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Bioorganic & Medicinal Chemistry Letters 13 (2003) 1673–1677

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

2,4-Disubstituted Pyrimidines: A Novel Class of KDR Kinase Inhibitors

Peter J. Manley,* Adrienne E. Balitza, Mark T. Bilodeau, Kathleen E. Coll, George D. Hartman, Rosemary C. McFall, Keith W. Rickert, Leonard D. Rodman and Kenneth A. Thomas

Departments of Medicinal Chemistry and Cancer Research, Merck Research Laboratories, West Point, PA 19486, USA

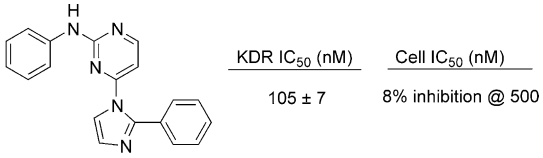
Received 7 January 2003; revised 10 March 2003; accepted 11 March 2003

Abstract—2,4-Disubstituted pyrimidines were synthesized as a novel class of KDR kinase inhibitors. Evaluation of the SAR of the screening lead compound **1** (KDR $IC_{50}=105$ nM, Cell $IC_{50}=8\%$ inhibition at 500 nM) led to the potent 3,5-dimethylaniline derivative **2d** (KDR $IC_{50}=6$ nM, cell $IC_{50}=19$ nM).

Neovascularization plays an important role in the pathology of several diseases including diabetic retinopathy,[1] rheumatoid arthritis,[2] psoriasis,[3] and cancer.[4] The process, know as angiogenesis, is controlled by a number of endogenous mitogenic proteins including vascular endothelial growth factor (VEGF). Up-regulation of VEGF and its subsequent signaling through the receptor tyrosine kinase KDR (VEGFR-2) is an important requirement for tumor growth and proliferation.[5,6] Interruption of this signaling cascade with anti-VEGF[7] and anti-KDR[8] antibodies as well as small molecule inhibitors of the KDR kinase domain[9] has resulted in the inhibition of angiogenesis as observed in tumor xenograft models. The interest in using small molecules as anti-angiogenic agents in human cancers has increased in recent years. Clinical trials have been initiated for a number of KDR kinase inhibitors from different structural classes, including indolin-2-ones, phthalazines, and quinazolines.[10]

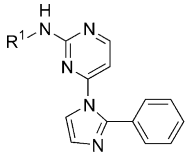
In our efforts to discover novel small molecule inhibitors of the KDR kinase domain,[11] a screening of the corporate sample collection resulted in the lead compound **1** (Fig. 1). While **1** exhibited moderate intrinsic potency (KDR $IC_{50}=105$ nM)[12] it lacked any significant cellular activity (cell $IC_{50}=8\%$ inhibition at 500 nM).[13]

Initially, we sought to investigate the SAR of the aniline portion of compound **1** with a focus on increasing intrinsic potency (Fig. 2). Table 1 describes the initial SAR trends of **2a–l**. In general, *ortho*- or *para*-substitution of the aniline ring of compound **1** decreased intrinsic activity regardless of the nature of the substituent. *meta*-Substitution, on the other hand, enhanced intrinsic potency relative to compound **1**. Symmetrical di-substitution with a slightly electron donating group (**2d**) or electron-rich (**2h**) substituents resulted in a further enhancement of intrinsic activity



1

Figure 1.



2a-l, **3a-d**, **4a-f**

Figure 2.

*Corresponding author. Tel.: +1-215-652-9466; fax: +1-215-652-7310; e-mail: peter_manley@merck.com

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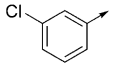
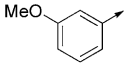
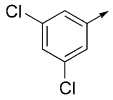
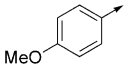
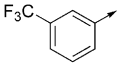
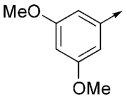
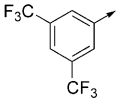
doi:10.1016/S0960-894X(03)00244-0

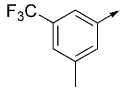
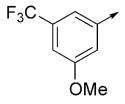
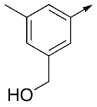
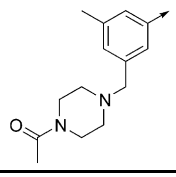
Table 1. KDR kinase activity of compounds **2**[12]

Compd	R^1	KDR IC_{50} (nM)	Compd	R^1	KDR IC_{50} (nM)	Compd	R^1	KDR IC_{50} (nM)
2a		3080	**2e**		289	**2i**		25±18
2b		18±3	**2f**		20±9	**2j**		26±10
2c		279	**2g**		392	**2k**		10±1
2d		6±1	**2h**		5±1	**2l**		77±17

relative to their mono-substituted analogue (**2b** and **2f**, respectively). However, symmetrical di-substitution with electron-withdrawing substituents (**2j** and **2l**) had little effect on intrinsic potency relative to their mono-substituted analogues (**2i** and **2k**).

