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

TrkA cell IC$_{50}$ 0.01μM
TrkB cell IC$_{50}$ 1.8μM
TrkC cell IC$_{50}$ 0.7μM
Peripherally restricted: C$_{b,u}$/C$_{p,u}$ < 2%

Steph McCabe
Wipf Group Current Literature
12$^{th}$ 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
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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
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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
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 \( \frac{C_{b,u}}{C_{p,u}} \leq 5\% \)
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

TrkA Selective/Allosteric Hit

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

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

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

![TrkA Selective/Allosteric Hit](image)

**Hit**

TrkA cell IC$_{50}$ 3.3 µM
TrkB/C cell IC$_{50}$ >50 µM
## Physio- and Biochemical Properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Criteria</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trk A IC$_{50}$ (nM)</td>
<td>Single digit nM ideal (potent) – they chose ≤15 nM</td>
<td></td>
</tr>
<tr>
<td>MW/logD$_{7.4}$</td>
<td>MW&lt;500 (for good oral bioavailability)✅</td>
<td>Log D$_{7.4}$ &lt; 1: high solubility, but permeability issues, susceptible to renal clearance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Log D$_{7.4}$ 1-3: optimal range (good balance between solubility and passive permeability)✅</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Log D$_{7.4}$ 3-5: low solubility (increased metabolic liability)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Log D$_{7.4}$ &gt; 5: very low solubility (high metabolic clearance)</td>
</tr>
<tr>
<td><strong>LipE</strong></td>
<td>Lipophilic efficiency (= pIC$_{50}$ – logD)</td>
<td>LipE &lt;3 = poor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LipE 3-5 = moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LipE 5-7 = good ✅</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E.g. lipE 6 (e.g. 9 (pIC$_{50}$) – 3(logP) for 1 nM inhibitor)</td>
</tr>
<tr>
<td>HLM Cl$_{int}$ (µL/min/mg protein)</td>
<td>HLM Cl$_{int}$ ≤ 8.6 µL = low ✅</td>
<td>HLM Cl$_{int}$ 8.6–47 = medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HLM Cl$_{int}$ ≥ 47 = high</td>
</tr>
<tr>
<td>hHeps Cl$_{int}$ (µL/min/ 10⁶ cells)</td>
<td>HLM Cl$_{int}$ ≤ 3.5 = low ✅</td>
<td>HLM Cl$_{int}$ 3.5–19 = medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HLM Cl$_{int}$ ≥ 19 = high</td>
</tr>
<tr>
<td>P-gp/BCRP ER (P$<em>{app}$B-A/P$</em>{app}$A-B)</td>
<td>≤1 = no significant efflux ✅ (+usually good)</td>
<td>2-3 = modest efflux</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ 3 = significant efflux [⚠️ for a peripherally restricted drug]</td>
</tr>
<tr>
<td>RRCK A-B P$_{app}$ (x 10⁻⁶ cms⁻¹)</td>
<td>P$_{app}$ &lt; 2 = low permeability</td>
<td>P$_{app}$ 2–20 = moderate permeability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P$_{app}$ &gt; 20 = high permeability ✅</td>
</tr>
<tr>
<td>Solubility (µM)</td>
<td>&lt;10 µM = low solubility</td>
<td>&lt;10 µg/mL = low solubility</td>
</tr>
<tr>
<td></td>
<td>10-100 µM = moderate solubility</td>
<td>10-60 µg/mL = moderate solubility</td>
</tr>
</tbody>
</table>
|                               | >100 µM = high solubility ✅                                            | >60 µg/mL = high solubility ✅
**TrkA Selective/Allosteric Hit**

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>TrkA cell IC\textsubscript{50} ((\mu\text{M}))</td>
<td>3.3</td>
<td>✓</td>
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<tr>
<td>TrkB/C cell IC\textsubscript{50} ((\mu\text{M}))</td>
<td>&gt;50 (\mu\text{M})</td>
<td>✓</td>
</tr>
<tr>
<td>MW/logD\textsubscript{7.4}</td>
<td>346✓/3.3~</td>
<td></td>
</tr>
<tr>
<td>LipE</td>
<td>2.2</td>
<td>✓</td>
</tr>
<tr>
<td>HLM Cl\textsubscript{int} ((\mu\text{L/min/mg}))</td>
<td>35</td>
<td>✓</td>
</tr>
<tr>
<td>hHeps Cl\textsubscript{int} ((\mu\text{L/min/mill}))</td>
<td>48</td>
<td>✓</td>
</tr>
<tr>
<td>P-gp/BCRP ER</td>
<td>1✓/ND</td>
<td></td>
</tr>
<tr>
<td>RRCK A-B P\textsubscript{app} ((x \times 10^{-6} \text{ cms}^{-1}))</td>
<td>30✓</td>
<td></td>
</tr>
<tr>
<td>PSA</td>
<td>47✓</td>
<td></td>
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</table>

