Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 1246–1249

Thienopyridine urea inhibitors of KDR kinase

H. Robin Heyman,[a,*] Robin R. Frey,[a] Peter F. Bousquet,[b] George A. Cunha,[b] Maria D. Moskey,[b] Asma A. Ahmed,[b] Niru B. Soni,[a] Patrick A. Marcotte,[a] Lori J. Pease,[a] Keith B. Glaser,[a] Melinda Yates,[a] Jennifer J. Bouska,[a] Daniel H. Albert,[a] Candace L. Black-Schaefer,[a] Peter J. Dandliker,[a] Kent D. Stewart,[a] Paul Rafferty,[b] Steven K. Davidsen,[a] Michael R. Michaelides[a] and Michael L. Curtin[a]

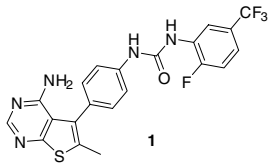
[a]*Global Pharmaceutical Research and Development, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-6100, USA*
[b]*Abbott Bioresearch Center, 100 Research Drive, Worcester, MA 01605-5314, USA*

Received 7 November 2006; revised 4 December 2006; accepted 4 December 2006
Available online 9 December 2006

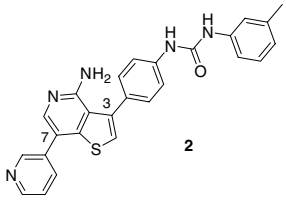
Abstract—A series of substituted thienopyridine ureas was prepared and evaluated for enzymatic and cellular inhibition of KDR kinase activity. Several of these analogs, such as **2**, are potent inhibitors of KDR (<10 nM) in both enzymatic and cellular assays. Further characterization of inhibitor **2** indicated that this analog possessed excellent in vivo potency (ED_{50} 2.1 mg/kg) as measured in an estradiol-induced mouse uterine edema model.

Reversible protein phosphorylation by protein kinases is one of the primary biochemical mechanisms mediating eukaryotic cell signaling.[1] A subset of these kinases, the receptor tyrosine kinases (RTKs), possess both extracellular and intracellular domains and selectively catalyze the phosphorylation of tyrosine hydroxyl groups in response to binding of certain extracellular growth factors.[2] RTK signaling pathways are normally highly regulated, yet their over-activation has been shown to promote the growth, survival, and metastasis of cancer cells, and has been associated with the progression of various human cancers.[3] The VEGF receptor family of RTKs, most notably VEGFR2 or KDR, mediates the biological function of vascular endothelial growth factor (VEGF) which is a regulator of vascular permeability and an inducer of endothelial cell proliferation, migration, and survival.[4] Accordingly, interruption of the KDR-mediated signaling cascade can provide an anti-angiogenic effect in human cancers as recently demonstrated by the FDA approval of the anti-VEGF antibody Avastin™ for the treatment of colorectal cancer.[5] In addition, the small-molecule KDR kinase inhibitors sorafenib and sunitinib have both been approved by the FDA for the treatment of patients with advanced renal cell carcinoma.[6]

In an ongoing effort at Abbott Laboratories to develop small-molecule RTK inhibitors, it was recently disclosed that a series of thienopyrimidine ureas were potent inhibitors of both the VEGFR and PDGFR families of RTKs;[7] for example, it was shown that compound **1** was a potent inhibitor of both KDR (IC_{50} = 6 nM)[8] and PDGFRβ (IC_{50} = 60 nM) with excellent cellular and in vivo activity. The ATP-mimic core of **1** complements other related nuclei including furanopyrimidine,[9] pyrrolopyrimidine,[10] and pyrazolopyrimidine[11] while the importance of the diphenyl urea moiety for tyrosine kinase inhibition is consistent with earlier observations regarding this structural motif.[12] In an effort to expand the scope of this series of inhibitors and potentially complement the kinase activities and ADME properties of **1**, SAR studies have been conducted on a series of thienopyridine 3-ureas. We wish to report that these compounds are potent inhibitors of KDR kinase and that 7-heteroarylation (e.g., **2**) provides compounds with excellent cellular and in vivo activity.[13]



