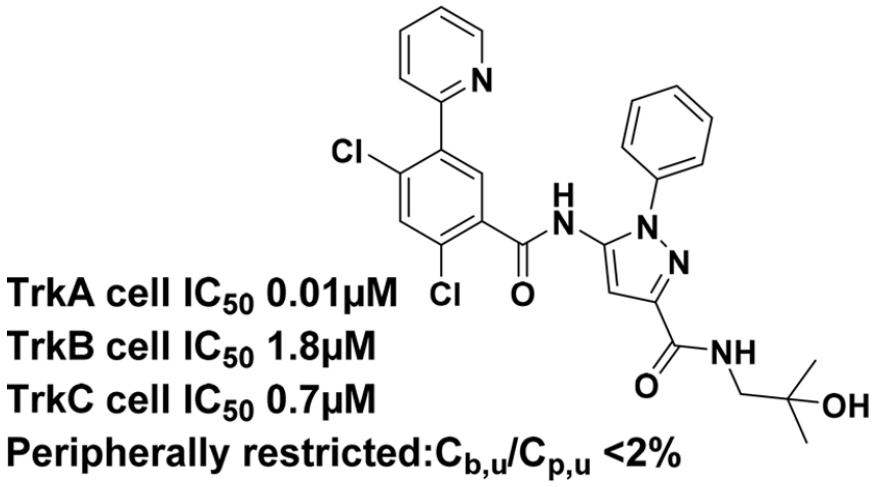
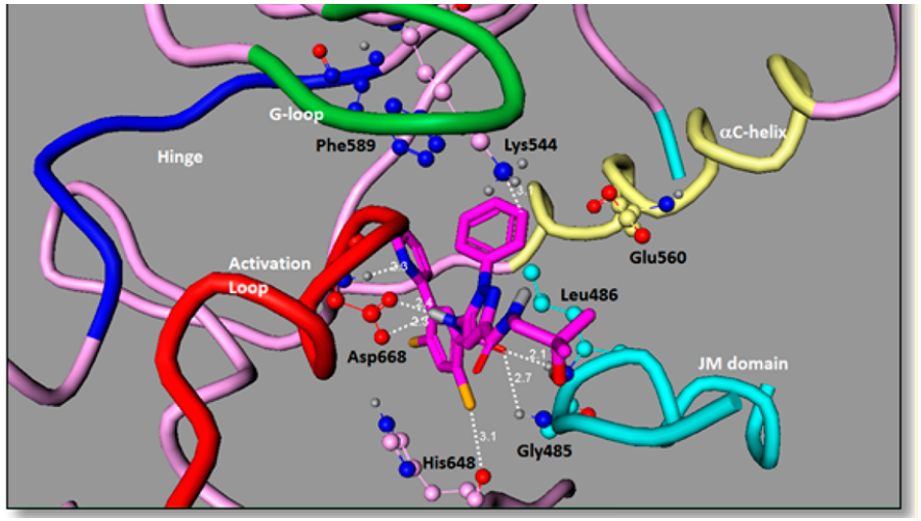
Discovery of Allosteric, Potent, Subtype Selective, and Peripherally Restricted TrkA Kinase Inhibitors

Sharan K. Bagal, Kiyoyuki Omoto, David C. Blakemore, *et al.*
J. Med. Chem., **2018**, Article ASAP





Steph McCabe
Wipf Group Current Literature
12th May 2016

Acute and Chronic Pain

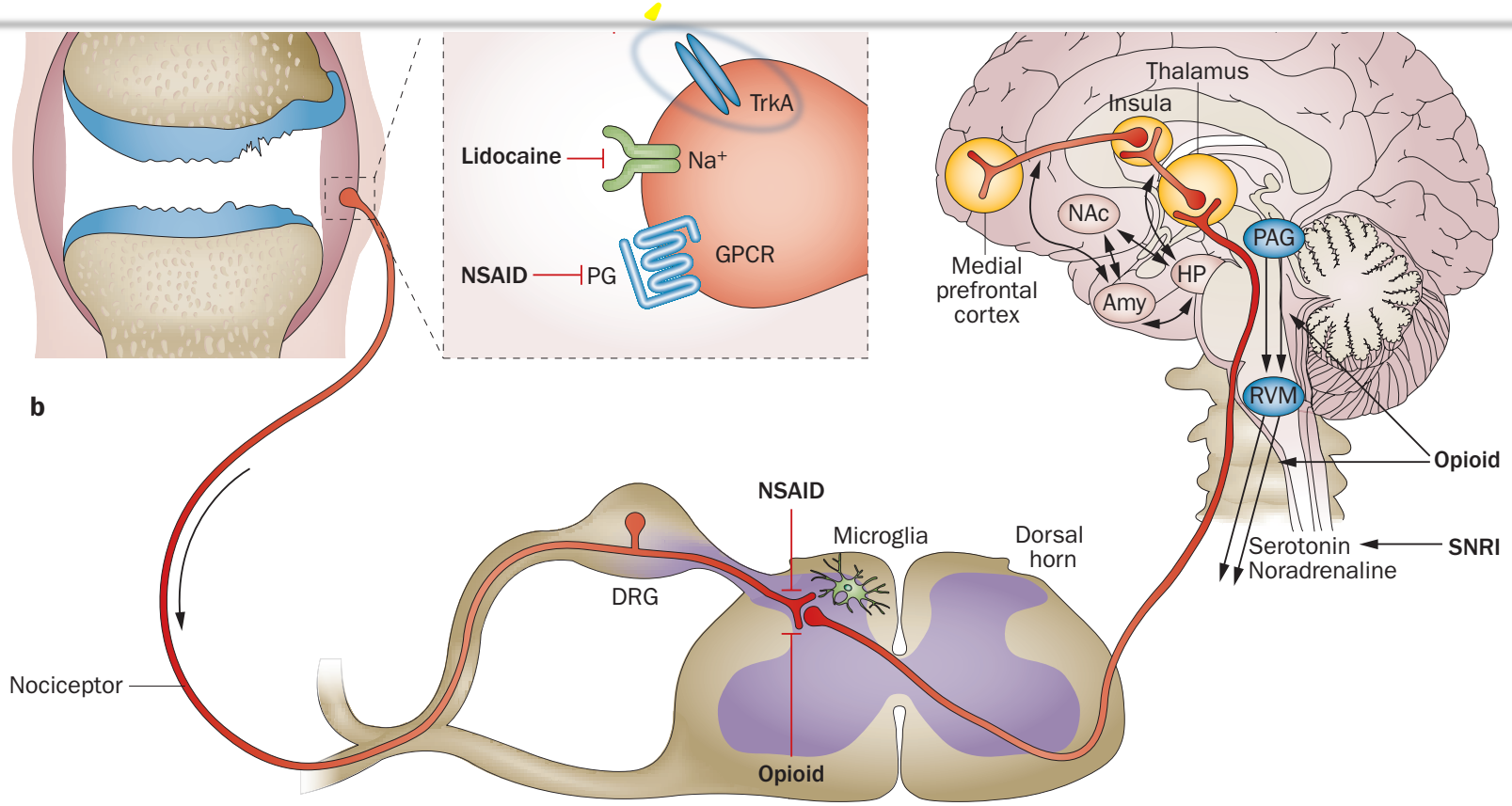
- **Pain:** is a symptom produced when inflammation or changes to the nervous system due to illness/ injury are transmitted to the brain, producing a physical sensation that alerts the brain that damage has occurred.

- **Chronic pain:** is generally defined as any pain lasting >12 weeks
  - Includes headache, lower back pain, cancer pain and arthritis

  - Chronic pain is the #1 reason Americans access the health care system.
  - Affects ~100 million adults in the US
  - Estimated annual cost of US $560–635 billion.