Table 2 describes the SAR of unsymmetrically di-substituted aniline derivatives of **1**. The combination of a slightly electron donating substituent with an electron-withdrawing one, as in **3a**, had no effect on intrinsic potency relative to the corresponding symmetrically substituted analogue **2d**. However, a substantial enhancement was observed when compared to the symmetrically substituted analogue **2l**. Combination of an electron-rich substituent with an electron-withdrawing one, as in **3b**, astonishingly resulted in a severe loss of intrinsic potency. In an effort to increase the overall polarity of the series, the addition of a hydroxyl group to one of the methyl substituents of **2d** resulted in **3c**, which suffered little loss in potency. Conversion of the hydroxymethyl to an aminomethyl, as in **3d**, resulted in a moderate loss of potency.

Table 2. KDR kinase activity of compounds **3**[12]

Compd	R^1	KDR IC_{50} (nM)
3a		5±3
3b		19,000
3c		9±1
3d		32±5

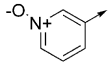
In a further effort to increase the overall polarity of the series, heterocyclic amines were used in place of aniline derivatives. Of those examined, all but the pyridine analogues (Table 3) resulted in a dramatic loss of intrinsic activity. Compound **4a**, as well as all the other heterocycles examined that contained a heteroatom adjacent to the pyrimidine core, had intrinsic potencies greater than 1 μM (data not shown). Potency enhancement of the most active compound in this series (**4c**) was achieved by the sequential addition of methyl groups, as in **4e** and **4f**, to mimic **2b** and **2d**; however, none of these analogues was as potent as the lead **1**.

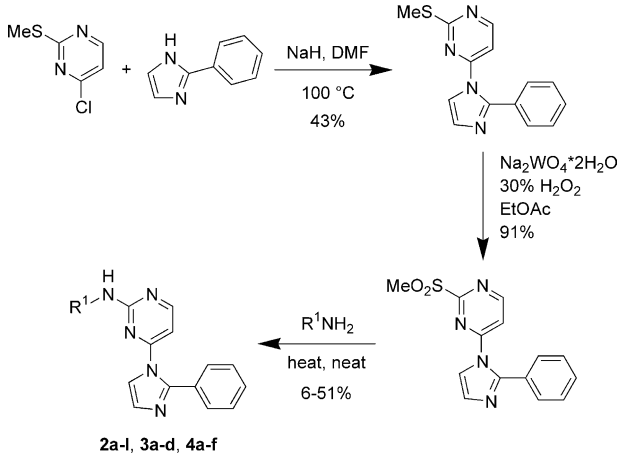
The synthesis of **2a–l**, **3a–d**, and **4a–f** is found in Scheme 1. Nucleophilic aromatic substitution of commercially available 4-chloro-2-methylthiopyrimidine with the sodium salt of 2-phenylimidazole in DMF at 100 °C gave 2-(methylthio)-4-(2-phenyl-1*H*-imidazol-1-yl)pyrimidine. Oxidation of the thioether with sodium tungstate dihydrate and 30% H_2O_2 in EtOAc gave 2-(methylsulfonyl)-4-(2-phenyl-1*H*-imidazol-1-yl)pyrimidine. Nucleophilic aromatic substitution of the methyl sulfone with the various aniline derivatives gave **2a–l**, **3a–d**, and **4a–f**.

Next, we focused our attention on the synthesis and SAR of the 2-position of the imidazole ring in **2d**. Nucleophilic substitution of the thiomethyl group in 2-(methylthio)pyrimidin-4(3*H*)-one with 3,5-dimethylaniline in diglyme at 170 °C gave 2-[(3,5-dimethylphenyl)amino]pyrimidin-4(3*H*)-one. Conversion of the pyrimidinone functionality to 4-chloro-*N*-(3,5-dimethylphenyl)pyrimidin-2-amine was accomplished in refluxing $POCl_3$. Nucleophilic aromatic substitution of the chloride with imidazole derivatives and Cs_2CO_3 in DMA at 140 °C gave compounds **5a–j** (Scheme 2).

