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Inhibitors of the tyrosine kinase EphB4. Part 4: Discovery and optimization of a benzylic alcohol series

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ABSTRACT

Optimization of our bis-anilino-pyrimidine series of EphB4 kinase inhibitors led to the discovery of compound **12** which incorporates a key *m*-hydroxymethylene group on the C4 aniline. **12** displays a good kinase selectivity profile, good physical properties and pharmacokinetic parameters, suggesting it is a suitable candidate to investigate the therapeutic potential of EphB4 kinase inhibitors.

The Eph receptors, which form the largest family of receptor tyrosine kinases (RTK), are implicated in embryogenesis, neural development, vascular development and adult neovascularisation. In particular, EphB4 is increasingly recognized as a potential target for treatment of solid cancers through angiogenesis inhibition.[1] Several studies have shown that both EphB4 and its only cognate ligand, EphrinB2, are essential to embryonic vasculature development. Disruption of the EphB4–EphrinB2 interaction with soluble extracellular domain of EphB4 has demonstrated anti-angiogenic activity and tumor growth inhibition.[2] More recently, similar effects have been reported using EphB4 monoclonal antibodies.[3] The EphB4 signaling could also interplay with the vascular endothelial growth factor (VEGF) signaling, as EphrinB2 has been found to control VEGF-induced angiogenesis and lymphangiogenesis.[4]

Unlike other RTKs, the EphB receptors sub-family can trigger a bi-directional signaling when binding to the cell membrane bound EphrinB1–3 ligands. Upon ligand-receptor interaction and subsequent dimerization, a 'forward' signaling is induced by auto-phosphorylation of the EphB receptor kinase domain while a 'reverse' signaling arises from the EphrinB ligand which contains a transmembrane domain. Because of the bi-directional nature of the EphB4–EphrinB2 signaling axis, the effect of a small molecule inhibitor of the EphB4 kinase on angiogenesis has remained unknown until researchers at Novartis recently described for the first time that such an agent inhibits VEGF driven angiogenesis in vivo,[5] suggesting new therapeutic opportunities for cancer or angiogenesis related disorders.

In addition there have only been a limited number of reports on small molecules specifically targeting EphB4 inhibition. We have previously described the discovery of a series of bis-anilino-pyrimidines displaying excellent inhibitory activity against the EphB4 kinase, such as the benzodioxole **1** (Fig. 1)[6] or the indazole **2**.[7] Other groups have reported structurally distinct inhibitors of the EphB4[8,9] or EphB2[10] kinases and the marketed drug dasatinib is also known to inhibit Eph kinases.[11] However, some of these molecules have a multiple kinase inhibitory profile which would possibly make difficult the interpretation of any anti-angiogenic activity.[12]

Compounds **1** and **2** have both shown a cytochrome P450 time dependant inhibition[7] (TDI) which can result in drug–drug interactions in patients. In order to circumvent this potential risk and expand the chemical space of our bis-anilino-pyrimidine series, we explored the structure–activity relationship further. Since we

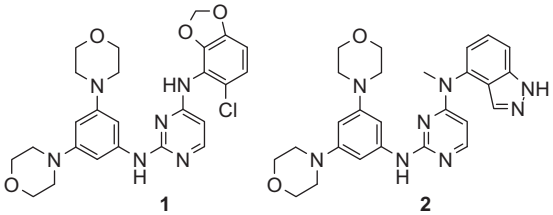

Figure 1. Structures of EphB4 kinase inhibitors **1** and **2**.

