Bioorganic & Medicinal Chemistry Letters 19 (2009) 6122–6126

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structure–activity relationship study of EphB3 receptor tyrosine kinase inhibitors

Lixin Qiao [a], Sungwoon Choi [a], April Case [a], Thomas G. Gainer [a,b], Kathleen Seyb [a], Marcie A. Glicksman [a], Donald C. Lo [b], Ross L. Stein [a], Gregory D. Cuny [a,*]

[a] Laboratory for Drug Discovery in Neurodegeneration, Harvard NeuroDiscovery Center, Brigham and Women's Hospital and Harvard Medical School, 65 Landsdowne Street, Cambridge, MA 02139, USA
[b] Center for Drug Discovery and Department of Neurobiology, Duke University Medical Center, 4321 Medical Park Drive, Suite 200, Durham, NC 27704, USA

ARTICLE INFO

Article history:
Received 3 August 2009
Revised 1 September 2009
Accepted 4 September 2009
Available online 9 September 2009

Keywords:
Structure–activity relationship
EphB3
Kinase
Inhibitor
Microsomal stability

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

* Corresponding author. Tel.: +1 617 768 8640; fax: +1 617 768 8606.
E-mail address: gcuny@rics.bwh.harvard.edu (G.D. Cuny).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.09.010

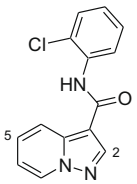


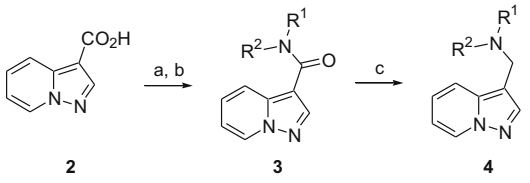
Figure 1. EphB3 inhibitor identified by HTS. Also shown is the numbering system for pyrazolo[1,5-*a*]pyridines.

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.

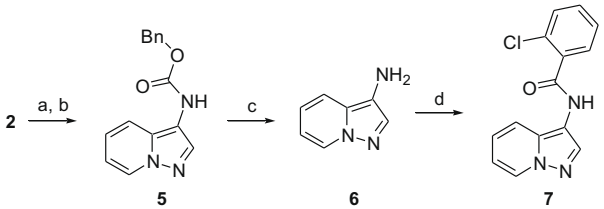
The synthesis of many pyrazolo[1,5-*a*]pyridine derivatives was accomplished according to Scheme 1 (Method A). Pyrazolo[1,5-*a*]pyridine-3-carboxylic acid, **2**, was converted to the corresponding acyl chloride and then treated with amines to give **3**. Derivatives incorporating other heterocycles 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**.

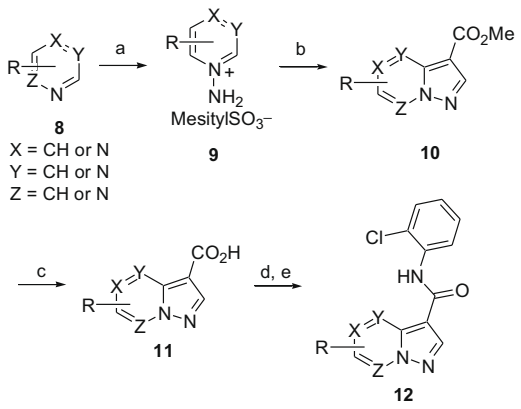
The synthesis of substituted pyrazolo[1,5-*a*]pyridine and derivatives that incorporate additional nitrogen atoms into the pyrazolo[1,5-*a*]pyridine is illustrated in Scheme 3 (Method C).



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.

