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Pyridinylimidazole inhibitors of Tie2 kinase

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Abstract—This communication details the evolution of the screening lead **SB-203580**, a known CSBP/p38 kinase inhibitor, into a potent and selective Tie2 tyrosine kinase inhibitor. The optimized compound **5** showed efficacy in an in vivo model of angiogenesis and a MOPC-315 plasmacytoma xenograft model.

Receptor tyrosine kinases (RTKs) are involved in the process of angiogenesis, defined as the formation of new capillaries from established blood vessels. In particular, angiogenesis is dependent on the vascular endothelial growth factor (VEGFR2/KDR) and Tie2.[1] Several studies have shown that many tumors are inhibited by the blockade of the VEGF/VEGF receptor pathway, while others are unaffected, suggesting that alternative pathways for vascular growth can drive tumor angiogenesis.[2,4,5] It has been demonstrated that blocking Tie-2 activation with a recombinant Tie2 receptor AdExTek inhibits tumor angiogenesis and tumor growth in vivo.[1,2] Therefore, there is an expectation that small molecule inhibitors of Tie2 kinase would also be attractive candidates as anti-angiogenic cancer chemotherapeutic agents.

Screening efforts in our laboratories identified two trisubstituted imidazoles, **SB-203580** and 2-naphthyl substituted compound **1** (Table 1), as Tie2 kinase inhibitors. **SB-203580** had poor intrinsic potency and no cellular activity, while compound **1** exhibited moderate potency (Tie2 IC_{50} = 300 nM) and poor cellular activity (cell IC_{50} = 30,000 nM).[7]

The binding model for compound **1** in Tie2[3,9] (Figs. 1 and 2) is based on the published co-crystal structures of **SB-203580** with p38 and mimicks many of the key interactions demonstrated to be crucial for binding.

For example in the **SB-203580** p38 co-crystal structure, the 4-pyridyl nitrogen forms a hydrogen bond with the backbone amide nitrogen of Met109. In analogy with what has been demonstrated in other kinase crystal structures, the 4-pyridyl nitrogen of compound **1** is believed to mimic the interaction of the $N-1$ of adenine in ATP with an NH of the amide backbone. Consistent with this role of the 4-pyridyl group, the phenyl analog of compound **1** was prepared and found to be inactive (IC_{50} > 100 μM) for Tie2 kinase (data not shown).

Initially, we sought to investigate the SAR of the imidazole 4-position (R^2 in Table 1) with the goals of increasing Tie2 potency and enhancing selectivity against p38 kinase. It was apparent from the initial SAR (data not shown) that only naphthyl substitution at the 4-position of the imidazole afforded Tie2 potency. This aspect of the SAR can be rationalized by examination of the Tie2/compound **1** docking model (Fig. 1). Thus, the naphthyl moiety is predicted to occupy the aryl specificity pocket, lined by residues L876, I886, L888, L900, and I902 in Tie2. The back pocket in Tie2 is considerably deeper than that of p38, and may therefore, favor increased potency for naphthyl-containing inhibitors against Tie2 kinase and conversely disfavor binding to p38. Further analysis of the docking model in Tie2 suggested that introduction of functional groups at the 6-position of the naphthyl ring might improve potency for Tie2 and enhance selectivity against p38.

Keywords: Tie2 kinase; Imidazole; Xenograft.
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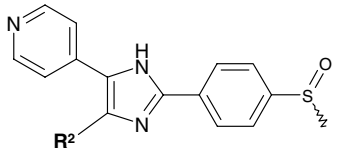
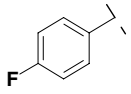
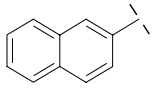
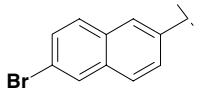
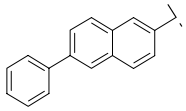
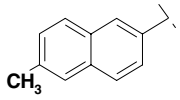
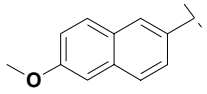
Table 1. Tie2 and p38 kinase activity of compounds **1**–**5**[7]

Compound		Tie2 IC_{50} (nM)	p38 IC_{50} (nM)
			