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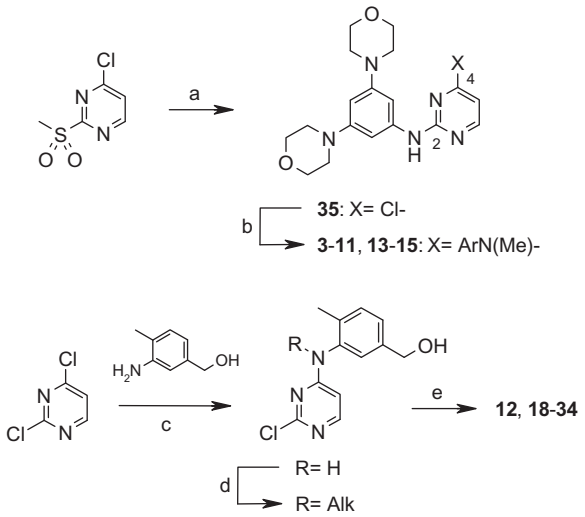
originally attributed the TDI effect of compounds **1** and **2** to their bicyclic ring,[7,13] we decided to investigate mono-cyclic replacements of the C4 aniline.

The general synthetic routes to the compounds described herein are outlined in Scheme 1. Compounds **3–11** and **13–15** have been obtained by addition of *N*-methyl anilines onto intermediate **35** under acidic catalysis. Compounds **12** and **18–32** have been prepared by a three steps sequence: addition of (3-amino-4-methylphenyl)-methanol onto 2,4-dichloropyrimidine followed by alkylation of the C4-nitrogen provided 2-chloropyrimidines which were condensed with 3,5-disubstituted anilines under acidic conditions. Compounds **33** and **34** have been prepared in a similar way but using Buchwald coupling conditions in the final stage. Further details on the synthesis and characterization of compounds **3–34** can be found in the patent literature.[14]

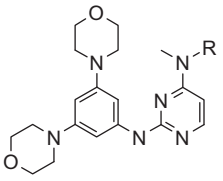


Scheme 1. Synthesis of compounds **3–15** and **18–34**. Reagents and conditions: (a) *N*-(3,5-dimorpholin-4-ylphenyl)formamide, NaH, THF, 0 °C to rt, 73%; (b) ArNH(Me), HCl (cat.), pentanol (68% yield for compd **7**); (c) TEA, ethanol, 39% (d) Alk-X, Cs_2CO_3, DMF, 26–61%; (e) $ArNH_2$, HCl (cat.), pentanol (62% yield for compd **12**); for compd **33–34**, (i) TBDMSCl, imidazole, DMF, (ii) $HetNH_2$, K_2CO_3, Xantphos, Pd_2dba_3, toluene, (iii) 2 N HCl, MeOH (35% overall yield).

The structure–activity relationship around the 4-anilino group of the pyrimidine core has been assessed with compounds **3–17** and the results are summarized in Table 1. The unsubstituted phenyl (compd **3**) provided a good activity in both the enzymatic and cellular assays, confirming the importance of the 3,5-bis-morpholino-aniline and the C4-nitrogen methylation for EphB4 activity, as already described in our previous publication.[7] However, we found that compound **3** also shows some activity against the FGFR1 and VEGFR2 kinases (Table 1). These two RTKs are implicated in the formation of new vasculature. In particular, VEGFR2 blockade by either antibodies or small molecule kinase inhibitors has been validated in the clinic as an anti-angiogenic approach. Recognizing the importance of avoiding such pharmacology, we pursued our lead optimization efforts to obtain EphB4 inhibitors with an improved selectivity profile.

