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Structure–activity relationship study of EphB3 receptor tyrosine kinase inhibitors

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ABSTRACT

A structure–activity relationship study for a 2-chloroanilide derivative of pyrazolo[1,5-*a*]pyridine revealed that increased EphB3 kinase inhibitory activity could be accomplished by retaining the 2-chloroanilide and introducing a phenyl or small electron donating substituents to the 5-position of the pyrazolo[1,5-*a*]pyridine. In addition, replacement of the pyrazolo[1,5-*a*]pyridine with imidazo[1,2-*a*]pyridine was well tolerated and resulted in enhanced mouse liver microsome stability. The structure–activity relationship for EphB3 inhibition of both heterocyclic series was similar. Kinase inhibitory activity was also demonstrated for representative analogs in cell culture. An analog (**32**, LDN-211904) was also profiled for inhibitory activity against a panel of 288 kinases and found to be quite selective for tyrosine kinases. Overall, these studies provide useful molecular probes for examining the in vitro, cellular and potentially in vivo kinase-dependent function of EphB3 receptor.

Erythropoietin-producing hepatocellular carcinoma (Eph) receptors are highly conserved transmembrane proteins composed of multiple domains that participate in an array of complex cell signaling pathways. Sixteen Eph receptors have been identified in vertebrates. They can be divided into two major classes (EphA and EphB) based on sequence similarity in the extracellular domain and binding characteristics. Mammals, including humans, have 14 Eph receptors (EphA1–EphA8, EphA10, EphB1–EphB4 and EphB6).[1]

The Eph receptors interact with cell surface ligands called Eph receptor interacting proteins (ephrins).[2] Currently, nine ephrins are known and are divided into two major classes (ephrin A1–6 and ephrin B1–3). Humans have all but ephrin A6. Following binding of the Eph receptors to the ephrin ligands, which requires cell–cell interactions, propagation of signaling occurs bi-directionally into both the Eph receptor and the ephrin presenting cells.[3] The signaling events resulting from these interactions are important in both neural development[4] and during adulthood. For example, the Eph receptors together with ephrins participate in axon guidance by providing repulsive cues during axonal neurogenesis.

The EphB3 receptor subtype is expressed during embryonic development and in discrete areas of the adult brain, including the cerebellum and hippocampus. It co-localizes to brain regions with high levels of ephrin B ligand expression.[5] EphB3 receptor expression also increases following central nervous system injury. However, it remains unclear if EphB3 is inhibitory to axonal regeneration or beneficial for axonal repair. For example, following adult optic nerve injury, EphB3 receptor appears and coincides with retinal ganglion cell axon sprouting and remodeling.[6] However, after spinal cord injury EphB3 expression increases and appears to contribute to restricted axonal regeneration and sprouting.[7] Increased EphB3 receptor expression has also been documented in pancreatic cancer cell lines,[8] squamous cell carcinoma,[9a] and rhabdomyosarcoma.[9b]

In addition to ligand binding domains, the Eph receptors have an intracellular tyrosine kinase domain, although EphA10 and EphB6 lack essential amino acid residues to enable catalysis. The Eph receptor's kinase activity is required for some, but not all, of the signal transduction pathways involving Eph receptors.[10]

Engagement of the ephrin ligands with the Eph receptors initially results in receptor dimerization followed by autophosphorylation of tyrosine residues in the juxtamembrane region of the receptor, which is located between the transmembrane and the kinase domains. These phosphorylation events result in kinase activation by dissociation of the juxtamembrane segment from the kinase domain.[11] Once fully active, the kinase domain then can bind and phosphorylate intracellular adaptor molecules perpetuating signaling.

Ligands that target different binding components of Eph receptors could serve as useful molecular probes to help elucidate the

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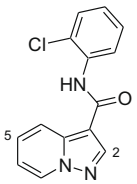


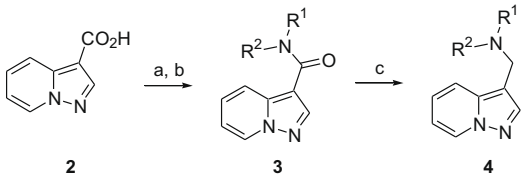
Figure 1. EphB3 inhibitor identified by HTS. Also shown is the numbering system for pyrazolo[1,5-*a*]pyridines.

cellular biology and in vivo physiology of Eph receptors.[12] These ligands could also be used to selectively modulate Eph receptor's kinase-dependent and independent functions.[13] Utilizing a recently developed high throughput screen (HTS) for EphB3 kinase activity, the pyrazolo[1,5-*a*]pyridine derivative **1** (Fig. 1)[14] was discovered as a moderately potent inhibitor (IC_{50} ~1 μM). Herein, we describe the results of a structure–activity relationship study to optimize EphB3 kinase inhibition and to increase mouse liver microsome stability. In addition, kinase inhibitory activity is also demonstrated in cell culture and a select analog is profiled against a broad panel of kinases.

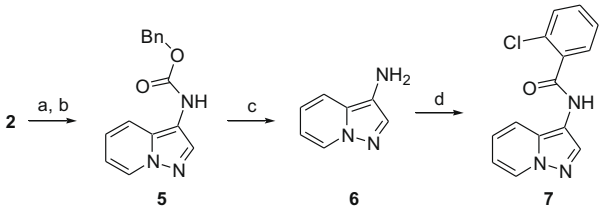
The synthesis of many pyrazolo[1,5-*a*]pyridine derivatives was accomplished according to Scheme 1 (Method A). Pyrazolo[1,5-*a*]pyridine-3-carboxylic acid, **2**, was converted to the corresponding acyl chloride and then treated with amines to give **3**. Derivatives incorporating other heterocycles in place of the pyrazolo[1,5-*a*]pyridine were prepared in a similar manner, unless otherwise noted. The amide could be reduced with $LiAlH_4$ to give amine **4**.