National Center for Health Statistics. Health, United States, 2012
National Institutes for Health: Pain Management: Fact Sheet, 2010

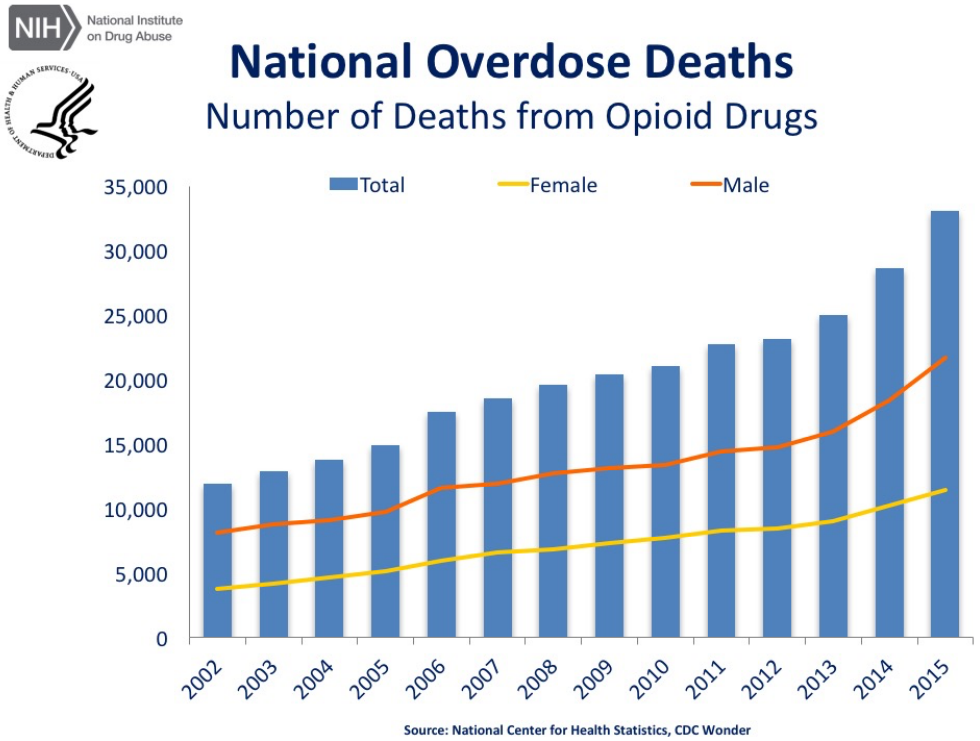
Pain Pathway/ Gold Standard Analgesics



- Non-steroidal anti-inflammatory drugs (NSAIDs) *e.g. aspirin, ibuprofen*
- Opioids *e.g. morphine, oxycodone*
- Antidepressants
- Anticonvulsants
- Local Anesthetics

Nat Rev Rheumatol. 2013, 9(11), 654

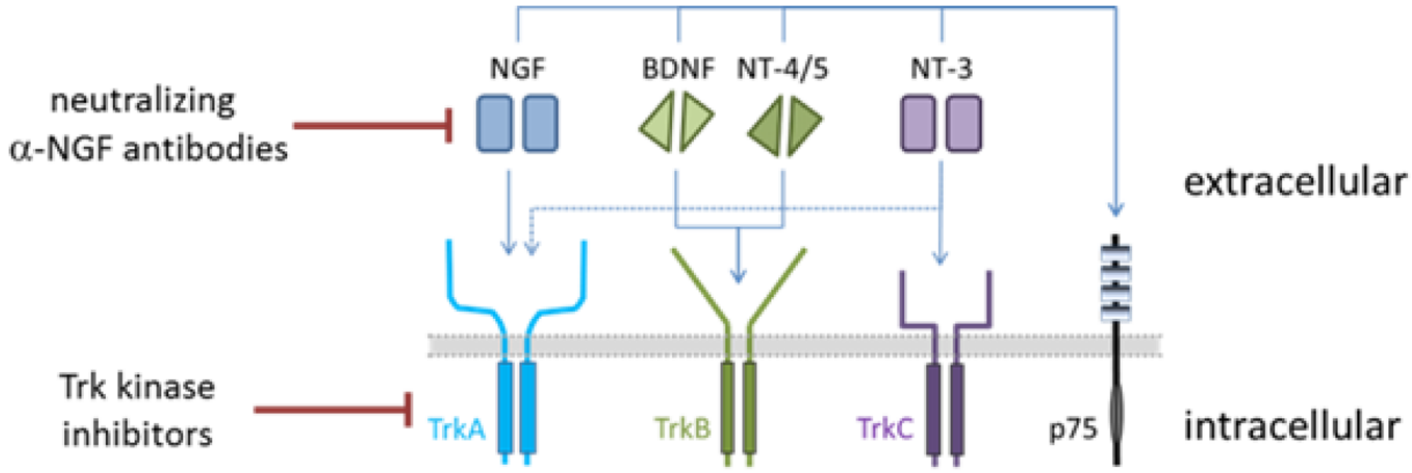
Unmet Need for Safe and Effective Pain Medication



- Current gold standard analgesics are often ineffective and/or have side effects (*e.g. GI/renal side-effects for NSAIDs and psychotropic for opioids*)
- Opioids killed more than 33 000 people in 2015, more than any year on record

National Center for Health Statistics, CDC: Overdose Death Rates

Role of NGF & TrkA in the Pathogenesis of Inflammatory Pain



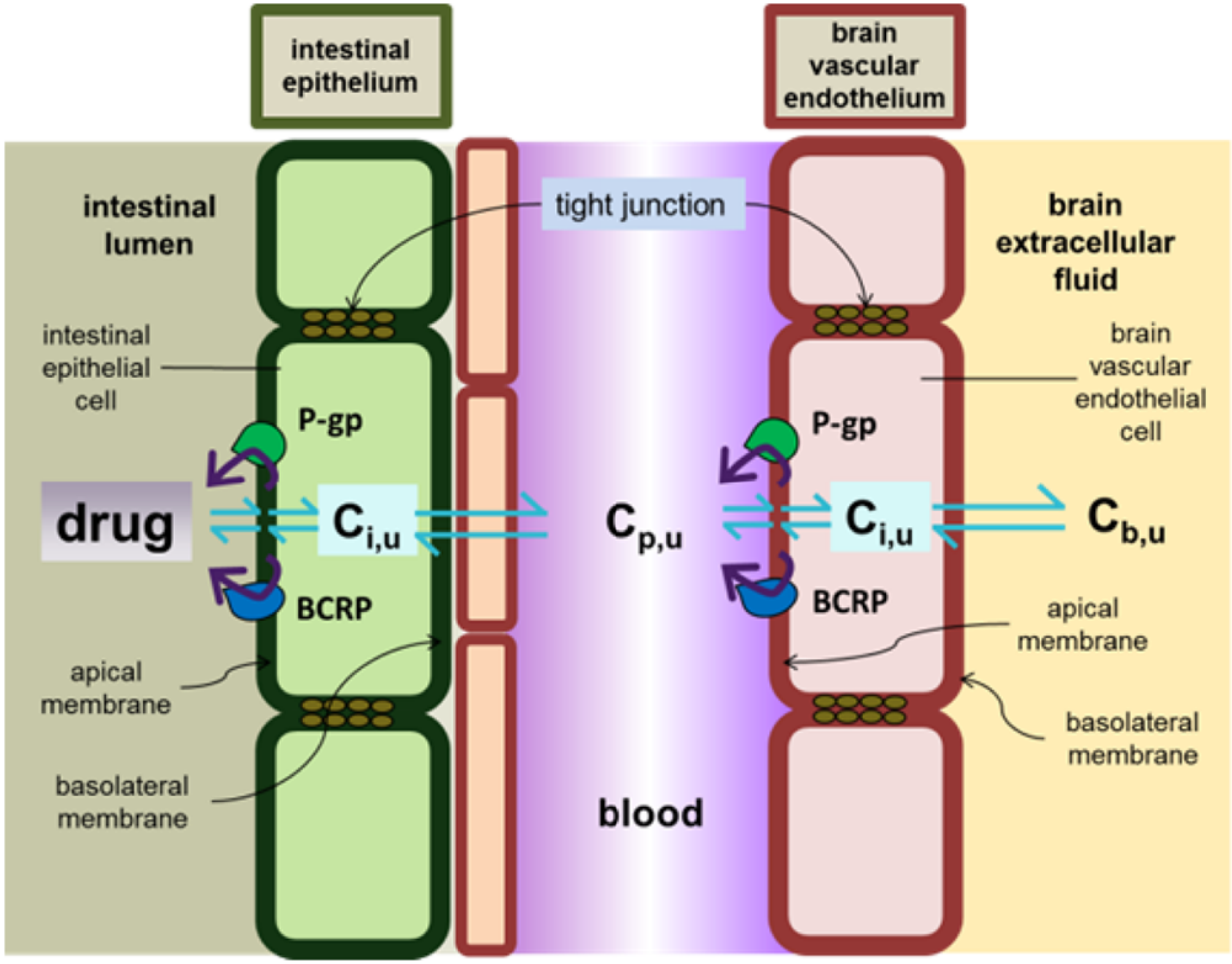
- NGF levels are elevated in response to chronic pain, injury and inflammation
- Administration of exogenous NGF induces pain in humans
- People with null mutations in TrkA and NGF genes develop congenital insensitivity to pain
- Inhibition of NGF function by anti-NGF antibodies and small molecule Trk inhibitors has shown efficacy in animal and human pain models *e.g. anti-NGF monoclonal antibody Tanezumab (Pfizer/Eli-Lily)*

