2-Hydroxy-4,6-diamino-[1,3,5]triazines: A Novel Class of VEGF-R2 (KDR) Tyrosine Kinase Inhibitors

Nand Baindur, Naresh Chadha, Benjamin M. Brandt, Davoud Asgari, Raymond J. Patch, Celine Schalk-HiHi, Theodore E. Carver, Ioanna P. Petrounia, Christian A. Baumann, Heidi Ott, Carl Manthey, Barry A. Springer, and Mark R. Player*

Drug Discovery, Johnson & Johnson Pharmaceutical Research and Development, L.L.C., 8 Clarke Drive, Cranbury, New Jersey 08512

Received August 4, 2004

Abstract: 2-Hydroxy-4,6-diamino-[1,3,5]triazines are described which are a novel class of potent inhibitors of the VEGF-R2 (flk-1/KDR) tyrosine kinase. 4-(Benzothiazol-6-ylamino)-6-(benzyl-isopropyl-amino)-[1,3,5]triazin-2-ol (**14d**) exhibited low nanomolar potency in the in vitro enzyme inhibition assay (IC_{50} = 18 nM) and submicromolar inhibitory activity in a KDR-induced MAP kinase autophosphorylation assay in HUVEC cells (IC_{50} = 280 nM), and also demonstrated good in vitro selectivity against a panel of growth factor receptor tyrosine kinases. Further, **14d** showed antiangiogenic activity in an aortic ring explant assay by blocking endothelial outgrowths in rat aortas with an IC_{50} of 1 μM.

Angiogenesis is the process by which new capillaries form from the established vasculature, and it is necessary for tumors to grow beyond a certain size.[1] The growth of new vessels supplies oxygen, nutrients, and growth factors to small tumors and removes the waste products of metabolism. There is also an association between tumor vascularization and metastatic capacity of a given tumor.[2] Thus, new means to retard angiogenesis have shown promise as potential cancer therapies. The angiogenic phenotype is also featured in a variety of pathological states, including diabetic retinopathy,[3] osteoarthritis,[4] and rheumatoid arthritis.[5]

The vascular endothelial growth factor (VEGF, permeability factor) is a primary regulator of physiological new vessel formation, particularly in wound healing,[6] embryogenesis,[7] and the female reproductive system.[8] It has also been shown to play a direct role in pathological neovascularization.[9] VEGF recognition, by the high-affinity receptor tyrosine kinases VEGF-R2 (flk-1/KDR) and VEGF-R1 (flt-1), is a key mitogenic stimulus resulting in endothelial cell proliferation, migration, and vascular permeability.[10] KDR is expressed selectively in endothelial cells.

