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The discovery of *N*-(1,3-thiazol-2-yl)pyridin-2-amines as potent inhibitors of KDR kinase

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Abstract—An azo-dye lead was modified to a novel *N*-(1,3-thiazol-2-yl)pyridin-2-amine series of KDR kinase inhibitors through the use of rapid analog libraries. This new class has been found to be potent, selective, and of low molecular weight. Molecular modeling has postulated an interesting conformational preference and binding mode for these compounds in the active site of the enzyme.

Abnormal angiogenesis is operative in the progression of diseases including diabetic retinopathy, rheumatoid arthritis, psoriasis, and cancer.[1] The growth of solid tumors in particular is dependent on the ingrowth of new blood vessels.[2] Vascular endothelial growth factor (VEGF) expression has been shown to be up-regulated in a variety of tumors and has been reported to be a prognostic indicator of tumor progression and/or decreased survival in a variety of tumors. KDR, a transmembrane tyrosine kinase-linked receptor for VEGF, mediates a mitogenic response, and is expressed by endothelial cells. Inhibition of VEGF activity or KDR kinase has been shown to inhibit tumor angiogenesis and the growth of tumors in animal models. Bevacizumab, a VEGF antibody was reported to be efficacious in Phase III clinical trials.[3] Clinical evaluations have also been undertaken with an antiKDR antibody,[4] a soluble VEGF decoy-receptor,[5] as well as small molecule inhibitors of KDR kinase activity.[6] The importance of KDR in the signaling pathway of VEGF makes this enzyme a particularly attractive target against tumor angiogenesis.

In searching for lead compounds that showed a high degree of selectivity for KDR inhibition over related kinases, we were intrigued by the potent and selective screening hit **1** (Table 1).[7] However the molecule possessed problematic functionality from a toxicology perspective, containing both an azo function and a nitroaromatic moiety. We therefore sought to explore changes in this series in a rapid analog format. In our initial effort, we explored amide libraries based on **1** where the planar amide function was utilized to replace the azo moiety. These efforts resulted in the discovery of the benzamide **2**, demonstrating that both of the problematic functionalities in **1** could be replaced, in this case with amide and phenyl moieties.[8] Although good potency was maintained with these changes, kinase selectivity was significantly decreased.

We continued to seek alternative templates eliminating the amide functionality by creating a library of aminothiazoles (Fig. 1). We employed a Hantzsch thiazole synthesis, reacting 33 thioureas consisting of alkyl, aromatic, and heteroaromatic examples with (1-bromo-2,2-dimethoxyethyl)benzene[9] to produce 5-phenyl substituted aminothiazoles. One compound stood out upon testing, namely the *N*-(1,3-thiazol-2-yl)pyridin-2-amine **3** ($IC_{50} = 7$ nM). This compound had significantly greater activity than any other members of the library. For the subsequent compounds discussed the

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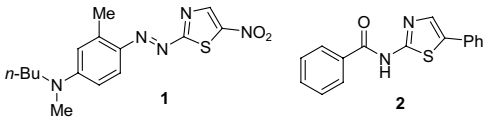
Table 1. Potency and selectivity profile of lead compounds



Compd	KDR IC_{50} (nM)[a]	Fold-selectivity versus KDR[b]				
		Flt-1	Flt-4	FGFR-1	FGFR-2	Src
1	151 ± 30	>130	1	>130	>130	>130
2	81 ± 23	4	1	72	14	>240

[a] For determinations where $n \geqslant 2$, standard deviation is given.
[b] Ratio of given enzyme IC_{50} divided by KDR IC_{50}.

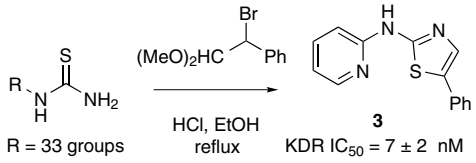


Figure 1. 2-Aminothiazole library.

