Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2767–2770

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Initial SAR Studies of 3,6-Disubstituted Pyrazolo[1,5-*a*]pyrimidines: A New Class of KDR Kinase Inhibitors

Mark E. Fraley,* William F. Hoffman, Robert S. Rubino, Randall W. Hungate, Andrew J. Tebben, Ruth Z. Rutledge, Rosemary C. McFall, William R. Huckle, Richard L. Kendall, Kathleen E. Coll and Kenneth A. Thomas

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

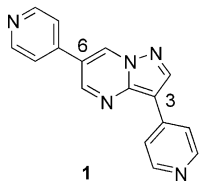
Received 27 March 2002; accepted 10 June 2002

Abstract—We have synthesized and evaluated the activity of 3,6-disubstituted pyrazolo[1,5-*a*]pyrimidines as a new class of KDR kinase inhibitors. Starting with screening lead **1**, potency against isolated KDR was fully optimized with 3-thienyl and 4-methoxyphenyl substituents at the 6- and 3-positions (**3g**, KDR $IC_{50} = 19$ nM), respectively. The synthesis and SAR of these compounds are described.

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 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 our efforts to identify small molecule inhibitors of KDR kinase, we discovered a new class whose central core is comprised of a pyrazolo[1,5-*a*]pyrimidine. Herein we describe the synthesis and initial structure–activity relationship (SAR) studies of this novel series.

Our chemistry efforts began with the goal of improving the activity of screening lead **1** (Fig. 1, KDR $IC_{50} = 224$ nM). Toward optimization of the substituents at the 3- and 6-positions of **1**, an initial set of 3,6-diarylpyrazolo[1,5-*a*] pyrimidines was prepared in combinatorial fashion

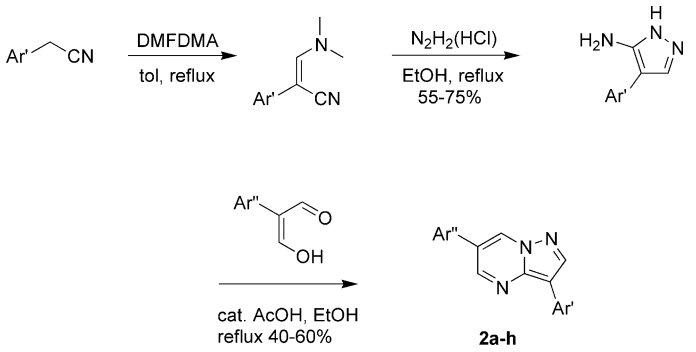


1

Figure 1.

*Corresponding author. Fax: +1-215-652-6345; e-mail: mark_fraley@merck.com

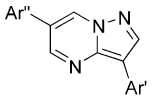
0960-894X/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00525-5

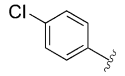
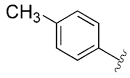
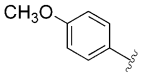
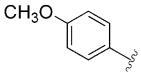
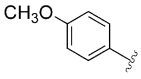
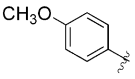


Scheme 1. Synthesis of compounds **2a**–**h**.[12]

through condensation reactions of a 3-amino-4-arylpyrazoles and commercially available 2-arylmalondialdehydes (Scheme 1). The starting 3-amino-4-arylpyrazoles were prepared in two steps from the corresponding acetonitriles in 55–75% yield. Heating solutions of the 3-amino-4-arylpyrazoles and 2-arylmalondialdehydes in the presence of acetic acid in ethanol followed by cooling furnished the 3,6-diarylpyrazolo[1,5-*a*]pyrimidines as yellow crystalline solids (40–60% yield).[11] KDR kinase inhibitory activity for a select set of compounds is presented in Table 1.