Disruption of VEGF signaling secondary to capture of VEGF[11] or its receptor[12] with antibody has been demonstrated to retard angiogenesis and inhibit tumor growth. A number of small molecule structural classes,[13] e.g. indolin-2-ones,[14] phthalazines,[15] quinolinones,[16] imidazopyridines,[17] benzimidazoles,[18] pyridines,[19] and quinazolines[20] have been disclosed as potent inhibitors of KDR in vitro or have demonstrated antiangiogenic
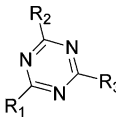
Scheme 1[a]
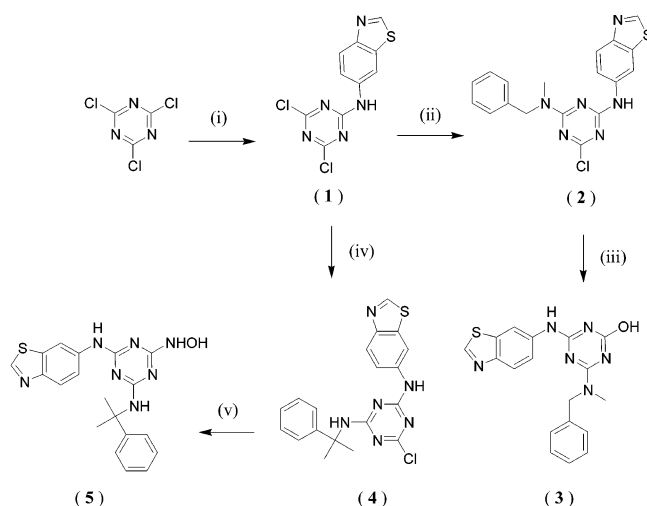
[a] Conditions: (i) 6-aminobenzothiazole, DIEA, THF, 30 min; (ii) *N*-methylbenzylamine, DIEA, THF; (iii) 95% TFA in DCM, 60 °C, 4 h; (iv) cumylamine, DIEA, THF; (v) hydroxylamine·HCl, DIEA, EtOH, 75 °C, 16 h.
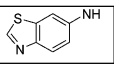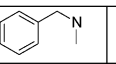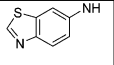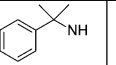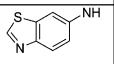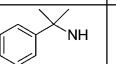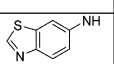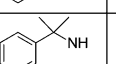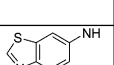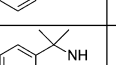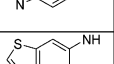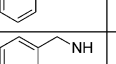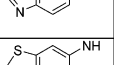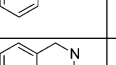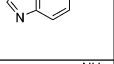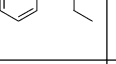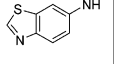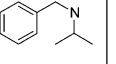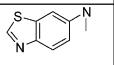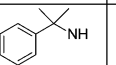
properties in vivo. In this letter, we describe a novel scaffold, composed of a 2-hydroxy-4,6-diamino-[1,3,5]-triazine nucleus.

The syntheses of certain 2-hydroxy-4,6-diamino-[1,3,5]-triazines has been recently described by two different research groups. In a study of 2,4,6-triamino-[1,3,5]-triazine inhibitors of the Erm family of methyltransferases (Erm), the 2-hydroxy-4,6-diamino-[1,3,5]triazine analogue was synthesized and found to be inactive (K_i > 100 uM for Erm-AM inhibition), in contrast to the corresponding 2-amino analogue (K_i = 8 μM for Erm-AM inhibition).[21] In a different study of a series of 2,4,6-triamino-[1,3,5]triazines as inhibitors of cholesterol ester transfer protein (CETP), the corresponding 2-hydroxy-4,6-diamino-[1,3,5]triazine analogue was prepared.[22] The triazine analogue showed only 11% inhibition of CETP at 50 μM, while the corresponding hydrazone exhibited a moderate degree of potency (IC_{50} = 10 μM for CETP inhibition).

Previous approaches for the synthesis of 2-hydroxy-4,6-diamino-[1,3,5]triazines have utilized solution-phase methodologies that involve consecutive nucleophilic displacement of cyanuric chloride. Acid-induced hydrolysis (6 N aqueous HCl reflux)[21] or base-induced hydrolysis (aqueous NaOH reflux)[22] of the final chlorine then affords the hydroxytriazine in low to moderate yields. The present triazines appear to exist in a mixture of enol and amide forms.[23] We found that solution-phase synthesis of this series (Scheme 1) proceeds most readily by initial treatment of cyanuric chloride with the moderately nucleophilic aromatic amine, 6-aminobenzothiazole, which gives access to the dichloro-arylamino-triazine (**1**) followed by treatment with the more nucleophilic secondary amine, *N*-methylbenzylamine. Finally, hydrolysis of the last chlorine was accomplished by heating with aqueous TFA to yield the desired hydroxytriazine analogue (**3**) in relatively good yield. Treatment of **1** with cumylamine afforded the 2-chloro analogue (**4**), which could be further converted to the

* To whom correspondence should be addressed. Phone: (609) 655-6950. Fax: (609) 655-6930. E-mail: mplayer@prdus.jnj.com.

