Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4371–4375

Hit-to-lead optimization of 1,4-dihydroindeno[1,2-*c*]pyrazoles as a novel class of KDR kinase inhibitors

Jürgen Dinges,[a,*] Irini Akritopoulou-Zanze,[a] Lee D. Arnold,[b] Teresa Barlozzari,[b] Peter F. Bousquet,[b] George A. Cunha,[b] Anna M. Ericsson,[b] Nobuhiko Iwasaki,[c] Michael R. Michaelides,[a] Nobuo Ogawa,[c] Kathleen M. Phelan,[a] Paul Rafferty,[b] Thomas J. Sowin,[a] Kent D. Stewart,[a] Ryukou Tokuyama,[c] Zhiren Xia[a] and Henry Q. Zhang[a]

[a]*Global Pharmaceutical Research and Development, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-6101, USA*
[b]*Abbott Bioresearch Center, 100 Research Drive, Worcester, MA 01605-5314, USA*
[c]*Hokuriku Seiyaku Co., Ltd, 37-1-1 Inokuchi, Katsuyama, Fukui, 911, Japan*

Received 27 March 2006; revised 16 May 2006; accepted 16 May 2006
Available online 5 June 2006

Abstract—A series of 1,4-dihydroindeno[1,2-*c*]pyrazoles was prepared and evaluated for their enzymatic inhibition of KDR kinase. Computer modeling studies revealed the importance of attaching a basic side chain in predicting the binding mode of those compounds. Further investigation of structure-activity relationships led to **19**, a lead compound with an acceptable selectivity profile, activity in whole cells, and good oral efficacy in an estradiol-induced murine uterine edema model of VEGF activity.

Multicellular organisms have to form new blood vessels to maintain a sufficient supply of nutrients and oxygen when they grow beyond a size of 1–2 mm. Initially blood vessels are assembled from endothelial precursors (vasculogenesis) and are then expanded through angiogenesis (sprouting and intussusception).[1] Angiogenesis is a tightly controlled process, which is balanced by a number of angiogenic growth factors and endogenous inhibitors.[2] While angiogenesis is an essential process, for example, in embryonic development, the menstrual cycle, and wound healing,[3] its imbalance has been implicated in various disease states such as rheumatoid arthritis, stroke, psoriasis, and in cancer.[4] At an early stage, solid tumors depend on angiogenesis to grow, survive, and metastasize. Without sufficient vascularization, however, they remain small and become necrotic or apoptotic. Members of the family of vascular endothelial growth factors (VEGFs), especially VEGF-A, were identified as major regulators of these events.[5] Expression of VEGF is stimulated by hypoxia and its biological effects are mediated by the receptor tyrosine kinases FLT1 (VEGFR1, Fms-like tyrosine kinase 1) and, more importantly, KDR (VEGFR2, kinase insert domain-containing receptor tyrosine kinase).[6] KDR is a transmembrane protein that is specifically expressed in vascular endothelial cells. Upon binding of VEGF to its extracellular ligand binding domain, KDR undergoes dimerization and trans-autophosphorylation of its intracellular kinase domain. This initiates a cascade of downstream signaling events, which ultimately lead to endothelial cell proliferation and migration. Its essential role in tumor progression has made the inhibition of KDR signaling a highly attractive target for therapeutic intervention in cancer.[7] In fact, small molecule KDR inhibitors have been shown to inhibit the growth of solid tumors derived from breast, colon, lung, and prostate in tumor xenograft models. Clinical validation for the general strategy can be derived from the recent FDA approval of Avastin™, a monoclonal antibody to VEGF, for the treatment of colorectal cancer,[8] as well as from the advancement of ZD6474[9] and PTK787[10] into phase III clinical trials.

High-throughput screening of our compound repository identified the 1,4-dihydroindeno[1,2-*c*]pyrazole **1** (Fig. 1) as a reversible ATP-competitive inhibitor (IC_{50} = 0.93 μM) of KDR kinase. In this report, we describe

Keywords: Cancer; Receptor tyrosine kinase; KDR kinase; Inhibitor; 1,4-Dihydroindeno[1,2-*c*]pyrazoles.
* Corresponding author. Tel.: +1 847 935 1448; e-mail: jurgen.dinges@abbott.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.05.052

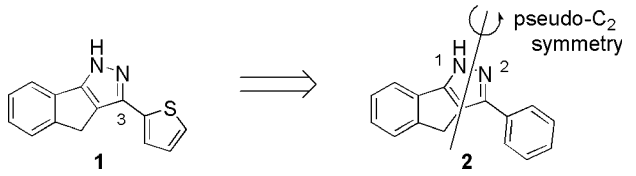


Figure 1. Structure of the high throughput screening hit **1** and demonstration of pseudo-C_2 symmetry in **2**.

an improved synthesis of 1,4-dihydroindeno[1,2-*c*]pyrazoles and their hit-to-lead evaluation as KDR kinase inhibitors.