1



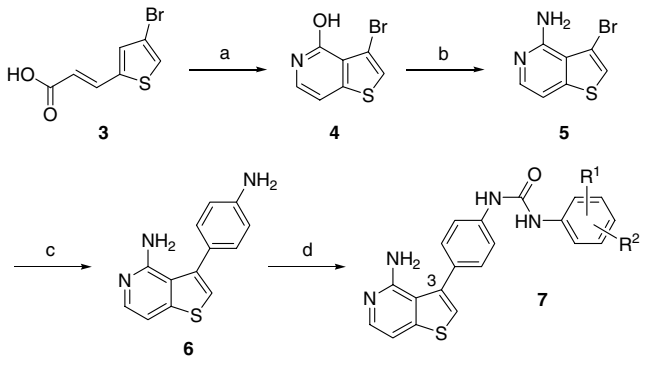
2

Keywords: KDR kinase; VEGF; Thienopyridine; Urea.
* Corresponding author. Tel.: +1 847 937 1885; fax: +1 847 935 5165; e-mail: robin.heyman@abbott.com

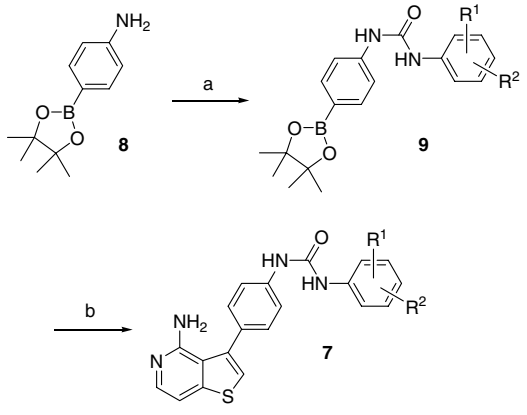
0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.12.015

The preparation of the 3-(diphenylurea)thienopyridine inhibitors began with the three-step conversion of carboxylic acid **3**, prepared by condensation of 4-bromo-2-thiophenecarboxaldehyde with malonic acid, to thienopyridinone **4** as reported previously for the desbromo substrate (Scheme 1).[14] Compound **4** was converted to aminopyridine **5** by high-pressure aminolysis of the corresponding chloropyridine intermediate. Suzuki coupling gave aniline **6** which was condensed with variously substituted phenyl isocyanates to afford thienopyridine ureas **7** in good yield (70–95%). Due to competing acylation of the aminopyridine moiety of **6**, the urea formation was usually conducted at low temperature (−20 °C) with a small excess (1.05 equiv) of isocyanate; the bis-urea by-product could then be easily removed by flash chromatography.

An alternate preparation of the urea analogs utilizes the commercially available aniline boronate **8** which was condensed with substituted phenyl isocyanates to give urea boronates **9** (Scheme 2). These intermediates would then undergo Suzuki coupling with bromide **5** to give the desired thienopyridine phenyl ureas (**7**) in 50–65% yield.



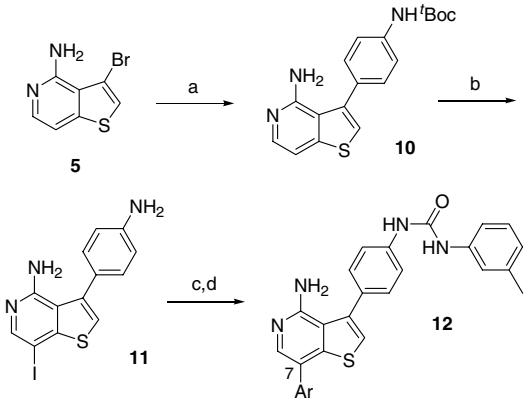
Scheme 1. Reagents and conditions: (a) $SOCl_2$, CH_2Cl_2; NaN_3, dioxane/H_2O; CH_2Cl_2, Ph_2O, reflux, 70%; (b) $POCl_3$; NH_3, dioxane, 290 psi, 160 °C, 61%; (c) 4-(tetramethyldioxaborolanyl)aniline, cat. $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O, 90 °C, 75%; (d) aryl isocyanate, NMM, DMF, −20 °C, 70–95%.



