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Optimization of triarylimidazoles for Tie2: Influence of conformation on potency

Neil W. Johnson,[a,*] Marcus Semones,[a] Jerry L. Adams,[a] Michael Hansbury[b] and Jim Winkler[c]

[a]*GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA*
[b]*Incyte Pharm., Wilmington, DE, USA*
[c]*Array Biopharma, Boulder, CO, USA*

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Abstract—In an effort to understand the effect of N-alkylation of triarylimidazoles on Tie2 inhibition, *ortho*-substituted C-2 aryl analogs were synthesized to investigate the effect of different torsion angles on potency. This exercise resulted in the identification of a potent and selective tetrasubstituted imidazole that was efficacious in an animal model of angiogenesis.

Several receptor tyrosine kinases (VEGF, FGF, PDGF) are known to be involved in the process of angiogenesis—the formation of new capillaries from established blood vessels. Similar to VEGFR2, the Tie family of receptors, Tie1 and Tie2, are expressed predominately on endothelial cells[1] and are essential for vessel formation. Interference of the Tie2 pathway with an extracellular receptor domain results in a significant inhibition of tumor growth.[2] Furthermore, targeted disruption of the *tie2* gene results in malformed vasculature in embryonic mice.[3] The recent approval of an anti-VEGF monoclonal antibody (Avastin™; Genentech, and Roche)[4] for the treatment of metastatic colorectal cancers has validated the earlier hypothesis that disruption of angiogenesis could be an effective strategy for the treatment of cancer. The involvement of Tie2 kinase in angiogenesis makes it an attractive target for investigation.

Our group has been investigating a series of triarylimidazoles as selective inhibitors of Tie2. As reported in the accompanying manuscript,[5] the incorporation of an *N*-alkyl substituent on the triarylimidazole core was found to improve Tie2 activity. The significant improvement in Tie2 inhibition upon methylation of the imidazole (Fig. 1, compound **1** vs compound **2**) has been difficult to rationalize.

To gain insight into the effect of N-alkylation of the imidazole, small molecule X-ray crystal structures were obtained for compounds **1** and **2**.[6] The torsion angles of the aryl rings attached to the imidazole showed that the C-2 aryl ring is slightly out of coplanarity with the imidazole in compound **1**, whereas in compound **2** it is out of plane by almost 50°. When the methyl group is

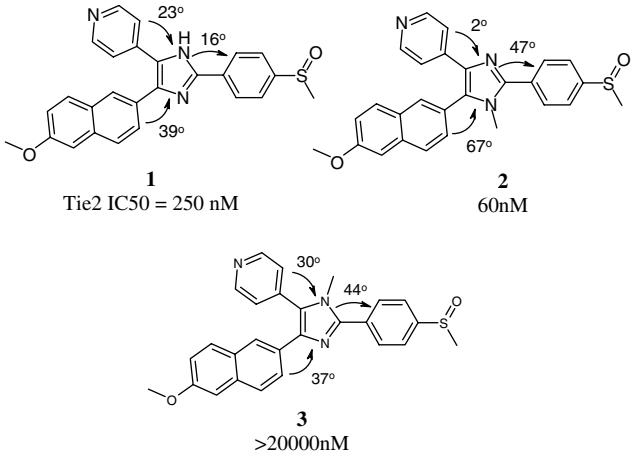

Figure 1. Representative triaryl imidazoles and their torsion angles with Tie2 IC_{50}'s.

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* Corresponding author. Tel.: +1 610 917 6292; fax: +1 610 917 6020; e-mail: neil.w.johnson@gsk.com

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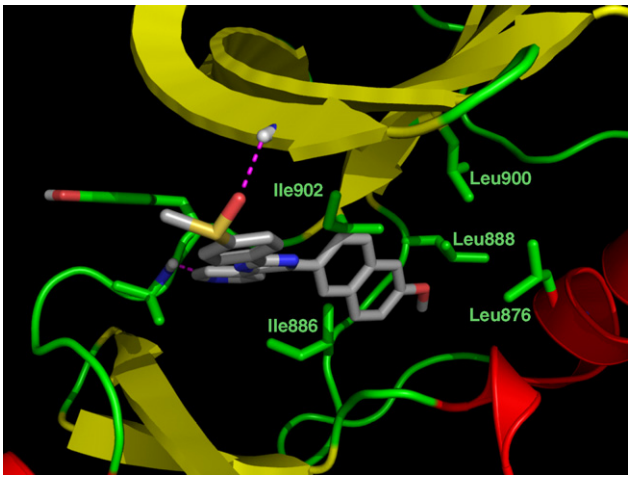


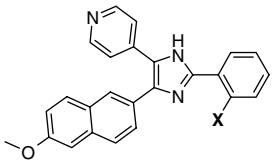
Figure 2. Docking model of Compound **1** in Tie2.