Table 1. In Vitro and Cellular Activities (μM) of Hydroxytriazines against KDR

| Compound	R1 (2-position)	R2 (4-position)	R3 (6-position)	IC$_{50}$ (μM)[a]	IC$_{50}$ (μM)[b]			
					HUVEC	CHO	NIH3T3	A431
3	OH	(benzothiazol-6-yl-NH)	(benzyl-N-methyl)	0.32	2.4	N.M.	N.M.	N.M.
4	Cl	(benzothiazol-6-yl-NH)	(cumylamine)	5.5	1.08	N.M.	N.M.	N.M.
5	NHOH	(benzothiazol-6-yl-NH)	(cumylamine)	0.040	0.84	> 10 μM	> 10 μM	> 10 μM
8	OMe	(benzothiazol-6-yl-NH)	(cumylamine)	6.1	>30	N.M.	N.M.	N.M.
14a	OH	(benzothiazol-6-yl-NH)	(cumylamine)	0.035	0.4	> 10 μM	> 10 μM	> 10 μM
14b	OH	(benzothiazol-6-yl-NH)	(benzyl-NH)	2.0	>30	N.M.	N.M.	N.M.
14c	OH	(benzothiazol-6-yl-NH)	(benzyl-N-ethyl)	0.049	0.6	> 10 μM	> 10 μM	> 10 μM
14d	OH	(benzothiazol-6-yl-NH)	(benzyl-N-isopropyl)	0.018	0.28	> 10 μM	> 10 μM	> 10 μM
14e	OH	(benzothiazol-6-yl-N-methyl)	(cumylamine)	1.6	9.9	N.M.	N.M.	N.M.
14f	OH	(indazol-6-yl-NH)	(cumylamine)	0.24	>20	N.M.	N.M.	N.M.

[a] Data represent the mean (3 determinations) of the concentration needed to inhibit enzyme activity by 50% (IC$_{50}$ value; μM) at 10 μM ATP. [b] Data represent the mean from three independent experiments of the concentration needed to inhibit the growth factor induced phosphorylation of p42/44 MAP kinase by 50% (IC$_{50}$ value; μM) by the methods detailed in the Supporting Information.

corresponding hydroxylamine (**5**) by treatment with hydroxylamine hydrochloride. The 2-methoxy analogue (**8**) could be accessed by an alternative synthetic route starting from the commercially available 4,6-dichloro-2-methoxy-[1,3,5]triazine (**6**). Thus, treatment of **6** with 6-aminobenzothiazole forms 1-(6-amino-benzothiazolyl)-3-chloro-5-methoxytriazine (**7**) and when followed by cumylamine yields **8** in relatively good yield.

In order to rapidly develop an SAR around **3** that would explore the diversity of the two amine substitutions on the triazine ring, an alternative synthetic strategy was desired which would provide facile access to a wide range of analogues (Scheme 2). Wang resin was treated with an excess of cyanuric chloride in anhydrous THF in the presence of DIEA to obtain the resin-bound dichlorotriazine (**9**); this in turn underwent nucleophilic displacement of a chlorine with aromatic amines (**10a**−**c**) in anhydrous THF at room temperature to yield the resin-bound monochlorotriazines (**11a**−**c**). Reaction utilizing a second set of amines (**12a**−**d**) under more forcing conditions (heating at 90 °C in anhydrous dioxane for 16 h) led to displacement of the final chlorine to yield the resin-bound diamino-triazines (**13a**−**f**). Finally, the desired hydroxytriazines (**14a**−**f**) were obtained by cleavage using TFA/DCM conditions (50% TFA/DCM, 3 h).

