Bioorganic & Medicinal Chemistry Letters 19 (2009) 5887–5892

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Phenylaminopyrimidines as inhibitors of Janus kinases (JAKs)

Christopher J. Burns [a], David G. Bourke [a,*], Laura Andrau [a], Xianyong Bu [a], Susan A. Charman [b], Andrew C. Donohue [a], Emmanuelle Fantino [a], Michelle Farrugia [a], John T. Feutrill [a], Max Joffe [a], Marcel R. Kling [a], Margarita Kurek [a], Tracy L. Nero [a], Thao Nguyen [a], James T. Palmer [a], Ian Phillips [a], David M. Shackleford [b], Harrison Sikanyika [a], Michelle Styles [a], Stephen Su [a], Herbert Treutlein [a], Jun Zeng [a], Andrew F. Wilks [a]

[a] Cytopia Research Pty Ltd, 576 Swan St, Richmond, VIC 3121, Australia
[b] Centre for Drug Candidate Optimisation, Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, 381 Royal Pde, Parkville, VIC 3052, Australia

ARTICLE INFO

Article history:
Received 10 June 2009
Revised 19 August 2009
Accepted 20 August 2009
Available online 23 August 2009

Keywords:
JAK2
Kinase
Inhibitor
Janus
CYT387
Myeloproliferative

ABSTRACT

A series of phenylaminopyrimidines has been identified as inhibitors of Janus kinases (JAKs). Development of this initial series led to the potent JAK2/JAK1 inhibitor CYT387 (*N*-(cyanomethyl)-4-[2-[[4-(4-morpholinyl)phenyl]amino]-4-pyrimidinyl]-benzamide). Details of synthesis and SAR studies of these compounds are reported.

The Janus kinases (JAKs), consisting of JAK1, JAK2, JAK3, and TYK2, are an important family of cytoplasmic tyrosine kinases as a consequence of their essential role in cytokine signal transduction.[1] Association of individual JAK proteins to activated cytokine receptors leads to autophosphorylation and subsequent phosphorylation of specific STAT (Signal Transducer and Activation of Transcription) proteins. The phosphorylated STAT proteins then dimerize and translocate to the cell nucleus, which leads to DNA transcription. Overactivation of JAK-STAT signaling, through genetic mutations or increased localized concentration of cytokines, has been identified in various inflammatory diseases and in a variety of cancers.[2] The discovery that a constitutively activating mutation in JAK2 (V617F) is central to the pathogenesis of myeloproliferative disorders (MPDs), including Polycythemia Vera[3] (PV), has accelerated the search for JAK2 inhibitors for the treatment of these and other diseases.[4]

Screening of Cytopia's internal kinase-focused compound library against isolated JAK2 enzyme and a JAK2 dependent engineered cell line (Baf3TEL-JAK2)[5,6] led to the identification of several sub-micromolar hits of the *N*-(4-morpholinophenyl)-4-arylpyrimidin-2-amine class. Table 1 illustrates examples of this chemotype with corresponding biochemical and cellular potency.

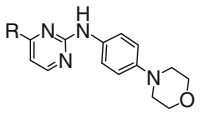
Table 1 indicates that compounds possessing an H-bond donor in the *para*-position showed a greater potency against JAK2. A model (Fig. 1) of the most active inhibitor (compound **12**) in the ATP binding site of the JAK2 enzyme, shows that the 2-amino NH and pyrimidine N1 of the compound most likely form H-bond interactions with the hinge region of JAK2 (Leu932). In the modeled binding mode, the *para*-hydroxy substituent of the 4-phenyl ring is located in a pocket formed by Lys857 to Ser862 of the Gly-rich loop, Gly993 and Asp994 of the DFG motif and residue Asn981. There are a number of potential interaction sites within 7 Å of the 4-phenyl *para*-position, including Asp994, which is within H-bond distance of the *para*-hydroxy group. The modeled binding mode of compound **12** suggested that larger 4-phenyl *para*-substituents would be accommodated within this pocket, under the Gly-rich loop, and allow exploration of additional interaction sites. Accommodation of larger *para*-substituents in this binding pocket is consistent with the data presented in Table 1 (compounds **5**, **7**, and **8**). However, the decreased inhibition of JAK2 for these compared to compound **12** indicated an opportunity for further optimization of this substituent.