Scheme 2. Reagents and condition: (a) aryl isocyanate, NMM, THF, 85–95%; (b) 3-bromothieno[3,2-*c*]pyridin-4-ylamine (**5**), cat. $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O, 90 °C, 50–65%.

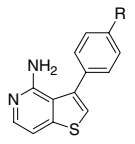
A series of 7-aryl thienopyridine ureas were made using the synthetic route in Scheme 3. Bromide **5** was coupled with *N*-Boc-protected aniline boronate to afford thienopyridine **10** which could then be selectively iodinated at the 7-position to give, after TFA deprotection, iodide **11** in 81% yield over three steps. Coupling with aryl or heteroaryl boronate/boronic acids using standard Pd^0 coupling conditions and urea formation as in Scheme 1 provided ureas **12** in 65–95% yield.

The SAR of the initial thienopyridine compounds is shown in Table 1 and is quite similar to that observed for the thienopyrimidine series of inhibitors.[7] The importance of the urea functionality was demonstrated by the low activity of non-urea analogs **6**, **13**, and **14** while the 3-methylphenyl urea (**7b**) was modestly more potent than phenyl urea **7a**. As with the isoindolinone series of KDR inhibitors,[12a] the external urea nitrogen was much more sensitive to modification (i.e., methylation or replacement with carbon) than the internal urea nitrogen as can be seen by comparing the potency of



Scheme 3. Reagents and conditions: (a) ^{t}Butyl *N*-[4-(tetramethyldioxaborolanyl)phenyl]carbamate, cat. $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O, 90 °C; (b) *N*-iodosuccinimide, DMF; TFA, 81% (three steps); (c) aryl boronate/boronic acid, cat. $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O, 90 °C, 45–70%; (d) 3-methylphenyl isocyanate, NMM, DMF, −20 °C, 65–95%.

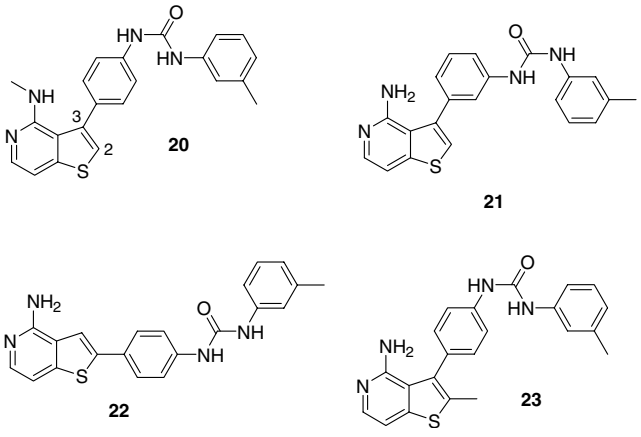
Table 1. KDR inhibitory potency of thienopyridine analogs



Compound	R	KDR IC_{50}[8] (nM)
13	H	> 12,500
6	NH_2	4900
14	–NHCOPh	12,000
7a	–NHCONHPh	56
7b	–NHCONH(3-MePh)	9
15	–NHCON(Me)(3-MePh)	4900
16	–N(Me)CONH(3-MePh)	23
17	$–NHCOCH_2$(3-MePh)	520
18	$–CH_2CONH$(3-MePh)	94
19	–NHC(S)NH(3-MePh)	410

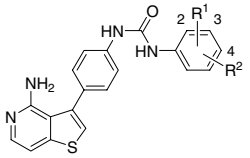
compounds **15** and **17** versus **16** and **18**. The thiourea **19** showed a significant decrease in activity compared to urea **7b**.