Steph McCabe @ Wipf Group
Hit to Lead

We recently reported the discovery of a potent pan-Trk inhibitor as a development clinical candidate. Ligand is an equipotent inhibitor of all three Trk kinases and was identified by following up a high-throughput screen (HTS) of the full Pfizer file using TrkA and TrkB cell based assays. It is a Type II inhibitor that binds to the inactive form of TrkA in a DFG-out conformation (Figure 5A). In order to find TrkA subtype selective hits, the HTS data set was reanalyzed to focus on compounds selective for TrkA over TrkB. The selection criteria were as follows: demonstration of some possibility for TrkA selectivity over TrkB in the existing data set (≥ 2-fold or not determined), chemotypes that were not known pan-Trk inhibitors from the Pfizer pan-Trk program, and lack of a common kinase hinge binder. Based on this analysis, a set of ∼250 compounds was prioritized for test at TrkA and TrkB in cell based assays at high concentration of 50 μM. The data generated suggested that the vast majority of these compounds were not active at TrkA (TrkA cell IC50 > 50 μM) or were nonselective over TrkB (<2-fold). However, a promising hit emerged that was active at TrkA (TrkA cell IC50 3.3 μM) and >10-fold selective over TrkB (TrkB cell IC50 > 50 μM) (Figure 5). The allosteric nature of this hit was confirmed via X-ray crystallography with a 1.93 Å cocrystal structure of the compound with TrkA protein. Compound was indeed a Type III binder.
### Lead Properties

<table>
<thead>
<tr>
<th></th>
<th>Hit</th>
<th>Lead</th>
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<tbody>
<tr>
<td><img src="hit.png" alt="Chemical Structure" /></td>
<td><img src="lead.png" alt="Chemical Structure" /></td>
<td>peripheral Restriction: $C_{b,u}/C_{p,u} \sim 4%$</td>
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</table>

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TrkA cell $IC_{50}$ (µM)</td>
<td>3.3 $\times$</td>
<td>0.050 $\times$</td>
</tr>
<tr>
<td>TrkB/C cell $IC_{50}$ (µM)</td>
<td>$&gt;50$ µM $\checkmark$</td>
<td>14/4.1 $\checkmark$</td>
</tr>
<tr>
<td>MW/logD$_{7.4}$</td>
<td>346 $\checkmark$/3.3 $\sim$</td>
<td>432 $\checkmark$/2.3 $\checkmark$</td>
</tr>
<tr>
<td>LipE</td>
<td>2.2 $\times$</td>
<td>5.0 $\checkmark$</td>
</tr>
<tr>
<td>HLM $Cl_{int}$ (µL/min/mg)</td>
<td>35 $\times$</td>
<td>&lt;8 $\checkmark$</td>
</tr>
<tr>
<td>hHeps $Cl_{int}$ (µL/min/mill)</td>
<td>48 $\times$</td>
<td>18 $\sim$</td>
</tr>
<tr>
<td>P-gp/BCRP ER</td>
<td>1 $\times$/ND</td>
<td>5 $\checkmark$/7 $\checkmark$</td>
</tr>
<tr>
<td>RRCK A-B $P_{app}$ (x 10$^{-6}$ cm$^{-1}$)</td>
<td>30 $\checkmark$</td>
<td>17 $\sim$</td>
</tr>
<tr>
<td>PSA</td>
<td>47 $\checkmark$</td>
<td>129 $\checkmark$</td>
</tr>
</tbody>
</table>
Hit to Lead Optimization.

Allosteric hit 2 exhibited the following profile: TrkA cell IC50 3.3 μM, ligand efficiency (LE) 0.33, and lipophilic efficiency (LipE) 2.2 (LipE = −Log(TrkA cell IC50) − LogD). While this LE provided a reasonable starting point, the potency and LipE required significant improvement. Moreover, the potential for drug action at this allosteric site represented a key question: will sufficient potency and LipE be achievable in this lipophilic binding site such that a clinical candidate is a viable proposition?