Table 1. KDR kinase activity of compounds **2a**–**h**[13]

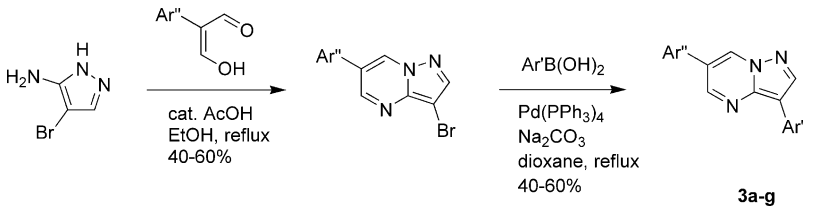


Compd	Ar″	Ar′	KDR IC_{50} (nM)
1			224
2a			113
2b			62
2c			37
2d			41
2e			1000
2f			71
2g			71
2h			88

The data in Table 1 indicate that substitution at the *para* position of the 6-aryl ring enhances inhibitory activity in the 3-(4-pyridyl) series. In the best case, potency was optimized 6-fold over lead compound **1** by the replacement of the 6-pyridyl substituent with a 4-methoxyphenyl group (**2c**). A number of more subtle findings are worthy of note. In the 6-methoxyphenyl series, the 3-(3-pyridyl) moiety provides nearly the same potency as the 3-(4-pyridyl) group (**2d** vs **2c**), while the 3-(2-pyridyl) arrangement (**2e**) is detrimental to activity. In the 3-phenyl series, very little difference in activity was observed between the 6-(4-methoxyphenyl) and 6-(4-pyridyl) ligands (**2f** vs **2g**), which is in marked contrast to the 3-(4-pyridyl series) as indicated. In addition, a 2-pyridyl ring at the 6-position of the ring system does not have the deleterious effect on activity as it did when placed in the 3-position (compare **2h** to **2e**).

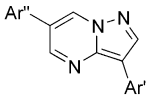
With the discovery of the 4-methoxy phenyl group as an advance in the optimization of the 6-position of the pyrazolo[1,5-*a*]pyrimidine nucleus, we next examined the SAR of the 3-position more closely. A convenient method for varying the substituent at the 3-position, which avoids repetitious 3-aminopyrazole synthesis, was employed in the assembly of a second set of analogues (Scheme 2). 3-Amino-4-bromopyrazole was utilized in the condensation reaction to provide 3-bromo pyrazolo[1,5-*a*]pyrimidines, which smoothly cross-coupled with aryl boronic acids under Suzuki conditions to afford 3,6-diarylpyrazolo[1,5-*a*]pyrimidines in 40–60% yield. Biochemical inhibition of KDR kinase activity is reported in Table 2 for a set of analogues wherein the 3-substituent was varied by this synthetic method.

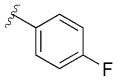
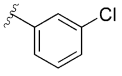
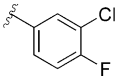
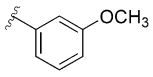
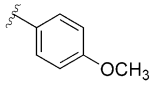
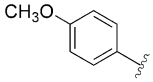
The data in Table 2 indicate that halogen and methoxyl substitution of the 3-aryl ring are detrimental to activity with the exception of the incorporation of a 4-fluorine atom (**3a** and **3c**). However, replacement of the 3-phenyl ring with a 3-thienyl group improves activity 3-fold in both the 6-(4-pyridyl) and 6-(4-methoxyphenyl) series (**3f** and **3g**, respectively). The data in Table 2 are consistent with docking studies using a KDR homology model that depicts the 3-substituent occupying the small and narrow hydrophobic region I of the ATP active site.[14] In addition, modeling and supporting SAR (not shown) suggest the nitrogen at the 1–position of the pyrazolo[1,5-*a*]pyrimidine makes in a key hydrogen bond with Cys 919 of the hinge region.



Scheme 2. Synthesis of compounds **3a**–**g**.

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

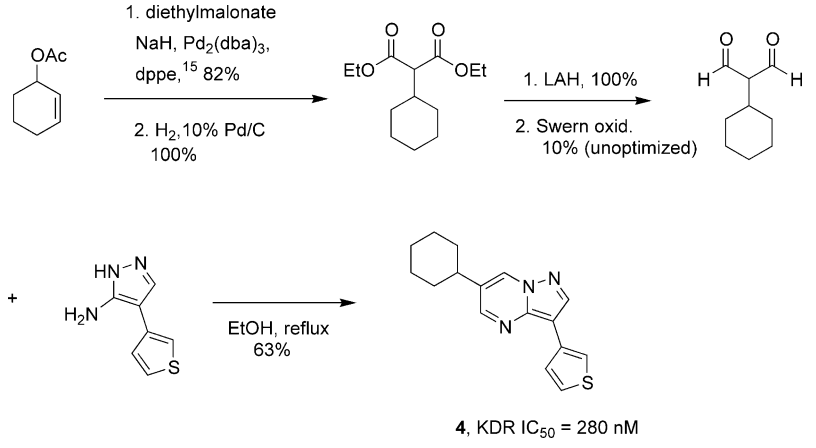


Compd	Ar″	Ar′	KDR IC_{50} (nM)
2g			71
3a			63
3b			350
3c			265
3d			510
3e			71% inh. @ 2500 nM
3f			24
3g			19

