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Bioorganic & Medicinal Chemistry Letters 13 (2003) 2485–2488

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design and Synthesis of 1,5-Diarylbenzimidazoles as Inhibitors of the VEGF-Receptor KDR

Mark T. Bilodeau,* April M. Cunningham, Timothy J. Koester, Patrice A. Ciecko, Kathleen E. Coll, William R. Huckle, Randall W. Hungate, Richard L. Kendall, Rosemary C. McFall, Xianzhi Mao, Ruth Z. Rutledge and Kenneth A. Thomas

Departments of Medicinal Chemistry and Cancer Research, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA

Received 5 March 2003; revised 7 May 2003; accepted 8 May 2003

Abstract—1,5-Diarylbenzimidazoles have been identified as potent inhibitors of KDR kinase activity. The series was developed with a goal of finding compounds with optimal drug-like properties. This communication describes structural modifications in the series that enhance solubility, lower protein binding, and provide compounds with excellent potency and pharmacokinetic profiles.

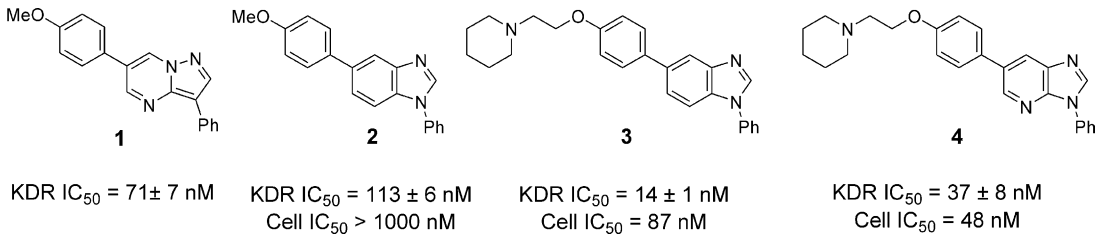
Angiogenesis, the formation of new capillaries from established blood vessels, is a normal developmental process that has also been shown to be operative in the progression of diseases such as diabetic retinopathy,[1] rheumatoid arthritis,[2] psoriasis,[3] and cancer.[4] In particular, the growth and metastasis of solid tumors has been shown to be dependent on the angiogenic process.[5] These observations have lead to widespread interest in the inhibition of angiogenesis as an approach for the treatment of cancer.

Among the molecular mechanisms elucidated in the angiogenic process, the role of vascular endothelial growth factor (VEGF) has elicited particular interest.[6] VEGF expression has been shown to be up-regulated in a variety of tumors and is an important factor in tumor angiogenesis. VEGF is a selective mitogen for endothelial cells and this signaling is mediated through the receptor tyrosine kinase KDR (VEGFR-2). A variety of inhibitors of VEGF-induced angiogenesis have been shown to be efficacious in in vivo tumor xenograft models. These inhibitors have included antibodies against VEGF[7] and its receptor KDR,[8] a soluble VEGF decoy-receptor[9] and small molecule inhibitors of KDR kinase activity.[10] Clinical trials have been underway for KDR kinase inhibitors derived from a number of different structural classes, including indolin-2-ones, phthalazines, and quinazolines.

As part of an effort to develop inhibitors of KDR, 3,6-diarylpyrazolo[1,5-*a*]pyrimidines such as **1** have been found to be potent inhibitors of KDR kinase activity ($IC_{50} = 71$ nM).[11,12] Compounds of this series had been hindered by poor physical properties, including low solubility, high logP and high protein binding, leading us to explore other templates to address these limitations. We sought different cores that could present the same key pharmacophore as these leads but address the problems of physical properties. Based on modeling, the core *N*-1 was important as a hydrogen bond acceptor and the aryl groups at positions 3- and 6-made crucial hydrophobic interactions. Thus, we considered benzimidazoles to be a likely replacement for the pyrazolo[1,5-*a*]pyrimidine core. The direct benzimidazole analogue of **1** (**2**, $IC_{50} = 113$ nM, Scheme 1)[13,14] maintained good intrinsic potency while significantly improving solubility in organic solvents.[15] However, **2** was still quite lipophilic, with poor cell potency.[16] In the 3,6-diarylpyrazolo[1,5-*a*]pyrimidine class, incorporation of a basic amine substituent modestly improved intrinsic potency and significantly improved cell potency. This was also the case for the benzimidazole class (see compound **3**) where enhanced activity was observed in an assay of VEGF-induced mitogenesis of human umbilical vein endothelial cells. Compound **3** showed significantly enhanced solubility relative to **2** although it is