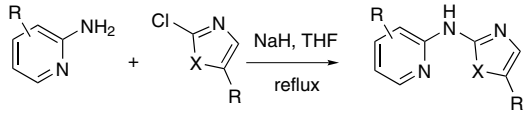


Figure 2. Alternate synthesis of aminothiazoles.

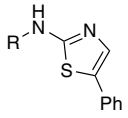
products were made according to the chemistry in Figure 1 or, alternately, according to Figure 2. In this latter sequence, aminothiazoles were made by nucleophilic substitution of aminoheterocycle and 2-chlorothiazoles.

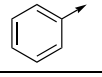
The SAR of this new lead class was first explored with aromatic substitution for the pyridine (Table 2). Heteroaromatics that place a basic nitrogen *ortho*- to the amino group tend to be required for a high level of potency. Hence the 2-pyrazine **4** and the 4-pyrimidine **5** had potency moderately lower than **3**. An exception was the 2-pyrimidine (**6**), which showed significantly less activity. The 3-pyridyl (**7**), 4-pyridyl (**8**), and phenyl analogs (**9**) showed similarly diminished levels of activity.

The SAR of the pyridine ring was generally tolerant of a wide variation of substitution (Table 3). Methyl group substitution was acceptable at every position (compounds **10**–**13**). In addition, electron withdrawing functionality was also well tolerated based on compounds **14** and **15**. An electron donating methoxy substituent at the 3-position (**16**) resulted in a drop in potency. Methoxysubstitution at the 4- or 6-position (**17** and **18**) was better tolerated.

We have explored substitution of the thiazoyl-5-phenyl moiety and found that in all cases substitution leads to lower potency (data not shown). Even relatively conservative changes, such as fluorine substitution are

Table 2. Heterocycle substitution of lead



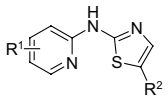
Compd	R	KDR IC_{50} (nM)[a]
4		23 ± 10
5		36 ± 8
6		137
7		240
8		259
9		124

[a] For determinations where $n \geqslant 2$, standard deviation is given.

poorly tolerated. Alternatively, we have sought to replace the phenyl moiety with smaller substituents. Halides and simple alkyl groups (see **19**, **20**, and **21**) provided analogs with significantly lower potency than the corresponding phenyl thiazoles. Remarkably, we found that replacement with cyano afforded analog **22** with only a moderate loss in potency. We had not anticipated that cyano would be a good substitution for a large lipophilic phenyl group but this modification provided a remarkably low molecular weight ($MW = 202$) and potent ($IC_{50} = 36\,nM$) inhibitor of KDR kinase.

Several changes to the core were made that are important to understanding the SAR of the series. Methylation of the central NH destroys potency. Replacement of the NH with an ether linkage also abolishes activity. The most remarkable observation has been that the

Table 3. Pyridine substituent SAR



Compd	R^1	R^2	IC_{50} (nM)[a]
3	H	Ph	7 ± 2
10	3-Me	Ph	13 ± 5
11	4-Me	Ph	6 ± 2
12	5-Me	Ph	19 ± 12
13	6-Me	Ph	10 ± 3
14	5-Cl	Ph	35 ± 25
15	6-Br	Ph	14 ± 5
16	3-OMe	Ph	46
17	4-OMe	Ph	11 ± 0
18	6-OMe	Ph	3 ± 2
19	H	Br	71 ± 26
20	H	Cl	194
21	H	Me	1274
22	H	CN	36 ± 8

[a] For determinations where $n \geqslant 2$, standard deviation is given.

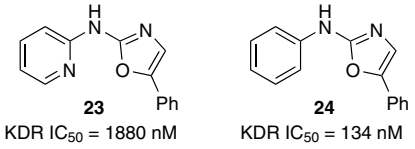


Figure 3. Oxazoles.

oxazole analog **23** is >250-fold less active than the thiazole **3** (Fig. 3). However, it is interesting to note that the phenyl oxazole **24** is equipotent with the phenyl thiazole **9**.