Over 1000 2-hydroxy-4,6-diaminotriazines were prepared using this solid-phase approach, varying both the

Scheme 2[a]
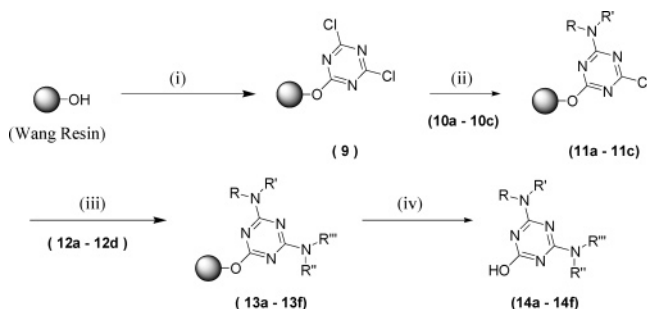
[a] Conditions: (i) cyanuric chloride, DIEA, THF; (ii) arylamines RNHR′ (**10a**−**c**), DIEA, THF, rt, 18 h; (iii) amines R″NHR‴ (**12a**−**d**), dioxane, 90 °C, 16 h; (iv) 50% TFA in DCM, rt, 3 h.

4- and 6-positions using aryl- and alkylamines at both positions. The compounds were initially evaluated for their inhibitory activity toward KDR using a fluorescence polarization competitive immunoassay, and the IC$_{50}$ values were computed (Table 1). At the outset, the structural requirements at the 2-position were examined. A H-bond donor at the 2-position such as hydroxy (**14a**) was found to be a key feature. Methylation of the 2-hydroxyl (**8**) or replacement with Cl (**4**) resulted in a greater than 50-fold loss of inhibitory potency. However, a 2-position hydroxylamine substitution (**5**) retained activity both in vitro and in the cell-based assay. At the 4-position, arylamines were found to be virtually essential for inhibitory activity.[24] Among the various

Table 2. IC$_{50}$s (μM) for the Inhibition of the Kinase Activities of KDR and Related Tyrosine Kinases[a] by Hydroxytriazines (**5**, **14a,c,d**)

kinase	**5**	**14a**	**14c**	**14d**
KDR	0.040	0.035	0.049	0.018
Tie-2	0.7	1.0	0.40	0.180
FGFR-1	1.8	>12.5	>12.5	>12.5
c-fms	14.0	>12.5	4.3	>12.5
c-src	1.8	4.3	3.1	>12.5
β-IRK	0.42	0.55	4.0	11 ± 5
PDGF-R2	3.9	8.0	2.5	>12.5

[a] IC$_{50}$ values are the average of 3 determinations. In all cases, the standard error was found to be less than 2% of the average value, with the exception of the inhibition of IRK by compound **14d** as shown.

arylamine substitutions, those displaying the best activities were 6-aminobenzothiazole (**14a**) and 6-aminoindazole (**14f**). Of these, **14a** (IC$_{50}$ = 35 nM) was superior to **14f** (IC$_{50}$ = 240 nM) in vitro. In addition, **14f** showed substantially weaker activity in the cell-based assay (IC$_{50}$ >20 μM), possibly as a result of impaired cellular permeability. N-Methylation of 6-aminobenzothiazole (**14e**) reduced activity almost 40-fold (IC$_{50}$ = 1.6 μM) in the in vitro assay. Interestingly, elaboration of the N-substituent to methyl (**3**) and then to ethyl (**14c**) each resulted in approximately 7-fold enhancement of the inhibitory potency, possibly as a reflection of favorable hydrophobic binding. An *N*-isopropyl substituent (**14d**) was subsequently found to be optimal and afforded the most potent compound in this series with an in vitro IC$_{50}$ of 18 nM as well as potency in the cell-based HUVEC assay (IC$_{50}$ = 289 nM). In the absence of a tertiary amine at the 6-position, rotationally hindered benzylamines such as cumylamine (**14a**) afforded good potency.

In order to assess the selectivity of these KDR inhibitors, inhibitory potencies were measured for compounds **5**, **14a**, **14c**, and **14d** against a panel of tyrosine kinases. C-fms and PDGF-R2 are members of the class III family of receptor tyrosine kinases, which includes KDR. Tie-2 is another receptor tyrosine kinase that is involved in vascular development and is potentially an important target for blocking angiogenesis at a secondary stage.[25] For the compounds tested, selectivity for KDR relative to the kinases in Table 2 was always at least 9-fold or greater. Compound **14d** in particular was found to have a potentially desirable selectivity profile, yielding significant inhibition against Tie-2 kinase only. This could be advantageous, as a dual KDR/Tie-2 kinase inhibitor may exhibit enhanced antiangiogenic activity in vivo.