*Corresponding author. Tel.: +1-215-652-5304; fax: +1-215-652-6345; e-mail: mark_bilodeau@merck.com

0960-894X/03/$ - see front matter
doi:10.1016/S0960-894X(03)00485-2



Scheme 1. Initial optimization of the benzimidazole core.

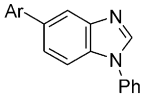
still quite lipophilic (LogP = 4.29).[17] This compound continued to possess good organic solubility but poor aqueous solubility at neutral pH (<0.00001 mg/mL). However compound **3** had an appreciable solubility at acidic pH (solubility of 0.120 mg/mL at pH 5.2).

Substitution of the 3-phenyl substituent (data not shown) lead to significantly lower activity in the series, even with relatively conservative fluorine substitutions. This observation was consistent with docking studies using a KDR homology model that depicted the bicyclic core bound in the adenine region of the ATP active site. In this model *N*-1 of the benzimidazole is engaged in a hydrogen bond with Cys919 of the hinge region, and the *N*-3 aryl substituent occupying the sterically confined hydrophobic region I.[18] Thus, all efforts to substitute this phenyl moiety lead to steric clashes within the hydrophobic pocket and decreased potency. According to this postulate the other benzimidazole 5-substituent should be open to substitution, hence the addition of basic amines off of the 5-aryl moiety.

Since the lipophilicity of the lead compound **3** was high, we began to incorporate more polarity into the core. This was accomplished by incorporating pyridyl functionality into the benzimidazole core itself as in the 7-azabenzimidazole derivative **4**. This compound had comparable intrinsic and improved cell potency relative to compound **3**. In addition, **4** had a significantly lower LogP of 3.55 and consequently an improved aqueous solubility (Table 2).

Other changes to improve the physical properties of the series focused on the 5-aryl substituent (Table 1).[17] Pyridyl functionality was incorporated into this position to produce the alkoxypyridine **5** and the aminopyridine **6**. While these changes tended to generally improve the physical properties of the series, these changes resulted in low cell potency. Additional heterocycles were investigated at this position, with the most dramatic improvement occurring upon incorporation of pyridone functionality. Thus, the synthesis of **7** resulted in a compound with good intrinsic potency and, in contrast to the previous compounds of this series good potency in cells. Most significantly, **7** is more polar with a LogP = 2.25. Compound **7** had minimal effect on the unstimulated growth of HUVECS at 2.5 μM and a 60-fold ratio for inhibition of bFGF-stimulated growth of

Table 1. SAR of the benzimidazole 5-substituent



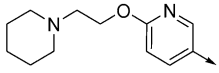
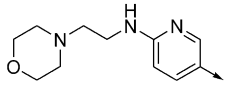
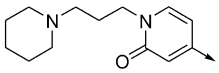
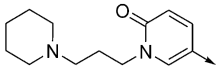
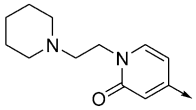
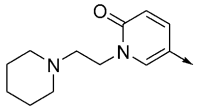
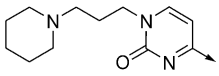
Compd	Ar	KDR IC_{50} (nM)	Cell IC_{50} (nM)
5		23±12	122
6		52±13	213
7		25±9	37
8		37±7	152
9		88	nd
10		270	nd
11		96	164

Table 2. Physical properties of selected compounds

Compd	LogP	Prot. Bind. (% bound)	Solubility @ pH 5.2 (mg/mL)	Solubility @ pH 7.4 (mg/mL)
3	4.29	99.4	0.120	<0.00001
4	3.55	97.4	6.46	0.021
7	2.25	80.7	33.9	8.4

Table 3. Pharmacokinetic data for **3**[a]

PK parameter	Rat[b]	Dog[c]
$t_{1/2}$ (h)	6.1	5.2
Cl (mL/min/kg)	14.4	4.6
V_{dss} (L/kg)	1.9	3.1
F (%)	76	nd

[a]Compound dosed iv as a solution in DMSO and p.o. as a suspension in 0.5% methocel.
[b]Average of four rats dosed at each of 5 mpk iv and 16 mpk po.
[c]Average of two dogs dosed 1 mpk iv.