Compound **1** already represents the minimum required pharmacophore for KDR activity. Without the thiophene substituent in 3-position the compound is inactive. Previous studies of 1,4-dihydroindeno[1,2-*c*]-pyrazoles bound to the PDGF[11] or CDK2[12] kinase active sites indicated the possibility of multiple binding modes based on the pseudo-C_2 symmetry of those molecules. The situation becomes more obvious if the 3-thiophene substituent is replaced by a phenyl group, as shown in **2**. Using the crystal structure of KDR kinase, this molecule then can be docked into the ATP binding site in such a way that its N(1)-H acts as a hydrogen bond donor to Glu 917 and its N(2) acts as a hydrogen bond acceptor to Cys 919 in the hinge region of KDR (mode A in Fig. 2). In this case, the phenyl group is oriented toward the solvent. The tautomeric form of **2**, however, is able to bind in the opposite direction such that the 3-phenyl substituent projects toward the specificity pocket (mode B). To further investigate this situation, we decided to attach a (4-methylpiperazin-1-yl)methyl side chain to the core **2**.

The general synthesis of 1,4-dihydroindeno[1,2-*c*]pyrazoles with basic side chains in the 5-, 6- or 7-position is exemplified with the synthesis of compound **10** (Scheme 1). Indanone **3** was protected in the form of its dioxolane **4**. Halogen–lithium exchange followed by formylation with DMF afforded aldehyde **5**, which

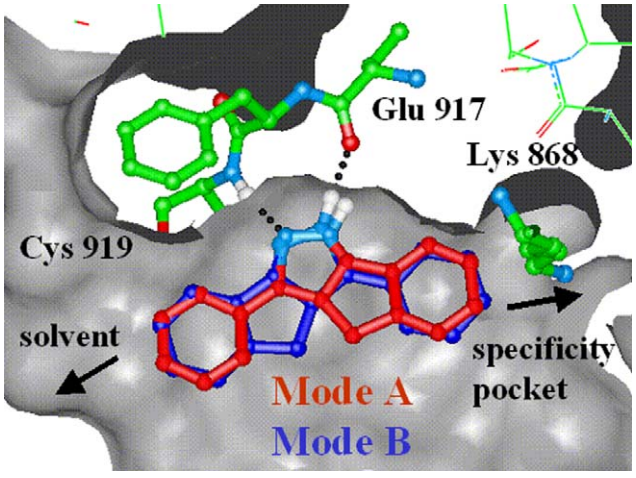
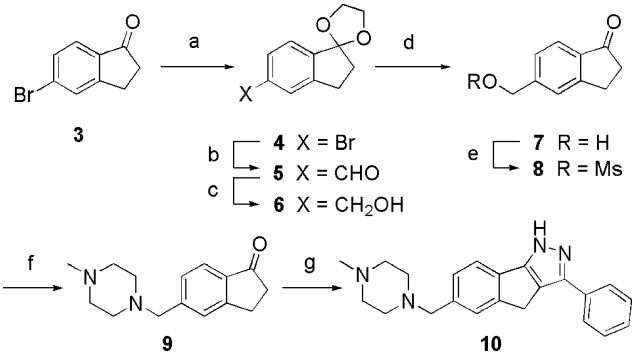


Figure 2. Diagram of compound **2** bound into active site of KDR kinase (PDB 1VR2) in two orientations.

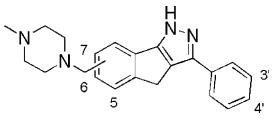


Scheme 1. Reagents and conditions: (a) ethylene glycol, *p*-TsOH, benzene, reflux (Dean–Stark trap); (b) i—*n*-BuLi, THF, −78 °C; ii—DMF, THF, −78 °C to rt; (c) $NaBH_4$, MeOH/THF (10:1), 0 °C to rt; (d) *p*-TsOH, acetone/H_2O (4:1), reflux; (e) MsCl, Et_3N, THF, 0 °C; (f) 1-methylpiperazine, K_2CO_3, EtOH, 0 °C to rt; (g) i—NaH, phenyl benzoate, benzene, reflux; ii—$H_2NNH_2{\cdot}H_2O$, HOAc, EtOH, reflux.