Since an inhibitor of KDR must bind to an intracellular kinase domain, the compounds were evaluated in a cellular assay using a HUVEC cell line, which endogenously expresses KDR. Inhibition of KDR activity in HUVECs was monitored through analysis of KDR-induced MAP kinase phosphorylation. Low passage (3–12) HUVEC cells were grown to confluence, serum starved, treated with compound, or DMSO and stimulated with VEGF. HUVECs were then lysed, and the lysates were subjected to SDS–PAGE followed by quantitative immunoblotting with a phospho-specific MAP kinase (pERK1/2) antibody. Inhibition of KDR-induced MAP kinase phosphosphorylation was quantified as a percent of the total VEGF-stimulated MAP

Table 3. Effect on Vessel Outgrowth in Rat Aortic Ring Assay[a]

compd	concn, μM	total tube length ± SEM
control	n/a	2060 ± 369
14d	30	36.3 ± 12.9
14d	3	348 ± 124
14d	0.3	1503 ± 227

[a] The total length (arbitrary units) of capillary-like sprouts emanating outward from individual aortic rings was measured following 7 days of culture in collagen gels containing 5 ng/mL VEGF. Values are the mean and SEM from six aortic rings.
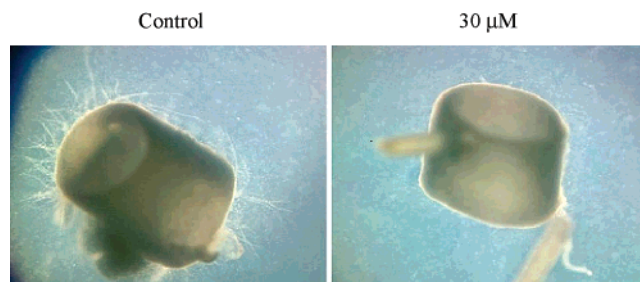
Figure 1. Inhibition of vessel formation in a rat aortic ring model (**14d**). A light micrograph (original 10×) of representative control and **14d**-treated aortic rings. Emanating from control rings were capillary-like sprouts, which are absent in the **14d**-treated cultures.

kinase phosphorylation in DMSO-treated control cells. Several of the best compounds inhibited VEGF-induced MAP kinase activation with IC$_{50}$s in the submicromolar range (Table 1). Because these compounds had some modest activity toward other kinases in vitro, we proceeded to evaluate cell-based specificity of the compounds toward a diverse set of receptor tyrosine kinases. We tested compound activity in cells expressing the human insulin receptor (CHO-IR), mouse FGFR1 (NIH 3T3 fibroblasts), and the human EGF receptor (A431). The compounds were evaluated for inhibition of IR, FGF-R1, and EGFR induced phosphorylation of MAP kinase in cells. This was done using a phosphorylation specific ELISA method that directly detects growth factor induced phosphorylation of MAP kinase in cells grown, treated, and fixed to 96-well tissue culture dishes as described in the Supporting Information.[26] Using this quantitative method compounds **5**, **12a**, **12c**, and **12d** all displayed IC$_{50}$s of greater than 10 μM in the cell-based counterscreens for EGF-R, IR, and FGF-R1 activation. Although compounds **5**, **12a**, **12c**, and **12d** displayed some activity toward the insulin receptor kinase in enzyme studies, the compounds lack significant cell-based insulin receptor inhibition. This is most likely due to either the cell permeability of the compounds or, because the compounds are ATP competitive, the increased ATP concentration in the cellular environment compared to the in vitro enzyme assay condition (1–2 mM and 10 μM respectively). Additionally, these results suggest that compounds **5**, **12a**, **12c**, and **12d** are not significantly affecting key kinases in the MAP kinase pathway downstream of most receptor tyrosine kinases.