SB-203580		20,000	42
1		300	300
2		339	4571
3		5500	16,500
4		219	741
5		250	50,000

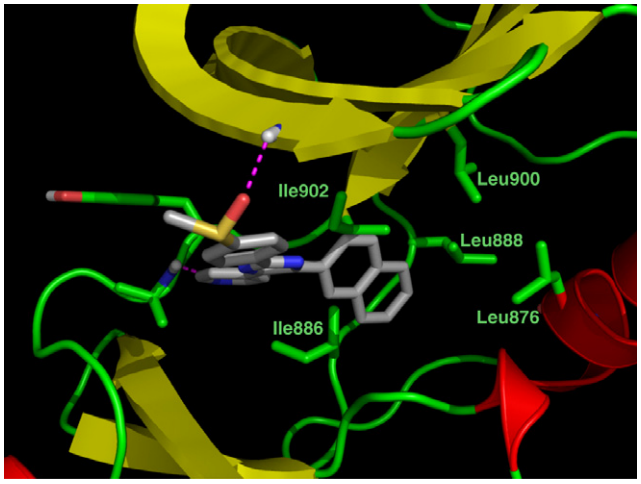


Figure 1. Tie2 docking model for compound **1**.[9]

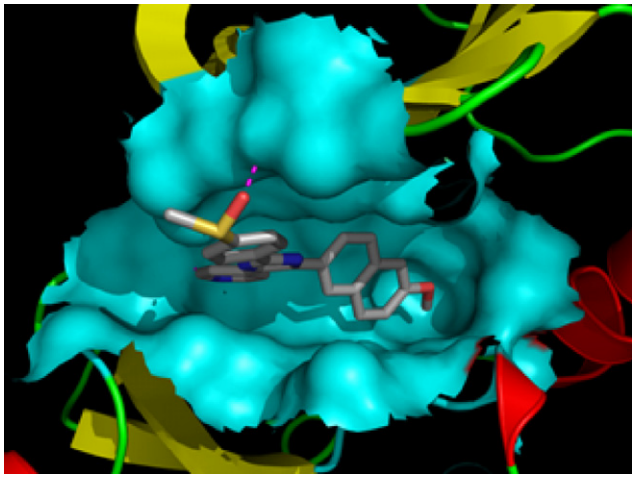
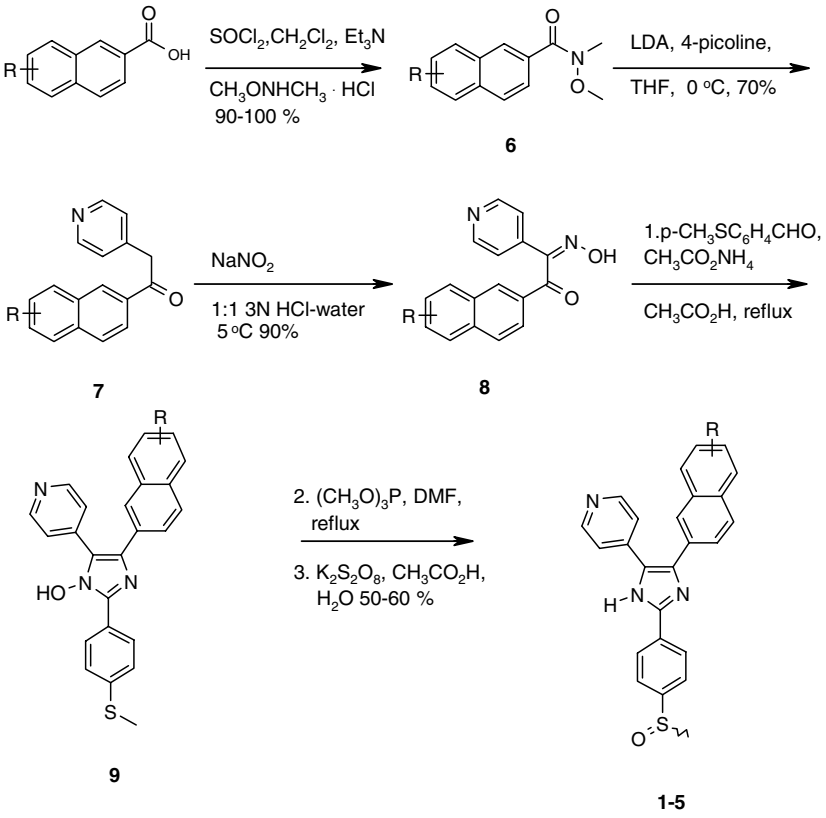