- TrkA kinase inhibitor efficacy is expected to be driven by target engagement in peripheral neurons.
- Trks are broadly expressed in the brain
  - Regulate cholinergic activity, excitatory signaling and feeding/ body weight
- Clinical CNS side effects (cognitive deficits, personality changes and sleep deprivation) have been noted in an oral pan-Trk/Tie2 kinase inhibitor.

CNS concerns/Peripheral Restriction

Goal: Orally bioavailable small molecule Trk inhibitor with minimal brain availability

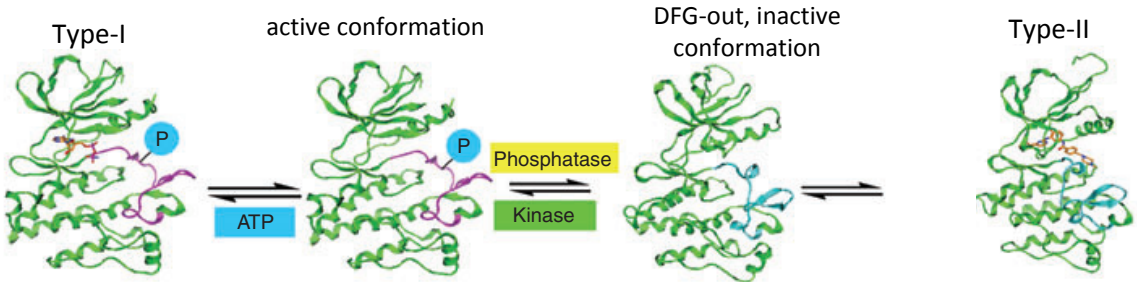
1. High absorption across the GI epithelium (MW<500, PSA<140, <10 rotatable bonds)
2. Good substrates for blood−brain barrier (BBB) efflux transporters (e.g. P-gp, BCRP)
3. Exquisite kinase selectivity (target an allosteric binding pocket)

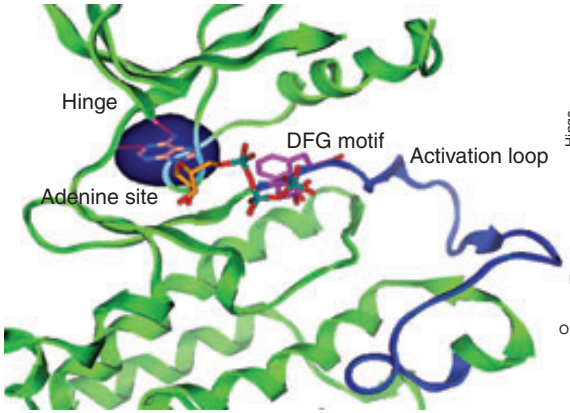


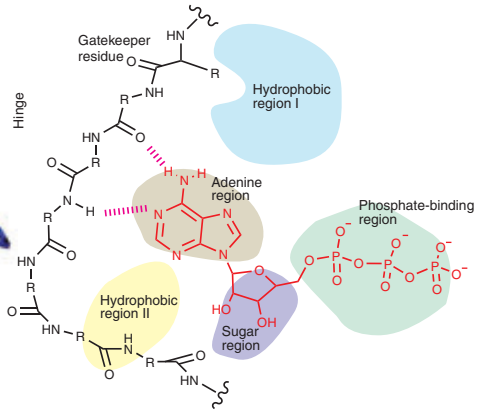
Desired peripheral restriction profile:
Free brain/ free plasma ratio ($C_{b,u}/C_{p,u}$) ≤ 5%

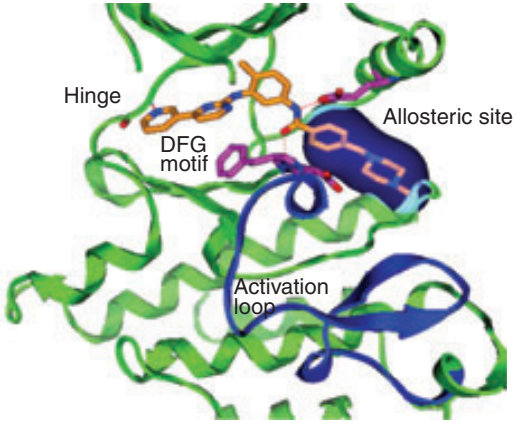
Classification of Kinase Inhibitors

Type I kinase inhibitors –bind to the active form (activation loop is phosphorylated)/ target the ATP binding site
Type II kinase inhibitors –bind to the inactive conformation/ target the ATP binding site and an allosteric 'DFG out' hydrophobic pocket immediately adjacent to the region occupied by ATP









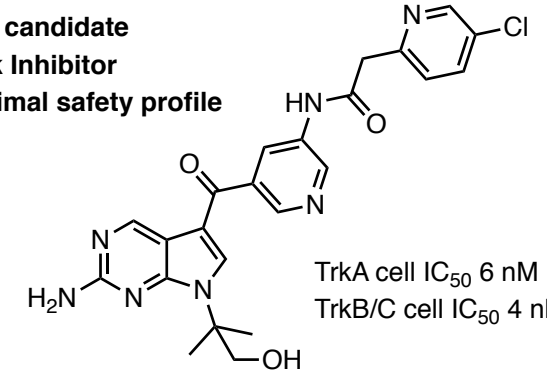
Type III kinase Inhibitors – bind next to the ATP binding pocket
Type IV-VI also known

- Type I and Type II binders tend to exhibit pan-Trk activity rather (no residue differences in the ATP binding site)
- Isoform selectivity has been achieved with type III allosteric ligands which do not interact with the conserved hinge region

