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Discovery and Evaluation of 3-(5-Thien-3-ylpyridin-3-yl)-1*H*-indoles as a Novel Class of KDR Kinase Inhibitors

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Abstract—We have discovered 3-(5-thien-3-ylpyridin-3-yl)-1*H*-indoles as potent inhibitors of KDR kinase activity. This communication details the evolution of this novel class from a potent screening lead of vastly different structure with an emphasis on structural modifications that retained activity and provided improvements in key physical properties. The synthesis and in-depth evaluation of these inhibitors are described.

Angiogenesis, the formation of new capillaries from established blood vessels, is an essential process for normal growth and development that has also been implicated in the pathogenesis of several diseases including diabetic retinopathy,[1] rheumatoid arthritis,[2] psoriasis,[3] and cancer.[4] Specifically, the growth and metastasis of solid tumors has been shown to be dependent on angiogenesis at an early stage,[5] while tumors that lack adequate vascularization become necrotic or apoptotic and do not grow beyond a limited size.[6] Interest in inhibition of angiogenesis as a new approach for the treatment of cancer has led to the elucidation of key underlying molecular mechanisms that control the angiogenic process. Angiogenesis is regulated by the expression of a variety of growth factors including vascular endothelial growth factor (VEGF), a selective mitogen for endothelial cells whose mitogenic signaling is mediated through the receptor tyrosine kinase KDR (VEGFR-2).[7] Several lines of evidence indicate that expression and signaling of VEGF are critical for tumor angiogenesis. Among these, antibodies against VEGF[8] and its receptor KDR[9] as well as small molecule inhibitors of KDR kinase activity[10] have been shown to inhibit angiogenesis in tumor xenograft models. Clinical trials have been initiated for KDR kinase inhibitors derived from a number of different structural classes, including indolin-2-ones, phthalazines, and quinazolines.[11]

In our efforts to discover novel small molecule inhibitors of KDR kinase activity, a screening of a large non-directed library of 5-acetamido-2,4-diaryloxazoles produced lead compound **1** (Fig. 1). Compound **1** exhibited good intrinsic potency (KDR $IC_{50} = 16\,nM$)[12] and reasonable cellular activity (cell $IC_{50} = 320\,nM$).[13]

However, we concluded from additional studies that the poor physical properties of **1**, including low aqueous solubility ($< 0.005\,mg/mL$) and high lipophilicity (logP > 4.1),[14] would hinder its development. Toward improving these physical properties, we initially investigated more polar replacements for the 2-hydroxy-3-naphthyl moiety at C-2 of the oxazole ring system. Of

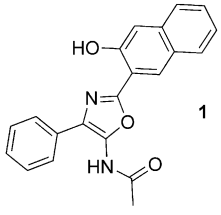

Figure 1. Screening lead **1**.

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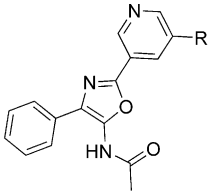
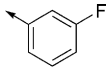
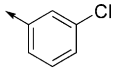
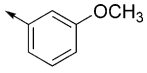
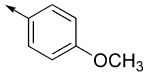
Table 1. KDR kinase activity of compounds **2a–f**



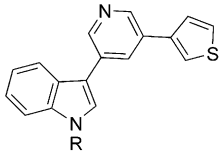
2a-f

Compd	R	KDR IC_{50} (nM)
2a		186
2b		575
2c		5700
2d		8320
2e		4860
2f		4040

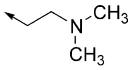
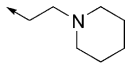
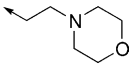
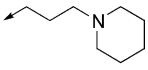
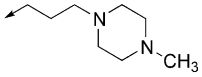
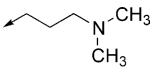
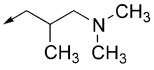
the wide range of aryl and heterocyclic substituents examined at C-2, all were relatively inactive with the exception of a small subset of *ortho*-phenols and, more interestingly, the 3-pyridyl ring system found in compound **2a** (Table 1).[15,16] The 3-fold greater potency provided by the thienyl substituent of **2a** compared to phenyl group within **2b** and dramatic loss in potency upon substitution of this phenyl group (cf **2c–f**) were reminiscent of the SAR established for the pyrazolo[1,5-*a*]pyrimidine class of KDR kinase inhibitors.[17] We proposed a similar binding mode that placed the pyridyl ring in the adenine binding region of the ATP active site and allowed the pyridyl nitrogen to engage in a hydrogen bond with Cys 919 of the hinge region.[18] We then envisioned the thienyl group fitting into the sterically confined hydrophobic region I and the oxazolyl moiety filling hydrophobic region II.