Consistent with the hypothesis that the thienopyridine nucleus is an ATP-mimic and that the aminopyridine acts as a 'hinge-binding' element,[15] N-methylation as in compound **20** gave a complete loss of activity. As initial modeling had suggested, replacement of the linking 1,4-phenyl of **7** with a 1,3-phenyl (**21**, KDR IC_{50} = 250 nM) gave a significant loss of potency while analogs bearing urea substitution at the 2-position of the thienopyridine nucleus as in **22** were inactive against KDR. However, it was found that 2-methylation of the thienopyridine, as in inhibitor **23** (KDR IC_{50} = 27 nM), was well tolerated.



The effect of substitution on the terminal phenyl of the urea is shown in Table 2. Aside from the modest potency difference between **7a** and **7b**, substitution of this ring at the 3- and 4-positions generally provided compounds (**7c**) that were roughly equipotent with **7a**, while 2-substitution (**7d**) led to a significant loss of enzymatic potency. A number of 3-substituents were allowed (**7e**–**h**) as were 3,4-disubstitution (**7i**) and 3,5-disubstitution (**7j**). It can be seen in Table 2 that most of these ureas had only a small difference (2- to 5-fold) between their enzymatic and cellular potencies.

Table 2. KDR enzymatic and cellular activity of ureas **7a**–**j**

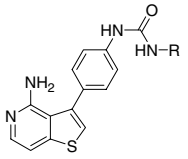


Compound	R^1	R^2	KDR IC_{50}[8] (nM)	KDR_{cell} IC_{50}[16] (nM)
7a	H	H	56	72
7b	3-Me	H	9	32
7c	4-Me	H	26	141
7d	2-Me	H	152	ND
7e	3-Et	H	25	32
7f	3-F	H	15	147
7g	3-Cl	H	22	56
7h	3-CN	H	45	198
7i	3-Me	4-Me	10	41
7j	3-Me	5-Me	16	48

The KDR inhibitory activities of thienopyridine urea analogs with non-phenyl terminal rings are shown in Table 3. It can be seen that while terminal alkyl rings such as cyclopentyl (**7k**) and cyclohexyl (**7l**) were not tolerated, heterocycles such as naphthyl (**7m**, **7n**) and thienyl (**7o**, **7p**) were potent KDR inhibitors.

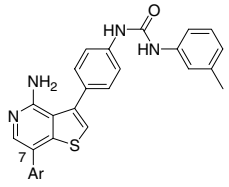
Initial modeling had suggested that substituents at the thienopyridine 7-position would project into a solvent accessible region of the active site and should be well tolerated. This substitution could potentially be used to modulate the pharmacodynamic properties of these inhibitors and offer an advantage over the thienopyrimidine series. Consistent with this hypothesis, arylation of the thienopyridine 7-position with phenyl (**12a**) or substituted phenyls such as in **12b** and **12c** provided analogs which were equipotent with **7b**, both in enzymatic and cellular assays (Table 4). Furthermore, it was found that heterocycles at this position, including 3-pyridyl (**2**), 4-pyridyl (**12d**), and 5-pyrimidyl (**12e**), gave a significant increase in cellular potency while the larger heterocycle 4-isoquinolyl (**12f**) had only modest enzymatic potency.