Nat. Chem. Biol. 2006, *7*, 358; *Pharmacol. Res.* 2016, *103*, 26

TrkA Selective/Allosteric Hit

clinical candidate
pan-Trk Inhibitor
suboptimal safety profile

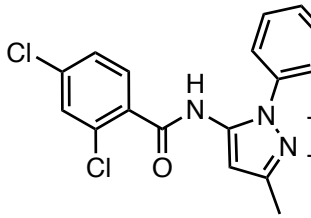


TrkA cell IC_{50} 6 nM
TrkB/C cell IC_{50} 4 nM

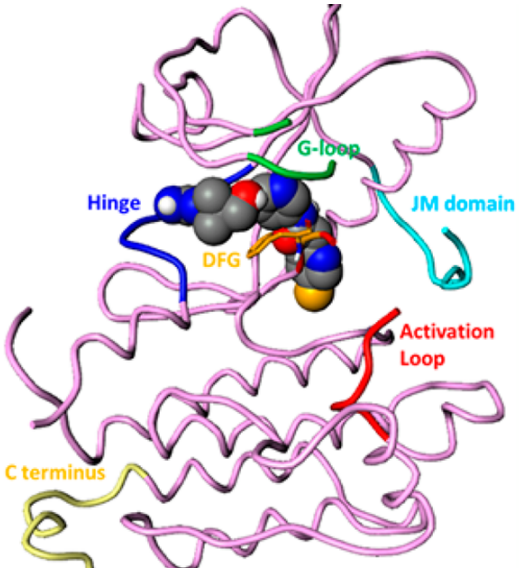
HTS reanalyzed

250 cpds tested in TrkA/B cell assays

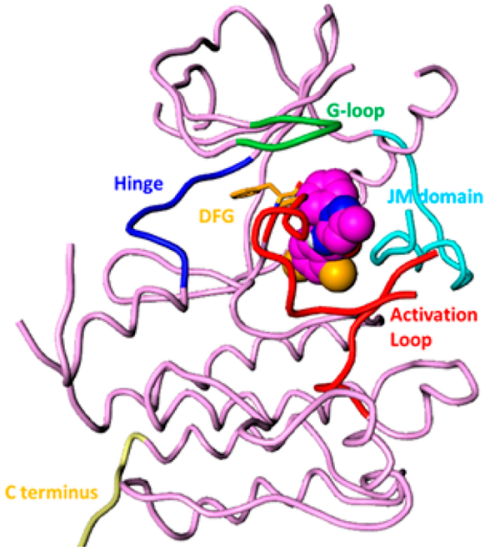
TrkA isoform selective hit



TrkA cell IC_{50} 3.3 μM
TrkB/C cell IC_{50} >50 μM



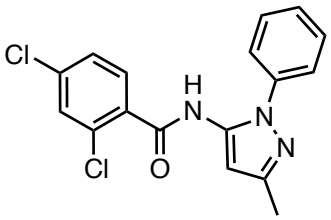
- Type II inhibitor
- Binds to DFG-out conformation
- Binds to the hinge region
- Extends into the DFG pocket



- Type III inhibitor
- Binds to DFG-out conformation
- Does not bind to the hinge region
- Binds to an allosteric hydrophobic pocket and interacts with the JM domain

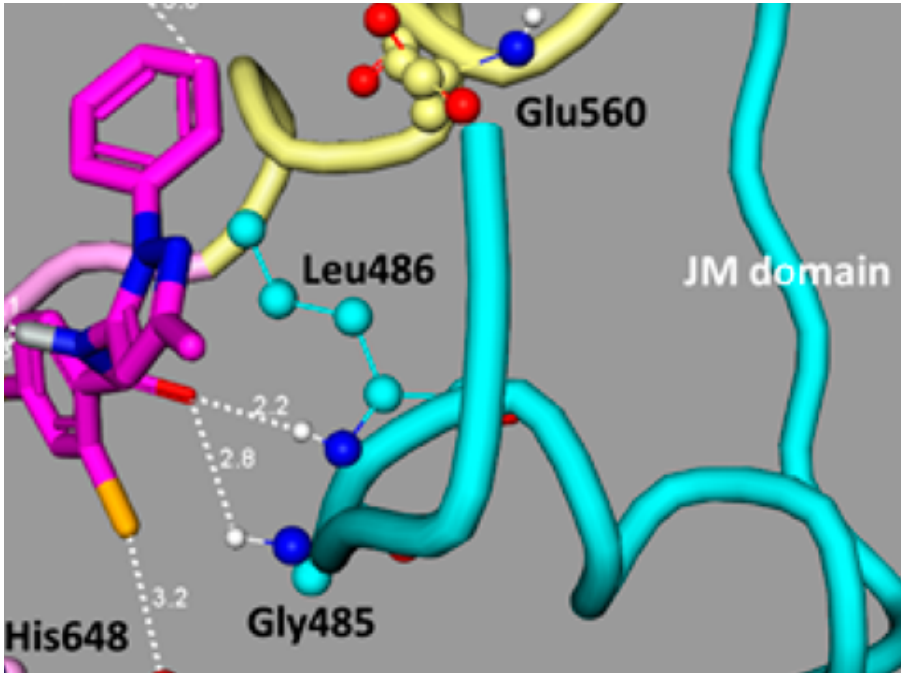
TrkA Selective/Allosteric Hit

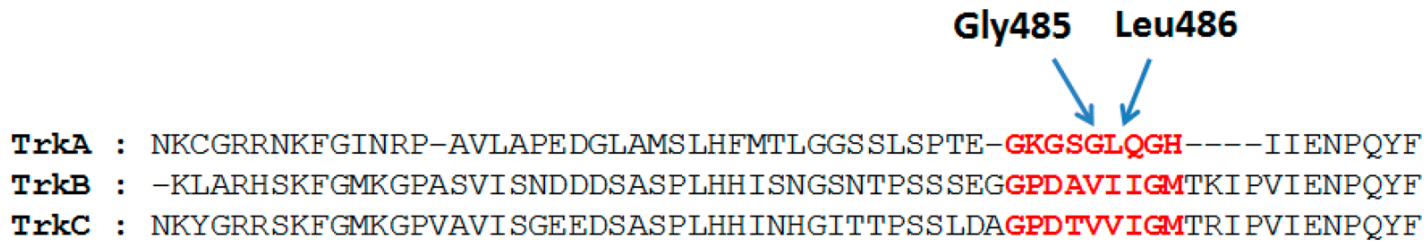
- The interaction of the JM domain with the ligand may explain isoform selectivity
- The JM domain is less conserved across Trks
- The JM domain of TrkA is shorter than TrkB/C so likely presents a different conformation to the ligand
- The cocrystal structure shows a H-bond between the main chain N-Hs of Leu486 and Gly485 with the C=O of the ligand



Hit

TrkA cell IC_{50} 3.3 μM
TrkB/C cell IC_{50} >50 μM

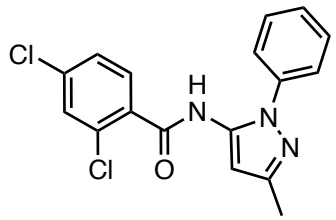




Physio- and Biochemical Properties

Trk A IC_{50} (nM)	Single digit nM ideal (potent) – they chose ≤15 nM	
MW/log$D_{7.4}$	MW<500 (for good oral bioavailability) ✔	Log $D_{7.4}$ < 1 high solubility, but permeability issues, susceptible to renal clearance Log $D_{7.4}$ 1-3 = optimal range (good balance between solubility and passive permeability)✔ Log $D_{7.4}$ 3-5 = low solubility (increased metabolic liability) Log $D_{7.4}$ > 5 = very low solubility (high metabolic clearance)
LipE	Lipophilic efficiency (= pIC_{50} – logD):	LipeE <3 = poor LipE 3-5 = moderate LipE 5-7 = good ✔ e.g. lipE 6 (e.g. 9 (pIC_{50}) – 3(logP) for 1 nM inhibitor
HLM Cl_{int} (µL/min/mg protein)	HLM Cl_{int} ≤ 8.6 µL = low ✔ HLM Cl_{int} 8.6–47 = medium HLM Cl_{int} ≥ 47 = high	
hHeps Cl_{int} (µL/min/10^6cells)	HLM Cl_{int} ≤ 3.5 = low ✔ HLM Cl_{int} 3.5–19 = medium HLM Cl_{int} ≥ 19 = high	
P-gp/BCRP ER (P_{app}B-A/P_{app}A-B)	▪ ≤1 = no significant efflux ✔ (+usually good) ▪ 2-3 = modest efflux ▪ ≥ 3 = significant efflux [✔ for a peripherally restricted drug)	
RRCK A-B P_{app} (x 10^{-6} cms^{-1})	▪ P_{app}< 2 = low permeability ▪ P_{app} 2–20 = moderate permeability ▪ P_{app}> 20 = high permeability ✔	
Solubility (µM)	▪ <10 µM = low solubility ▪ 10-100 µM = moderate solubility ▪ >100 µM = high solubility ✔	▪ <10 µg/mL = low solubility ▪ 10-60 µg/mL = moderate solubility ▪ >60 µg/mL = high solubility ✔