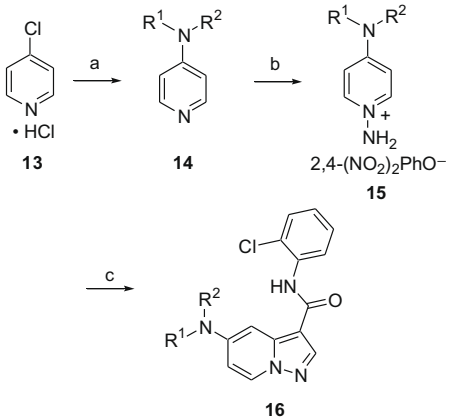


Scheme 3. Method C: (a) mesityl SO_3NH_2, DCM, 0 °C to rt, 30 min, 81%; (b) $HC{\equiv}CCO_2Me$, K_2CO_3, DMF, 50 °C, 72 h, 14%; (c) LiOH, $MeOH/H_2O$, rt, 2 h, then 1 N HCl, 85–90%; (d) $SOCl_2$, 80 °C, 1 h; (e) 2-$ClPhNH_2$, pyridine, rt, 16 h.

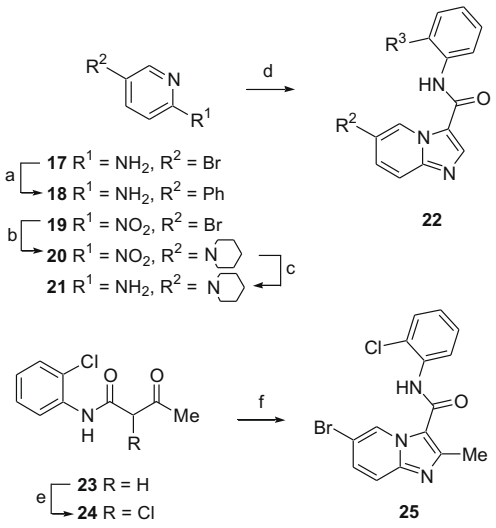
Heterocycles **8** were converted to the *N*-amino derivatives **9** with mesityl SO_3NH_2.[16] Cycloaddition with methyl propiolate in the presence of potassium carbonate gave **10** in low yield.[17] Hydrolysis of the esters yielded **11**. Conversion of the carboxylic acids to the corresponding acyl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



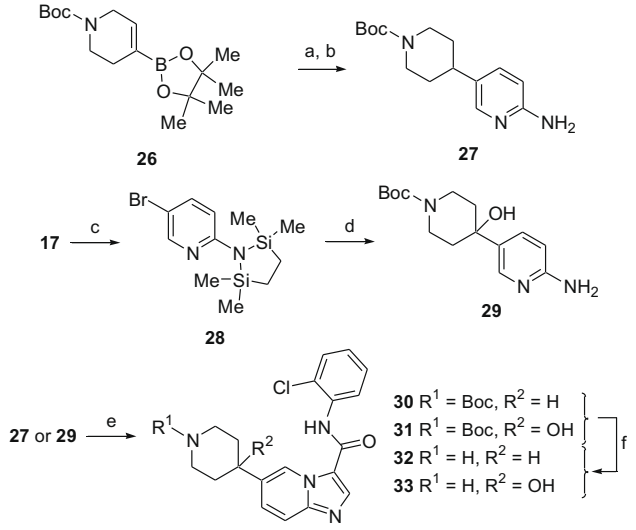
Scheme 4. Method D: (a) HNR^1R^2, H_2O, MW, 130 °C, 30 min, 90%; (b) 2,4-$(NO_2)_2PhONH_2$, CH_3CN, Δ, 16 h, 81%; (c) $HC{\equiv}CC(O)NH$-2-ClPh, K_2CO_3, DMF, 50 °C, 72 h.



Scheme 5. Method E: (a) $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, CH_3CN, H_2O, 90 °C, 16 h, 98%; (b) piperidine, DMSO, *n*-Bu_4NI, K_2CO_3, 95 °C, 16 h, 84%; (c) H_2 (1 atm), 10% Pd/C, EtOH/MeOH, rt, 16 h, 100%; (d) $Me_2NCH(OMe)_2$, toluene, Δ, 6 h then $BrCH_2C(O)NH$-2-R-Ph (where R = Cl or OMe), MeOH, Δ, 16 h, 17–45%; (e) SO_2Cl_2, 0 °C to rt; (f) **17**, toluene, $NaHCO_3$, 120 °C, 3 d, 10%.

