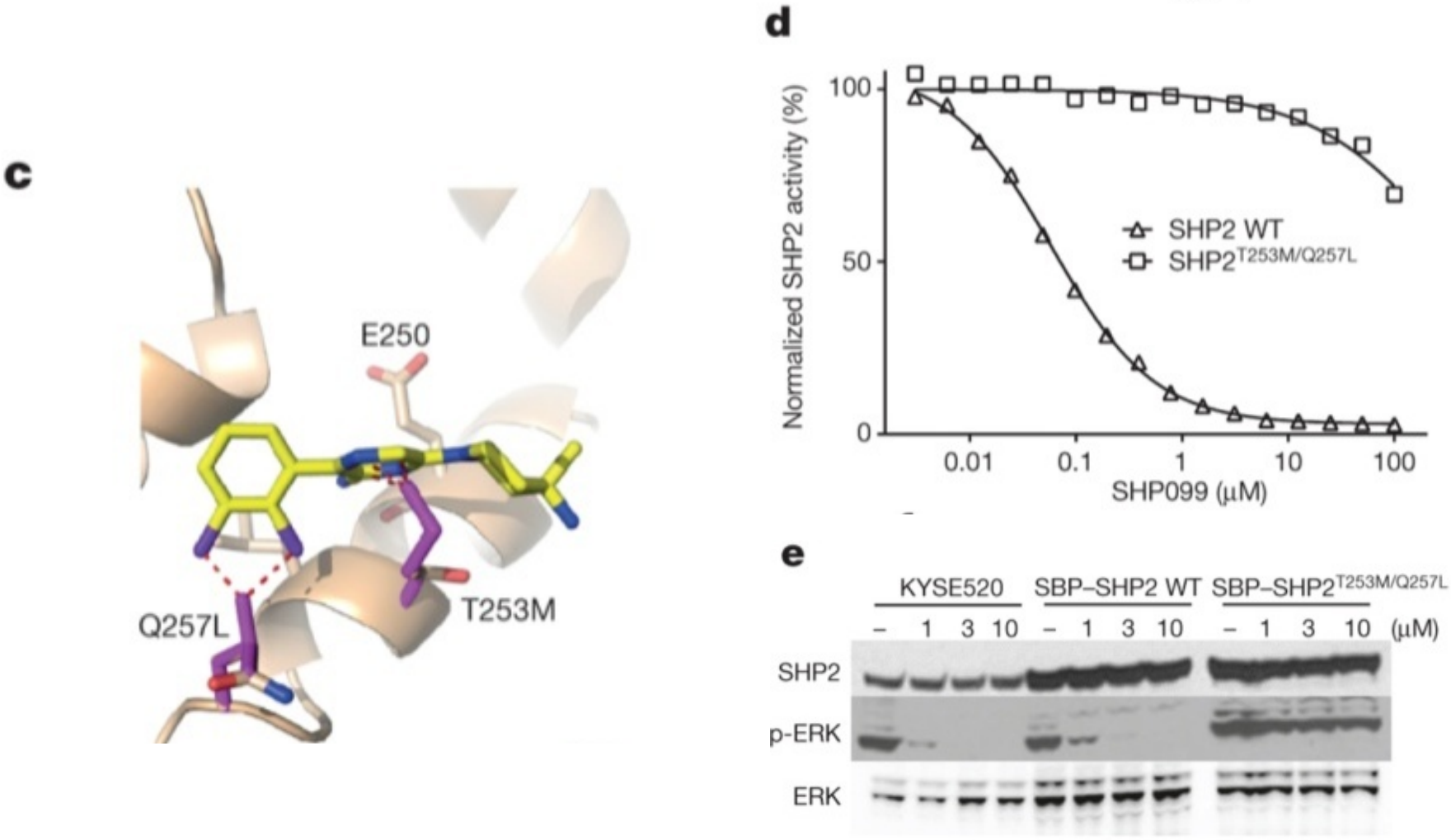
PDB: 5EHR

Protein-Ligand Interactions

- Conserved -NH2 H-bonding with Glu250 backbone
- New H-bond between pyrazine N and Arg111 sidechain
- Dichlorophenyl resides in a hydrophobic pocket (Leu254, Gln257, Pro491)
- Cationic-Pi interaction with dichlorophenyl ring Arg111
- New H-bonding between piperidine amine and F113 backbone

