Bioorganic & Medicinal Chemistry Letters 21 (2011) 2958–2961

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

In vitro and in vivo evaluation of 6-aminopyrazolyl-pyridine-3-carbonitriles as JAK2 kinase inhibitors

Tao Wang [a,*,†], Stephanos Ioannidis [a,†], Lynsie Almeida [a], Michael H. Block [a], Audrey M. Davies [a], Michelle L. Lamb [a], David A. Scott [a], Mei Su [a], Hai-Jun Zhang [a], Marat Alimzhanov [b], Geraldine Bebernitz [b], Kirsten Bell [b], Michael Zinda [b]

[a] Department of Cancer Chemistry, Oncology Innovative Medicines Unit, AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, MA 02451, USA
[b] Department of Cancer Bioscience, Oncology Innovative Medicines Unit, AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, MA 02451, USA

ARTICLE INFO

Article history:
Received 22 February 2011
Revised 14 March 2011
Accepted 15 March 2011
Available online 21 March 2011

Keywords:
JAK2 kinase inhibitors
Myeloproliferative neoplasms

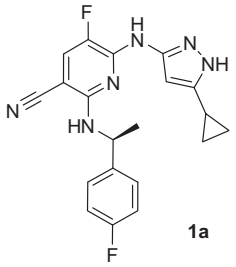
ABSTRACT

Synthesis and biological evaluation of a series of 6-aminopyrazolyl-pyridine-3-carbonitriles as JAK2 kinase inhibitors was reported. Biochemical screening, followed by profile optimization, resulted in JAK2 inhibitors exhibiting good kinase selectivity, pharmacokinetic properties, physical properties and pharmacodynamic effects.

Myeloproliferative neoplasms (MPNs) are a group of clonal hematopoietic stem cell disorders that include polycythemia vera (PV), essential thrombocythemia (ET), and idiopathic myelofibrosis (IMF), all of which have the potential to transform to acute myeloid leukemia (AML).[1,2] Several research groups have independently identified a single point mutation in the Janus-associated Kinase 2 (JAK2) encoding a valine-to-phenylalanine change (V617F).[3–5] JAK2 belongs to a family that comprises four different cytoplasmic protein tyrosine kinases: JAK1, JAK2, JAK3 and TYK2. JAKs play an important role in cellular survival, proliferation, and differentiation.[6] JAK2 V617F is constitutively phosphorylated and able to activate downstream signaling in the absence of cytokine stimulation when transfected into factor-dependent cell lines.[7] This mutation has been found in around 50% in ET and IMF patients, 95% in PV patients.[8–10]

There are a number of inhibitors against JAK2 along with other kinases[11–18] and several JAK2 kinase inhibitors are currently in clinical development against MPNs.[19–21] In the context of our interest in this target,[22–24] a subset screening of the AstraZeneca compound collection identified compound **1a** (Fig. 1) as a JAK2 inhibitor (JAK2 kinase IC_{50}: 0.003 μM). Compound **1a** belongs to the 6-aminopyrazolyl-pyridine-3-carbonitrile structural class.

With such scaffolds we anticipate that the pyrazol-3-ylamine occupies the adenine binding-site while the C_5 substituent (cyclopropyl for compound **1a**) on the pyrazole ring is packed against the methionine gatekeeper of JAK2 kinase. As described previously,[23] the steric bulk of this substituent affects the potency against JAK2 and selectivity versus other kinases. As the data in Table 1 demonstrates, replacing the cyclopropyl group with larger lipophilic groups such as an O^{i}Pr group (**1b**) resulted in a drop in JAK2 enzyme potency. An analogue with a smaller R^1 group such as methyl (**1c**, AZ960)[25,26] showed less than 3 nM potency against JAK2 enzyme. While analogues with the cyclopropyl and methyl substituents at R^1 showed comparable potencies at biochemical level (e.g., **1a** vs **c**), in a TEL-JAK2 proliferation assay in Ba/F3 cells where the fusion of oligomerization domain TEL with the kinase domain of JAK2 resulted in constitutive kinase activity, **1c** had potency of 0.024 μM versus 0.17 μM for the cyclopropyl analogue **1a**. These compounds were 3- to 50-fold selective against JAK3 in the enzyme assay.