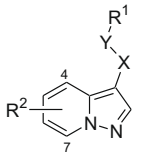
The synthesis of several other 5-substituted imidazo[1,2-*a*]pyridine derivatives was accomplished according to the method outlined in Scheme 6 (Method F). A Pd-mediated coupling of boronic ester **26** with **17** followed by hydrogenation of the alkene yielded **27**. Amine **17** was also converted to intermediate **28**,[20] which was not isolated, but subjected to halogen metal exchange with *n*-BuLi followed by addition of *N*-Boc-4-piperidone to give **29** after an acidic work-up. Both **27** and **29** were converted to **30** and **31**, respectively, utilizing the procedure described in Method E.[19] Finally, removal of the carbamate protecting groups from **30** and **31** with 30% TFA in dichloromethane produced amines **32** and **33**, respectively.

Evaluation of EphB3 kinase inhibitory activity for the various compounds was accessed by measuring ^{33}P incorporation into



Scheme 6. Method F: (a) **17**, $Pd(PPh_3)_4$, Na_2CO_3, CH_3CN, H_2O, 90 °C, 6 h; (b) 10% Pd/C, H_2 (1 atm), EtOH, 16 h, 67% for two steps; (c) *n*-BuLi (2 equiv), $(Me_2SiClCH_2)_2$, THF, −78 °C, 1 h; (d) *n*-BuLi (1 equiv), *N*-Boc-4-piperidone, −78 °C to rt, 18 h, 58%; (e) DMF-DMA, 85 °C, toluene 8 h, then $BrCH_2C(O)NH$-2-Cl-Ph, MeOH, 85 °C, 18 h, 26–30%; (f) 30% TFA in DCM, 1 h, rt, 82%.

Table 1
IC_{50} determinations for inhibiting EphB3 phosphorylation of BTK-peptide



Cmpd	X	Y	R^1	R^2	Method	IC_{50} (μM)
1	C=O	NH	2-Cl-Ph	H	—	1.0
34	C=O	NH	Ph	H	A	NA
35	C=O	NH	2-F-Ph	H	A	~20
36	C=O	NH	3-Cl-Ph	H	A	NA
37	C=O	NH	4-Cl-Ph	H	A	NA
38	C=O	NH	2,3-Cl_2-Ph	H	A	~20
39	C=O	NH	2-OMe-Ph	H	A	NA
40	C=O	NH	2-CF_3-Ph	H	A	>20
41	C=O	NH	2-CN-Ph	H	A	>20
42	C=O	NH	2-$MeSO_2$Ph	H	A	NA
43	C=O	NMe	2-Cl-Ph	H	A	NA
44	C=O	NH	CH_2-2-Cl-Ph	H	A	NA
7	NH	C=O	2-Cl-Ph	H	B	>20
45	CH_2	NH	2-Cl-Ph	H	A	>20
46	C=O	NH	2-Cl-Ph	4-Me	C	~15
47	C=O	NH	2-Cl-Ph	5-Me	C	0.26
48	C=O	NH	2-Cl-Ph	6-Me	C	~15
49	C=O	NH	2-Cl-Ph	7-Me	C	NA
50	C=O	NH	2-Cl-Ph	4-Cl	C	~20
51	C=O	NH	2-Cl-Ph	5-Cl	C	2.0
52	C=O	NH	2-Cl-Ph	6-Cl	C	>20
53	C=O	NH	2-Cl-Ph	5-OMe	C	0.19
54	C=O	NH	2-Cl-Ph	5-Ph	C	0.066
55	C=O	NH	2-Cl-Ph	5-NMe_2	C	0.077
56	C=O	NH	2-Cl-Ph	5-Pyrr	D	0.20
57	C=O	NH	2-Cl-Ph	5-Morph	D	0.24
58	C=O	NH	2-Cl-Ph	5-Pip	D	0.063
59	C=O	NH	2-Cl-Ph	5-OBn	C	~20

NA: not active at 20 μM; Pyrr = *N*-pyrrolidinyl; Morph = *N*-morpholinyl; Pip = *N*-piperidinyl.