Table 3. KDR enzymatic and cellular activity of ureas **7k**–**p**



Compound	R	KDR IC_{50}[8] (nM)	KDR_{cell} IC_{50}[16] (nM)
7k	cC_5H_9	420	ND
7l	cC_6H_{11}	491	ND
7m	1-Naphthyl	75	895
7n	2-Naphthyl	21	15
7o	2-Thienyl	30	48
7p	3-Thienyl	38	61

Table 4. KDR enzymatic and cellular activity of ureas **2** and **12a**–**f**



Compound	Ar	KDR IC_{50}[8] (nM)	KDR_{cell} IC_{50}[16] (nM)
12a	Ph	13	33
12b	4-(OH)Ph	11	29
12c	3-(CONHMe)Ph	11	56
2	3-Pyridyl	9	4
12d	4-Pyridyl	5	4
12e	5-Pyrimidyl	8	1
12f	4-Isoquinolyl	388	ND

Table 5. KDR fold-potency versus a series of tyrosine kinases

Compound	FLT1	FLT3	KIT	PDGFRβ	TIE2	FGFR	SRC
7b	0.6	1	2	10	40	>1000	>1000
2	1	0.5	2	13	5	>440	>440

The selectivity of analogs **7b** and **2** for KDR versus other tyrosine kinases as a ratio of enzymatic IC_{50} values is shown in Table 5. These inhibitors were equipotent against the kinases most homologous to KDR (FLT1, FLT3, and KIT), modestly potent against PDGFRβ and TIE2, and much less active against FGFR and the cytosolic kinase SRC.

Further characterization of these inhibitors indicated that, while 7-unsubstituted analogs such as **7b** were only modestly active in vivo (40% inhibition, 30 mg/kg dose, po), as measured in an estradiol-induced mouse uterine edema model,[7] inhibitors such as **2** possessed excellent potency (ED_{50} 2.1 mg/kg). However, it was determined that **2** had a disappointing pharmacokinetic profile in mouse ($t_{1/2}$ 0.4 h, 3 mg/kg dose, iv) with low exposure after oral dosing (1.1 μmol h/L, 10 mg/kg dose, po) which precluded its assessment in a mouse tumor model. Pyridyl **2** was also a potent inhibitor of Cyp3A4 (IC_{50} ~250 nM).

Homology modeling[17] of inhibitor **2** bound to the ATP-binding site of the 'inactive' conformation of KDR is reminiscent of our earlier analyses of urea KDR inhibitors (Fig. 1).[7,12a] In this analysis, two hydrogen bonds were created with the kinase hinge: the exocyclic amino group of **2** with the backbone carbonyl of Glu917; and the proximal ring nitrogen of the thienopyridine with the backbone N–H of Cys919. In this bound conformation the urea unit accessed the back hydrophobic pocket adjacent to the ATP-binding site with the urea N–H bonds interacting with Glu885 of KDR. The terminal tolyl group rested in a hydrophobic region comprised of the side chains of Ile888, Leu889, Leu1019, and Val898. As mentioned, the 7-pyridyl of inhibitor **2** projects into a solvent-accessible region of the active site without any specific contact with the protein and is consistent with the fact that most of the 7-substituted analogs (**2** and **12a–f**) are equipotent with **7b**.

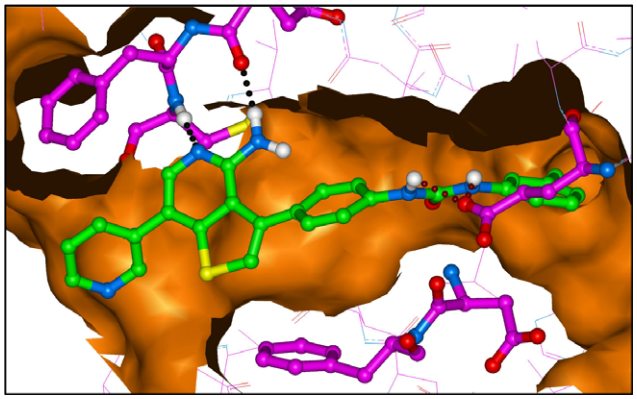


Figure 1. Model of thienopyridine **2** (green) bound to active site of KDR (model, inactive conformation, in purple) with hinge hydrogen bonds to Glu917 C=O and Cys919 N–H in black and urea hydrogen bonds to Glu885 in brown.

