Strategies involved in the construction of novel benzotriazine inhibitors of Src

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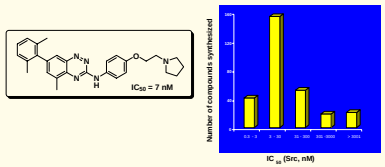
Src activation in cancer

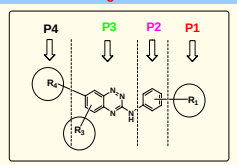
- Protein kinases are families of enzymes that catalyze the phosphorylation of specific residues in proteins.
- One family of cytoplasmic tyrosine kinases capable of communicating with a large number of different receptors is the Src tyrosine kinase family. The c-Src proto-oncogene plays a major role in the development, protein growth, progression, and metastasis of a wide variety of human cancers.
- Src kinase modulates signal transduction through multiple oncogenic pathways, including EGFR, Her2/neu, PDGFR, FGFR, and VEGFR.
- Since the activation and perhaps over-expression of Src has been implicated in cancer, osteoporosis, stroke, myocardial infarction, and vascular leak, among other diseases, a small molecule inhibitor of c-Src can be beneficial for the treatment of several disease states.
- TargeGen has designed and optimized potent Src inhibitors using structure-based drug design (see MEDI 65 and 66).

TargeGen benzotriazine Src inhibitors



- Potent compounds rapidly synthesized by a small group of medicinal chemists within 9-12 months

Design of novel benzotriazines



P1: solvent exposed region

P2: hydrophobic region 2

P3: hinge (core)

P4: hydrophobic region 1

1. Involve synthesis of 3 major fragments:

A. Synthesis of benzotriazine core (P3)

B. Synthesis of P4 moiety

C. Synthesis of P2-P1 solubilizing fragment

2. Assemble P4-P3 fragments

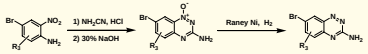
3. Assemble P4-P3 fragments with P2 and P1:

A. Linear synthesis

B. Convergent syntheses

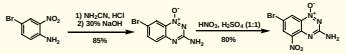
Step 1A: Synthesis of benzotriazine core (P3)

- **Constructing the core:**



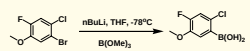
R3 = 5-CH3 (yield in 2-steps: 65%); 6-CH3 (60%)
6-OCH3 (67%); 5-CO2H (95%) ; 5-OH (67%); 5-H (80%)

- **Introducing a functional group on the core:**

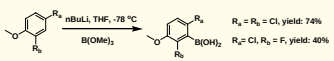


Step 1B: Synthesis of P4 moiety

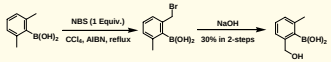
1. **Preparation of aryl boronic acids from aryl bromide:**



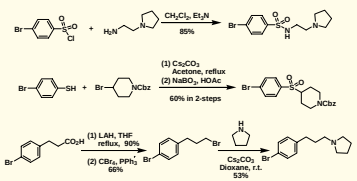
2. **Preparation of boronic acids by directed-metalation:**



3. **Functionalization of a boronic acid:**



Step 1C: Synthesis of P1-P2 solubilizing fragment



Step 2: Suzuki cross coupling

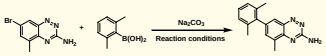
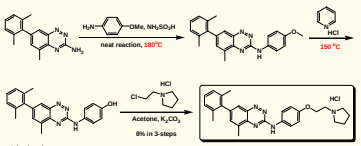


Table 1. Optimization of Suzuki coupling reactions

Entry	Reaction Conditions	Yield (%)
1	boronic acid (1.1 Equiv.), $Pd(PPh_3)_4$, DMF, reflux	30-40
2	boronic acid (1.5 Equiv.), $Pd(PPh_3)_4$, DME, reflux	40-70
3	boronic acid (1.5 Equiv.), $Pd(PPh_3)_4$, Toluene/EtOH, reflux	50-60
4	boronic acid (1.1 Equiv.), $Pd_2(PPh_3)_2Cl_2$, Toluene/EtOH, reflux	60-70
5	boronic acid (1.3 Equiv.), $Pd_2(PPh_3)_2Cl_2$, Toluene/EtOH, reflux	68-73
6	boronic acid (1.2 Equiv.), $Pd(PPh_3)_4$, DME/EtOH/H_2O, reflux	> 80

Step 3A: Assemble P4-P3 with P2 and P1 using linear synthesis



Limitations:

--- Three consecutive low yield reactions (8% in 3-steps)
--- Melting of sulfamic acid and pyridine HCl requires heating at high temperature