adjacent to the naphthyl ring (compound **2**) this forces the ring out of plane by 67° allowing the pyridine to be nearly coplanar with the imidazole. According to modeling studies, alkylating adjacent to the pyridine ring, as in compound **3**, would force the pyridine ring to be 30° out of plane and all other torsion angles to be almost equivalent. In our proposed binding mode[7] (Fig. 2), the pyridine hydrogen bonds Ala905 in the hinge region of the ATP pocket with the naphthyl ring occupying a lipophilic back pocket. As shown above, methylating adjacent to the naphthyl ring is allowed and preferred for Tie2 activity. The increase in the torsion angle of the naphthyl group along with the pyridine ring reaching coplanarity appears to be an optimal conformation for binding to Tie2. What remains unclear from this assessment is whether modifying the torsion angle of the C-2 aryl ring has any impact on Tie2 activity. In order to independently evaluate this torsion angle an *ortho*-methyl aryl analog of compound **1** was synthesized.

The requisite *ortho*-substituted benzaldehyde was synthesized from commercially available 4-bromo-2-methyl benzonitrile **4**, as shown in Scheme 1. The bromide was displaced with sodium thiomethoxide followed by reduction of the nitrile with DIBAL-H to afford the desired 2-methyl-4-thiomethyl benzaldehyde **5**. Using standard conditions for the formation of imidazoles,[8] condensation of benzaldehyde **5** with keto-oxime **6**[5] gave the triarylimidazole *N*-oxide that was then reduced to the imidazole by heating with trimethylphosphite in DMF. The resulting sulfide was oxidized with potassium persulfate to give the *ortho*-methyl C-2 aryl analog **7**. This compound was shown to inhibit Tie2 with an IC_{50} of 36 nM, an almost 2-fold improvement in potency versus compound **2** and a 10-fold improvement over compound **1**. After separation of the sulfoxide enantiomers via chiral HPLC, a small molecule X-ray was determined for the S-enantiomer of compound **7**.[6] While the S-enantiomer was not as active as the R (95 nM vs 22 nM, respectively) it still showed similar activity to compound **2** and thus a 3-fold improvement over compound **1**. The torsion angle of the C-2 aryl group was found to be 45°. The pyridine (30°) and naphthyl (48°) torsion angles were similar to those seen for compound **1**. Hence, the increase in potency can be partially attributed to the C-2 aryl group being forced out of plane with the imidazole.

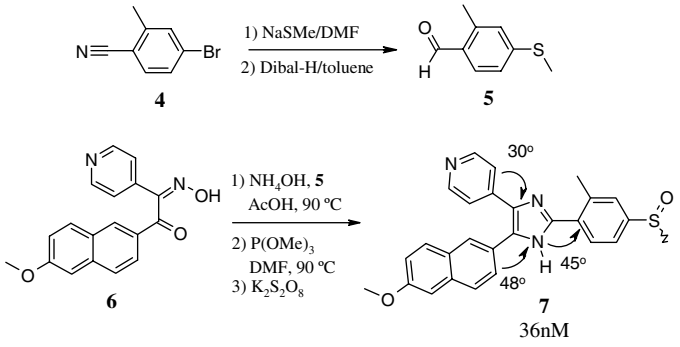
Imidazole condensations were then performed with a series of benzaldehydes and the pyridinyl 6-methoxy-naphthyl keto-oxime **6** to investigate the SAR for *ortho* substituted phenyl rings. Results are shown in Table 1. The *ortho*-methyl phenyl analog **9** is significantly more

Table 1. Tie2 IC_{50}'s for ortho-substituted C-2 phenyl triarylimidazoles

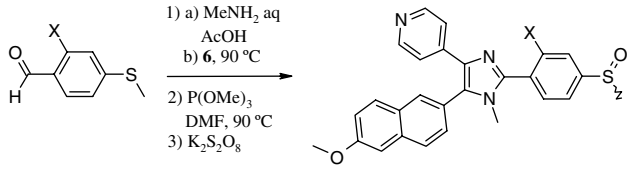


Compound	X	Tie2 IC_{50}, nM
8	H	60% at 10 μM
9	Me	387
10	F	1600
11	Cl	157
12	Br	82
13	CF_3	260
14	2,6-DiCl	78
15	Ethyl	346
16	*i*-Propyl	836

Values are means of at least two experiments.