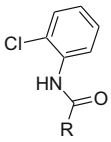
the BTK-peptide in the presence of varying test compound concentrations. Progress curves for production of phospho-BTK were linear for at least 30 min, allowing the calculation of observed velocities. IC_{50} values were then determined using a two parameter fit.[14]

The 2-chloro substituent on the aniline ring was necessary for kinase inhibitory activity (Table 1). Removal of this group, replacing it with other electron withdrawing or donating substituents, or transposing it to the 3- or 4-positions were all detrimental. In addition, methylation of the amide (**43**), insertion of a methylene to give a benzylamine derivative (**44**), inverting the amide functional group (**7**) or reduction of the amide to an amine (**45**) all resulted in diminished EphB3 kinase inhibitory activity.

Interestingly, addition of a methyl substituent to the 4-, 6-, or 7-positions of the pyrazolo[1,5-*a*]pyridine was also detrimental. However, placement of the methyl substituent in the 5-position (**47**) resulted in increased activity. Similarly, introduction of a chlorine to the 5-position (**51**) was also tolerated, but not at the 4- or 6-positions. Based on these results, other substituents were examined at the 5-position of the pyrazolo[1,5-*a*]pyridine. Introduction of a phenyl (**54**), dimethylamino (**55**) or *N*-piperidinyl (**58**) further increased activity resulting in IC_{50} values <100 nM. However, a larger benzyloxy (**59**) substituent was not tolerated at the 5-position.

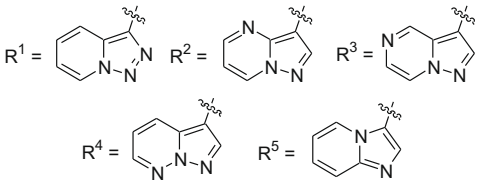
Various replacements of the pyrazolo[1,5-*a*]pyridine were next examined (Table 2). Most of the heterocycles, including indoles (**60** and **61**), a quinoline (**62**), a 1,2-benzisoxazole (**63**), an indazole (**64**) and pyrazolo[1,5-*a*]pyridines incorporating an additional nitrogen atom into the heterocycle (**65**–**68**) did not inhibit EphB3 kinase activity at concentrations <15 μM. However, replacement of the

Table 2
IC_{50} determinations for inhibiting EphB3 phosphorylation of BTK-peptide



Cmpd	R	Method	IC_{50} (μM)
60	3-Indolyl	[a]	NA
61	*N*-Methyl-3indolyl	A	NA
62	4-Quinolinyl	A	NA
63	3-(1,2-Benzisoxazolyl)	C	NA
64	3-(1H-indazolyl)	C[b]	>20
65	R^1	C	>20
66	R^2	C[b]	NA
67	R^3	C	~15
68	R^4	C	NA
69	R^5	C[b]	0.46

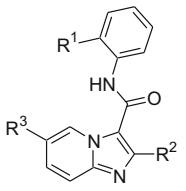
[a] Prepared by EDCI mediated coupling of indole-3-carboxylic acid with 2-chloroaniline.
[b] Method C, steps d and e; NA: not active at 20 μM.



pyrazolo[1,5-*a*]pyridine with an imidazo[1,2-*a*]pyridine (**69**) retained activity with an IC_{50} = 0.46 μM.