Analysis of the cocrystal structure shown in Figure 6 determined the optimization strategy for hit 2. A number of ligands that bind to kinases in the DFG-out conformation interact with the backbone NH of the DFG motif, for example, pan-Trk ligand 1,19,41 imatinib, 47 and BIRB 796. 48 The corresponding interaction with the main chain Asp668 NH could be targeted by building on the meta position of the dichlorophenyl unit of 2 (Figure 6A). The pyrazole methyl group has a vector toward the kinase exit, which is solvent exposed. This vector could be used to append polarity and decrease the lipophilicity of ligand 2 (LogD 3.3). Bound water molecules identified by crystallography were also analyzed using WaterMap (Figure 6B), which maps the locations and thermodynamic properties of water molecules that solvate protein binding sites.49,50 A number of relatively unstable water molecules calculated to have an unfavorable free energy and enthalpy relative to solvent water (colored red, Figure 6B) were identified in the direction of the kinase hinge. Such water molecules have been reported to be successfully replaced by lipophilic groups.49,50 A cluster of water molecules that appeared to be more stable (colored blue, Figure 6B) could be identified at the pyrazole methyl, consistent with the methyl pointing toward solvent. Based upon the considerations outlined above, ligand 2 was optimized to lead molecule 3 (Scheme 1). A series of virtual molecules was designed based on a Suzuki coupling reaction with commercially or internally available acids (Scheme 2). Eleven thousand molecules were created and docked against the X-ray structure of 2 (see Experimental Section). The molecules were then prioritized based on docking score as well as presence/absence of hydrogen bond with backbone NH of Asp668 in a docking pose. One hundred molecules were selected for synthesis and tested in the TrkA and TrkB cellular assays. As a result, 3 showed a significant improvement in potency (TrkA cell IC50 50 nM) and LipE (5) and maintained >80-fold TrkA selectivity over TrkB/C in cell based assays (Scheme 1).
Hit to Lead Optimization

Strategy

Lead to Candidate Optimization

Steph McCabe @ Wipf Group
**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%

<table>
<thead>
<tr>
<th>Entry</th>
<th>R$^1$</th>
<th>R$^2$</th>
<th>R$^3$</th>
<th>TrkA cell IC$_{50}$ (µM)</th>
<th>LogD</th>
<th>LipE</th>
<th>HLM</th>
<th>HLM UGT</th>
<th>hHep</th>
<th>RRCK P$_{app}$</th>
<th>P-gp ER</th>
<th>BCRP ER</th>
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<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>0.014 ✔</td>
<td>3 ✔</td>
<td>4.9 ✔</td>
<td>&lt;8 ✔</td>
<td>&lt;1.9 ✔</td>
<td>17 ~</td>
<td>17 ~</td>
<td>4.2 ✔</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>CH$_3$</td>
<td>H</td>
<td>0.041 X</td>
<td>2.6 ✔</td>
<td>4.8 ~</td>
<td>46 X</td>
<td>ND</td>
<td>36 X</td>
<td>12 ~</td>
<td>4.6 ✔</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>Cl</td>
<td>NH$_2$</td>
<td>0.031 X</td>
<td>0.94 X</td>
<td>6.6 ✔</td>
<td>9  ~</td>
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<td>3.2 ✔</td>
<td>0.72 X</td>
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<td>OH</td>
<td>0.014 ✔</td>
<td>2.8 ✔</td>
<td>5.1 ✔</td>
<td>&lt;8 ✔</td>
<td>ND</td>
<td>9  ~</td>
<td>12 ~</td>
<td>30 ✔</td>
<td>ND</td>
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<tr>
<td>5</td>
<td>H</td>
<td>Cl</td>
<td>OH</td>
<td>0.010 ✔</td>
<td>3.3 ~</td>
<td>4.7 ~</td>
<td>&lt;8 ✔</td>
<td>ND</td>
<td>4.7 ~</td>
<td>16 ~</td>
<td>28 ✔</td>
<td>103 ✔</td>
</tr>
</tbody>
</table>
Synthesis of Candidate Molecule

Cl\(\text{CH}_2\text{Cl}\)\(\text{OH}\) → MeOH, \(\text{H}_2\text{SO}_4\)
reflux; 98%

Cl\(\text{CH}_2\text{Cl}\)\(\text{OH}\) \(\rightarrow\) (Bpin)\(_2\), [[Ir(cod)OMe]\(_2\)]
THF, reflux, 1-7 h

Pd(PPh\(_3\))\(_4\), \(\text{K}_2\text{CO}_3\), \(\text{H}_2\text{O}\)
THF, reflux, 2 h

\(\text{Cl}\text{H}_2\text{Cl}\)\(\text{OH}\) + \(\text{H}_2\text{N}\\text{Bu}\)
\(\text{T}_3\text{P}, \text{DIPEA}\)
2-Me-THF; 77%

\(\text{Cl}\text{H}_2\text{Cl}\)\(\text{OH}\) + \(\text{H}_2\text{N}\text{Bu}\)
\(\text{DIPEA, DMF, rt, 2 h; 75%}\)

>300 g synthesized
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

**p.o. administration (rat)**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Cpd</th>
<th>Dose (mg/kg)</th>
<th>$T_{1/2}$ (h)</th>
<th>Oral $F$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>lead</td>
<td>3</td>
<td>0.5</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>candidate</td>
<td>2</td>
<td>4</td>
<td>72</td>
</tr>
</tbody>
</table>

**i.v. administration (rat)**

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

- **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

- **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

- 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.