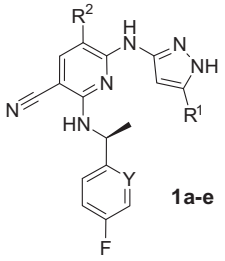
1a

Figure 1. 6-Aminopyrazolyl-pyridine-3-carbonitrile **1a**.

* Corresponding author. Tel.: +1 781 839 4734; fax: +1 781 839 4230.
E-mail address: tao.wang@astrazeneca.com (T. Wang).
† These authors have contributed equally to the preparation of this manuscript.

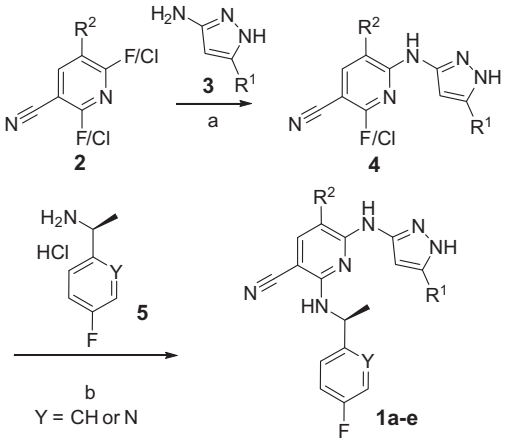
0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.03.053

Table 1
6-Aminopyrazolyl-pyridine-3-carbonitriles structure–activity relationships



Compd	$R^1/R^2/Y$	JAK2 IC_{50}[a] (μM)	JAK3 IC_{50}[a] (μM)	TEL-JAK2[b] (μM)	TEL-JAK3[b] (μM)
1a	Cyclo-Pr/F/CH	0.003	0.183	0.17	1.00
1b	O^iPr/F/CH	0.688	1.735	NT[c]	NT[c]
1c	Me/F/CH	<0.003	0.009	0.024	0.35
1d	Me/F/N	<0.003	0.007	0.036	1.30
1e	Me/Cl/N	<0.003	0.021	0.04	0.43

[a] At K_m ATP concentration.
[b] Protocols for the cellular assays in Ref. 22
[c] Denotes not tested.



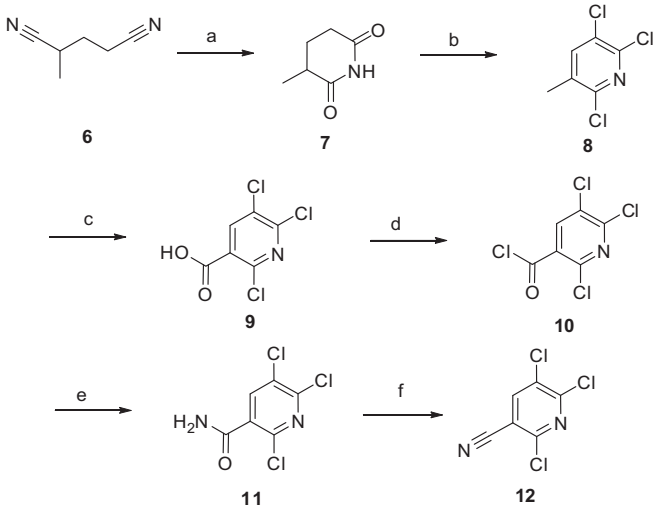
Scheme 1. Preparation of 6-aminopyrazolyl-pyridine-3-carbonitriles. Reagents and conditions: (a) Et_3N, CH_3CN or THF, room temperature to 80 °C; (b) DIPEA, *n*-BuOH, 150 °C, microwave.