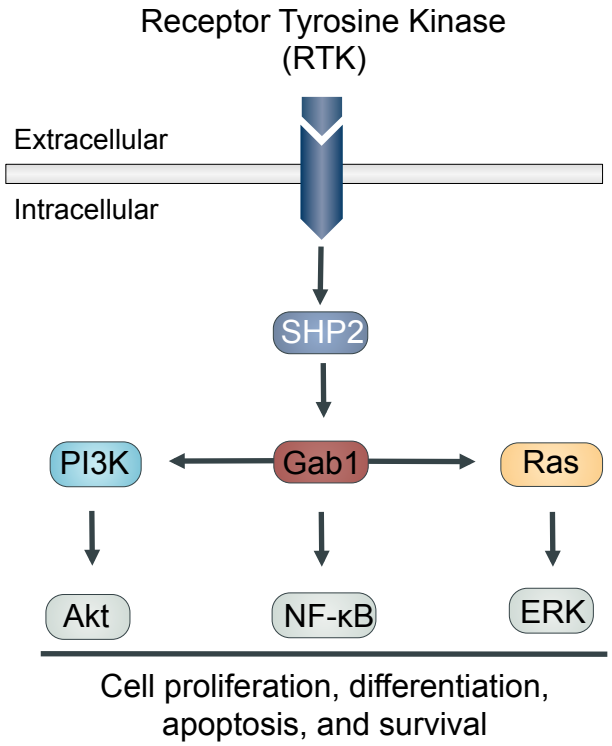
Nature **2016**, 535, 148.

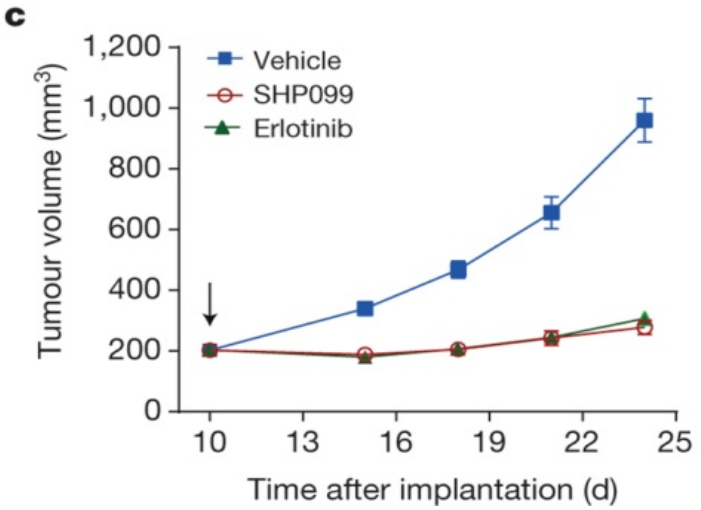
Biological Selectivity

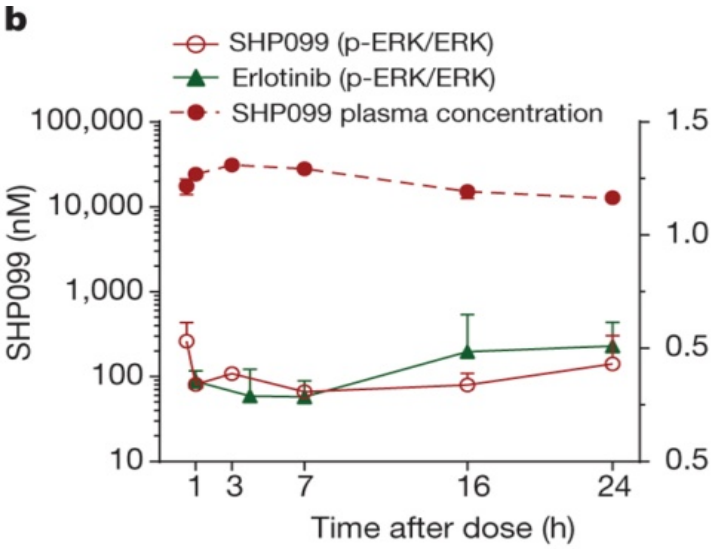


Nature **2016**, 535, 148.

In-Vivo Characterization of SHP099 in EGFR-Amplified KYSE-520 Cells

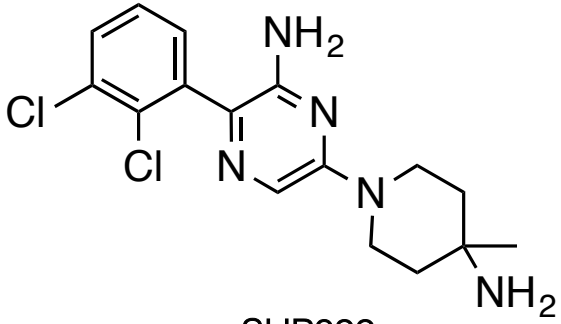






Nature **2016**, 535, 148.

Conclusion



SHP099
SHP2 Allosteric Modulator
SHP2 IC_{50} = 0.07 uM

- Identified an allosteric site that is influential in the enzymatic activity of SHP2, providing a platform for future drug discovery efforts.
- Developed a potent allosteric modulator of SHP2 that exhibits selectivity over other protein phosphatases.
- Pharmacological inhibition of SHP2 demonstrates a novel therapeutic approach to target RTK-dependent cancers.