We have profiled these inhibitors for selectivity versus a series of closely related kinases (see Table 4). Compound **3** has no selectivity versus Flt-1, Flt-4, or PDGFRβ and displays respectable levels of selectivity versus FGFR1, FGFR2, and Src. Certain pyridyl analogs have shown enhanced selectivity, including the 3-methyl substituted compound **10**, which shows enhanced levels of selectivity versus the FGFRs and Src. The 6-Me compound **13** has a similar profile, while the 4- and 5-Me substituents (**11** and **12**) show levels of selectivity similar to the parent **3**. Electron withdrawing groups such as 5-Cl (**14**) and 6-Br (**15**) tend to show even more dramatic improvements in the levels of selectivity versus the FGFRs and Src. Interestingly, the 5-cyano thiazole **22** provided a very different profile, which is more analogous to the nitrothiazole lead **1**. It has significantly enhanced levels of selectivity versus two of the most closely related kinases, Flt-1, and PDGFRβ, while still providing little selectivity versus Flt-4 and maintaining good selectivity versus the FGFRs and Src.

Although the lead compound **3** has excellent activity in the enzyme assay, poor activity in a cell-based assay of KDR inhibition was observed (Fig. 4).[10] However we have found that the pyridine moiety can be substituted with a basic amine, which serves to improve the physical properties and cell potency of the resulting analogs. The pyrrolidine containing molecule **25**, had excellent cell potency (cell $IC_{50} = 28$ nM), while showing no loss of enzyme activity relative to **3**.

We initiated modeling studies to understand the conformational preferences of these molecules and develop a theory of their binding modes.[11] Computational analysis resulted in four relative energy minima that are all close to planar in conformation (see dihedral angles). The lowest energy conformations for the thiazole series, regardless of substitution pattern, are conformers A and B (Table 5). Conformers C and D were found to be significantly higher in energy. Conformer A is likely to be the active conformer since 3-pyridine substitution (compound **10**) does not affect potency, while it would be expected to disfavor conformer B but not conformer A. This argument led us to a bias for A as the active conformation. A notable validation of this view is found

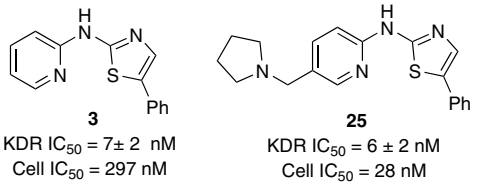
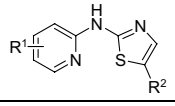


Figure 4. Activity of thiazoles in a cell-based assay.

Table 4. Kinase fold selectivity versus KDR[a]



Compd	R^1	R^2	Flt-1	Flt-4	PDGFRβ	FGFR1	FGFR2	Src
3	H	Ph	3	2	0.3	164	51	280
10	3-Me	Ph	16	3	1	820	200	1200
11	4-Me	Ph	7	2	0.5	220	50	190
12	5-Me	Ph	5	1	0.3	220	70	570
13	6-Me	Ph	8	3	0.3	600	210	760
14	5-Cl	Ph	6	1	0.1	>580	>580	>580
15	6-Br	Ph	12	3	0.3	1620	400	840
22	H	CN	30	4	68	214	107	678

[a] Ratio of the given enzyme IC_{50} divided by the KDR IC_{50}.