Replacement of the phenyl ring with a 2-pyridyl group generated compounds **1d–e** that had equivalent activities at enzymatic and cellular levels as compared to **1c** (Table 1). A compound with a 5-chloro substituent was as potent as the 5-fluoro analogue (**1e** vs **d**).

Compounds depicted in Table 1 were prepared via the general synthetic sequence shown in Scheme 1. Commercially available 2,6-dihalo-pyridine-3-carbonitriles **2** were reacted with pyrazol-3-ylamines **3** in the presence of a base to give intermediates **4**. Nucleophilic aromatic substitutions of **4** with amines **5** (usually as the hydrochloride salts) were achieved by heating under basic conditions in microwave reactors to provide the target compounds **1a–e**.

For the 5-chloropyridine core that was not readily available, synthesis commenced from 2-methylpentanedinitrile **6**. As shown in Scheme 2, hydrolysis of **6** gave piperidine-dione **7** that was then chlorinated to provide the corresponding 2,3,6-trichloropyridine **8**. Oxidation of the methyl group on **8** generated acid **9**, which was subsequently transformed to nitrile **12** via acid chloride **10** and amide **11** intermediates.

To further investigate the overall kinase selectivity of the 6-aminopyrazolyl-pyridine-3-carbonitrile scaffold, compound **1d**



Scheme 2. Preparation of 2,5,6-trichloropyridine-3-carbonitrile. Reagents and conditions: (a) H_2SO_4, AcOH; (b) PCl_5, 150 °C; (c) $KMnO_4$, 2 days, HCl; (d) oxalyl chloride, DCM; (e) NH_4OH, dioxane, 0 °C; (f) $POCl_3$, 90 °C.

Table 2
Kinase selectivity data for compound **1d**[a]

Kinase	IC_{50} (μM)	Kinase	IC_{50} (μM)
JAK2	<0.001	CDK2/cyclinE	0.174
TrkA	0.003	Lck	0.215
JAK3	0.015	MuSK	0.224
Ret	0.019	EGFR	>10
Flt3 (D835Y)	0.082	EphB2	>10
FGFR3	0.115	InsR	>10
Pyk2	0.129		

[a] All kinases above are of human sequence.

was screened against a selected group of kinases.[27] As the data in Table 2 indicates, **1d** has >170-fold selectivity against kinases such as CDK2 and EGFR. Over 15-fold selectivity against JAK3 was again observed. TrkA kinase was inhibited in this assay; however, in a cellular context, the inhibitory activity of **1d** against TrkA (MCF-10A TrkAΔ)[28] was found to be 0.276 μM. For those kinases whose IC_{50}'s fall below 100 nM, it is difficult to extrapolate the selectivity due to the inhibition of JAK2 enzyme below the detection limit of the assay. The kinase selectivity of **1c** has been previously reported,[25] and **1c** and **d** shared some common kinase hits such as FGFR and Flt family members. They also revealed some differences in that **1d** inhibited, for example, JAK3 and Ret, while **1c** instead, inhibited Aurora A, Ark and ALK.

Having achieved sub-100 nM in vitro enzyme activity and cell potency against JAK2, we then evaluated the pharmacokinetic properties of the lead compounds **1c–e**. As shown in Table 3, these compounds were found to have less than 7.5 mL/min/kg for in vivo clearances and between 2 and 6 h for half-lives in rats when dosed intravenously. A rough correspondence between rat microsomal and in vivo clearance can be seen in Table 3, suggesting that the in vivo metabolism of these compounds is mainly cytochrome P450-mediated, at least in rats. Furthermore, these analogues were orally bioavailable in rats (39–47%). In a dog pharmacokinetic study, compound **1d** had an in vivo clearance of 7.4 mL/min/kg, a half life of 3.6 h and was 100% orally bioavailable. Regarding their physical properties, compounds **1d** and **e** exhibited aqueous solubilities of 303 and 100 μM, respectively, as compared to 13 μM for **1c**.[29] The difference of aqueous solubilities could be attributed

Table 3
Rat microsomal stability and rat pharmacokinetic profile of lead compounds[a]

Compd	Clint (μL/min/mg)	CL (mL/min/kg)	$T_{1/2}$ (h)	V_{dss} (L/kg)	F (%)
1c	17	7.5	5.7	2.6	44
1d	5	4.8	2.2	0.84	39
1e	5	2.3	2.7	0.52	47

[a] Han Wistar rat male; 10 mg/kg po (0.1% HPMC); 3 mg/kg iv (DMA/PEG/saline = 40:40:20).