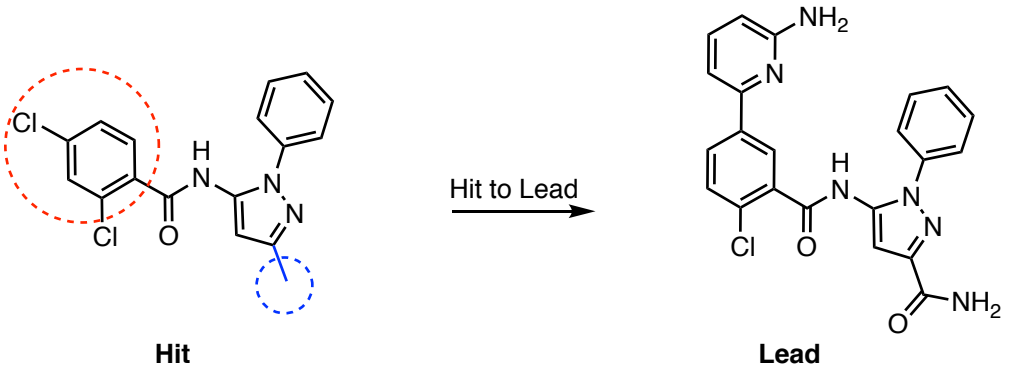
TrkA Selective/Allosteric Hit



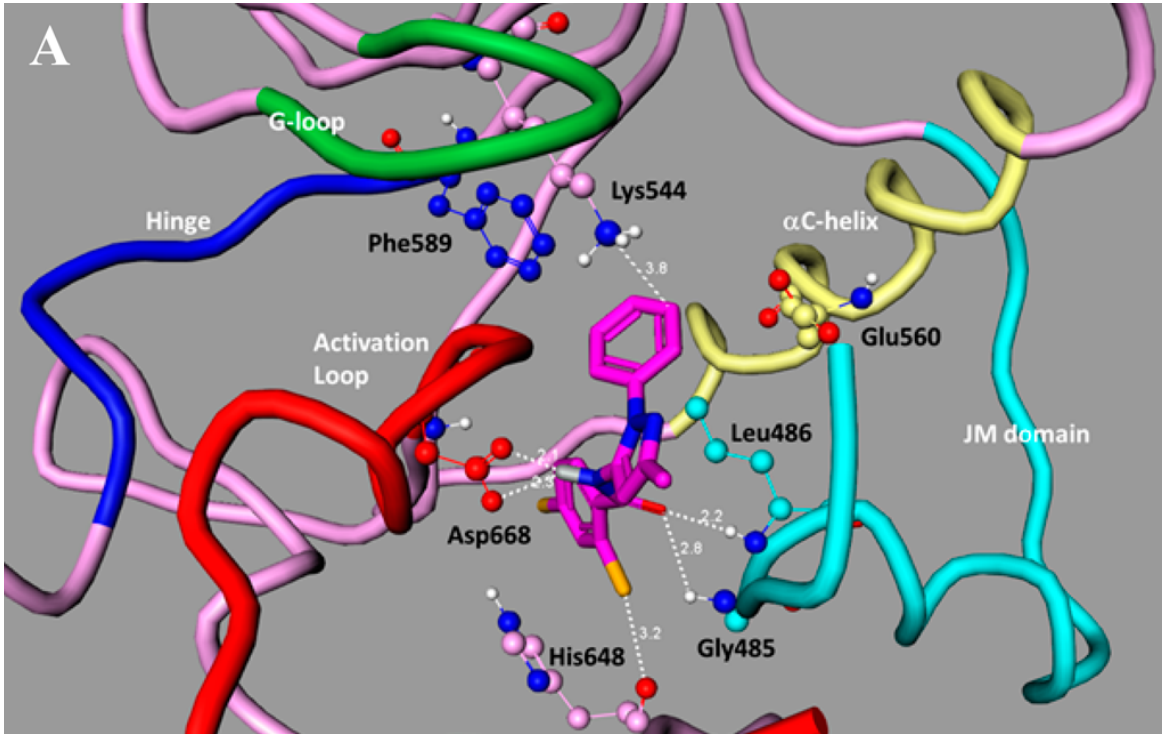
Hit

TrkA cell IC_{50} (µM)	3.3 ✗
TrkB/C cell IC_{50} (µM)	>50 µM ✔
MW/log$D_{7.4}$	346 ✔/3.3~
LipE	2.2 ✗
HLM Cl_{int}(µL/min/mg)	35 ✗
hHeps Cl_{int}(µL/min/mill)	48 ✗
P-gp/BCRP ER	1 ✗/ND
RRCK A-B P_{app} (x 10^{-6} cms^{-1})	30 ✔
PSA	47 ✔

Hit to Lead



Hit **Lead**



11 000 virtual ligands
(generated by changing the substituent at *p*-Cl)

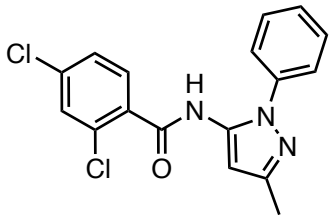
Docking Study (prioritized by docking score/ H-bond to NH of Asp668)

100 ligands

synthesized and tested in TrkA/B cellular assays

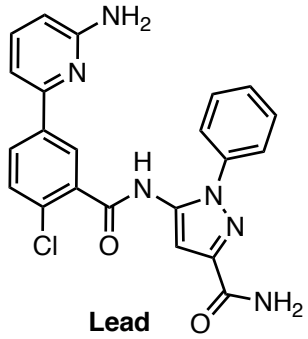
lead ligand

Lead Properties



Hit

Hit to Lead



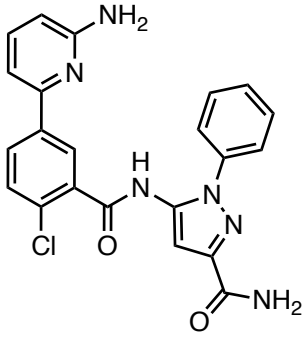
Lead

peripheral Restriction: $C_{b,u}/C_{p,u}$ ~ 4%

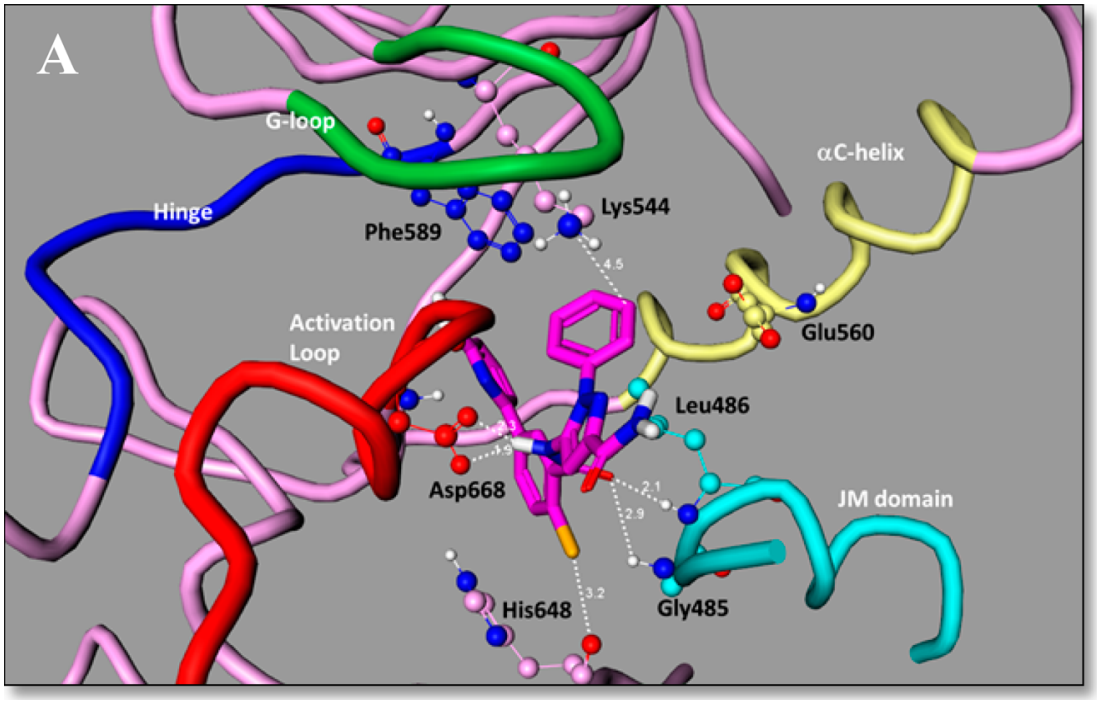
TrkA cell IC_{50} (μM)	3.3 ✗
TrkB/C cell IC_{50} (μM)	>50 μM ✔
$MW/logD_{7.4}$	346 ✔/3.3~
LipE	2.2 ✗
HLM Cl_{int}(μL/min/mg)	35 ✗
hHeps Cl_{int}(μL/min/mill)	48 ✗
P-gp/BCRP ER	1 ✗/ND
RRCK A-B P_{app} (x 10^{-6} cms^{-1})	30 ✔
PSA	47 ✔

