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Potent 2-[(pyrimidin-4-yl)amine}-1,3-thiazole-5-carbonitrile-based inhibitors of VEGFR-2 (KDR) kinase

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Abstract—Pyrimidino-thiazolyl carbonitriles were prepared that are potent VEGFR-2 (KDR) kinase inhibitors. The modification of lead structures resulted in **3m** which exhibited the best overall profile in KDR inhibitory activity, iv/po pharmacokinetics, and reduced hERG affinity.

Vascular endothelial growth factor (VEGF) activation is a critical rate-limiting step in physiological and pathological angiogenesis.[1] The biological effects of VEGF are mediated by two receptor tyrosine kinases, VEGFR-1[2] kinase and VEGFR-2 kinase [also known as kinase domain region (KDR kinase)][3], and it has been demonstrated that the disruption of VEGF signaling can retard angiogenesis and inhibit tumor growth.[4] A number of VEGF inhibitors are currently undergoing advanced-stage clinical trials for the treatment of cancer, including Bevacizumab, a recently marketed antibody against VEGF[4] and a soluble VEGF decoy receptor.[5] In addition, small molecule inhibitors of KDR kinase[6] are being investigated that have been shown to be efficacious in in vivo tumor xenograft models.

Previous work from our laboratories[7] has shown compound **1** (Fig. 1) to have good potency against KDR kinase (KDR $IC_{50} = 36$ nM). Exploring the SAR in the analogous pyridine thiazole series revealed that the phenyl moiety could be replaced with a cyano group with no loss in potency (compound **2a**, KDR $IC_{50} = 36$ nM). Further SAR revealed that the pyridine ring could also be substituted with a basic amine, as in compound **2b**, to improve the physical properties and the cell potency of the analogs.[8] Unfortunately, some analogs of compound **2b** were shown to be potent binders to the potassium channel encoded by the human ether-a-go-go-related gene (hERG). This delayed rectifier potassium channel is involved in ventricular repolarization and underlies the rapid component of the

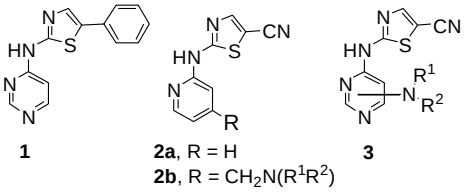

Figure 1. Lead structures.

Keywords: KDR; VEGFR-2.
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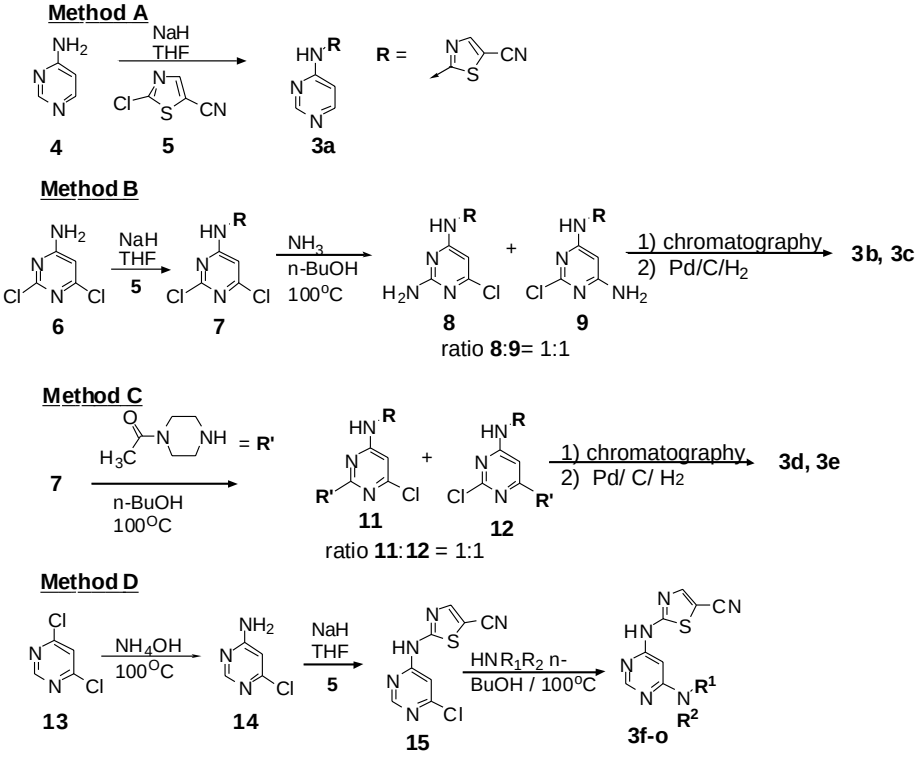
delayed rectifier K+ current (IKr). Blockade of this potassium channel has been shown to lead to QT prolongation and to potentially fatal ventricular arrhythmias.[9] The hERG binding was addressed in the pyridine thiazole series (analogs of compound **2b**) by modifying the physical properties and basicity of the molecules. In a similar effort to design novel and potent KDR kinase inhibitors with reduced hERG channel binding in the pyrimidine series, we merged some of the features of compounds **1** and **2**, and focused on the synthesis of compounds of generic structure **3**. We targeted a series of pyrimidines in which the side chain was directly attached to the pyrimidine ring (structure **3**) and was engaged in a program of modifying the basicity of this side chain in an effort to address the hERG binding issues. In this paper, we describe our efforts to further refine these leads and report the discovery of 2-[(pyrimidin-4-yl)amine}-1,3-thiazole-5-carbonitriles as a new structural class of potent KDR kinase inhibitors.