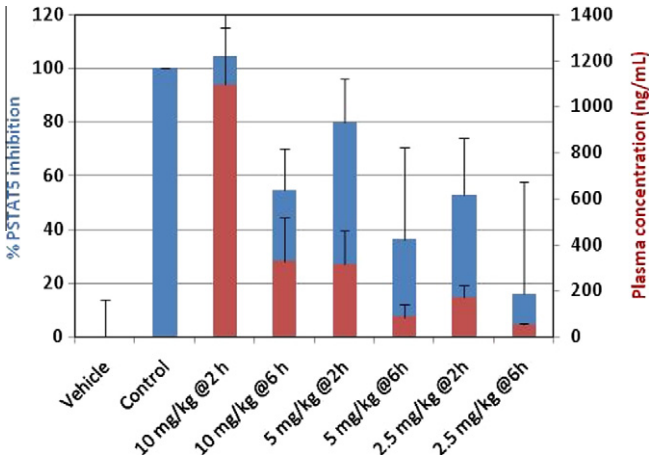


Figure 2. Pharmacodynamic effect of compound **1d** on PSTAT5 inhibition (blue) in the Ba/F3 TEL-JAK2 mouse model and relationship with plasma pharmacokinetics (concentration shown in red bars). PSTAT5 inhibitions (n = 3 for each dose and each time point) are calculated using vehicle and control inhibitor for maximum and minimum value estimation. Mice used in these studies were maintained under specific pathogen-free conditions and were used in compliance with protocols approved by the Institutional Animal Care and Use Committees of AstraZeneca, which conform to institutional and national regulatory standards on experimental animal usage.

to the lower lipophilicity of a pyridine ring (in **1d** and **e**) compared to a phenyl group (in **1c**).

The pharmacokinetic profile of the lead compounds prompted us to investigate the pharmacodynamic effects of **1d** in splenic tissues of nude mice bearing TEL-JAK2 transfected Ba/F3 cells. As shown in Figure 2, three different doses (10, 5 and 2.5 mg/kg) were given and the percentage (%) inhibition of STAT5 phosphorylation (PSTAT5) was determined at 2 and 6 h post dose. STAT5 is the downstream target of JAK2 and an essential component in the JAK-STAT signaling pathway. After 10 mg/kg oral dose, compound **1d** showed complete inhibition of STAT5 phosphorylation for at least 2 h which decreased to 54% at 6 h. Similar trends were observed at the two lower doses (5 and 2.5 mg/kg). When different doses were compared at 2 h time point, the PSTAT5 inhibitions exhibited a dose-dependent pattern. The plasma concentrations of the drug correlated roughly with the PSTAT5 inhibition level in this study (Fig. 2). For example, a 10 mg/kg oral dose resulted in a 1096 ng/mL total plasma concentration of the compound at 2 h, decreasing to 328 ng/mL at 6 h. For simplicity, it is assumed here that the mouse free unbound level is similar to that measured in human plasma (fu = 5.3%) and the cellular proliferation GIC_{50} tracks the PSTAT5 EC_{50} (data not shown), the free drug concentration at 6 hours time point with this dose would be 0.049 μM, still above its cellular GIC_{50} (0.036 μM). This data suggested that the 10 mg/kg dose under an optimized dosing schedule would provide adequate drug coverage to test the therapeutic potential of JAK2 inhibition in mice.