Several of the SAR findings established for the pyrazolo[1,5-*a*]pyridine derivatives were next examined for the imidazo[1,2-*a*]pyridine series (Table 3). Introduction of substituents, such as bromo (**25**), phenyl (**70**) and *N*-piperidinyl (**71**), at the 5-position again resulted in a significant increase in EphB3 inhibitory activity with IC_{50} values <100 nM, whereas replacing the 2-chloroanilide with a 2-methoxyanilide (**72**) or introduction of a methyl substituent at the 2-position of the imidazo[1,2-*a*]pyridine (**73**) abolished inhibitory activity. The 4-piperidinyl analog (**32**) retained significant EphB3 inhibitory activity, while also offering greater aqueous solubility due to the presence of a basic amine. However, the inhibitory activity was eroded with further introduction of a hydroxyl group onto the 4-piperidinyl ring (**33**).

Table 3
IC_{50} determinations for inhibiting EphB3 phosphorylation of BTK-peptide



Cmpd	R^1	R^2	R^3	Method	IC_{50} (μM)
25	Cl	H	Br	E	0.173
70	Cl	H	Ph	E	~0.060[a]
71	Cl	H	Pip	E	0.087
72	OMe	H	Ph	E	>20
73	Cl	Me	Ph	E	>20
32[b]	Cl	H	R^4	F	0.079
33	Cl	H	R^5	F	0.228

Pip = *N*-piperidinyl.
[a] An accurate IC_{50} value was difficult to obtain due to poor compound solubility at higher concentrations.
[b] Oxylate salt.

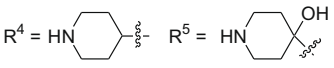


Table 4
Mouse liver microsome stability half-life and clearance values

Compound	$t_{1/2}$ (min)	CL_{int} (μL/min/mg protein)
1	5.3	261 ± 18
32[a]	348	4 ± 1.4
54	27	51 ± 2.1
58	8.3	167 ± 4.2
69	3.8	361 ± 64
70	53	26 ± 0.80
71	20	71 ± 2.0

[a] Oxylate salt.

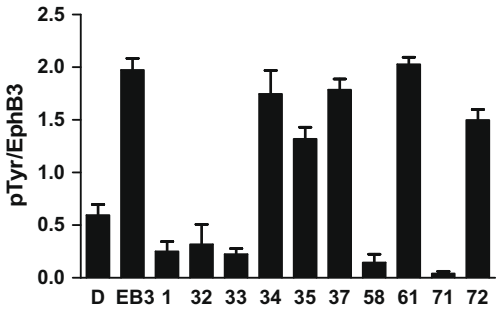


Figure 2. Inhibition assay of EphB3-induced autophosphorylation in HEK293 cells. D = DMSO, EB3 = ephrinB3, [compound] = 10 μM, *N* = 3. Note: **32** was the oxylate salt.

Next, several derivatives were assessed for in vitro metabolic stability in pooled mouse liver microsomes.[14,21] The results of these studies are shown in Table 4. Both pyrazolo[1,5-*a*]pyridine and imidazo[1,2-*a*]pyridine analogs either without substitution or with an amino group attached through a N–C bond at the 5-position of the heterocycle (**1**, **58**, **69**, and **71**) demonstrated poor stability ($t_{1/2} \leqslant 20$ min). However, introduction of a substituent through a C–C bond at the 5-position (**54** and **70**) resulted in increased stability, with **32** demonstrating the best stability with a $t_{1/2}$ of 348 min and a CL_{int} of 4 μL/min/mg protein.

A subset of analogs was also evaluated for inhibition of EphB3 receptor autophosphorylation in HEK293 cells following stimulation with ephrinB3 (Fig. 2).[14] Several compounds (**34**, **35**, **37**, **61**, and **72**) that were inactive or weakly active in the in vitro biochemical kinase assay were found to be inactive or weakly active in this cell-based assay. In contrast, derivatives (**1**, **32**, **33**, **58**, and **71**) that were active in the biochemical assay again demonstrated potent activity in cells.