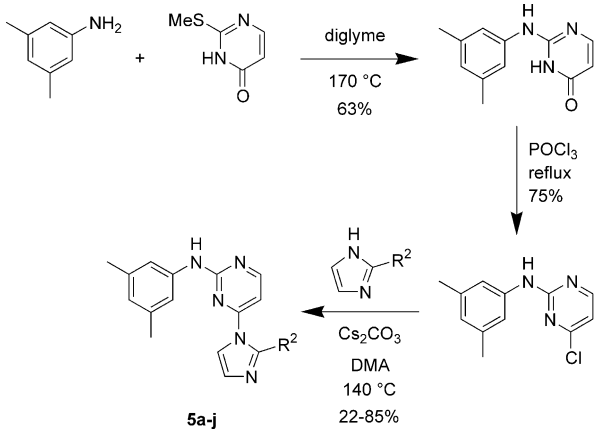
Table 4 describes the SAR of these compounds. While all suffered a loss in intrinsic potency relative to **2d**, compounds **5d**, **5e**, **5g**, and **5j** showed comparable

Table 3. KDR kinase activity of compounds **4**[12]

Compd	R^1	KDR IC_{50} (nM)
4a		6830
4b		340
4c		273
4d		3570
4e		276.5
4f		135

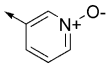


Scheme 1. Synthesis of compounds **2a–l**, **3a–d**, and **4a–f**.[14]



Scheme 2. Synthesis of compounds **5a–j**.

Table 4. KDR kinase activity of compounds **5**[12]

Compd	R^2	KDR IC_{50} (nM)
5a		1610
5b		2390
5c		1410
5d		149
5e		45±7
5f		1870
5g	–Cl	60
5h	–Me	765
5i	$-NH_2$	4220
5j	–CN	232

intrinsic activity to the lead **1**. These data suggest that the unsubstituted phenyl ring of **2d** might fit tightly in the H-1 hydrophobic pocket of the ATP active site of the KDR kinase domain[15] since even the relatively small electronic perturbation found in compound **5e** resulted in nearly a 10-fold loss in potency. Conversely, the addition of functionality onto one of the methyl groups of the aniline ring in **2d**, as in **3c** and **3d**, had little to no effect on intrinsic potency. This suggests that the aniline portion of **2d** is exposed to solvent and is consistent with our binding hypothesis.

Other modifications of **2d** included movement of the aniline portion of the molecule from the 2-position to the 6-position of the pyrimidine core (**6**), deletion of *N*-3 in the pyrimidine ring (**7**), and the addition of a methyl group in the 4-position of the imidazole ring (**8**, Fig. 3). In each case a drastic loss in intrinsic potency was observed relative to **2d** (KDR IC_{50} = 1808, 892, and 450, respectively).

The cellular activity of a number of potent compounds can be found in Table 5. As with intrinsic activity, the addition of *meta*-functionality on the aniline ring of **1** increased potency. The addition of polar functionality gave mixed results. Alcohol **3c** maintained its cellular potency while the amine **3d** suffered a 20-fold loss in potency relative to **2d**. The latter is contrary to reports in other series from our laboratories[11] where the addition of a basic amine had little effect on intrinsic potency but did have a beneficial effect on cellular potency.

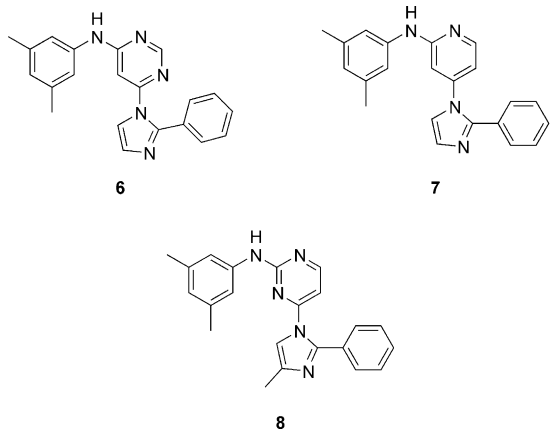


Figure 3.[16,17]

Table 5. Comparison on intrinsic and cellular potency of compounds **1**, **2b**, **2d**, **2f**, **2h**, **2k**, **3a**, **3c**, and **3d**[12,13]

Compd	KDR IC_{50} (nM)	Cell IC_{50} (nM)
1	105±7	8% inhibition @ 500
2b	18±3	151
2d	6±1	19
2f	20±9	88
2h	5±1	22
2k	10±1	303
3a	5±3	26
3c	9±1	23
3d	32±5	396

Table 6. Selectivity profile of compounds **1**, **2b**, and **2d**

Compd	PDGFRβ	FLT-1	FLT-4	FGFR-1	FGFR-2	SRC
1	nd	13	5	295	nd	11
2b	22	103	13	1023	686	11
2d	6	30	5	606	261	10

nd, not determined.