As discussed previously, JAK2 V617F is the causative mutation for a major population of MPNs, and it is therefore interesting to see whether these compounds are efficacious in a cell line that carries such a mutation. Compound **1c** was tested in a SET-2 cell line, which is heterozygous to JAK2 V617F mutation, and did show growth inhibition (GI_{50} = 0.033 μM).[25] The PK and PD coupled with the cellular potency shown above suggested that compounds such as **1c** (or **1d**) might be useful probe compounds in disease models with this key mutation.

In summary, a series of 6-aminopyrazolyl-pyridine-3-carbonitriles has been evaluated as JAK2 kinase inhibitors. Compounds such as **1c**–**e** offered the potential to test the modulation of JAK2 kinase activity in vivo.

Acknowledgments

The authors would like to thank Ethan Hoffmann for PK studies, David Ayres for PK sample analysis and Array BioPharma team (John Josey, Yongxin Han, Bin Wang and Peter Mohr) for synthetic support. Special thanks to Susan Ashwell and Dennis Huszar for useful recommendations during the preparation of this letter.

References and notes

1. Campbell, P. J.; Green, A. R. *N. Eng. J. Med.* **2006**, *355*, 2452.
2. Morgan, K. J.; Gilliland, D. G. *Annu. Rev. Med.* **2008**, *59*, 213.
3. James, C.; Ugo, V.; Le Couedic, J. P.; Staerk, J.; Delhommeau, F.; Lacout, C.; Garcon, L.; Raslova, H.; Berger, R.; Bennacaur-Griscelli, A.; Villeval, J. L.; Constantinescu, S. N.; Casadevall, N.; Vainchenker, W. *Nature* **2005**, *434*, 1144.
4. Baxter, E. J.; Scott, L. M.; Campbell, P. J.; East, C.; Fourouclas, N.; Swanton, S.; Vassiliou, G. S.; Bench, A. J.; Boyd, E. M.; Curtin, N.; Scott, M. A.; Erber, W. N.; Green, A. R. *Lancet* **2005**, *365*, 1054.
5. Levine, R. L.; Wadleigh, M.; Cools, J.; Ebert, B. L.; Wernig, G.; Huntly, B. J. P.; Boggon, T. J.; Wlodarska, I.; Clark, J. J.; Moore, S.; Adelsperger, J.; Koo, S.; Lee, J. C.; Gabriel, S.; Mercher, T.; D'Andrea, A.; Fröhling, S.; Döhner, K.; Marynen, P.; Vandenberghe, P.; Mesa, R. A.; Tefferi, A.; Griffin, J. D.; Eck, M. J.; Sellers, W. R.; Meyerson, M.; Golub, T. R.; Lee, S. J.; Gilliland, D. G. *Cancer Cell* **2005**, *7*, 387.
6. Rane, S. G.; Reddy, E. P. *Oncogene* **2000**, *19*, 5662.
7. Vainchenker, W.; Constantinescu, S. N. *Hematol. Am. Soc. Hematol. Educ. Program* **2005**, 195–200.
8. Tefferi, A.; Gilliland, D. G. *Cell Cycle* **2005**, *4*, 1053.
9. Tefferi, A.; Vardiman, J. W. *Leukemia* **2008**, *22*, 14.
10. Nelson, M. E.; David, P.; Steensma, D. P. *Leukemia Lymphoma* **2006**, *47*, 177.
11. Thompson, J. E.; Cubbon, R. M.; Cummings, R. T.; Wicker, L. S.; Frankshun, R.; Cunningham, B. R.; Cameron, P. M.; Meinke, P. T.; Liverton, N.; Weng, Y.; DeMartino, J. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1219.