was directly reduced with sodium borohydride to give the hydroxymethyl compound **6**. After deprotection of the keto functionality, the hydroxyl group in **7** was mesylated to afford **8**. Indanone **9** was then obtained via nucleophilic substitution with 1-methylpiperazine, using potassium carbonate as the base. The yield in the subsequent formation of the tricyclic ring system in **10** is very sensitive to the nature of the substituent in 3-position. Therefore, we developed an improved 'one-pot' procedure,[13] which is more amenable to elucidate structure–activity relationships (SARs) in this position. The 1,4-dihydroindeno[1,2-*c*]pyrazoles with basic side chains in 3′- or 4′-position were produced in analogy to Scheme 1, starting from 2-indanone and the appropriate 4-methyl piperazinomethyl benzoate.[14]

The activity of the produced compounds to inhibit the phosphorylation of a peptide substrate (biotin–Ahx–AEEEYFFLFA–amide) by KDR kinase was assessed in an HTRF® assay at 1.0 mM concentration of adenosine 5′-triphosphate (ATP).[15] Table 1 shows that having the polar side chain attached to the 5-position (**11**), directed toward the ribose binding pocket, was not well tolerated. Attachment in 6- (**10**) and 7-position (**12**) produced about equipotent compounds with a slight improvement of activity compared to the parent compound **2**, and substitution in 3′- (**13**) and 4′-position

Table 1. KDR inhibitory activity of parent compound **2** and its (4-methylpiperazin-1-yl)methyl substituted derivatives **10**–**14**

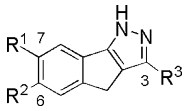


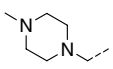
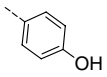
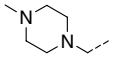
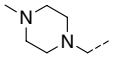
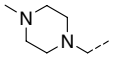
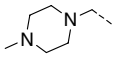
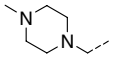
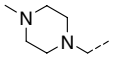
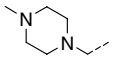
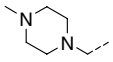
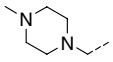
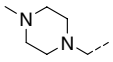
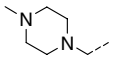
Compound	Substituent in position	KDR IC_{50}[a] (μM)
2	No substituent	1.2
11	5	14.1
10	6	0.7
12	7	0.6
13	3′	3.4
14	4′	1.4

[a] Values are means of two experiments.

(**14**) resulted in a slight loss of potency. Based on our modeling studies, compound **12** cannot bind to KDR in mode A because of severe clashes between the basic side chain and the protein. On the other hand, compound **14** is unable to bind in mode B because of the same reason. However, both compounds have reasonable potencies. Together with the fact that polar groups, such as 4-methylpiperazine, will likely prefer the solvent-accessible region of the active site, we therefore assume that the position of the basic side chain will determine the binding mode of our compounds. Overall, adding a polar side chain improved the solubility of our compounds and had a positive effect on their potency in those cases where the substituent was attached in the 6- or 7-position. Both forms were considered for further studies.

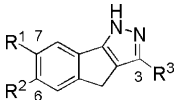
Table 2. KDR inhibitory activity of a selected set of 1,4-dihydroindeno[1,2-*c*]pyrazoles with modifications in 3-position **15**–**26**

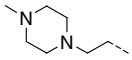
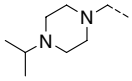
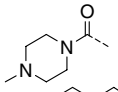
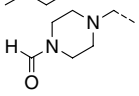


Compound	R^1	R^2	R^3	KDR IC_{50}[a] (μM)
15	H			0.50
16	H			9.20
17	H			1.05
18	H			0.40
19		H		0.18
20	H			2.84
21	H			7.48
22	H			16.64
23	H			5.47
24	H			12.54
25	H			>50.00
26	H			>50.00

[a] Values are means of two experiments.

Table 3. KDR inhibitory activity of a selected set of 1,4-dihydroindeno[1,2-*c*]pyrazoles with modifications in 6- and 7-position **27**–**35**



Compound	R^1	R^2	R^3	KDR IC_{50}[a] (nM)
27	H			1005
28		H		143
29		H		824
30		H		190
31		H		4081
32		H		665
33		H		190
34		H		108
35		H		147

[a] Values are means of two experiments.