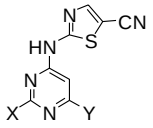
Scheme 1 outlines the synthesis of target compounds **3a**–**o** (Table 1). Alkylation of 4-aminopyrimidine **4** in THF with sodium hydride and 2-chloro-5-cyanothiazole **5** (Method A) provided compound **3a**. In a similar manner, compound **7** was obtained by treating 4-amino-2,6-dichloropyrimidine **6** with NaH and chloride **5**. Compound **7** was treated with ammonium hydroxide in *n*-butanol at 100 °C in a sealed tube to provide regioisomers **8** and **9**. Compounds **8** and **9** were individually hydrogenolyzed to yield compounds **3b** and **3c**, respectively (Method B). Compounds **11** and **12** were prepared (Method C) by exposing compound **7** to 1-acetylpiperazine in *n*-butanol at 100 °C, followed by hydrogenolysis of each product to yield **3d** and **3e**, respectively. Finally, compounds **3f**–**o** were prepared (Method D) by treating **13** with ammonium hydroxide in a sealed tube at 100 °C to give 4-amino-6-chloropyrimidine **14** as the only product. None of the bis-amino displacement products was observed. Alkylation of **14** with sodium hydride and 2-chloro-5-cyanothiazole **5** in THF provided compound **15**. Exposure of **15** to various amines in *n*-butanol at 100 °C afforded the desired compounds **3f**–**o** as filterable solids.

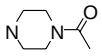
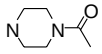
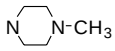
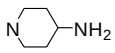
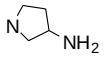
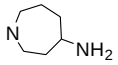
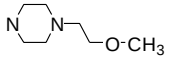
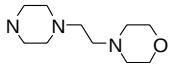
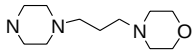
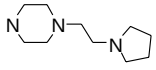
Table 1 summarizes the in vitro data for compounds **3a**–**o**. Target compounds were evaluated for their ability to inhibit the KDR kinase enzyme in a standard enzyme assay as previously described.[10] Compounds were also evaluated in a cell-based KDR autophosphorylation inhibition assay (KDR Phos.)[11] in HEK293 cells stably transfected to express high levels of full length human KDR. The affinity of compounds for the hERG channel was also evaluated in radioligand competition experiments analogous to [^{3}H]-dofetilide binding assays.[12] Previous work in our laboratories[8] had shown that some lipophilic, basic amine-containing analogs of compound **2b** had high levels of potency in our hERG binding assay, and it was critical to track this activity as we synthesized new compounds. Initially, we synthesized the unsubstituted pyrimidino-5-cyanothiazole **3a** (Table 1) and found it to be 30-fold less potent in the KDR kinase assay than was **1** and the pyridino-5-cyanothiazole **2a**.[7] Interestingly, this simple, unsubstituted compound (**3a**) had some hERG binding activity, indicating that hERG activity might be an issue with this series of compounds.



Scheme 1.

Table 1. Properties of selected compounds



Compound	X	Y	KDR IC_{50} (nM)	KDR Phos IC_{50} (nM)	HERG binding IP (nM)	Method
3a	H	H	990	ND	2932	A
3b	H_2N	H	557	ND	>10000	B
3c	H	NH_2	41	ND	>10000	B
3d		H	153	ND	ND	C
3e	H		13 ± 4	44	3291	C
3f	H		16	34	21,500	D
3g	H		10 ± 2	23	923	D
3h	H		10 ± 3	59	6643	D
3i (rac)	H		16	21	3891	D
3j (rac)	H		9	15	1012	D
3k	H		27 ± 8	32	2951	D
3l	H		9 ± 3	22	4029	D
3m	H		12 ± 5	19 ± 4	5000	D
3n	H		10 ± 2	23	4003	D
3o	H		9 ± 4	11	383	D

For determinations where $n > 2$, the standard deviation is given.

In analogy to the earlier pyridine-based series of compounds of generic structure **2**, we wanted to examine the meta substitution pattern in an attempt to enhance the potency of these inhibitors. In an effort to simplify the synthesis of our target compounds as well as to remove the potentially metabolically labile methylene group of compound **2b**, we chose to pursue pyrimidines that were substituted directly with basic side chains as indicated in the generic structure **3**. Entries **3b–e** in Table 1 summarize our efforts to discern the optimal substitution pattern on the pyrimidine ring to maximize activity against KDR. Comparing the results from compounds **3b** and **3d** versus those for **3c** and **3e** clearly indicates that the 4,6-pyrimidine substitution pattern results in more potent analogs than the 2,4-pyrimidine series in the enzymatic assay. Further elaboration of the amine-containing side chains led to a series of compounds with a 2- to 5-fold enhancement of their inhibitory potency. Simple cyclic side chains such as those in compounds **3e–k** all provided potent inhibitory activity in both the enzymatic and cell-based assays. Activity in the hERG assay varied, with the 4-alkyl piperazine **3g**