12. Hexner, E. O.; Serdikoff, C.; Jan, M.; Swider, C. R.; Robinson, C.; Yang, S.; Angeles, T.; Emerson, S. G.; Carroll, M.; Ruggeri, B.; Dobrzanski, P. *Blood* **2008**, *111*, 5663.
13. Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri, P.; Davis, M. I.; Edeen, P. T.; Faraoni, R.; Floyd, M.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P. *Nat. Biotechnol.* **2008**, *26*, 127.
14. Manshouri, T.; Quintas-Cardama, A.; Nussenzveig, R. H.; Gaikwad, A.; Estrov, Z.; Prchal, J.; Cortes, J. E.; Kantarjian, H. M.; Verstovsek, S. *Cancer Sci.* **2008**, *99*, 1265.
15. Purandare, A. V.; Lorenzi, M. V.; Lombardo, L. J. *Annu. Rep. Med. Chem.* **2010**, *45*, 211.
16. Verstovsek, S.; Pardanani, A. D.; Shah, N. P.; Sokol, L.; Wadleigh, M.; Gilliland, D. G.; List, A. F.; Tefferi, A.; Kantarjian, H. M.; Bui, L. A.; Clary, D. O. *Blood* (ASH Annual Meeting Abstracts) **2007**, *110*, Abst. 553.
17. Geron, I.; Abrahamsson, A. E.; Barroga, C. F.; Kavalerchik, E.; Gotlib, J.; Hood, J. D.; Durocher, J.; Mak, C. C.; Noronha, G.; Soll, R. M.; Tefferi, A.; Kaushansky, K.; Catriona, H. M.; Jamieson, C. H. M. *Cancer Cell* **2008**, *13*, 321.
18. Pissot-Soldermann, C.; Gerspacher, M.; Furet, P.; Gaul, C.; Holzer, P.; McCarthy, C.; Radimerski, T.; Regnier, C. H.; Baffert, F.; Drueckes, P.; Tavares, G. A.; Vangrevelinghe, E.; Blasco, F.; Ottaviani, G.; Ossola, F.; Scesa, J.; Reetz, J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2609.
19. Verstovsek, S. *Clin. Cancer Res.* **2010**, *16*, 1988.
20. Kiss, R.; Sayeski, P. P.; Keseru, G. M. *Expert Opin. Ther. Patents* **2010**, *20*, 471.
21. Ruben, A.; Mesa, R. A.; Tefferi, A. *Expert Opin. Emerging Drugs* **2009**, *14*, 471.
22. Ioannidis, S.; Lamb, M. L.; Davies, A. M.; Almeida, L.; Su, M.; Bebernitz, G.; Ye, M.; Bell, K.; Alimzhanov, M.; Zinda, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6524.
23. Ioannidis, S.; Lamb, M. L.; Almeida, L.; Guan, H.; Peng, B.; Bebernitz, G.; Bell, K.; Alimzhanov, M.; Zinda, M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1669.
24. Ioannidis, S.; Lamb, M. L.; Wang, T.; Almeida, L.; Block, M. H.; Davies, A. M.; Peng, B.; Su, M.; Zhang, H.-J.; Hoffmann, E.; Rivard, C.; Green, I.; Howard, T.; Pollard, H.; Read, J.; Alimzhanov, M.; Bebernitz, G.; Bell, K.; Ye, M.; Huszar, D.; Zinda, M. *J. Med. Chem.* **2011**, *54*, 262.

25. Gozgit, J. M.; Bebernitz, G.; Patil, P.; Ye, M.; Wu, J.; Su, N.; Wang, T.; Ioannidis, S.; Davies, A. M.; Huszar, D.; Zinda, M. *J. Biol. Chem.* **2008**, *283*, 32334.
26. Yang, J.; Ikezoe, T.; Nishioka, C.; Furihata, M.; Yokoyama, A. *Mol. Cancer Ther.* **2010**, *9*, 3386.
27. http://www.millipore.com/drugdiscovery/dd3/KinaseProfiler.
28. Thress, K.; MacIntyre, T.; Wang, H.; Whitston, D.; Liu, Z.-Y.; Hoffmann, E.; Wang, T.; Brown, J. L.; Webster, K.; Omer, C.; Zage, P. E.; Zeng, L.; Zweidler-McKay, P. A. *Mol. Cancer Ther.* **2009**, *8*, 1818.
29. Solubility measurement described in Ref. 24