Next, we examined the SAR of substituents in 3-position of the 1,4-dihydroindeno[1,2-*c*]pyrazole system (Table 2). Substitutions on the phenyl ring generally resulted in a loss of potency, only the two analogs with a hydroxyl group in 3′- (not shown) and 4′-position (**15**) maintained activities comparable to the parent compound **10**. Replacing the phenyl group with six-membered aromatic heterocycles also proved to be detrimental. For example, the 3′-pyridyl derivative **16**, as the most active representative of this subseries, showed about a 13-fold loss in KDR enzymatic potency. Attempts to attach aliphatic residues in 3-position rendered the compounds completely inactive. Only thiophenes, even in comparison with other five-membered aromatic heterocycles, gave promising results. As the comparison of the KDR IC_{50}-values for compounds **17** and **18** shows, attaching a thiophene in its 3′-position is better tolerated than attachment in 2′-position. A boost in potency, compared to the parent compound **10**, was then achieved by switching the basic side chain from the 6- into the 7-position (**19**). In modeling **19** into the active side of KDR, we note the proximity of the thiophene sulfur relative to the sulfur of Cys 1045 of the enzyme. 'Soft–soft' molecular interactions between the two sulfur atoms may provide a possible

Table 4. Kinase inhibition profiles of 1,4-dihydroindeno[1,2-*c*]pyrazoles **19** and **35**

Compound	IC_{50}[a] (nM)									
	KDR	FLT1	FLT4	cKit	PDGFR	Tie2	FGFR	EGFR	LCK	cMET
19	178	970	1573	145	>50,000	47,793	>50,000	>50,000	>50,000	>50,000
35	148	601	169	103	>50,000	17,641	34,355	>50,000	21,141	>50,000

[a] Values were determined in HTRF® assays.

rationale for the preferred binding of the thiophene analogs over other aromatic rings. We also speculate that, depending on its position, the basic side chain could help to orient the molecule toward this interaction. To further expand on the SAR of the thiophene substituent, we introduced an additional heteroatom into the system. However, the assay results for compounds **20**–**22** demonstrate that this change was not very well tolerated. A 'methyl-walk' around the 2′-thienyl group was carried out to investigate the possibility of attaching larger substituents, which could reach into the hydrophobic specificity pocket (compounds **23**–**25**). Here it was found that, in comparison, the 5′-position would be best suited (**23**), but even the attachment of a methyl group already resulted in about a 5-fold loss in potency. Annulation of an additional phenyl ring as in **26** only produced inactive compounds.

Concluding that a 3′-thiophene substituent is optimal for achieving potent KDR activity, we focused on the optimization of the basic side chain (Table 3). Elimination of the methylene spacer in compound **17** (Table 2) had no effect on the KDR activity (**27**), while extending this tether to an ethyl group (**19** vs **28**) slightly gained potency but then turned out to be detrimental in the in vivo efficacy model. Opening of the 1-methylpiperazine ring and elimination of one of the basic nitrogens was unfavorable as demonstrated with **29**. Extension of the *N*-alkyl group, exemplified with **30**, was tolerated but did not result in any significant improvement. Reduction of the basicity of either nitrogen by introduction of a neighboring carbonyl functionality (**31** and **32**) showed that the internal nitrogen has the more significant contribution to the overall potency of the inhibitors, but also that both basic nitrogens are required for optimum potency. While considering aromatic systems, we identified several five-membered heterocycles of interest. The 1*H*-1,2,3-triazol substituted compound **33** almost retained the potency of **19**, although being a lot less basic. Changing to its 1*H*-1,2,4-triazol isomer **34** then even led to an improvement of in vitro activity. This find was of great interest to us because through modulating the overall basicity of our compounds, we were able to reduce the volume of distribution in their PK profile. Unfortunately, we found that the in vivo efficacy decreased with the decreasing pK_a values of our KDR inhibitors. As a consequence, the imidazole-containing compound **35** was produced and showed a KDR IC_{50} of 147 nM. To determine its whole cell activity, **35** (KDR cell IC_{50} = 293 nM) was evaluated in an enzyme linked immunosorbent assay (ELISA) for its ability to inhibit VEGF-induced phosphorylation of KDR in full length human KDR-transfected NIH3T3 cells.[15] The compound was then tested in an estradiol-induced murine uterine edema model of VEGF activity[15] to determine its in vivo efficacy and systemic oral exposure. Here **35** achieved an ED_{50} of 20 mg/kg. Table 4 shows the inhibitory potencies of **35** and its parent compound **19** against a panel of kinases. The compounds are most active against the kinases of the VEGFR family as well as cKit, and overall display a selectivity profile that is unique, compared to other known classes of KDR inhibitors.