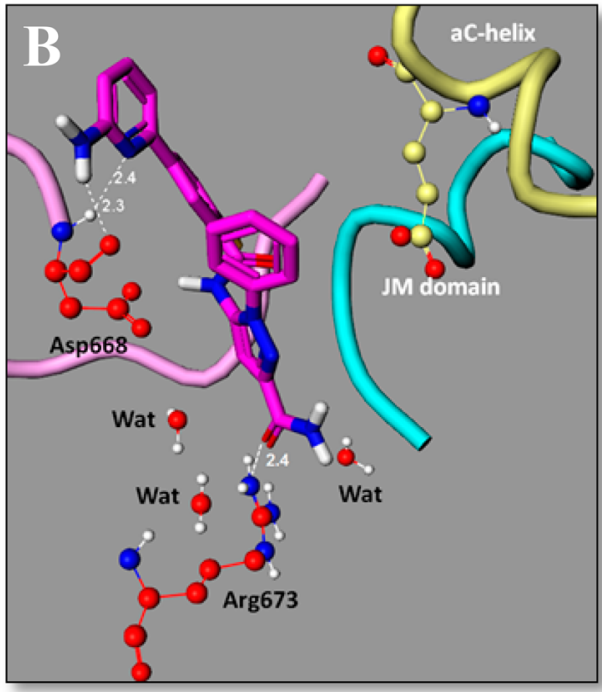
TrkA cell IC_{50} (μM)	0.050 ✗
TrkB/C cell IC_{50} (μM)	14/4.1 ✔
$MW/logD_{7.4}$	432 ✔/2.3 ✔
LipE	5.0 ✔
HLM Cl_{int}(μL/min/mg)	<8 ✔
hHeps Cl_{int}(μL/min/mill)	18 ~
HLM UGT Cl_{int}(μL/min/mg)	29 ✗
P-gp/BCRP ER	5 ✔/7 ✔
RRCK A-B P_{app} (x 10^{-6} cms^{-1})	17 ~
PSA	129 ✔

Cocrystal Structure of Lead Molecule Bound to TrkA

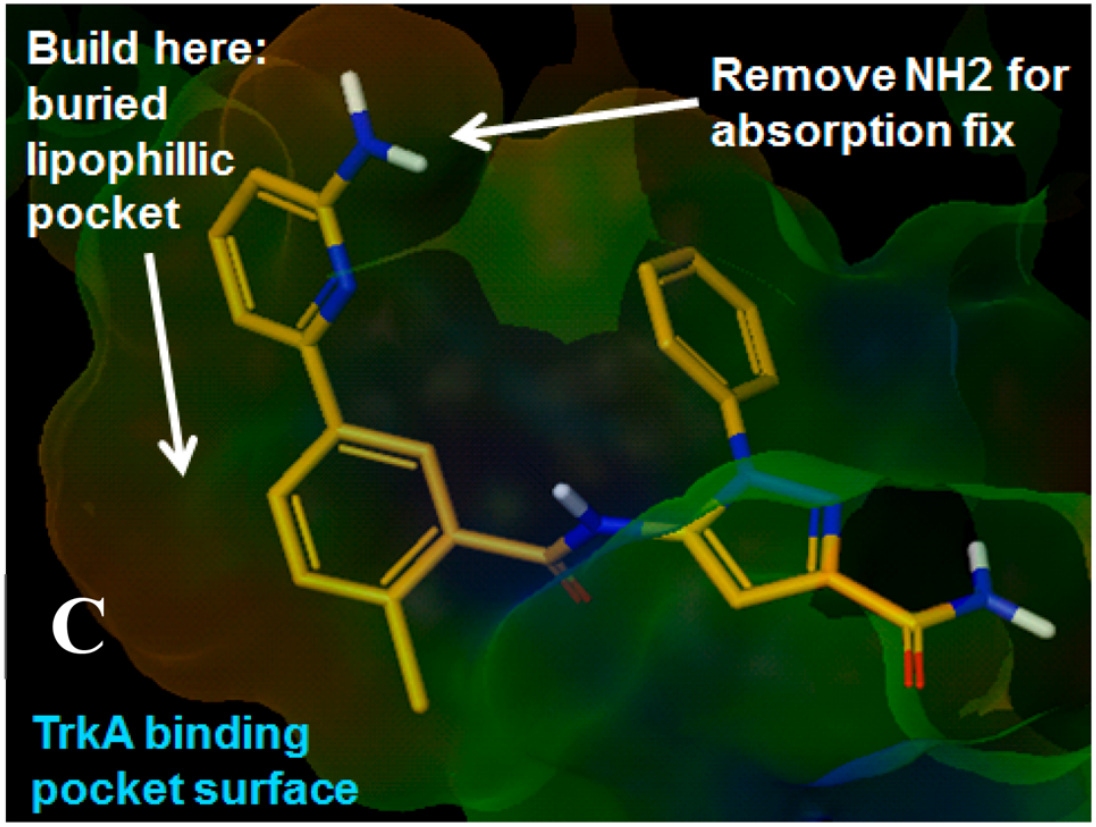


- H-bond pyridine N/Asp688 NH
- H-bond amino NH_2/ main chain C=O Asp668
- H-bond amide C=O/ NH_2 Arg673



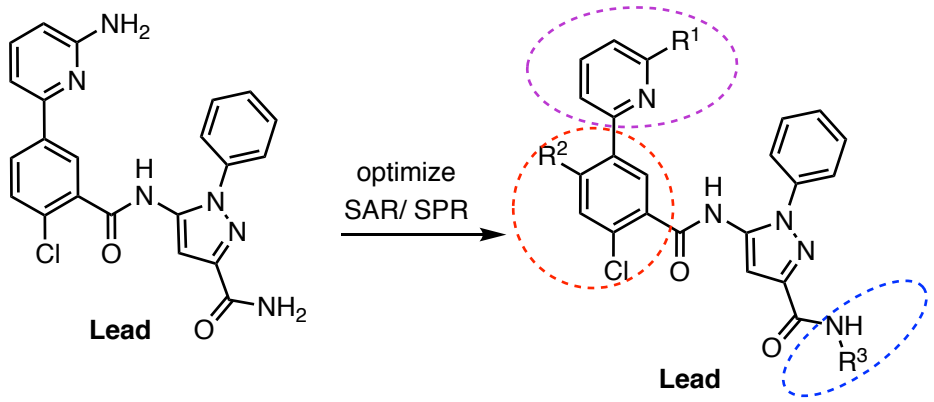


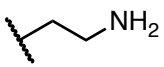
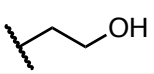
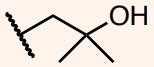
Lead to Candidate Optimization Strategy