Figure 2. Tie2 docking model for compound **5**.[9]

To probe this hypothesis, compounds **2**–**5** containing substituents at the 6-position of the naphthyl group were synthesized according to the synthetic route outlined in Scheme 1. Conversion of the naphthoic acid to the acid chloride followed by reaction with *N*,*O*-dimethylhydroxylamine gave the desired Weinreb amide **6**. Nucleophilic addition of lithiated 4-picoline to the latter provided ethanone **7** that was readily converted to the keto-oxime **8** as a mixture of geometric isomers. Cyclodehydration to the desired imidazol-1ol was accomplished by in situ generation of the imine from a suitable aldehyde [in this case, 4-(methylthio) benzaldehyde] and ammonium acetate, followed by addition of the keto-oxime in acetic acid. Finally, reduction of the crude *N*-oxide **9** with trimethyl phosphate followed by oxidation of the thiomethyl group with potassium persulfate afforded the requisite racemic imidazoles.



Scheme 1. The synthesis of compounds **1**–**5**.

Compounds **4** and **5** with a methyl and a methoxy substituents at the 6-position of the naphthyl group, respectively, showed only a slight improvement in Tie2 inhibition; however, a remarkable improvement in selectivity over p38 versus compound **1** (13- and 200-fold increase) was achieved. A substantial decrease in potency against Tie-2 was observed with the 6-phenyl substituted naphthyl group (**3** vs **1**).

Next, we investigated what effect an *N*-alkyl group placed on the triarylimidazole core would have on selectivity and potency of this series for Tie2 over p38. Synthesis of the tetra-substituted imidazoles **10**–**14** was accomplished by N-alkylation of imidazole **5** with an appropriate electrophile (Cs_2CO_3, THF °C 2 h). The resultant mixtures of regioisomers were separated by preparative reverse phase HPLC and fully characterized by ^{1}H NMR and small molecule X-ray crystallography.[6]

The data in Table 2 indicate a clear preference for *N*-methyl substitution on the imidazole ring adjacent to the naphthyl group (compound **10** vs **11**). To our delight, regioisomer **10** also displayed a fourfold increase in potency over the parent tri-substituted imidazole **5**. Alkyl groups larger than methyl were not as well tolerated. Our Tie2 homology model places the *N*-methyl group in a space occupied by the catalytic lysine relative to p38. The conserved lysine is pushed further back in the pocket in Tie2, allowing the methyl group to reside in that area without hindering binding to the kinase. Further analysis of this observation will be addressed in the following paper.

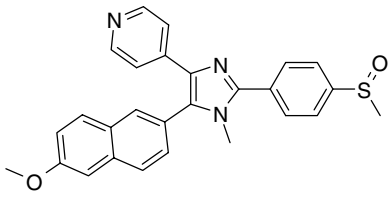
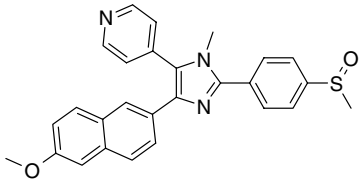
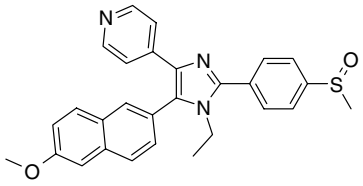
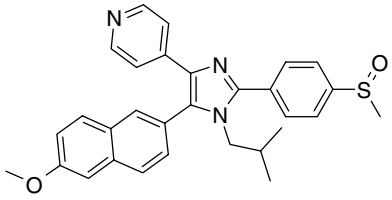
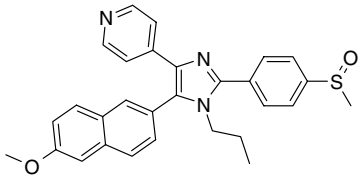
Compounds **5** and **10** were found to have moderate to excellent cellular activities (cell IC_{50} = 232 nM and 24.4 nM, respectively). Compounds **5** and **10** displayed similar pharmacokinetic parameters in rodents and were advanced into an in vivo angiogenesis model.