Table 4. Pharmacokinetic data for **7**[a]

PK parameter	Rat[b]	Dog[c]
$t_{1/2}$ (h)	2.9	10.4
Cl (mL/min/kg)	17.3	8.9
V_{dss} (L/kg)	3.9	7.9
F (%)	73	nd

[a]Compound dosed iv as a solution in DMSO and po as a suspension in 0.5% methocel.
[b]Average of four rats dosed at each of 5 mpk iv and 20 mpk po.
[c]Average of two dogs dosed 1 mpk iv.

Table 5. Fold-selectivity versus a series of kinases

Compd	Flt-1	Flt-4	PDGFRβ	FGFR-1	FGFR-2	c-Src
3	5	6	0.5	60	47	62
4	6	5	0.4	38	25	63
5	4	3	0.5	51	42	50
7	11	6	1	97	57	181

HUVECs, consistent with specificity of action through KDR and moderate selectivity against FGFRs (Table 5).

Changing the basic amine side-chain as in **8** leads to a moderate loss of intrinsic activity and in this case **9** is significantly less active in cells. The length of he chain is also critical for potency as **9** and **10** demonstrate (compare with **7** and **8** respectively). Again, other heterocycles such as the pyrimidone **11** were less active than the pyridone **7**.

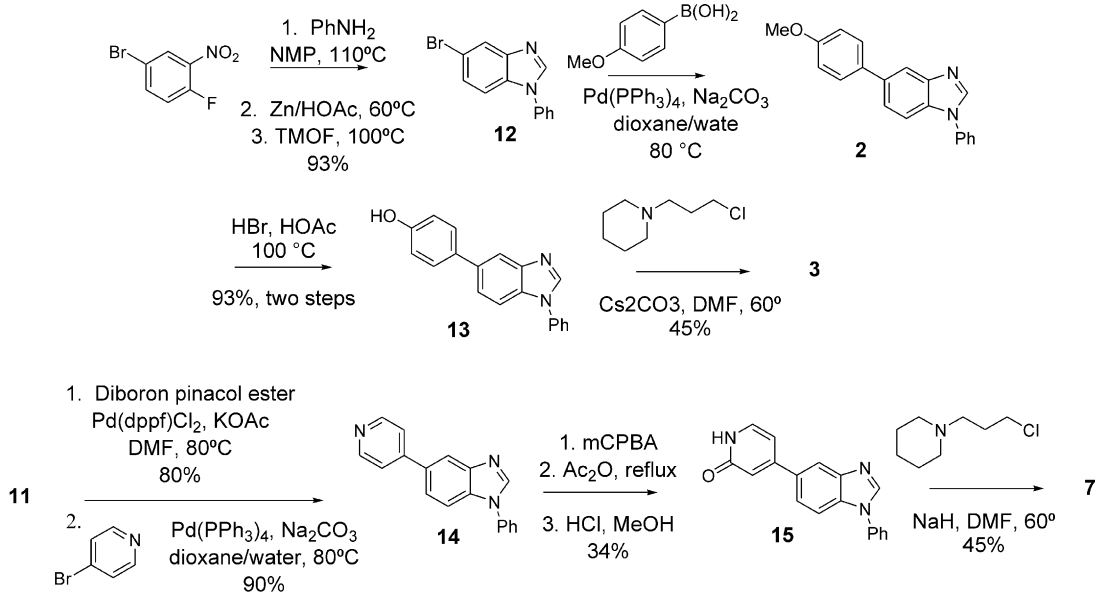
Table 2 highlights the physical property improvements achieved in this series.[17] Compound **3** is highly lipophilic with high human plasma protein binding. Introduction of the basic functionality in **4** lowered LogP and protein binding while dramatically improving solubility. A further significant improvement in all of these parameters has been observed with the introduction of pyridone functionality as in **7**. These changes are not due primarily to the three carbon chain between heteroatoms and potential changes in amine pK_a as **10** displays comparable protein binding (85.0%) and solubility (>1 mg/mL @ pH 7.4) as **7.**

Compound **3** displayed excellent pharmacokinetic behavior in rats and dogs (Table 3). It showed a good half-life with moderate clearance and excellent oral bioavailability in rats. Also, the iv pharmacokinetics for **3** was excellent in dogs. It exhibited a long half-life and low clearance.