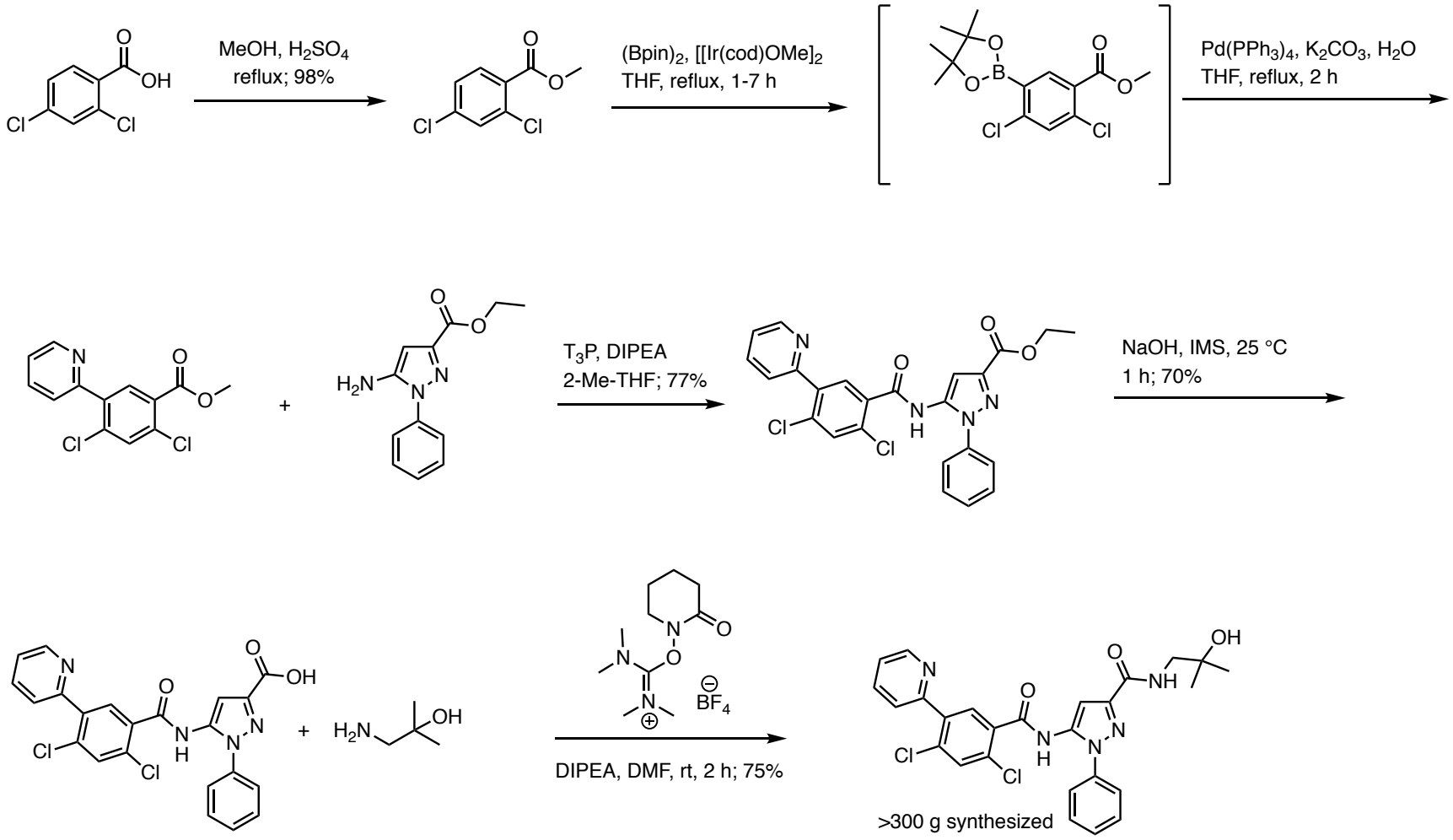
SAR/ SPR: Summary

TrkA cell IC_{50} 0.050 μM
TrkB/C cell IC_{50} 14/4.1 μM
LipE 5.0
LogD 2.3
RRCK 17
HLM <8, hUGT 29, hHep 18
P-gp ER 5
BCRP ER 7
Peripheral restriction C_{bu}/C_{pu} ~ 4%

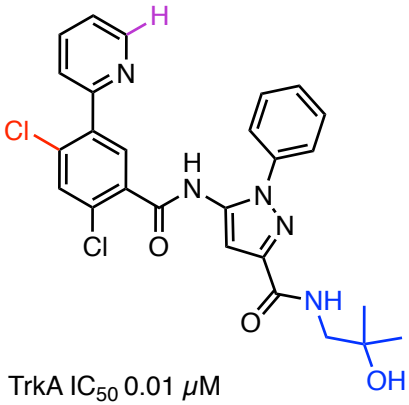


Entry	R^1	R^2	R^3	TrkA cell IC_{50} (μM)	LogD	LipE	HLM	HLM UGT	hHep	RRCK P_{app}	P-gp ER	BCRP ER
1	H	Cl	H	0.014 ✔	3 ✔	4.9 ✔	<8 ✔	<1.9 ✔	17 ~	17 ~	4.2 ✔	ND
2	H	CH_3	H	0.041 ✗	2.6 ✔	4.8 ~	46 ✗	ND	36 ✗	12 ~	4.6 ✔	ND
3	H	Cl		0.031 ✗	0.94 ✗	6.6 ✔	9 ~	ND	3.2 ✔	0.72 ✗	12 ✔	ND
4	H	Cl		0.014 ✔	2.8 ✔	5.1 ✔	<8 ✔	ND	9 ~	12 ~	30 ✔	ND
5	H	Cl		0.010 ✔	3.3 ~	4.7 ~	<8 ✔	ND	4.7 ~	16 ~	28 ✔	103 ✔

