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Optimization of a Pyrazolo[1,5-*a*]pyrimidine Class of KDR Kinase Inhibitors: Improvements in Physical Properties Enhance Cellular Activity and Pharmacokinetics

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Abstract—We have introduced solubilizing functionality to a 3,6-disubstituted pyrazolo[1,5-*a*]pyrimidine series of KDR kinase inhibitors to improve the physical properties of these compounds. The addition of a basic side-chain to the 6-aryl ring, introduction of 3-pyridyl groups, and most significantly, incorporation of a 4-pyridinonyl substituent at the 6-position of the core are modifications that maintain and often enhance the intrinsic potency of this class of inhibitors. Moreover, the improvements in physical properties result in marked increases in cellular activity and more favorable pharmacokinetics in rats. The synthesis and SAR of these compounds are described.
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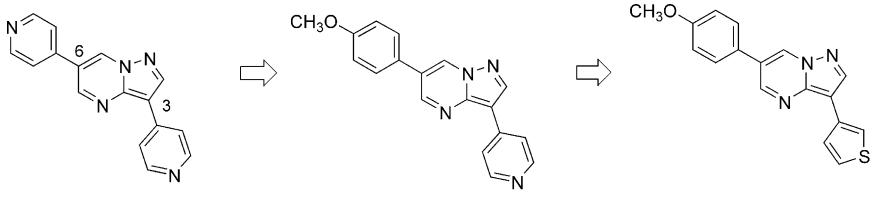
Angiogenesis, the formation of new capillaries from established blood vessels, contributes to the pathogenesis of a number of disease states such as diabetic retinopathy,[1] rheumatoid arthritis,[2] psoriasis,[3] and cancer.[4] At an early stage in the development of solid tumors, angiogenesis is triggered when blood perfusion from the existing vasculature becomes insufficient for sustaining tumor cell growth.[5] Under hypoxic conditions, the tumor cells elicit an angiogenic response by expressing a variety of growth factors, including vascular endothelial growth factor (VEGF), whose mitogenic signaling is mediated through the receptor tyrosine kinase VEGFR-2 (KDR).[6] Several lines of evidence indicate that expression and signaling of VEGF are critical for tumor angiogenesis. Among these, antibodies against VEGF[7] and its receptor KDR[8] as well as small molecule inhibitors of the KDR kinase domain[9] have been shown to inhibit angiogenesis in tumor models. Recently, the therapeutic potential of small molecule inhibitors of KDR kinase for use as anti-angiogenic



1, KDR IC_{50} = 224 nM
ECMA IC_{50} = 622 nM
LogP = 2.5

2, KDR IC_{50} = 37 nM
ECMA IC_{50} = 150 nM
LogP = 4.0

3, KDR IC_{50} = 19 nM
ECMA IC_{50} = 387 nM
LogP >> 4.0

Figure 1. Initial optimization of screening lead **1**.

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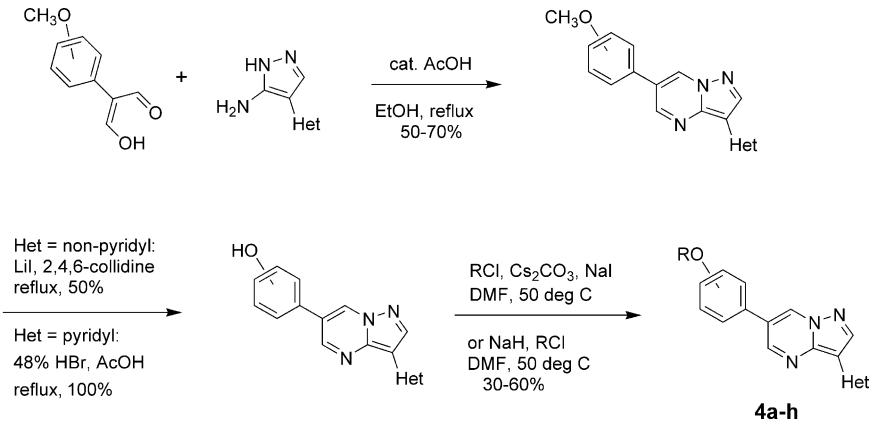
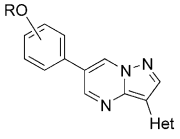