In summary, 1,4-dihydroindeno[1,2-*c*]pyrazoles were identified as potent KDR inhibitors. Attachment of a basic side chain allowed the prediction of the binding mode of these compounds into the active site of KDR, and affords improved pharmacological properties. Further hit-to-lead optimization studies resulted in lead compound **19**, which shows an acceptable selectivity profile, activity in whole cells, and good efficacy when dosed orally in a primary in vivo efficacy model. Follow-up work in this series will be presented in due time.

References and notes

1. Carmeliet, P.; Jain, R. K. *Nature* **2000**, *407*, 249.
2. Tonini, T.; Rossi, F.; Claudio, P. P. *Oncogene* **2003**, *22*, 6549.
3. Risau, W. *Nature* **1997**, *386*, 671.
4. Folkman, J. *Nat. Med.* **1995**, *1*, 27.
5. Ferrara, N. *Semin. Oncol.* **2002**, *29*, 10.
6. Jansen, M.; de Witt Hamer, P. C.; Witmer, A. N.; Troost, D.; van Noorden, C. J. F. *Brain Res. Rev.* **2004**, *45*, 143.
7. Paz, K.; Zhu, Z. *Front. Biosci.* **2005**, *10*, 1415.
8. Hurwitz, H.; Fehrenbacher, L.; Cartwright, T.; Hainsworth, J.; Heim, W.; Berlin, J.; Baron, J.; Griffing, S.; Holmgren, E.; Ferrara, N.; Fyfe, G.; Rogers, B.; Ross, R.; Kabbinavar, F. *N. Eng. J. Med.* **2004**, *23*, 2335.
9. Wedge, S. R.; Ogilvie, D. J.; Dukes, M.; Kendrew, J.; Chester, R.; Jackson, J. A.; Boffey, S. J.; Valentine, P. J.; Curwen, J. O.; Musgrove, H. L.; Graham, G. A.; Hughes, G. D.; Thomas, A. P.; Stokes, E. S. E.; Curry, B.; Richmond, G. H. P.; Wadsworth, P. F.; Bigley, A. L.; Hennequin, L. F. *Cancer Res.* **2002**, *62*, 4645.
10. Thomas, A. L.; Morgan, B.; Drevs, J.; Unger, C.; Wiedemann, B.; Vanhoefer, U.; Laurent, D.; Dugan, M.; Steward, W. P. *Semin. Oncol.* **2003**, *30*, 32.
11. Ho, C. H.; Ludovici, D. W.; Maharoof, U. S. M.; Mei, J.; Sechler, J. L.; Tuman, R. W.; Strobel, E. D.; Andraka, L.; Yen, H. K.; Leo, G.; Li, J.; Almond, H.; Lu, H.; DeVine, A.; Tominovich, R. M.; Baker, J.; Emanuel, S.; Gruninger, R. H.; Middleton, S. A.; Johnson, D. L.; Galemmo, R. A. *J. Med. Chem.* **2005**, *48*, 8163.
12. Furet, P.; Meyer, T.; Strauss, A.; Raccuglia, S.; Rondeau, J. M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 221.
13. A mixture of the indanone **9**, phenyl benzoate (1 equiv), and sodium hydride (2.5 equiv) in benzene was heated to reflux for 2.5 h to give a dicarbonyl intermediate. The

reaction was quenched by addition of 50% aq HOAc and the reaction mixture was evaporated in high vacuum. For the ring closure, the crude residue was taken up in ethanol, hydrazine (1.5 equiv) and HOAc (3 equiv) were added and the mixture was refluxed for 4 h.

14. Sairam, P.; Puranik, R.; Kelkar, A. S.; Sasikiran, S.; Veerender, M.; Parvathi, A. *Synth. Commun.* **2003**, *33*, 3597.
15. The conditions for all biological assays are described in: Dai, Y.; Guo, Y.; Frey, R. R.; Ji, Z.; Curtin, M. L.; Ahmed, A. A.; Albert, D. H.; Arnold, L.; Arries, S. S.; Barlozzari, T.; Bauch, J. L.; Bouska, J. J.; Bousquet, P. F.; Cunha, G. A.; Glaser, K. B.; Guo, J.; Li, J.; Marcotte, P. A.; Marsh, K. C.; Moskey, M. D.; Pease, L. J.; Stewart, K. D.; Stoll, V. S.; Tapang, P.; Wishart, N.; Davidsen, S. K.; Michaelides, M. R. *J. Med. Chem.* **2005**, *48*, 6066.