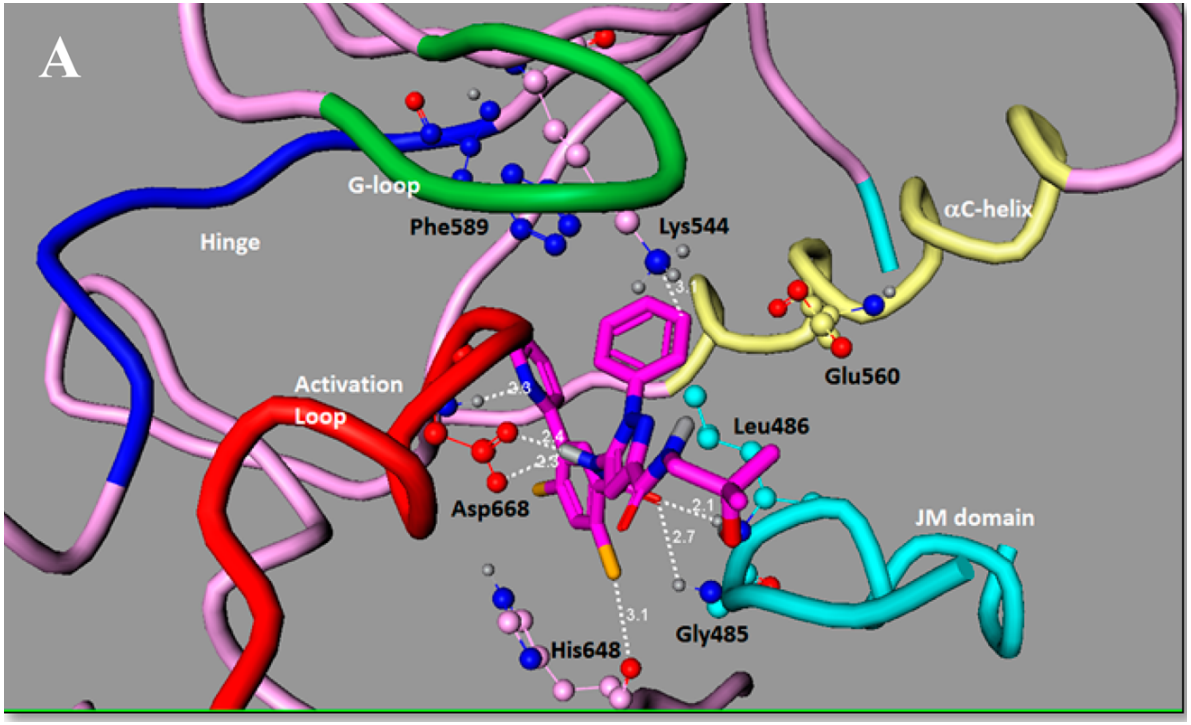
Synthesis of Candidate Molecule

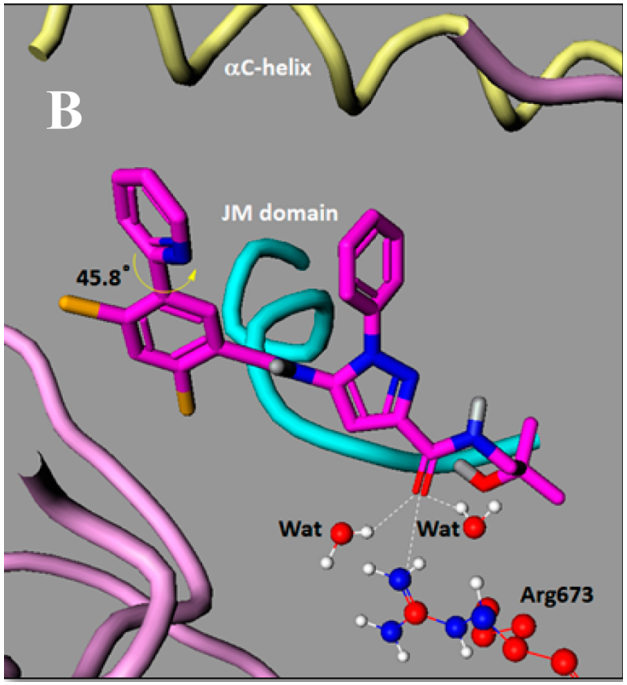


Cocrystal Structure of Candidate Bound to TrkA



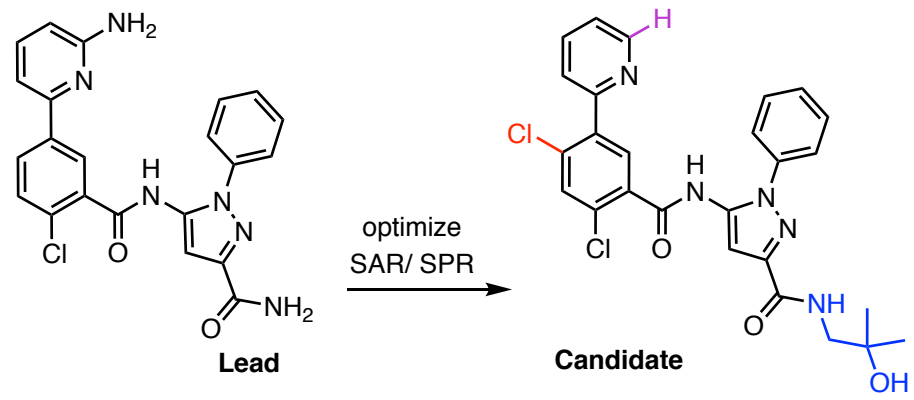
TrkA IC_{50} 0.01 μM
TrkB IC_{50} 1.8 μM (180-fold)
TrkC IC_{50} 0.7 μM (70-fold)





- H-bond Pyridine N/ Asp668 NH
- 2,4-dichloroaryl moiety occupies the lipophilic pocket
- The 4-Cl substituent results in an optimal dihedral angle of 45.8 ° between the pyridyl/aryl rings
- H-bond terminal amide C=O/ Arg673 NH_2
- Tertiary alcohol interacts with water network

PK Properties



Volume of distribution	V*d*	>10 L/kg = high <1 L/kg = low
Plasma clearance	Cl	Rat: >45 mL/min/kg = high Rat: <10 mL/min/kg = low
Half-life	$T_{1/2}$	Rat: > 3 h = high Rat: < 1 h = low
Oral bioavailability	%F	>50% = high <20% = low
Peripheral restriction	$C_{b,u}/C_{p/u}$	< 0.05 = peripherally restricted

p.o. administration (rat)

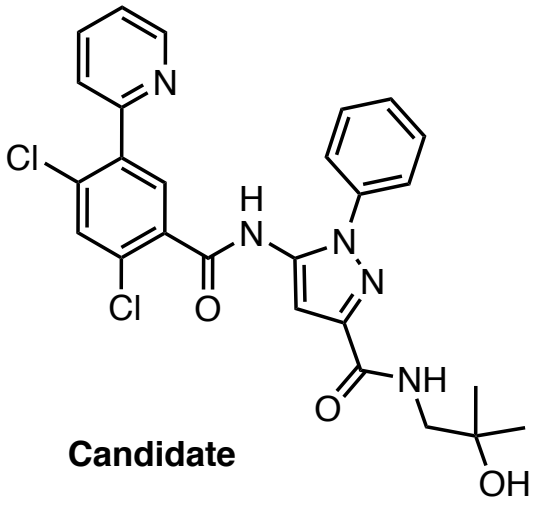
Entry	Cpd	Dose (mg/kg)	$T_{1/2}$ (h)	Oral *F* (%)
1	lead	3	0.5	6
2	candidate	2	4	72

i.v. administration (rat)

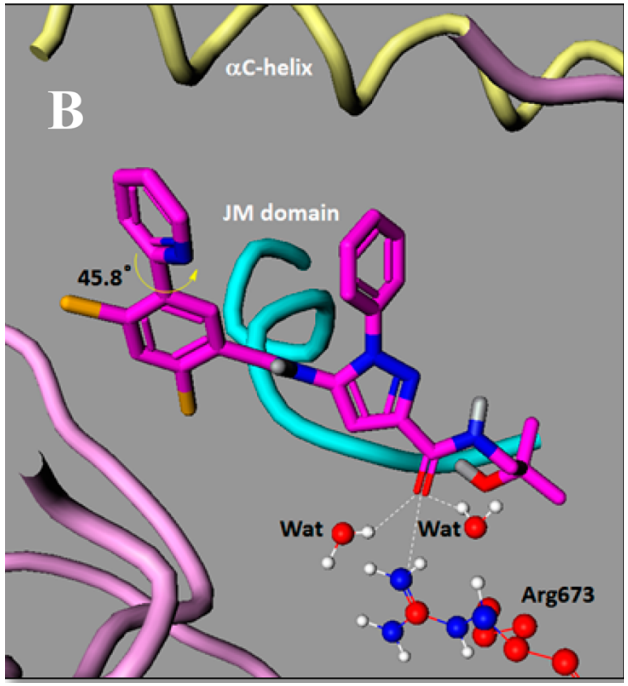
Entry	Cpd	Dose (mg/kg)	$T_{1/2}$ (h)	Plasma CL (mL/min/kg)	V_d (L/kg)	$C_{b,u}/C_{p/u}$
1	lead	1	1.6	31.5	1.3	0.041
2	candidate	0.5	2.7	11.9	2.8	0.014

- Excellent kinome selectivity (392 kinases <15% inhibition at 10 µM)
- Ligand profiling in 84 target assays (off-target liability)
  (no hits with inhibition >40% at 10 µM)

Summary/ Outlook



Candidate



- The tyrosine receptor kinase tropomyosin related kinase A (TrkA) is an important target in pain therapy
- TrkA isoform selectivity was achieved by developing a type III allosteric ligand
- The optimized TrkA inhibitor was highly potent, isoform-selective, orally bioavailable and peripherally restricted and nominated as a candidate for clinical development for the treatment of pain