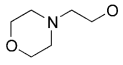
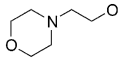
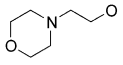
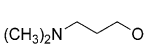
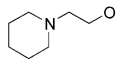
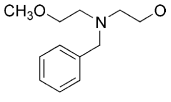
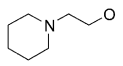
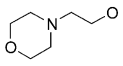
Scheme 1. Synthesis of compounds **4a–h**.[14]

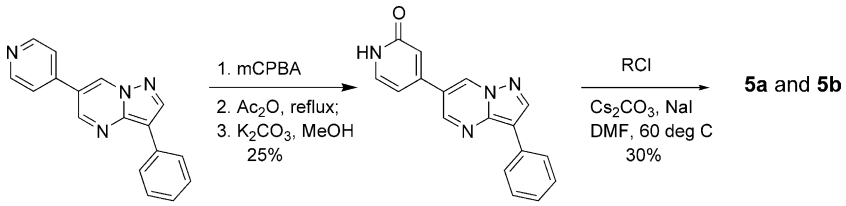
agents for the treatment of cancer has come under much attention. Clinical trials have been initiated for KDR kinase inhibitors derived from a number of different structural classes, including indolin-2-ones, phthalazines, and quinazolines.[10] This approach to cancer therapy differs from the traditional use of cytotoxic agents in that the target tissue is the vasculature encompassing the neoplasm, not tumor cells, and relies on 'starvation' of the tumor for growth inhibition rather than direct cell-cycle arrest.

In a previous paper,[11] we reported our initial optimization studies of a newly discovered pyrazolo[1,5-*a*]pyrimidine class of KDR kinase inhibitors. In that work, we identified a number of replacements for the 4-pyridyl groups of screening lead **1** which offered marked improvements in the biochemical activity of this series. Potency was fully optimized with 4-methoxyphenyl and 3-thienyl groups at the 6- and 3-positions, respectively, of the pyrazolo[1,5-*a*]pyrimidine, as illustrated in the progression of **1** to **3** (Fig. 1).[12] Results from a VEGF-stimulated endothelial cell mitogenesis assay (ECMA) indicated that compounds **1** and **2** which bear solubilizing pyridine groups have enhanced cellular inhibitory activity (a lower ECMA to KDR IC_{50} ratio) than the

Table 1. KDR kinase and cellular activity of **4a–h**[17]



Compound	Side-chain position	RO	3-Het/Ar	KDR IC_{50} (nM)	ECMA IC_{50} (nM)
4a	*para*			4	22
4b	*para*			9	32
4c	*meta*			14	49% inhibition @ 100 nM
4d	*para*			10	23
4e	*para*			3	18
4f	*para*			19	31% inhibition @ 100 nM
4g	*para*			20	29
4h	*para*			11	31

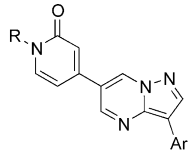


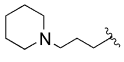
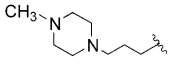
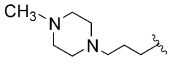
Scheme 2. Synthesis of compounds **5a** and **5b**.

less soluble and more lipophilic compound **3**.[13] These data suggested that cellular activity in the mitogenesis assay was improved by increasing the solubility and polarity of these inhibitors. In this paper, we describe structural modifications that improved the physical properties of inhibitors within this series, and thereby enhanced the cellular activity and improved pharmacokinetics in rats.

Toward improving the physical properties of our initial leads, we first introduced solubilizing basic amines to the *meta* and *para* positions of the 6-aryl ring via an ether linkage utilizing the chemistry outlined in Scheme 1. Thus, 6-(3- and 4-methoxyphenyl)pyrazolo[1,5-*a*]pyrimidines were assembled in good yield via condensation reactions of the corresponding 2-(3- and 4-methoxyphenyl)malondialdehydes and 3-amino-4-heteroarylpyrazoles.[15] Demethylation of the 6-aryl ethers was accomplished in one of two ways depending on the nature of the 3-heteroaryl group as depicted.[16] Alkylation of the resulting phenols with a variety of mustard-like chloroalkyl amines proceeded in moderate yield to provide analogues **4a–h** (Table 1).