Because compound **2a** showed little improvement in physical properties (aq soln <0.005 mg/mL, logP >4.0) over **1**, we continued to focus on major structural modifications. Based on our limited understanding of the binding mode of **2a**, we speculated that potency would be maintained by replacement of the oxazolyl appendage at C-3 of the pyridyl ring system with a planar substituent of lower molecular weight. A survey of a set of aryl and heterocyclic groups at C-3 culminated in the identification of the 3-indolyl substituent (**3a**, Table 2) as the optimal group in terms of retaining activity and providing an appropriate point of attachment for solubilizing functionality, via the indolyl nitrogen. We took advantage of this key feature in the design and synthesis of compounds **3b–h** (Scheme 1). Briefly, palladium-catalyzed cross-coupling of 3-bromo-5-thien-3-

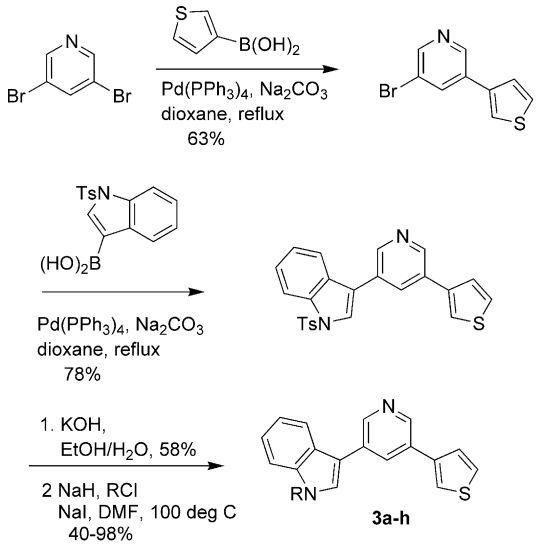
Table 2. KDR kinase activity of compounds **3a–h**



3a-h

Compd	R	KDR IC_{50} (nM)
3a	H	126
3b		115
3c		111
3d		60
3e		38
3f		16
3g		64
3h		46

ylpyridine and 1-[(4-methylphenyl)sulfonyl]-1H-indol-3-ylboronic acid[19] under Suzuki conditions proceeded smoothly to give 1-[(4-methylphenyl)sulfonyl]-3-(5-thien-3-ylpyridin-3-yl)-1H-indole. Hydrolysis of the tosyl group with potassium hydroxide afforded **3a**. Deprotonation of **3a** with sodium hydride and alkylation of the resultant anion with the corresponding mustard-like amines proceeded in moderate to excellent yield to furnish compounds **3b–h**.[20]



Scheme 1. Synthesis of compounds **3a–h**.

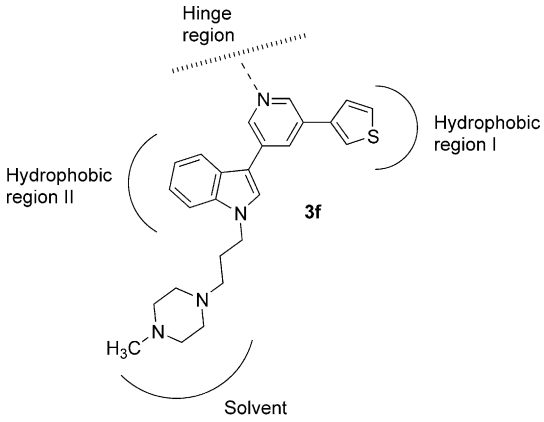


Figure 2. Proposed binding mode of **3f**.

The addition of the alkyl amines to **3a** was well-tolerated for compounds **3b** and **3c**, and modestly potency-enhancing for **3d–h**, with **3f** exhibiting the greatest potency (KDR $IC_{50} = 16$ nM). The three-carbon methylene spacer in **3e** and **3g** conferred 2-fold greater potency relative to the two-carbon linker in analogues **3c** and **3b**, respectively. That inhibitory activity was relatively insensitive to the nature of the basic side chain is consistent with the proposed binding mode depicted in Figure 2 wherein the side chain extends away from the binding cleft and is solvent exposed. Further SAR studies supported this model. As was the case in the oxazolyl series (compounds **2a–f**), replacement of the thienyl group with a substituted phenyl ring led to nearly complete loss of activity in this series, presumably the result of steric clashes within hydrophobic region I. The indolyl ring system proved more tolerant of substitution in terms of retaining activity. For example, placing a chloro, cyano, or methoxyl group at the indolyl C-5 position of **3f** resulted in only a 3-fold decrease in potency.

Cell data indicated that the basic side chain was required for achieving submicromolar levels of inhibitory activity in this series (Table 3). A modest shift (5- to 20-fold) was observed in cellular IC_{50} compared to the corresponding biochemical IC_{50} value. This effect may be due, in part, to high protein binding, as measured for **3f** (99.8% bound to human plasma protein).