An initial evaluation of the antiangiogenic activity of this novel class of KDR inhibitors was carried out using an aortic ring explant assay.[27] In this assay, rings cut from aortas were embedded and cultured in collagen gels and gave rise over 7 days to endothelial outgrowths resembling microcapillary sprouts. Nicosia et al. reported previously that at least 70% of the angiogenic

response can be blocked by neutralizing antibody against VEGF,[27] underscoring the importance of VEGF signaling in this assay system. We found that compound **14d** blocked vascular sprout formation by 83% and 27% at 3 and 0.3 μM, respectively (Table 3 and Figure 1). The approximate IC$_{50}$ for compound **14d** in the aortic ring explant assay (1 μM) is somewhat higher than the IC$_{50}$ for blocking HUVEC proliferation (0.28 μM). This may reflect potential species differences in compound potency or compound binding to collagen gels.

In summary, we have described a novel series of 2-hydroxy-4,6-diamino-[1,3,5]triazines, which are potent inhibitors of KDR kinase, and which have comparable selectivities to other reported small molecule inhibitors in preclinical development.

Supporting Information Available: Experimental procedures and spectra for the research described in this letter. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Carmeliet, P.; Jain, R. K. Angiogenesis in cancer and other diseases. *Nature* **2000**, *401*, 249−257. Ferrara, N. VEGF and the quest for tumour angiogenesis factors. *Nat. Rev. Cancer* **2002**, *2*, 795−803. Martiny-Baron, G.; Schlaeppi, J. M.; Wood, J. M. Therapies directed at vascular endothelial growth factor. *Expert Opin. Invest. Drugs* **2002**, *11*, 1715−1736.

(2) McDonnell, C. O.; Hill, D. A.; McNamara, D. A.; Walsh, T. N.; Bouchier-Hayes, D. J. Tumor micrometastases: the influence of angiogenesis. *Eur. J. Surg. Oncol.* **2000**, *26*, 105−115.

(3) Patel, N.; Sun, L.; Moshinsky, D.; Chen, H.; Leahy, K. M.; Le, P.; Moss, K. G.; Wang, X.; Rice, A.; Tam, D.; Laird, A. D.; Yu, X.; Zhang, Q.; Tang, C.; McMahon, G.; Howlett, A. A selective and oral small molecule inhibitor of vascular epithelial growth factor receptor (VEGFR)-2 and (VEGFR)-1 inhibits neovascularization and vascular permeability. *J. Pharmacol. Exp. Ther.* **2003**, *306*, 838−845.

(4) Enomoto, H.; Inoki, I.; Komiya, K.; Shiomi, T.; Ikeda, E.; Obata, K.; Matsumoto, H.; Toyama, Y.; Okada, Y. Vascular endothelial growth factor isoforms and their receptors are expressed in human osteoarthritic cartilage. *Am. J. Pathol.* **2003**, *162*, 171.

(5) Clavel, G.; Bessis, N.; Boissier, M. C. Recent data on the role of angiogenesis in rheumatoid arthritis. *Joint Bone Spine* **2003**, *70*, 321−326.

(6) Tonnesen, M. G.; Feng, X.; Clark, R. A. Angiogenesis in wound healing. *J. Invest. Dermatol. Symp. Proc.* **2000**, *5*, 40−46.

(7) Zhou, Y.; Genbacev, O.; Fisher, S. J. The human placenta remodels the uterus by using a combination of molecules that govern vasculogenesis or leukocyte extravasation. *Ann. N.Y. Acad. Sci.* **2003**, *995*, 73−83.

(8) Reynolds, L. P.; Grazul-Bilska, A. T.; Redmer, D. A. Angiogenesis in the female reproductive organs: pathological implications. *Int. J. Exp. Pathol.* **2002**, *83*, 151−163.

(9) Kim, K. J.; Li, B.; Winer, J.; Armanini, M.; Gillett, N.; Phillips, H. S.; Ferrara, N. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. *Nature* **1993**, *362*, 841−844.