Finally, **32** was profiled for functional inhibitory activity against a panel of 288 kinases at 5 μM.[22] The results demonstrated that this compound was quite selective for tyrosine kinases (Table S1 and Fig. S1).[14] The only noted exceptions were the three serine/threonine kinases p38α, p38β, and Qik. In addition, the compound only showed moderate selectivity among the tyrosine kinases and little selectivity verses other EphA and EphB subtypes, except for EphA6 and EphA7.

In conclusion, a structure–activity relationship study of the pyrazolo[1,5-*a*]pyridine **1**, identified as an EphB3 kinase inhibitor utilizing a recently developed HTS assay, revealed that increased inhibitory activity could be accomplished by retaining the 2-chloroanilide and introducing a phenyl or small electron donating substituent to the 5-position of the pyrazolo[1,5-*a*]pyridine. In addition, replacement of the pyrazolo[1,5-*a*]pyridine with an imidazo[1,2-*a*]pyridine was well tolerated and the resulting struc-

ture–activity relationship of both heterocyclic series was similar. However, the imidazo[1,2-*a*]pyridines offered enhanced in vitro metabolic stability as assessed in mouse liver microsomes. In particular, **32** (LDN-211904) was a potent EphB3 inhibitor, displaying excellent liver microsome stability and had enhanced aqueous solubility due to incorporation of a more basic secondary amine. EphB3 inhibitory activity was also demonstrated for representative analogs in cell culture and a correlation with in vitro biochemical activity was demonstrated. Finally, **32** was profiled for inhibitory activity against a panel of kinases and found to be quite selective for tyrosine kinases. These studies provide useful molecular probes for examining the in vitro and cellular kinase-dependent function of EphB3. Continued optimization of this compound series to further increase selectivity could afford additional analogs useful for exploring the in vivo pharmacology of EphB3 kinase inhibition in various animal disease models where EphB3 receptor expression occurs, either endogenously or as a result of injury or disease.

Acknowledgments

The authors thank the Harvard NeuroDiscovery Center for financial support. This work was also supported in part by an NIH grant (U24 NS049339). We also would like to thank Dr. Bing-Cheng Wang (Case Western Reserve University School of Medicine, Cleveland, OH) for the HEK293 cells expressing wild-type or kinase-inactive EphB3.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.09.010.