Carboxylic acid **2** was also allowed to react with $(PhO)_2P(O)N_3$ to generated the corresponding acyl azide, which upon heating in the presence of benzyl alcohol underwent a Curtius rearrangement followed by alcohol addition to the intermediate isocyanate to produce **5** (Scheme 2, Method B).[15] Deprotection of the benzyl carbamate in the presence of hydrogen (1 atm) and 10% Pd/C yielded amine **6**, which upon treatment of 2-chlorobenzoyl chloride gave **7**.

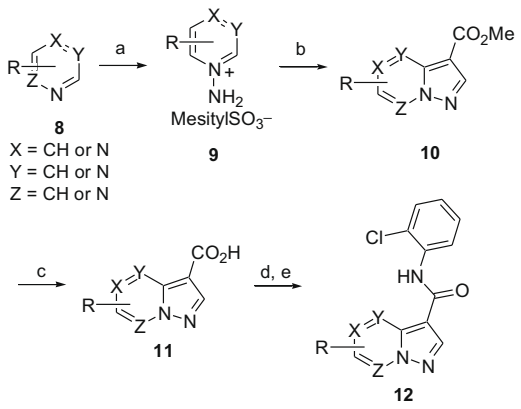
The synthesis of substituted pyrazolo[1,5-*a*]pyridine and derivatives that incorporate additional nitrogen atoms into the pyrazolo[1,5-*a*]pyridine is illustrated in Scheme 3 (Method C).



Scheme 1. Method A: Reagents and conditions: (a) $(C{=}O)_2Cl_2$, DCM, cat. DMF, 0 °C to rt, 2 h; (b) R^1R^2NH, DCM, DIPEA, rt, 18 h; (c) $LiAlH_4$, THF, Δ, 2 h.



Scheme 2. Method B: (a) $(PhO)_2P(O)N_3$, THF, DIPEA, rt, 16 h; (b) BnOH, Δ, 12 h, 80% for two steps; (c) H_2 (1 atm), 10% Pd/C, MeOH/EtOAc (1:1), 45 min; (d) 2-Cl-PhC(O)Cl, DCM, DIPEA, rt, 16 h, 36% over two steps.

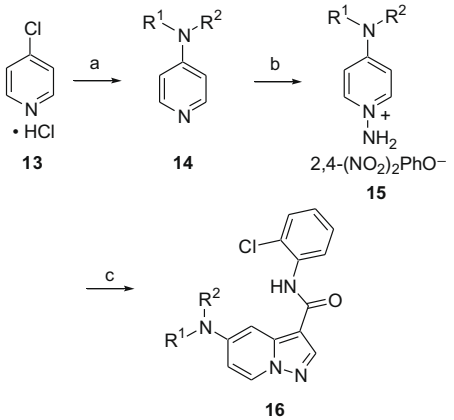


Scheme 3. Method C: (a) mesityl SO_3NH_2, DCM, 0 °C to rt, 30 min, 81%; (b) $HC{\equiv}CCO_2Me$, K_2CO_3, DMF, 50 °C, 72 h, 14%; (c) LiOH, $MeOH/H_2O$, rt, 2 h, then 1 N HCl, 85–90%; (d) $SOCl_2$, 80 °C, 1 h; (e) 2-$ClPhNH_2$, pyridine, rt, 16 h.

Heterocycles **8** were converted to the *N*-amino derivatives **9** with mesityl SO_3NH_2.[16] Cycloaddition with methyl propiolate in the presence of potassium carbonate gave **10** in low yield.[17] Hydrolysis of the esters yielded **11**. Conversion of the carboxylic acids to the corresponding acyl chlorides with thionyl chloride followed by treatment with 2-chloroaniline in pyridine gave **12**.

The synthesis of 5-amino substituted pyrazolo[1,5-*a*]pyridine derivatives is outlined in Scheme 4 (Method D). 4-Chloropyridinium hydrochloride, **13**, was treated with an amine to generate **14**, via a nucleophilic aromatic substitution. Conversion of **14** to the *N*-aminopyridine **15** with 2,4-$(NO_2)_2PhONH_2$ followed by cycloaddition with *N*-(2-chlorophenyl)-2-propynamide[18] produced **16**.

The synthesis of imidazo[1,2-*a*]pyridine derivatives was accomplished according to Scheme 5 (Method E). 2-Amino-5-bromopyridine, **17**, was coupled with phenyl boronic acid to give **18**. 5-Bromo-2-nitropyridine, **19**, was treated with piperidine to give **20**, which was subsequently hydrogenated in the presence of 10% Pd/C to yield **21**. The 2-aminopyridines **18** or **21** were heated at reflux in toluene with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) and then treated with 2-bromo-*N*-arylacetamides (prepared by coupling anilines with 2-bromoacetyl chloride) to give **22**.[19] β-Ketoamide **23** was chlorinated with sulfuryl chloride yielding **24**, which was used without purification. Heating **24** in the presence of **17** and sodium bicarbonate gave the 2-methyl imidazo[1,2-*a*]pyridine derivative **25**.



Scheme 4. Method D: (a) HNR^1R^2, H_2O, MW, 130 °C, 30 min, 90%; (b) 2,4-$(NO_2)_2PhONH_2$, CH_3CN, Δ, 16 h, 81%; (c) $HC{\equiv}CC(O)NH$-2-ClPh, K_2CO_3, DMF, 50 °C, 72 h.