(10) Ferrara, N.; Gerber, H. P. The role of vascular endothelial growth factor in angiogenesis. *Acta Haematol.* **2001**, *106*, 148−156.

(11) Kabbinavar, F.; Hurwitz, H. I.; Fehrenbacher, L.; Meropol, N. J.; Novotny, W. F.; Lieberman, G.; Griffing, S.; Bergsland, E. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J. Clin. Oncol.* **2003**, *21*, 60−65. Yang, J. C.; Haworth, L.; Sherry, R. M.; Hwu, P.; Schwartzentruber, D. J.; Topalian, S. L.; Steinberg, S. M.; Chen, H. X.; Rosenberg, S. A. A randomized trial of bevacizumab, an antivascular endothelial growth factor antibody, for metastatic renal cancer. *New Engl. J. Med.* **2003**, *349*, 427−434.

(12) Prewett, M.; Huber, J.; Li, Y.; Santiago, A.; O'Connor, W.; King, K.; Overholser, J.; Hooper, A.; Pytowski, B.; Witte, L.; Bohlen, P.; Hicklin, D. J. Antivascular endothelial growth factor receptor (fetal liver kinase 1) monoclonal antibody inhibits tumor angiogenesis and growth of several mouse and human tumors. *Cancer Res.* **1999**, *59*, 5209−5218.

(13) For a review of KDR inhibitors, see: Boyer, S. J. Small molecule inhibitors of KDR (VEGFR-2) kinase: an overview of structure activity relationships. *Curr. Top. Med. Chem.* **2002**, *2*, 973−1000.

(14) Sun, L.; Tran, N.; Liang, C.; Tang, F.; Rice, A.; Schreck, R.; Waltz, K.; Shawver, L. K.; McMahon, G.; Tang, C. Design, synthesis, and evaluations of substituted 3-[(3- or 4-carboxyethylpyrrol-2-yl)methylidenyl]indolin-2-ones as inhibitors of VEGF, FGF, and PDGF receptor tyrosine kinases. *J. Med. Chem.* **1999**, *42*, 5120−5130. Sun, L.; Tran, N.; Liang, C.; Hubbard, S.; Tang, F.; Lipson, K.; Schreck, R.; Zhou, Y.; McMahon, G.; Tang, C. Identification of substituted 3-[(4,5,6,7-tetrahydro-1*H*-indol-2-yl)methylene]-1,3-dihydroindol-2-ones as growth factor receptor inhibitors for VEGF-R2 (Flk-1/KDR), FGF-R1, and PDGF-Rβ tyrosine kinases. *J. Med. Chem.* **2000**, *43*, 2655−2663.

(15) Bold, G.; Altmann, K. H.; Frei, J.; Lang, M.; Manley, P. W.; Traxler, P.; Wietfeld, B.; Bruggen, J.; Buchdunger, E.; Cozens, R.; Ferrari, S.; Furet, P.; Hofmann, F.; Martiny-Baron, G.; Mestan, J.; Rosel, J.; Sills, M.; Stover, D.; Acemoglu, F.; Boss, E.; Emmenegger, R.; Lasser, L.; Masso, E.; Roth, R.; Schlachter, C.; Vetterli, W. New anilinophthalazines as potent and orally well absorbed inhibitors of the VEGF receptor tyrosine kinases useful as antagonists of tumor-driven angiogenesis. *J. Med. Chem.* **2000**, *43*, 2310−2323.

(16) Fraley, M. E.; Arrington, K. L.; Buser, C. A.; Ciecko, P. A.; Coll, K. E.; Fernandes, C.; Hartman, G. D.; Hoffman, W. F.; Lynch, J. J.; McFall, R. C.; Rickert, K.; Singh, R.; Smith, S.; Thomas, K. A.; Wong, B. K. Optimization of the indolyl quinolinone class of KDR kinase inhibitors: effects of 5-amido- and 5-sulfonamido-indolyl groups on pharmacokinetics and hERG binding. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 351−355.