The selectivity profile of **1**, **2b**, and **2d** is found in Table 6. The data, expressed as a ratio of IC_{50} to KDR kinase, suggests modest selectivity versus the highly homologous kinases PDGFRβ, Flt-1, Flt-4, and SRC kinases and high selectivity versus FGFR-1 and FGFR-2. These data are representative for the entire series.

In this series, the optimized compound **2d** exhibits excellent intrinsic activity and cell potency with modest kinase selectivity. **2d** has the following physical and pharmacokinetic properties: a logP of 3.74[18] with a high clearance (Cl = 30.10±1.27 mL/min/kg), volume of distribution (Vdss = 1.11±0.01 L/kg), and short half-life ($t_{1/2}$ = 0.71±0.01 h) in dogs.

In conclusion we have investigated the SAR for KDR inhibition of lead compound **1**. *meta*-Substitution of the aniline portion of compound **1** led to potency enhancements both with the enzyme and in cells.

Acknowledgements

We thank Dr. Art Coddington, Dr. Chuck Ross, and Dr. Harri Ramjit for mass spectral analyses, and Matt Zrada for logP determinations. We also thank Dr. Cory Theberge and Irma Escudero for their synthesis of intermediates.

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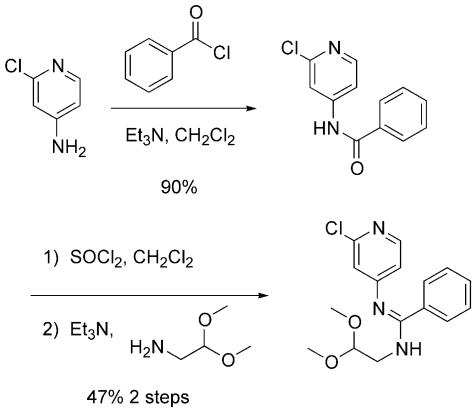
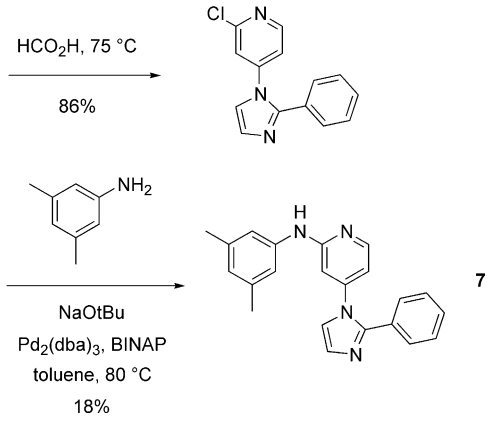
12. The KDR IC_{50} value represents biochemical inhibition of phosphorylation of a poly-Glu/Tyr (4:1) peptide substrate by isolated KDR kinase (cloned and expressed as a GST-fusion protein); see: Kendall, R. L.; Rutledge, R. Z.; Mao, X.; Tebben, A. L.; Hungate, R. W.; Thomas, K. A. *J. Biol. Chem.* **1999**, *274*, 6453. Values are reported as single determinations or as the average of at least two determinations±standard deviation.
13. The Cell IC_{50} value represents the inhibition of VEGF-stimulated mitogenesis as determined in human umbilical vein endothelial cells.
14. All target compounds were fully characterized by ^{1}H NMR and mass spectroscopy.
15. For a description of the ATP binding region of protein tyrosine kinases, see: Traxler, P.; Furet, P. *Pharmacol. Ther.* **1999**, *82*, 195.
16. **6** was synthesized according to Scheme 1 starting with commercially available 4,6-dichloropyrimidine. **8** was synthesized according to Scheme 2 using 4(5)-methyl-2-phenylimidazole as the imidazole in the last step.

17.





18. Partition coefficients were determined by HPLC analysis in octanol/phosphate buffer (pH 7.4).