and the amino azepine **3j** having moderately potent activity. The 4-substituted piperazine derivatives **3l–n** all possessed a good balance of KDR kinase inhibitor activity, cell potency, and decreased affinity for the hERG channel. Compound **3o** was highly potent in both the enzymatic and cell-based assays, however this compound had very high affinity for the hERG channel. Attempts at directly correlating basicity and/or lipophilicity to hERG activity were unsuccessful. It appears that the overall properties of the molecules are critical, and the combination of lipophilicity and basicity is a warning sign but not necessarily predictive for potential hERG binding activity. While it was not always apparent how to design a compound that lacked hERG channel activity, by preparing a series of compounds of varying side chain basicities one could modulate the hERG activity and find compounds of varying affinities. Employing this strategy provided a variety of potent inhibitors of KDR kinase, a number of which showed greatly reduced levels of hERG activity.

Based on their biological profiles, several compounds were chosen for pharmacokinetic evaluation in dogs following iv administration (Table 2). Compound **3h**, which bears a primary amine substituent on the side chain, showed low AUC, high clearance, and relatively short half-life. Analogs containing 4-substituted piperazine side chains (**3l–o**) consistently exhibited better pharmacokinetic profiles, with higher exposures and longer half-lives. In the case of compound **3m**, low clearance was likely a major contributor to the long iv half-life observed for this compound. However, in the case of compounds **3n–o** where the observed clearances were higher, large increases in volume of distribution were likely responsible for the long iv half-lives observed for these analogs. This may be attributable to the more highly basic amines present in the side chain of compounds **3n–o**, as well as the slightly higher lipophilicity of these two analogs.

Based on its overall excellent in vitro and iv pharmacokinetic performance, compound **3m** was selected for further pharmacokinetic evaluation. The pharmacokinetic profile of **3m** in rat, dog, and rhesus monkey after iv/po dosing is detailed in Table 3. The compound exhibited moderate to long half-life, low to moderate clearance, and excellent oral bioavailability in all three species.

Compound **3m** was also evaluated against a panel of related tyrosine kinases (Table 4). Data are expressed as a ratio of the given kinase IC_{50} to the KDR kinase IC_{50}. In general, the compound showed low levels of selectivity versus the closely related Fit and Flk families of kinases, but more moderate levels of selectivity versus other tyrosine kinases.

In summary, we have developed a new class of KDR kinase inhibitors based on the 2-[(pyrimidin-4-yl)-amine}-1,3-thiazole-5-carbonitrile template. Compounds within this series are potent in enzyme and cell-based assessments of KDR kinase inhibitory activity. Modulating the basicity of the side chain in the 4-position of the pyrimidine ring provided compounds of varying affinities for the hERG channel. Representative compounds from this series possess good pharmacokinetic profiles after iv and po dosing. Compound **3m** possessed the best overall profile of KDR inhibitory activity, reduced hERG affinity, and iv/po pharmacokinetics, and became a second-generation lead structure for further work in this series. These compounds represent a novel class of KDR kinase inhibitors and are the target of further efforts in our laboratories to obtain potent inhibitors that have good pharmacokinetic profiles and greatly reduced affinity for the hERG channel. The results of these efforts will be reported in due course.

Table 2. Dog iv PK data for selected compounds

Compound	AUC (μM h)	V_d (L/kg)	Cl (mL/min/kg)	$T_{1/2}$ (h)
3h	1.91	3.65	30.20	1.34
3l	4.81	1.31	10.50	2.00
3m	10.20	2.40	4.11	10.50
3n	3.08	14.80	13.10	7.97
3o	6.07	14.05	7.26	23.00

(Beagles, 0.25 mg/kg iv, DMSO) Average of two dogs, individual determinations are within ±25% of the mean unless noted otherwise.

Table 3. Pharmacokinetic data for **3m**[a] in three species

	Dog[b]	Rat[c]	Rhesus[d]
$T_{1/2}$	11.26	1.58	3.23
Cl (mL/min/kg)	4.11	38.67	22.70
V_{dss} (L/kg)	2.72	3.32	10.12
F (%)	51	>99	54

[a] Dosed iv as a DMSO solution and po as a solution in 0.05 M citric acid.
[b] Average of two dogs dosed at 1 mg/kg iv and 1 mg/kg po. The individual determinations are within ±25% of the mean.
[c] Average of three rats dosed at 2 mg/kg iv and 10 mg/kg po. Individual determinations are within ±25% of the mean.
[d] Average of two rhesus dosed at 1 mg/kg iv and 1 mg/kg po. Individual determinations are within ±25% of the mean.

Table 4. Kinase selectivity of compound **3m**

Kinase	**3m**
Flk-1	5
Flt-1	6.5
Flt-3	4
Flt-4	2
PDGFRβ	15
c-kit	35
c-fms	13
FGFR1	52
FGFR2	14
Src	26

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