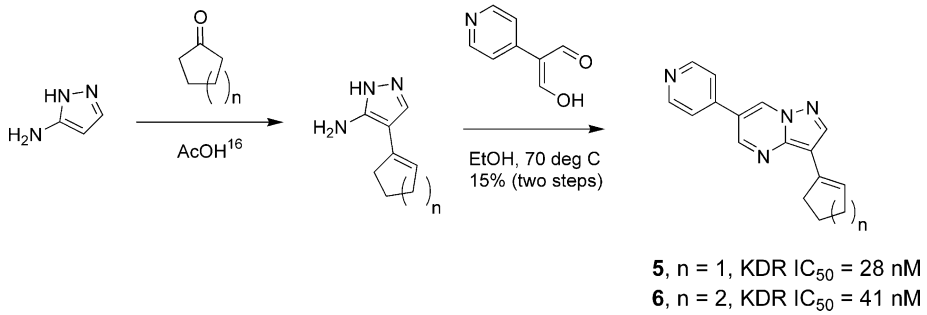
In subsequent studies, we have found that a number of modifications to the substitution pattern about the pyrazolo[1,5-*a*]pyrimidine nucleus have deleterious effects on kinase inhibitory activity. For example, deletion of the 3- or 6-aryl group from the core leads to complete loss of activity, as does independently shifting the 6-aryl group to the 7- or 5-position, or moving the 3-aryl group to the 2-position. The addition of a methyl group at the 7- or 2-position of the pyrazolo[1,5-*a*]pyrimidine also produces inactive compounds. In addition, substitution of the 3- or 6-aryl ring at a position *ortho* to the carbon appended to the bicyclic core is detrimental to activity. These latter results suggest a nearly planar ring system for optimized binding and are consistent with kinetics studies which indicate that these compounds bind in the narrow ATP binding cleft of the kinase domain.

We next examined the SAR of fully and partially saturated ring systems at the 6- and 3-positions of the central nucleus. Incorporation of a cyclohexyl ring at the 6-position in compound **4** led to a 15-fold loss in activity (Scheme 3). Activity was mostly maintained, however, with cyclopentenyl (**5**) and cyclohexenyl (**6**) substitution at the 3-position (Scheme 4). A fully saturated cyclohexyl ring or small alkyl substituents at the 3-position, such as a 3-ethyl group, were not well-tolerated (KDR IC_{50}s > 5 μM).

The selectivity for KDR kinase inhibition was determined for **1**, **2c**, **3f**, and **3g** via screening against a small panel of highly homologous receptor tyrosine kinases



Scheme 3. Synthesis and KDR kinase activity of compound **4**.



Scheme 4. Synthesis and KDR kinase activity of compounds **5** and **6**.

Table 3. KDR kinase selectivity (fold) of compounds **1**, **2c**, **3f**, and **3g**

Compd	PDGFRβ	FLT-1	FLT-4	FGFR-1	SRC
1	0.4	4.6	2.7	59	20
2c	0.5	5.7	2.2	54	27
3f	11.4	7.1	9.2	104	146
3g	1.8	10.0	10.0	>100	>100

Table 4. ECMA IC_{50}s for compounds **1**, **2c**, **3f**, and **3g**

Compd	KDR IC_{50} (nm)	ECMA IC_{50} (nm)	Log P[17]
1	224	622	2.5
2c	29	150	4.0
3f	24	127	—
3g	19	387	>>4.0

and SRC kinase (Table 3). The data, expressed as the biochemical IC_{50} ratios to KDR kinase, indicate that this set of inhibitors is relatively non-selective for KDR versus PDGFRβ, FLT-1, and FLT-4 kinases, but moderately KDR selective versus FGFR-1 and SRC kinases. The data also suggest that the 3-thienyl ring at the 3-position of the pyrazolo[1,5-*a*]pyrimidine enhances KDR selectivity in comparison to the 4-pyridyl ligand.