The EphB4 inhibitory activity was maintained at a good level with a range of anilines bearing small *ortho* or *meta* substituents (compd **4–14**) as well as 2- or 3-pyridyl groups (compd **15–17**).Introduction of a methyl on the 2- or 3- position of the aniline (**4**, **5**) slightly improved cellular activity over **3** with little impact on FGFR1 or VEGFR2. Similarly a 3-chloro or 3-methoxy group (**6**, **7**) improved cellular activity but inhibition of FGFR1 and VEGFR2 was maintained. Combining substituents in a 2,5 pattern (**8**, **9**) was also well tolerated and the 2,5-dimethylphenyl **8** was particularly interesting since the selectivity over FGFR1 and VEGFR2 was increased. We found that a 3-hydroxymethyl group was also tolerated since compound **10** displayed the same enzymatic activity as its methyl analogue **5**. Although the cellular activity had decreased, it was an interesting option to reduce the lipophilicity of the C4-aniline. Again, combining two substituents on the phenyl ring proved advantageous with compounds **11–13** being more potent than **10** in the cellular assay. Consistent with our observation with **8**, compound **12** had a significantly improved selectivity profile over **10** with a 800-fold selectivity vs. FGFR1 and 200-fold versus VEGFR2 when comparing enzymatic IC_{50}. Expanding the size of the benzyl alcohol was detrimental to EphB4 activity, as demon-

Table 1
EphB4, FGFR1 and VEGFR2 kinase inhibition and EphB4 cellular activity for compounds **3–17**



Compound	R	EphB4 enz. IC_{50}[a] (μM)	FGFR1 enz. IC_{50}[a] (μM)	VEGFR2 enz. IC_{50}[a] (μM)	*p*-EphB4 IC_{50}[a,b](μM)
3	Phenyl	0.017	0.313	0.094	0.084
4	*o*-Tolyl	0.010	0.662	0.092	0.028
5	*m*-Tolyl	0.008	0.501	0.115	0.028
6	3-Chlorophenyl	0.006	0.112	0.027	0.012
7	3-Methoxyphenyl	0.002	0.183	0.100	0.010
8	2,5-Dimethylphenyl	0.019	1.58	0.965	0.038
9	2-Methyl-5-methoxyphenyl	0.010	0.350	0.231	0.021
10	3-(Hydroxymethyl)-phenyl	0.010	0.738	0.141	0.114
11	3-(Hydroxymethyl)-5-methoxyphenyl	0.002	0.447	0.190	0.007
12	2-Methyl-5-(hydroxymethyl)-phenyl	0.004	3.34	0.818	0.012
13	3-(Hydroxymethyl)-4-chlorophenyl	0.002	0.056	0.022	0.013
14	2-Methyl-5-(1-hydroxyethyl)-phenyl	0.035	1.55	0.589	0.180
15	4-Methoxy-2-pyridyl	0.013	1.38		0.041
16	6-Methoxy-2-pyridyl	0.006	0.998	0.989	0.028
17	5-Methoxy-3-pyridyl	0.002	0.140	0.430	0.030

[a] $n \geqslant 2$, standard error less than 0.3 log unit.
[b] Inhibition of ephB4 autophosphorylation (engineered CHO-K1 cell line stably expressing EphB4).

strated with the racemic compound **14** which is approximately 10 times less potent than **12** in enzymatic and cellular assays. Finally, the methoxy-pyridyl derivatives **15**–**17** had an interesting activity and selectivity profile although they were slightly inferior to compound **12** in the cellular assay.

We pursed our optimization work around **12** by modifying one of the morpholino groups on the C2-aniline. Replacement of morpholine with other cyclic amines (Table 2, compd **18**–**22**) maintained a good level of cellular activity but no improvement over **12** has been achieved. Only piperidine **19** was as potent as **12** but the increased lipophilicity was not desirable. Homologation of the morpholine into a benzylic amine (**23**) induced a small drop in activity with no impact on log *D*. In contrast, a carbonyl linker (**24**) had a significant effect on log *D* but this modification led to a large decrease in EphB4 cellular activity. Introduction of a phenyl ether instead of a morpholine provided reasonably active compounds (**25**–**27**) but more lipophilic than **12**. Interestingly, the methylsulfone **28** was as active as **12** with no increase in log *D*. The methyl on the 4-amino group of the pyrimidine could be extended to other small alkyl chains (**29**–**31**) without loss of activity. This was especially interesting for compound **31** as it is slightly less lipophilic than **12**. However, **31** was less selective than **12** with an IC_{50} against FGFR1 and VEGFR2 of 0.72 and 0.39 μM, respectively. Compound **32** confirmed the interesting replacement of the morpholine with a methylsulfone since it is as potent and less lipophilic compared to its analogue **30**.