In summary, a series of potent KDR thienopyridine urea inhibitors has been identified. Optimal urea and 7-substitution afforded analogs (e.g., **2**) with significant enzymatic and cellular potency as well as selectivity against the non-VEGF tyrosine kinases FGFR and SRC. Homology modeling suggested that the predominant interactions include hydrogen-bonds between the thienopyridine nucleus and the protein backbone as well as the urea functionality with a glutamate residue and a hydrophobic pocket.

References and notes

1. Hunter, T. *Cell* **2000**, *100*, 113.
2. (a) Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. *Science* **2000**, *298*, 1912; (b) Robinson, D. R.; Wu, Y. M.; Lin, S. F. *Oncogene* **2000**, *19*, 5548.
3. Blume-Jensen, P.; Hunter, T. *Nature* **2001**, *411*, 355.
4. Ferrara, N.; Gerber, H. P.; LeCouter, J. *Nat. Med.* **2003**, *9*, 669.
5. Dvorak, H. F. *J. Clin. Oncol.* **2002**, *20*, 4368.
6. Morabito, A.; De Maio, E.; Di Maio, M.; Normanno, N.; Perrone, F. *Oncologist* **2006**, *11*, 753.
7. Dai, Y.; Guo, Y.; Frey, R. R.; Ji, Z.; Curtin, M. L.; Ahmed, A. A.; Albert, D. H.; Arnold, L.; 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**, 6066.
8. KDR and PDGFRβ IC_{50} values were determined using an ATP concentration of 1 mM as described in Ref. 7.
9. Miyazaki, Y.; Matsunaga, S.; Tang, J.; Maeda, Y.; Nakano, M.; Philippe, R. J.; Shibahara, M.; Liu, W.; Sato, H.; Wang, L.; Nolte, R. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2203.
10. Burchat, A. F.; Calderwood, D. J.; Hirst, G. C.; Holman, N. J.; Johnston, D. N.; Munschauer, R.; Rafferty, P.; Tometzki, G. B. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2171.
11. Burchat, A. F.; Calderwood, D. J.; Friedman, M. M.; Hirst, G. C.; Li, B.; Rafferty, P.; Ritter, K.; Skinner, B. S. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1687.
12. (a) Curtin, M. L.; Frey, R. R.; Heyman, H. R.; Sarris, K. A.; Steinman, D. H.; Holmes, J. H.; Bousquet, P. F.; Cunha, G. A.; Moskey, M. D.; Ahmed, A. A.; Pease, L. J.; Glaser, K. B.; Stewart, K. D.; Davidsen, S. K.; Michaelides, M. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4505; (b) Dumas, J.; Smith, R. A.; Lowinger, T. B. *Curr. Opin. Drug Discov. Dev.* **2004**, *7*, 600.
13. A related series of KDR inhibitors have recently been reported: Miyazaki, Y.; Nakano, M.; Sato, H.; Truesdale, A. T.; Stuart, J. D.; Nartey, E. N.; Hightower, K. E.; Kane-Carson, L. *Bioorg. Med. Chem. Lett.* **2006**. doi:10.1016/j.bmcl.2006.09.050.
14. New, J. S.; Christorpher, W. L.; Yevich, J. P.; Butler, R.; Schlemmer, R. F.; VanderMaelen, C. P.; Cipollina, J. A. *J. Med. Chem.* **1989**, *32*, 1147.
15. Toledo, L. M.; Lydon, N. B.; Elbaum, D. *Curr. Med. Chem.* **1999**, *6*, 775.
16. The cellular IC_{50} values represent inhibition of KDR phosphorylation in NIH3T3 cells stably transfected with full length human KDR. The procedure was followed as described in Ref. 12.
17. The computational analysis used to create this homology model with a recently published crystal structure of KDR-homolog KIT kinase (PDB entry 1T46) is described in Ref. 7.