Table 3. Cell activity for selected compounds

Compd	KDR IC_{50} (nM)	Cell IC_{50} (nM)
3a	126	37% inhib. @ 2500 nM
3d	60	1410
3e	38	495
3f	16	328
3h	46	256

Table 4. Physical properties of compounds **3d–f**

Compd	LogP	Solubility @ pH 5.2 (mg/mL)	Solubility @ pH 7.4 (mg/mL)
3d	3.3	0.024	0.005
3e	N.D.	0.177	<0.005
3f	3.7	18.17	>0.205

N.D., not determined.

Table 5. Rat pharmacokinetic data for compounds **3d–f**[a]

Compd	Cl (mL/min/kg)	$t_{1/2}$ (h)	Vdss (L/kg)	%F
3d	65	1.9	7.2	35
3e	15	1.6	1.8	28
3f	13	1.8	1.7	33

[a]Compound dosed 5 mg/kg iv as a solution in DMSO and 20 mg/kg po as a suspension in aqueous 0.5% methyl cellulose (pH 5).

Table 6. KDR kinase selectivity (fold)[a] of **3f**

PDGFRβ	Flt-1	Flt-4	FGFR-1	FGFR-2	c-Src
7	20	3	246	136	147

[a]Expressed as the biochemical IC_{50} (nM) ratios to KDR.

Physical properties were determined for selected compounds (Table 4). Gratifyingly, compounds **3d–f** showed greater aqueous solubility and lower lipophilicity relative to compounds **1** and **2a**, leading us to evaluate their pharmacokinetic properties.

Compounds **3d–f** exhibited moderate pharmacokinetic behavior in rats with $t_{1/2}$ ranging from 1.6 to 1.9 h and oral bioavailability ca. 30% (Table 5).

The selectivity of **3f** for inhibition of KDR kinase versus several closely related receptor tyrosine kinases and a non-receptor tyrosine kinase (c-Src) is presented in Table 6 and representative of the class. In general, these compounds showed low levels of selectivity for KDR against the highly KDR-homologous kinases PDGFRβ, Flt-1, and Flt-4 and higher levels against FGF-1, FGF-2, and c-Src.

In conclusion, we have described the evolution of a novel 3-(5-thien-3-ylpyridin-3-yl)-1H-indolyl class of KDR kinase inhibitors from a potent oxazole-based lead. Compounds within this new series retained high activity and exhibited improved physical properties and reasonable pharmacokinetics in rats.

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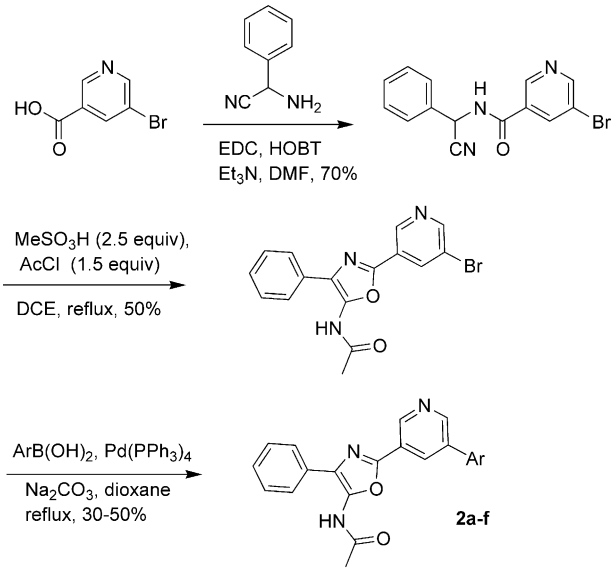
12. The KDR IC_{50} value represents biochemical inhibition of phosphorylation of poly-Glu/Tyr (4:1) peptide substrate by isolated KDR kinase (cloned and expressed as a GST-fusion protein); see: Kendall, R. L.; Rutledge, R. Z.; Mao, X.; Tebben, A. J.; Hungate, R. W.; Thomas, K. A. *J. Biol. Chem.* **1999**, *274*, 6453. Values are reported as the average of at least two determinations, except for compound **3c** ($n=1$). Standard deviations are typically within 25% of the IC_{50} value.
13. The cell IC_{50} value represents the inhibition of VEGF-stimulated mitogenesis as determined in human umbilical vein endothelial cells. Refer to US Patent 6,265,403 for assay details. Values are reported as the average of three determinations for compounds **1** and **3d**, and single determinations for **3a**, **3e**, **3f**, and **3h**.
14. Partition coefficients were determined by HPLC analysis in octanol/water.
15. All target compounds were fully characterized by ^1H NMR and mass spectroscopy.
16. Compounds **2a**–**f** were prepared by the following sequence; for related chemistry; see: Lipshutz, B. H.; Hungate, R. W.; McCarthy, K. E. *J. Am. Chem. Soc.* **1983**, *105*, 7703.



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20. Additional examples as well as experimental details of the synthetic methods employed in this work can be found in US Patent 6,265,403.