We have been able to obtain a crystal structure[15] of **32** bound to the kinase domain of ephB4 and the binding mode shown on Figure 2 is consistent with our previously reported crystal structures[6a,b,7] of related bis-anilinopyrimidines. It is noteworthy that the benzylic hydroxyl group makes two hydrogen bonds with the carboxylate side chain of E664 and the backbone NH of D758. This may help to explain why the compounds retain affinity for the kinase despite the presence of a polar group in the relatively lipophilic environment of the back pocket of the ATP site.

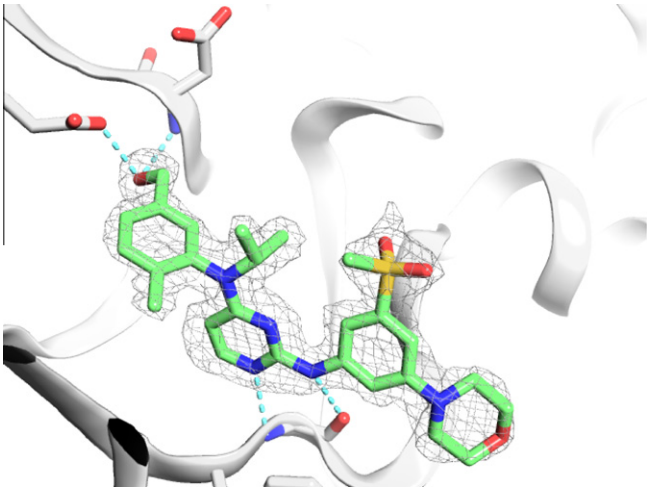
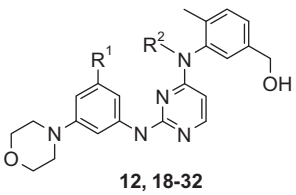


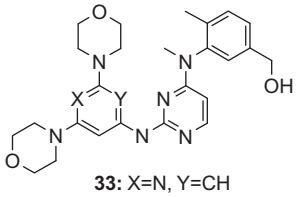
Figure 2. Crystal structure of compound **32** bound to EphB4 kinase domain. The compound is colored with green carbon atoms. Hydrogen bonds are shown as blue dotted lines. The protein carbon atoms are shown in white and the backbone is depicted as a cartoon. Electron density ($2f_o f_c$) is shown as a wire mesh and contoured at 1σ. The pyrimidine group is engaged in the canonical hydrogen bond donor acceptor motif with the hinge region of the protein at the bottom of the view. The hydroxyl group of **32** at the top of the view makes hydrogen bonds with the carboxylate side chain of E664 and the backbone NH of D758 from the DFG motif. Picture produced using PYMOL.[20]

Direct analogues of **12** in which the C2-aniline is replaced by the corresponding 4-aminopyridine (**33**) or the 4-aminopyrimidine (**34**) have also been prepared. Compound **33** was remarkably potent with an IC_{50} of 8 nM whereas the pyrimidine **34** was significantly less potent in the cellular assay. Surprisingly, the measured log *D* of these compounds indicate that lipophilicity increases from phenyl (3.1) to pyridine (3.4) to pyrimidine (3.9), which is opposite to what is typically observed with such modifi-

Table 2
EphB4 kinase inhibition, cellular activity and log *D* for compounds **18–34**