The ability of compounds **1**, **2c**, **3f**, and **3g** to inhibit VEGF-stimulated mitogenesis was determined in human umbilical vein endothelial cells. The inhibition constants determined in the biochemical assay are compared to those measured in the endothelial cell mitogenesis assay (ECMA) in Table 4. Compounds **1**, **2c**, and **3f**, which bear a solubilizing 4-pyridyl substituent, demonstrated a lower ratio of cell to biochemical activity compared to compound **3g**, whose activity may be limited by poor cell penetration due to unfavorable physical properties.

In conclusion, we have identified a new and synthetically accessible class of potent KDR kinase inhibitors comprised of a pyrazolo[1,5-*a*]pyrimidine core. Within this series, potent kinase activity is limited to 3,6-disubstituted analogues that may adopt planar orientations.

Acknowledgements

We thank Dr. Art Coddington, Dr. Chuck Ross, and Dr. Harry Ramjit for mass spectral analyses, Matt Zrada, Ken Anderson, and Patrice Ciecko for logP determinations, and Dr. George Hartman and Elaine Walker for editing and assistance in the preparation of this manuscript.

References and Notes

1. Adamis, A. P.; Shima, D. T.; Yeo, K. T.; Yeo, T. K.; Brown, L. F.; Berse, B.; D'Amore, P. A.; Folkman, J. *Biochem. Biophys. Res. Commun.* **1993**, *193*, 631.
2. Giatromanolaki, A.; Sivridis, E.; Athanassou, N.; Zois, E.; Thorpe, P. E.; Brekken, R. A.; Gatter, K. C.; Harris, A. L.; Koukourakis, I. M.; Koukourakis, M. I. *J. Path.* **2001**, *194*, 101.
3. Detmar, M. *Dermatol. Sci.* **2000**, *24*, S78.
4. For reviews, see (a) Carmeliet, P.; Jain, R. K. *Nature* **2000**, *407*, 249. (b) Folkman, J. *Nature Med.* **1995**, *1*, 27.
5. (a) Hanahan, D.; Folkman, J. *Cell* **1996**, *86*, 353. (b) Holmgen, L.; O'Reilly, M. S.; Folkman, J. *Nature Med.* **1995**, *1*, 149.
6. (a) Veikkola, T.; Karkkainen, M.; Claesson-Welsh, L.; Alitalo, K. *Cancer Res.* **2000**, *60*, 203. (b) Thomas, K. A. *J. Biol. Chem.* **1996**, *271*, 603.
7. Kim, K. J.; Li, B.; Winer, J.; Armanini, M.; Gillett, N.; Phillips, H. S.; Ferrara, N. *Nature* **1993**, *362*, 841.
8. Witte, L.; Hicklin, D. J.; Zhu, Z.; Pytowski, B.; Kotanides, H.; Rockwell, P.; Bohlen, P. *Cancer Metastasis Rev.* **1998**, *17*, 155.
9. (a) Fong, T. A. T.; Shawyer, L. K.; Sun, L.; Tang, C.; App, H.; Powell, T. J.; Kim, Y. H.; Schreck, R.; Wang, X.; Risau, W.; Ullrich, A.; Hirth, K. P.; McMahaon, G. *Cancer Res.* **1999**, *59*, 99. (b) Drevs, J.; Hofmann, I.; Hugenschmidt, H.; Wittig, C.; Madjar, H.; Müller, M.; Wood, J.; Martiny-Baron, G.; Unger, C.; Marmé, D. *Cancer Res.* **2000**, *60*, 4819.
10. For a current review, see Sun, L.; McMahon, G. *Drug Discov. Today* **2000**, *5*, 344.
11. (a) Muhmel, G.; Hanke, R.; Breitmaier, E. *Synthesis* **1982**, *8*, 673. (b) Reichardt, C.; Ferwanah, A.-R.; Pressler, W.; Yun, K.-Y. *Liebigs Ann. Chem.* **1984**, 649.
12. All target compounds were fully characterized by ^{1}H NMR and mass spectroscopy.
13. 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. J.; Hungate, R. W.; Thomas, K. A. *J. Biol. Chem.* **1999**, *274*, 6453.
14. Traxler, P.; Furet, P. *Pharmacol. Ther.* **1999**, *82*, 195.
15. Fiaud, J.; Malleron, J. *Tetrahedron Lett.* **1980**, *21*, 4437.
16. Winters, G.; Sala, A.; DePaoli, A.; Conti, M. *Synthesis* **1984**, 1050.
17. Partition coefficients were determined in octanol/water.