Table 5. Relative conformational energies (RHF/6-31G**) and dihedral angles

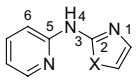
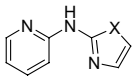
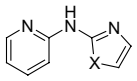
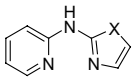
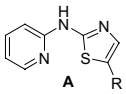
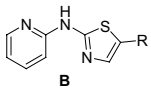
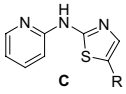
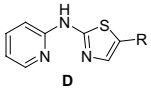
	 A	 B	 C	 D
Thiazole (X = S)	0.00 kcal/mol	0.03 kcal/mol	3.15 kcal/mol	8.00 kcal/mol
$\tau 1$ (N_1–C_2–N_3–H_4)	0.0°	−179.9°	11.7°	−148.6°
$\tau 2$ (H_4–N_3–C_5–C_6)	0.0°	180.0°	−168.8°	16.4°
Oxazole (X = O)	5.78 kcal/mol	0.00 kcal/mol	2.23 kcal/mol	7.72 kcal/mol
$\tau 1$ (N_1–C_2–N_3–H_4)	8.3°	179.9°	0.0°	179.9
$\tau 2$ (H_4–N_3–C_5–C_6)	21.6°	180.0°	180.0°	0.0°

Table 6. Relative conformational energies (RHF/6-31G**) for substituted thiazoles

	 A	 B	 C	 D
R = Ph	0.00 kcal/mol	0.065 kcal/mol	3.16 kcal/mol	8.06 kcal/mol
R = CN	0.00 kcal/mol	1.63 kcal/mol	4.30 kcal/mol	9.05 kcal/mol

in the analysis of the oxazole (Table 5). The oxazole series conformer A is remarkably higher in energy than the lowest energy conformer B (by 5.78 kcal/mol). This conformational argument accounts for the dramatically lower potency of the oxazole **23**. The equal activity of the phenyl amino thiazole **9** and the phenylaminooxazole **24** is further evidence that the lack of activity in **23** is due to a conformational bias away from the active conformer rather than an inherent low enzyme affinity for the oxazole moiety.

In examining the effect of the 5-thiazole substitution on these conclusions, the phenyl moiety creates only slight changes to the relative energies (Tables 5 and 6). However, the nitrile substituent creates a remarkable bias toward the single conformer A. The energetic cost to achieve conformation B in the nitrile case is 1.63 kcal/mol (Table 6). This may in part account for the surprising level in potency for the nitrile **23**, as it has greater bias towards the postulated active conformer.

These considerations lead us to postulate the following enzyme binding mode (Fig. 5). Based on the SAR of the series and the fact that the inhibitors are ATP-competitive (data not shown), we have postulated that the aminothiazole engages in hydrogen bonding with the Cys919 in the enzyme active site, with the 5-phenyl moiety occupying the H1 hydrophobic pocket.[12] Only conformers A and C allow the thiazole nitrogen to hydrogen bond with the backbone amide proton of Cys919 and the amine proton to hydrogen bond to the backbone carbonyl oxygen of Cys919. But conformer C is significantly higher in energy than A (by 3.15 kcal/mol). This model also accounts for the loss of potency upon substitution of the thiazole 5-phenyl as well as for the ability to substitute the pyridine ring at the 4-, 5-, and 6-positions without diminishing activity as these positions point towards solvent.

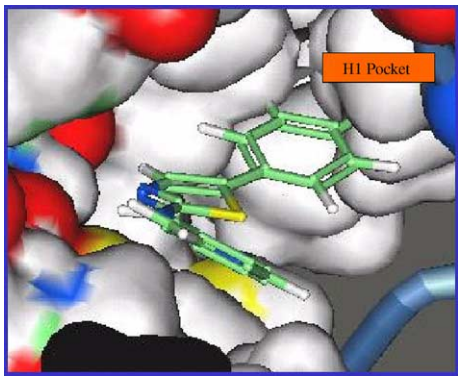


Figure 5. Proposed binding mode of **3** in the KDR kinase active site.

From a structurally undesirable lead we have developed very potent, small molecule inhibitors of KDR kinase. These compounds exhibit promising levels of selectivity versus closely related kinases. We have found a very low molecular weight molecule that surprisingly replaces a nitrile function for a phenyl moiety. This compound has been shown to be a potent and selective inhibitor. From molecular modeling, a consistent picture has emerged that indicates an interesting conformational preference of these molecules and a preferred binding mode in the enzyme active site. This model also helps account for SAR observed in the series. Future communications will discuss the optimization of this novel lead series.

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