12, 18-32



33: X=N, Y=CH
34: X=N, Y=N

Compound	R^1	R^2	EphB4 enz. IC_{50}[a] (μM)	p-EphB4 IC_{50}[a,b] (μM)	Log *D*
12	Morpholin-4-yl	Me	0.004	0.012	3.1
18	Pyrrolidin-1-yl	Me	0.016	0.027	3.8
19	Piperidin-1-yl	Me	0.010	0.011	4.2
20	1,4-Oxazepan-4-yl	Me	0.009	0.028	3.3
21	4-Methyl-piperazin-1-yl	Me	0.004	0.032	2.6
22	4-Hydroxy-piperidin-1-yl	Me	0.007	0.045	2.8
23	(Morpholin-4-yl)-methyl	Me	0.006	0.025	3.0
24	(Morpholin-4-yl)-carbonyl	Me	0.017	0.459	2.2
25	(Tetrahydropyran-4-yl)-oxy	Me	0.008	0.023	3.7
26	2-Methoxyethoxy	Me	0.008	0.035	3.3
27	Methoxy	Me	0.005	0.032	3.9
28	Methylsulfonyl	Me	0.003	0.011	2.9
29	Morpholin-4-yl	Et	0.004	0.014	3.8
30	Morpholin-4-yl	*i*-Pr	0.005	0.013	3.9
31	Morpholin-4-yl	2-methoxyethyl	0.012	0.012	2.8
32	Methylsulfonyl	*i*-Pr	0.002	0.007	3.4
33	—	-	0.014	0.008	3.4
34	—	-	0.015	0.106	3.9

[a] $n \geqslant 2$, standard error less than 0.3 log unit.
[b] Inhibition of ephB4 autophosphorylation (engineered CHO-K1 cell line stably expressing EphB4).

Table 3
DMPK properties of compounds **12**, **28** and **33**

Compound	Human plasma	Rat pharmacokinetics [a]			Dog pharmacokinetics [b]		
	Fu (% free)	Cl (%lbf)	V_{dss} (L/kg)	*F*%	Cl (%lbf)	V_{dss} (L/kg)	*F*%
12	11	11	0.5	78	51	2.1	58
28	8.5	16	0.4	17	—	—	—
33	5.2	9	0.7	64	57	1.6	22

[a] Male Han Wistar rats dosed at 1 or 2.5 μmol/kg iv and 5 or 10 μmol/kg p.o.
[b] Mean values for male and female beagle dogs dosed at 2 μmol/kg iv and 5 μmol/kg p.o.

cations. We believe this is a consequence of the higher sp^2 character of the morpholine nitrogen atom when its lone pair is conjugated with a heterocycle, thus reducing its solvation state in water. The increased lipophilicity of **33** versus **12** is also apparent from their relative protein binding in human plasma (see Table 3).

Compounds **12**, **28** and **33** have been further evaluated in vivo and Table 3 summarizes their pharmacokinetic properties. Compound **28** demonstrated a modest oral bioavailability in the rat despite a low plasma clearance whereas **12** and **33** displayed good oral exposure. In the dog, both **12** and **33** had a moderate plasma clearance but **12** proved to be superior to **33** with a better oral bioavailability. When dosed orally in nude mouse, compound **12** displayed a very good exposure at 100 and 200 μmol/kg, with a greater than proportional increase in AUC from a 10 μmol/kg dose (Table 4).

The broad kinase selectivity profile of **12** was assessed by measuring its inhibitory activity in a panel of 70 protein kinases at a concentration of 10 μM. Only three kinases were inhibited by more than 80%, namely Src, LCK and CSK. These activities have been confirmed with a dose–response and **12** was found to be more potent on Src and LCK (IC_{50} <0.001 μM for both kinases) than on CSK (IC_{50} 0.08 μM). The activity of **12** against Src was further evaluated in cell by measuring inhibition of proliferation of c-Src transfected 3T3 cells[16] (IC_{50} 0.010 μM). Therefore compound **12** can be considered as a dual ephB4-Src kinase inhibitor with an excellent overall selectivity profile.[17]