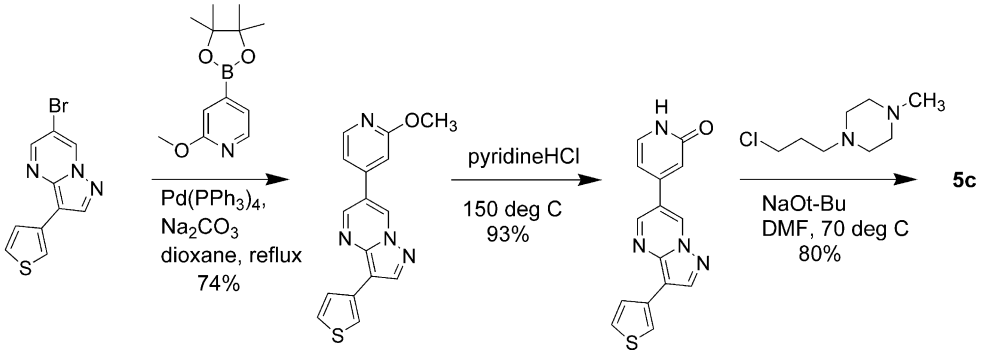
Table 2. KDR kinase and cellular activity of **5a–c**



Compound	R	3-Ar/Het	KDR IC_{50} (nM)	ECMA IC_{50} (nM)	LogP
5a			14	70	2.4
5b			13	80	2.0
5c			7	20	1.6

The screening data from this set of inhibitors revealed that addition of the basic side-chain enhanced the intrinsic potency and produced the predicted increase in cellular activity. For example, compound **4a** was 5-fold more potent against isolated KDR and nearly 20 times more active in the mitogenesis assay compared to **3**. Consistent with the structure–activity relationships (SARs) established in our preliminary work in this series, compounds possessing a 3-thienyl group at the 3-position of the pyrazolo[1,5-*a*]pyrimidine were 2-fold more potent biochemically than the corresponding 3-phenyl analogues (**4a** vs **4b**). Likewise, compounds possessing *para*-substituted basic ethers on the 6-aryl ring were 3-fold more active than the *meta*-substituted analogues (**4c** vs **4a**). In this data set, inhibitory activity appeared relatively insensitive to the nature of the basic side-chain (cf., **4a**, **4d**, and **4e**). This observation was consistent with docking studies using a KDR homology model that depicted the bicyclic core bound in the adenine region of the ATP active site, with N-1 of the pyrazolo[1,5-*a*]pyrimidine engaged in a hydrogen bond with Cys 919 of the hinge region, and the side-chain extending away from the cleft and exposed to solvent.[18] Although less potent, compound **4f** showed elevated KDR kinase specificity (see below). Lastly, 3- and 4-pyridyl substituents at the 3-position of the nucleus were well-tolerated (**4g** and **4h**) and provided greater solubility and polarity (e.g., logP **4g** = 3.4 vs logP **4e** > 4.0).

We next incorporated a 4-pyridinonyl substituent at the 6-position of the pyrazolo[1,5-*a*]pyrimidine, a modification



Scheme 3. Synthesis of compound **5c**.

that had been shown to greatly enhance physical properties in a related benzimidazole class of KDR kinase inhibitors. The chemistry in the 3-phenyl series involved oxidation of the corresponding 4-pyridyl ring followed by rearrangement of the resulting pyridine *n*-oxide under standard conditions (Scheme 2). Selective alkylation of the 4-pyridinonyl nitrogen provided compounds **5a** and **5b** in modest yield (Table 2). Introduction of the 6-(4-pyridinonyl) group in the more potent 3-(3-thienyl) series required an alternative synthesis due to the oxidatively sensitive 3-thiophene ring (Scheme 3). Key steps in the synthesis of **5c** included the Suzuki cross-coupling reaction of 6-bromo-3-(3-thiophenyl)pyrazolo[1,5-*a*]pyrimidine[19] with the 4-pinacolboronic ester of 2-methoxypyridine,[20] hydrolysis of the 2-methoxypyridine intermediate,[21] and the selective *N*-alkylation of the penultimate pyridinone under the optimized conditions shown.