We used the Matrigel model of angiogenesis in mice to test the in vivo efficacy of our optimized compound.[7] Angiogenesis is stimulated with bFGF and two doses of compound are administered. The two control groups are non-treated mice and mice that are not given bFGF. The mice are sacrificed after 6 days and the Heme content of the Matrigel plug is measured. At doses of 25 and 50 mg/kg ip b.i.d, compound **5** showed a reduction of 41% and 70%, and compound **10** showed a reduction of only 5% and 30%, respectively, of angiogenesis in this model.

Based on the encouraging result from the angiogenesis model, compound **5**[7,8] was advanced into a MOPC-315 plasmacytoma xenograft model. Figure 3 below shows that compound **5** induced a modest dose dependent delay in tumor growth.

In summary, we have optimized **SB-203580**, a known CSBP/p38 kinase inhibitor, into a potent and selective Tie2 tyrosine kinase inhibitor. The optimized compound **5** showed efficacy in an in vivo model of angiogenesis and a MOPC-315 plasmacytoma xenograft model.

Table 2. Tie2 and p38 kinase activity of alkylated compounds

Compound	Tetra-substituted imidazole	Tie2 IC_{50} (nM)	p38 IC_{50} (nM)
10		60	16,000
11		20,000	16,000
12		5370	>16,000
13		3890	>16,000
14		437	>16,000

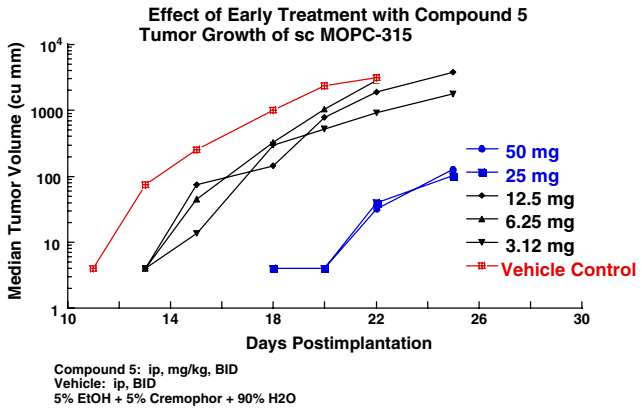


Figure 3. MOPC-315 xenograft model for compound **5**.[7]

References and notes

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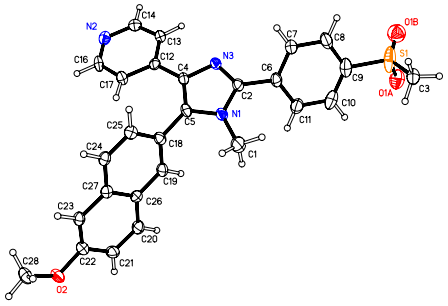
6. An ortep view of compound **10**. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic

Data Centre as supplementary publication numbers CCDC 644904. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].



7. Compound **1** was tested in our Tie2 receptor signal transduction assay and our Tie2 autophosphorylation assay. Compounds **5** and **10** were tested in our Tie2 autophosphorylation assay. For assay conditions see Semones, M. A. PCT Int. Appl. (2002), WO 2002060382 and Adams, J. L.; Kasparec, J.; Silva, D.; Yuan, C. C. K. PCT Int. Appl. (2003), WO 2003029209.
8. Compound **5** has a >10-fold selectivity against VEGFR2, VEGFR3, and PDGFR1β.
9. The homology model of Tie2 was generated in the program MOE using the crystal structure FGFR-2 kinase (PDB code 1oec) as the structural template; compounds **1** and **5** were manually docked into the homology model based on the bound conformation of **SB-203850** in crystal structure 1a9u. Figure 2 depicts the manual docking model for compound **5** in a second-generation homology model of an active form of Tie2; this second-generation homology model was generated in the program MOE using the crystal structures of inactive Tie2 (PDB code 1fvr) and active FGFR-2 kinase (PDB code 1oec) as structural templates. Figures were generated using the program pymol.