Finally, compound **12** was assessed in our CYP450 TDI assay, with the expectation that removal of the bicyclic aniline present in **1** and **2** would alleviate the tendency for such compounds to induce TDI. To our surprise, **12** displayed a similar effect on 3A4 compared to our previous leads,[18] invalidating our first hypothesis. The 3,5-bis-morpholinoaniline being a common feature to all three compounds, we suspect it could actually be the cause of the TDI effect. Effectively, it has been reported in the literature that oxidative metabolism on a morpholine ring, producing a reactive species, was responsible for a TDI effect of CYP 3A4.[19] Although we did not produce experimental evidence to support this hypothesis, this suggests that a similar process might occur with our compounds. Compound **12** also showed a modest reversible inhibition of CYP3A4 when assessed in a fluorometric assay (7 μM against 3A4, >10 μM against 1A2, 2C9, 2C19 and 2D6).

In conclusion, we have identified compound **12** as a potent and selective inhibitor of the EphB4 and Src kinase families with good pharmacokinetic properties in mouse, rat and dog. While **12** still carries a risk of drug-drug interaction, this compound is potentially useful to investigate the in vivo pharmacology of inhibitors of the Eph receptor tyrosine kinases.

Table 4
Oral exposure of compound **12** in nude mouse.

Dose (μmol/kg)	AUC (μM h)	C_{max} (μM)
10	1.6	1.9
100	63	15
200	155	17

Acknowledgements

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References and notes

1. For recent reviews see: (a) Pasquale, E. B. *Nat. Rev. Cancer* **2010**, *10*, 165; (b) Noren, N. K.; Pasquale, E. B. *Cancer Res.* **2007**, *67*, 3994.
2. Kertesz, N.; Krasnoperov, V.; Reddy, R.; Leshanski, L.; Kumar, S. R.; Zozulya, S.; Gill, P. S. *Blood* **2006**, *107*, 2330.
3. Krasnoperov, V.; Kumar, S. R.; Ley, E.; Li, X.; Scehnet, J.; Liu, R.; Zozulya, S.; Gill, P. S. *Am. J. Pathol.* **2010**, *176*, 2029.
4. Wang, Y.; Nakayama, M.; Pitulescu, M. E.; Schmidt, T. S.; Bochenek, M. L.; Sakakibara, A.; Adams, S.; Davy, A.; Deutsch, U.; Luethi, U.; Barberis, A.; Benjamin, L. E.; Maekinen, T.; Nobes, C. D.; Adams, R. H. *Nature* **2010**, *465*, 483.
5. Martiny-Baron, G.; Holzer, P.; Billy, E.; Schnell, C.; Brueggen, J.; Ferretti, M.; Schmiedeberg, N.; Wood, J. M.; Furet, P.; Imbach, P. *Angiogenesis* **2010**, *13*, 259.
6. (a) 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; (b) Bardelle, C.; Coleman, T.; 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*, 5717.
7. Bardelle, C.; Barlaam, B.; Brooks, N.; Coleman, T.; Cross, D.; Ducray, R.; Green, I.; Lambert-van der Brempt, C.; Olivier, A.; Read, J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6242.
8. Mitchell, S. A.; Danca, M. D.; Blomgren, P. A.; Darrow, J. W.; Currie, K. S.; Kropf, J. E.; Lee, S. H.; Gallion, S. L.; Xiong, J.-M.; Pippin, D. A.; DeSimone, R. W.; Brittelli, D. R.; Eustice, D. C.; Bourret, A.; Hill-Drzewi, M.; Maciejewski, P. M.; Elkin, L. L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6991.
9. Lafleur, K.; Huang, D.; Zhou, T.; Caflisch, A.; Nevado, C. *J. Med. Chem.* **2009**, *52*, 6433.
10. Choi, Y.; Syeda, F.; Walker, J. R.; Finerty, P. J.; Cuerrier, D.; Wojciechowski, A.; Liu, Q.; Dhe-Paganon, S.; Gray, N. S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4467.
11. See Supplementary data in: 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, M.; 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.
12. The compounds described in reference 8 are also inhibitors of the VEGFR2 and Tie2 receptor tyrosine kinases, both of which are linked to angiogenic mechanisms.
13. The benzodioxole ring is known to produce mechanism based inhibition of P450 enzymes, see: Fontana, E.; Dansette, P. M.; Poli, S. M. *Curr. Drug Met.* **2005**, *6*, 413.
14. PCT international applications WO/2008-104754 (for compd **3**–**14**, **18-32**), WO/2008-132505 (for compd **33**–**34**), and WO/2009-010794 (for compd **15**–**17**).
15. Human EphB4 (598–892; Y774E) was crystallized as described in Ref. 6b. Detailed protein preparation, crystallization and freezing protocols are included in the Supplementary data of Ref. 6b. Diffraction data for complex of EphB4 with **32** were collected on a Rigaku FRe X-ray generator equipped with a Saturn 944 CCD detector, using a CuKα wavelength of 1.54178 Å, focused using Osmic Varimax HF mirrors at 100 K. Data were processed using d*trek (Pflugrath, J.W. *Acta Crystallogr., Sect. D* **1999**, *55*, 1718) and reduced using CCP4 software (*Acta Crystallogr., Sect. D* **1994**, *50*, 760). The structures were solved by molecular replacement using coordinates of the EphB4 kinase domain[6b] as a trial model using CCP4 software. Protein and inhibitor were modeled into the electron density using, COOT (Emsley, P.; Cowtan, K. *Acta*