The pharmacokinetics for compound **7** were also good in both rats and dogs (Table 4). It also had a moderate clearance and good half life in rats. In dogs, it had a long half-life with a moderate clearance and higher volume of distribution relative to compound **3**.

We have examined the selectivity of these compounds for KDR versus other closely related receptor tyrosine kinases and a non-receptor tyrosine kinase. Fold selectivities for several compounds are shown for this selection of kinases versus KDR in Table 5. In general the compounds show low levels of selectivity against the most KDR-homologous kinases; Flt-1, Flt-4 and PDGFβ. Higher levels of selectivity are observed against FGF-1, FGF-2 and c-Src.

Scheme 2 outlines the synthesis of compounds **3** and **7**.[19] 4-Bromo-1-fluoro-2-nitrobenzene was condensed with aniline and this was followed by reduction and benzimidazole formation to provide 5-bromobenzimidazole **12**. A Suzuki reaction provided **2** and the methyl ether was subsequently deprotected to provide **13**. Alkylation of **13** provided **3**. For the synthesis of **7**, **12** was converted to a pinacol boronate and that intermediate was coupled with 4-bromopyridine to provide the pyridylbenzimidazole **14**. Following a standard pyridine *N*-oxidation and rearrangement sequence the pyridone **15** was obtained. The pyridone was then alkylated to



Scheme 2. Synthesis of compounds **3** and **7**.

provide **7**. This alkylation provides a 4:1 ratio of *N*- and *O*-alkylation products.

We have described the development of 1,5-diarylbenzimidazoles that are potent and selective KDR inhibitors. These compounds significantly improved physical properties relative to the corresponding 3,6-diarylpyrazolo[1,5-a]pyrimidines. In particular, **3** and **7** have excellent potency and pharmacokinetic profiles. The pyridone **7** additionally has a significantly improved LogP and solubility, properties which may confer advantages in vivo.

Acknowledgements

We thank Matthew M. Zrada and Kenneth D. Anderson for LogP, solubility and protein binding determinations.

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13. All target compounds were fully characterized by ^{1}H NMR and mass spectroscopy.
14. 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.
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16. The Cell IC_{50} value represents the inhibition of VEGF-stimulated mitogenesis as determined in human umbilical vein endothelial cells. For the key compounds **3**, **4** and **7** the averages are based on 3, 34 and 7 determinations respectively.
17. Partition coefficients were determined by adding an aliquot of a methanolic sample solution to equal volumes of 1-octanol and pH 7.4 buffer and measuring the concentration in each after an equilibration period of 8 h. Buffer solubilities were determined by comparing the HPLC peak area of a filtered, saturated solution of compound in pH adjusted buffer to peaks from standard methanolic solutions. Protein binding to human plasma is determined by equilibrating buffer solutions of test compounds and human plasma and using ultrafiltration for separation. The free compound concentration is measured by HPLC.
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19. Compound **4** was prepared according to the synthesis of **3**, beginning with 5-bromo-2-chloro-3-nitropyridine. Compounds **5**, **8** and **10** were derived from alkyation of 5-iodopyridin-2-ol and subsequent coupling with the boronate ester in Scheme 2. Compound **6** was prepared by coupling of the boronate ester with 5-bromo-2-fluoropyridine and displacement of the resulting fluoride with (2-morpholin-4-ylethyl)-amine. Compound **9** was prepared according to the synthesis of **7**, using the homologous alkylating reagent. Compound **11** was prepared by coupling of the boronate ester with 4-chloro-2-(methylthio)pyrimidine, followed by hydrolysis of the thiomethyl-functionality and alkylation according to Scheme 2.