Scheme 1. Synthesis of triaryl imidazole **7.**



Scheme 2. Regioselective *N*-Methyl imidazoles.

potent than the unsubstituted phenyl analog **8** and still has modest Tie2 inhibitory activity without the *para*-methylsulfoxide. The *ortho*-fluoro analog **10** was much less active, possibly due to its small size allowing a conformation similar to the unsubstituted analog **8**. Potency was improved with *ortho*-chloro (compound **11**) and even further with *ortho*-bromo and 2,6-dichloro (compounds **12** and **14**) vs *ortho*-methyl (compound **9**). A modest improvement in Tie2 activity was observed for the *ortho*-CF_3 (compound **13**) while the *ortho*-ethyl was equivalent in activity and the *ortho*-isopropyl had a negative impact on activity (compounds **13** and **15**, respectively). Given the increase in size and lipophilicity of adding a bromo or 2,6-dichloro substituent, the chloro and methyl substituents were chosen as the best compromise between potency and size.

We then wanted to combine the *para*-methylsulfoxide with the optimized *ortho*-methyl and *ortho*-chloro C-2 phenyl rings as well as examine the effect of these changes in the N-methylated triarylimidazoles. A regioselective synthesis of the *N*-methyl analogs was developed to simplify the synthesis of these compounds. The appropriate benzaldehydes were synthesized using a route similar to that used in Scheme 1. The benzaldehyde was stirred in acetic acid and aqueous methyl amine (Scheme 2) to generate the corresponding methyl imine. The keto-oxime **6** was then added and the reaction mixture was heated at 90 °C. This condensation method resulted in a single regioisomer of the imidazole *N*-oxide. The remainder of the synthesis went forward as performed previously.

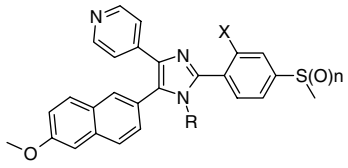
The corresponding *para*-sulfide, sulfoxide and sulfone analogs were tested along with the *N*-methylimidazole analogs of the *ortho*-methylphenyl derivatives (Table 2). For comparison, data for compounds **1**, **2**, and **7** are included in the table. There was very little difference in potency between the sulfones and sulfoxides (compound **7** vs **18** and **20** vs **21**), except with compound **23** vs **24** where there was a nearly 10-fold difference. Compound **23**, which possesses both the preferred *ortho*-methyl and *N*-methyl imidazole, showed an additional 5-fold increase in potency over the original *N*-methyl imidazole **2**, and a 3-fold improvement in potency compared to compound **7**. Unlike the earlier result with monosubstituted phenyls in Table 1, the *ortho*-chloro analogs were not more potent than the *ortho*-methyl analogs (i.e., compound **20** vs **7**). The combined effect of increasing the torsion angles of the naphthyl group and the C-2 phenyl group is favorable for Tie2 inhibition.[9]

Compound **23**, our most potent Tie2 inhibitor, was examined against a panel of kinases. Representative data are given in Table 3. Very high selectivity is shown against both tyrosine kinases and serine/threonine kinases. Even for the most sensitive kinases in this panel, c-fms and Alk5, selectivity remains good, being 25-fold for Alk5 and 50-fold for c-fms.

The in vivo efficacy of our optimized compound **23** was tested in the matrigel model of angiogenesis in mice.[10] In this model, angiogenesis is stimulated with bFGF and two doses of compound are given. The two control groups are untreated 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 two doses of compound **23** (25 and 50 mg/kg p.o., b.i.d.) inhibition of angiogenesis (35 and 80%, respectively) was observed in this model.

In summary, we have successfully exploited a conformational hypothesis to optimize a series of triarylimidazoles

Table 2. Combination of *ortho* and *para*-substituents



Compound	R	X	*n*	Tie2 IC_{50}, nM
1	H	H	2	250
2	Me	H	1	60
17	H	Me	0	1300
7	H	Me	1	36
18	H	Me	2	40
19	H	Cl	0	1500
20	H	Cl	1	51
21	H	Cl	2	59
22	Me	Me	0	335
23	Me	Me	1	12
24	Me	Me	2	114

Values are means of at least two experiments.

Table 3. Kinase selectivity data for compound **23**

Kinase	IC_{50}, nM
IGF1-R	>30,000
INS-R	>30,000
FLT3	>30,000
KIT	>30,000
PDGFR-α	>30,000
PDGFR-β	>30,000
VEGFR-2	1500
FGF-R1	9700
MET	>30,000
ABL	>30,000
PKC-β1	>30,000
p38	4100
c-FMS	550
CDK2	>30,000
GSK3	>30,000
ALK5	296
AKT1	>30,000

for Tie2 potency. The alkylation of the imidazole adjacent to the naphthyl ring and incorporation of a C-2 *ortho*-aryl substitution to increase its torsion angle relative to the imidazole resulted in a potent and selective Tie2 inhibitor. The optimized compound **23** showed efficacy in an in vivo model of angiogenesis.

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6. 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 646583 and 646584. 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. 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**–**3** 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 **1** 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.
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