Crystallogr., Sect. D **2004**, *60*, 2126) and AFITT (OpenEye). The model was refined using Refmac (Murshudov, G. N.; Vagin, A. A.; Dodson, E. J. *Acta Crystallogr., Sect. D* **1997**, *53*, 240). Atomic coordinates[21] and structure factors for the EphB4 complex with compound **32** has been deposited in the Protein Data Bank (2xvd) together with structure factors and detailed experimental conditions.

16. For a description of this assay see: Ple, P. A.; Green, T. P.; Hennequin, L. F.; Curwen, J.; Fennell, M.; Allen, J.; Lambert-van der Brempt, C.; Costello, G. *J. Med. Chem.* **2004**, *47*, 871.
17. In addition to ephB4, compound **12** is likely to inhibit other members of the Eph receptors family, given the high structural homology of their kinase domains.
18. Time dependant inhibition (TDI) of CYP450 (isoforms 1A2, 2C9, 2C19, 2D6 and 3A4) was evaluated by pre-incubating the compound (10 μM) with human liver microsomes for 30 min in the presence vs. absence of NADPH, followed by incubation with a specific CYP substrate with NADPH. Compound **12** gave 50% TDI on CYP3A4 with no significant effect on the 4 other isoforms. In the same assay, **1** gave, respectively, 40% and 42% TDI against CYP2C9 and 3A4 and **2** gave 62% TDI against CYP3A4 (see Ref. 7).
19. Zhang, X. et al *J. Med. Chem.* **2008**, *51*, 7099.
20. DeLano, W. L. *The PyMOL Molecular Graphics System*; DeLano Scientific: San Carlos, CA, USA, 2002.
21. Crystallographic statistics for the EphB4/compound **32** complex are as follows: Space group $P2_1$, unit cell 46.7, 53.3, 61.3 Å, β 111.7°, Resolution 43–1.7 (1.76–1.7) Å, 28,154 unique reflections with an overall redundancy of 3.3(2.1) give 90.9(46.9)% completeness with R_{merge} of 4.6(36.3)% and mean I/σ(I) of 14.3(2.0). The final model containing 2078 protein, 217 solvent, and 36 compound atoms has an R-factor of 17.5% (R_{free} 20.9%) using 5% of the data. Mean temperature factors for the protein and the ligand are 34 and 47 Å^2, respectively.