(17) Wu, Z.; Fraley, M. E.; Bilodeau, M. T.; Kaufman, M. L.; Tasber, E. S.; Balitza, A. E.; Hartman, G. D.; Coll, K. E.; Rickert, K.; Shipman, J.; Shi, B.; Sepp-Lorenzino, L.; Thomas, K. A. Design and synthesis of 3,7-diarylimidazopyridines as inhibitors of the VEGF-receptor KDR. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 909−912.

(18) Bilodeau, M. T.; Cunningham, A. M.; Koester, T. J.; Ciecko, P. A.; Coll, K. E.; Huckle, W. R.; Hungate, R. W.; Kendall, R. L.; McFall, R. C.; Mao, X.; Rutledge, R. Z.; Thomas, K. A. The discovery of N-(1,3-thiazol-2-yl)pyridin-2-amines as potent inhibitors of KDR kinase. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2485−2488.

(19) Bilodeau, M. T.; Rodman, L. D.; McGaughey, G. B.; Coll, K. E.; Koester, T. J.; Hoffman, W. F.; Hungate, R. W.; Kendall, R. L.; McFall, R. C.; Rickert, K. W.; Rutledge, R. Z.; Thomas, K. A. The discovery of N-(1,3-thiazol-2-yl)pyridin-2-amines as potent inhibitors of KDR kinase. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2941−2945.

(20) Hennequin, L. F.; Thomas, A. P.; Johnstone, C.; Stokes, E. S.; Pl_, P. A.; Lohmann, J. J.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Curwen, J. O.; Kendrew, J.; Lambert-van der Brempt, C. Design and structure−activity relationship of a new class of potent VEGF receptor tyrosine kinase inhibitors. *J. Med. Chem.* **1999**, *42*, 5369−5389. Hennequin, L. F.; Stokes, E. S.; Thomas, A. P.; Johnstone, C.; Plé, P. A.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Kendrew, J.; Curwen, J. O. Novel 4-anilinoquinazolines with C-7 basic side chains: design and structure activity relationship of a series of potent, orally active, VEGF receptor tyrosine kinase inhibitors. *J. Med. Chem.* **2002**, *45*, 1300−1312.

(21) Hadjuk, P. J.; Dinges, J.; Schkeryantz, J. M.; Janowick, D.; Kaminski, M.; Tufano, M.; Augeri, D. J.; Petros, A.; Nienaber, V.; Zhong, P.; Hammond, R.; Coen, M.; Beutel, B.; Katz, L.; Fesik, S. W. Novel Inhibitors of Erm Methyltransferases from NMR and Parallel Synthesis. *J. Med. Chem.* **1999**, *42*, 3852−3859.

(22) Xia, Y.; Mirzai, B.; Chackalamannil, S.; Czarniecki, M.; Wang, S.; Clemmons, A.; Ahn, H.-S.; Boykow, G. C. Substituted 1,3,5-Triazines as Cholesterol Ester Transfer Protein Inhibitors. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 919−922.

(23) See Supporting Information (IR spectra **3**) and the following: Socrates, G. *Infrared Characteristic Group Frequencies*; George Wiley and Sons: New York, 1994; pp 136−137.

(24) Data not shown.

(25) Thurston, G. Role of Angiopoietins and Tie receptor tyrosine kinases in angiogenesis and lymphangiogenesis. *Cell Tissue Res.* **2003**, *314*, 61−8.

(26) Versteeg, H. H.; Nijhuis, E.; van der Brink, G. R.; Evertzen, M.; Pynaert, G. N.; van Deventer, S. J. H.; Coffer, P. J.; Peppelenbosch, M. P. A new phosphospecific cell-based ELISA for the p42/44 mitogen-activated (MAPK), p38 MAPK and protein kinase B and cAMP-response-element binding protein. *Biochem. J.* **2000**, *350*, 717−722.

(27) Nicosia, R. F.; Lin, Y. J.; Hazelton, D.; Qian, X. Endogenous regulation of angiogenesis in the rat aorta model. Role of vascular endothelial growth factor. *Am. J. Pathol.* **1997**, *151*, 1379−1386.