Biochemical, cellular, and partition coefficient data are summarized for compounds **5a–c** in Table 2. A significant increase in polarity and aqueous solubility was observed with the incorporation of the 4-pyridinonyl group within **5a** (logP = 2.4) as compared to the highly lipophilic and relatively insoluble homologue **4e** (logP > 4.0). Physical properties and potency were further optimized in this series with addition of the 4-methylpiperazinyl and 3-thienyl appendages found in **5c**.

The KDR kinase selectivity profiles for compounds **4e–g** and **5c** against a panel of kinases are expressed as biochemical IC_{50} (nM) ratios to KDR in Table 3.[22] In general, these inhibitors showed modest selectivity for KDR kinase versus the highly homologous kinases PDGFRβ, Flt-1, and Flt-4 and moderate to high selectivity versus FGFR-1, FGFR-2, and SRC kinases. Of note, the branched side-chain within **4f** offered enhancement in KDR selectivity versus the FGF receptor kinases and SRC kinase.

The pharmacokinetic parameters of compounds **4e**, **4g**, and **5c** in rats are summarized in Table 4. The data show reductions in clearance and volume of distribution as well as improvements in bioavailability with the more polar analogues **4g** and **5c** compared to the highly lipophilic, less soluble derivative **4e**.

Table 3. KDR kinase selectivity (fold) of compounds **4e–g** and **5c**

Compound	PDGFRβ	FLT-1	FLT-4	FGFR-1	FGFR-2	SRC
4e	2.0	7.7	12.9	103	—	52
4f	2.2	3.9	3.3	>1000	370	>1000
4g	5.3	9.5	5.0	51	26	80
5c	4.6	8.5	7.3	242	86	358

Table 4. Rat pharmacokinetic parameters of **4e**, **4g**, and **5c**[23]

Compound	CI (mL/min/kg)	$t_{1/2}$ (h)	Vdss (L/kg)	%F
4e	80	5.1	16	18
4g	23	3.3	6.0	42
5c	28	3.4	7.9	56

In conclusion, we have identified a number of modifications to the pyrazolo[1,5-*a*]pyrimidine class of KDR kinase inhibitors that have improved the physical properties of these compounds over those of the initial leads. Modifications such as the addition of a basic side-chain to the 6-aryl ring, introduction of 3-pyridyl groups, and incorporation of a 4-pyridinonyl substituent at the 6-position of the nucleus afforded enhanced cellular potency and more favorable pharmacokinetics in rats.

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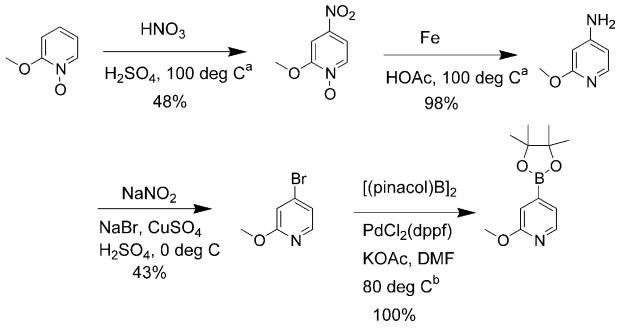

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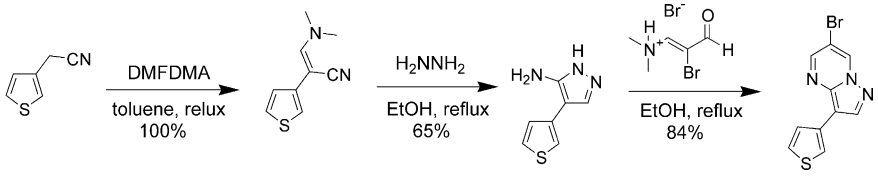

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