Step 3B: Assembly P1-P2 to P3-P4 by Buchwald reactions

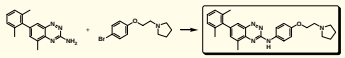


Table 2. Optimization of Buchwald coupling reaction

Entry	Reaction Conditions	Yield (%)
1	$Pd(PPh_3)_4$, BINAP, Cs_2CO_3, Toluene, reflux, 20 h	No reaction
2	$Pd(PPh_3)_4$, Xantphos, Cs_2CO_3, Dioxane, reflux, 20h	10
3	$Pd_2Cl_2(PPh_3)_2$, Xantphos, Cs_2CO_3, Dioxane, reflux, 20h	5
4	$Pd_2(dba)_3$, Xantphos, Cs_2CO_3, Dioxane, reflux, 20h	60-70

Advantages:

--- Reduce number of steps (convergent synthesis)
--- Overall higher yields (60-70% in 1-step vs. 8% in 3-steps)

Step 3B: Assembly P1-P2 to P3-P4 by Sandmeyer reactions

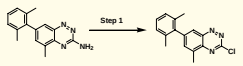


Table 3. Optimization of Sandmeyer reactions (Step 1)

Entry	Reaction Conditions	Yield (%)	Notes
1	$NaNO_2$ (1.5 Equiv.), CuCl, H_2O	32%	Low yield
2	$NaNO_2$ (1.5 Equiv.), CuCl, H_2O, $CHCl_3$	50-55%	10% SM unrecacted
3	$NaNO_2$ (2.0 Equiv.), CuCl, H_2O, $CHCl_3$	55%	10% SM unrecacted
4	$NaNO_2$ (3.0 Equiv.), CuCl, H_2O, $CHCl_3$	65-70%	Complete conversion

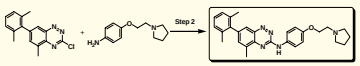


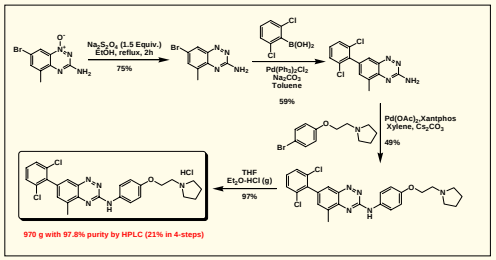
Table 4. Optimization of fragment coupling reactions (Step 2)

Entry	Reaction Conditions	Yield (%)	Notes
1	K_2CO_3, DMF, heat	15-20	Most SM decomposed
2	K_2CO_3, EtOH, heat	0	Most SM decomposed
3	K_2CO_3, Pd(OAc) $_2$, BINAP, Dioxane	25	Many by-products
4	*t*-BuOK, DMF, heat	15	Most SM decomposed
5	n-BuLi (1.2 Equiv.), THF, -60 to -70 ºC	50	Incomplete reaction
6	n-BuLi (1.5 Equiv.), THF, -60 to -70 ºC	> 60	Scalable process with simple purification

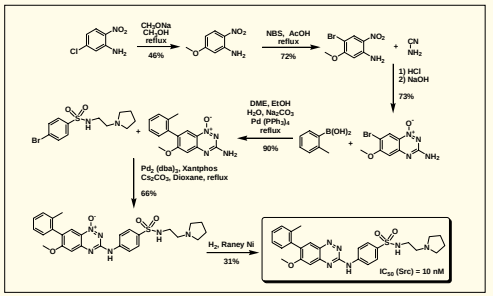
Advantage:

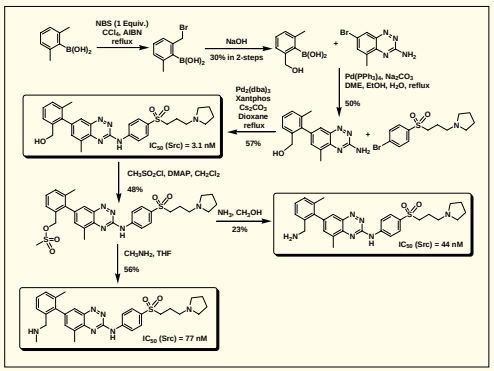
--- Good alternative to Buchwald coupling reaction

Kilogram scale process route



Examples of syntheses of novel benzotriazine Src inhibitors





Highlights

- **Over 300 benzotriazines; 200+ compounds under 30 nM synthesized by a small group of medicinal chemists within 9-12 months.**
- **Multiple synthetic routes; scalable chemistry via convergent routes.**
- **Extensive SAR understanding for Src and other kinases (see MEDI 65, 66 and 251).**

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