References and notes

1. Pasquale, E. B. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 462.
2. Kullander, K.; Klein, R. *Nat. Rev. Mol. Cell. Biol.* **2002**, *3*, 475.
3. (a) Pasquale, E. B. *Cell* **2008**, *133*, 38; (b) Himanen, J. P.; Saha, N.; Nikolov, D. B. *Curr. Opin. Cell. Biol.* **2007**, *19*, 534; (c) Aoto, J.; Chen, L. *Brain Res.* **2007**, *1184*, 72.
4. Holder, N.; Cooke, J.; Brennan, C. *Eur. J. Neurosci.* **1998**, *10*, 405.
5. Willson, C. A.; Foster, R. D.; Onifer, S. M.; Whittemore, S. R.; Miranda, J. D. *J. Mol. Hist.* **2006**, *37*, 369.
6. Liu, X.; Hawkes, E.; Ishimaru, T.; Tran, T.; Sretavan, D. W. *J. Neurosci.* **2006**, *26*, 3087.
7. (a) Willson, C. A.; Miranda, J. D.; Foster, R. D.; Onifer, S. M.; Whittemore, S. R. *Cell Transplant.* **2003**, *12*, 279; (b) Miranda, J. D.; White, L. A.; Marcillo, A. E.; Willson, C. A.; Jagid, J.; Whittemore, S. R. *Exp. Neurol.* **1999**, *156*, 218.
8. Farivar, R. S.; Gardner-Thorpe, J.; Ito, H.; Arshad, H.; Zinner, M. J.; Ashley, S. W.; Whang, E. E. *J. Surg. Res.* **2003**, *115*, 219.
9. (a) Kang, J. U.; Koo, S. H.; Kwon, K. C.; Park, J. W.; Kim, J. M. *BMC Cancer* **2009**, *9*, 237; (b) Berardi, A. C.; Marsilio, S.; Rofani, C.; Salvucci, O.; Altavista, P.; Perla, F. M.; Diomedi-Camassei, F.; Uccini, S.; Kokai, G.; Landuzzi, L.; McDowell, H. P.; Dominici, C. *Anticancer Res.* **2008**, *28*, 763.
10. (a) Miao, H.; Strebhardt, K.; Pasquale, E. B.; Shen, T.-L.; Guan, J.-L.; Wang, B. *J. Biol. Chem.* **2005**, *280*, 923; (b) Kullander, K.; Mather, N. K.; Diella, F.; Dottori, M.; Boyd, A. W.; Klein, R. *Neuron* **2001**, *29*, 73.
11. Binns, K. L.; Taylor, P. P.; Sicheri, F.; Pawson, T.; Holland, S. J. *Mol. Cell Biol.* **2000**, *20*, 4791.
12. For an example of small molecules inhibiting ephrin binding to the EphA2 and EphA4 receptors see: Noberini, R.; Koolpe, M.; Peddibhotla, S.; Dahl, R.; Su, Y.; Cosford, N. D. P.; Roth, G. P.; Pasquale, E. B. *J. Biol. Chem.* **2008**, *283*, 29461.
13. For other Eph receptor kinase inhibitors see: (a) Choi, Y.; Syeda, F.; Walker, J. R.; Finerty, P. J., Jr.; Cuerrier, D.; Wojciechowski, A.; Liu, Q.; Dhe-Paganon, S.; Gray, N. S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4467; (b) Bardelle, C.; Cross, D.; Davenport, S.; Kettle, J. G.; Ko, E. J.; Leach, A. G.; Mortlock, A.; Read, J.; Roberts, N. J.; Robins, P.; Williams, E. J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2776; (c) Kolb, P.; Kipouros, C. B.; Huang, D.; Caflisch, A. *Proteins* **2008**, *73*, 11; (d) Miyazaki, Y.; Nakano, M.; Sato, H.; Truesdale, A. T.; Stuart, J. D.; Nartey, E. N.; Hightower, K. E.; Kane-Carson, L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 250; (e) Caligiuri, M.; Molz, L.; Liu, Q.; Kaplan, F.; Xu, J. P.; Majeti, J. Z.; Ramos-Kelsey, R.; Murthi, K.; Lievens, S.; Tavernier, J.; Kley, N. *Chem. Biol.* **2006**, *13*, 711.
14. See supplementary data for details.
15. Ninomiya, K.; Shioiri, T.; Yamada, S. *Tetrahedron* **1974**, *30*, 2009.
16. Tamura, Y.; Minamikawa, J.; Miki, Y.; Matsugashita, S.; Ikeda, M. *Tetrahedron Lett.* **1972**, *40*, 4133.
17. Tamura, Y.; Sumida, Y.; Miki, Y.; Ikeda, M. *J. Chem. Soc., Perkin Trans. 1* **1975**, 406.
18. Coppola, G. M.; Damon, R. E. *Synth. Commun.* **1993**, *23*, 2003.
19. Georgescu, F.; Georgescu, E.; Draghici, C.; Iuhas, P. C.; Filip, P. I. *Rev. Roumaine Chim.* **2005**, *50*, 349; *Chem. Abstr.* **2005**, *144*, 350628.
20. Mayer, A.; Leumann, C. J. *Eur. J. Org. Chem.* **2007**, 4038.
21. Baranczewski, P.; Stańczak, A.; Sundberg, K.; Svensson, R.; Wallin, A.; Jannson, J.; Garberg, P.; Postlind, H. *Pharmacol. Rep.* **2006**, *58*, 453.
22. Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri, P.; Davis, M. I.; Edeen, P. T.; Faraoni, R.; Floyd, R.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P. *Nat. Biotechnol.* **2008**, *26*, 127.