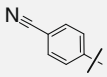
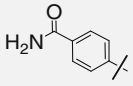
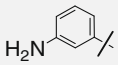
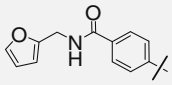
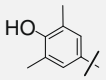
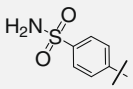
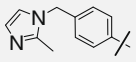
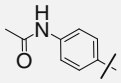
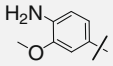
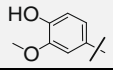
As phenols are known to be rapidly glucuronidated in vivo, the isosteric replacement of phenol with sulfonamide led us to explore

* Corresponding author. Tel.: +61 3 9208 4243; fax: +61 3 9208 4299.
E-mail addresses: cytopia@cytopia.com.au, bourkda213@hotmail.com (D.G. Bourke).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.08.071

Table 1
SAR about the 4-aryl substituent



Compd	R	JAK2 IC_{50} (nM)	Baf3TEL-JAK2 IC_{50} (nM)
1		533	>20,000
2		467	ND
3		326	5559
4		322	8518
5		202	5220
6		88	3525
7		83	ND
8		81	3730
9		67	3110
10		33	7134
11		16	5624
12		3	380

ND—not determined.

a series of *N*-phenylmethanesulfonamide analogues (Table 2). We prepared this series[8] via a two-step procedure (Scheme 1); first, a regioselective Suzuki reaction[9], followed by aniline displacement under acidic conditions[10] (Scheme 1, path b) or a palladium catalyzed Buchwald reaction[11] (Scheme 1, path c). The route taken for each example was dependent on the basicity of the substituent on the aniline used; anilines with weakly basic groups were preferably condensed with the 2-chloropyrimidine under acidic conditions. The modeled binding mode of compound **12** (Fig. 1) suggested that substitution in the 3- or 4-position of the *N*-phenyl ring would be tolerated and solvent exposed; consequently, we investigated substitutions at both positions.

We assessed the sulfonamide series for JAK2 inhibition and also for selectivity against the homologous JAK3 enzyme, as JAK3 inhibition has the functional consequence of immune supression.[2] Table 2 indicates biochemical potency for the most potent members of this series, along with inhibition of proliferation of both a JAK3 dependent cell line (Baf3TEL-JAK3) and a JAK1/JAK3 dependent cell line (IL-2 stimulated CTLL-2).[5] As a measure of functional

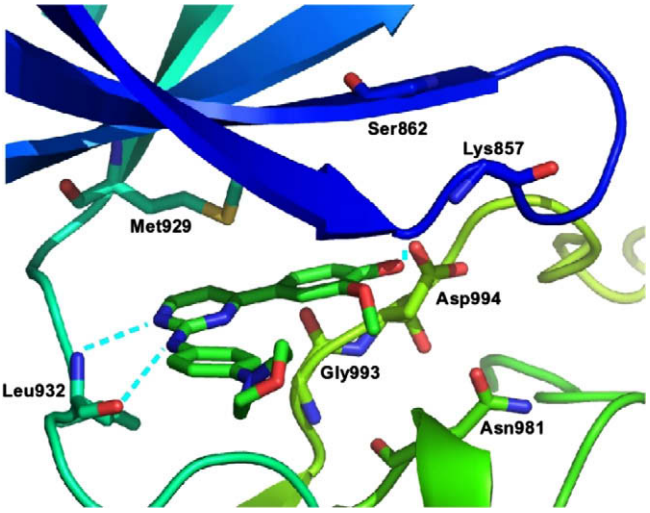


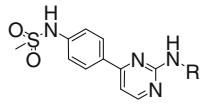
Figure 1. Model of compound **12** in the ATP binding site of JAK2.[7]

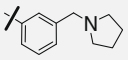
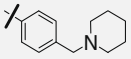
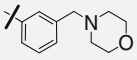
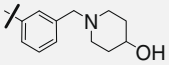
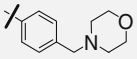
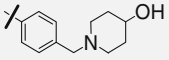
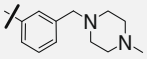
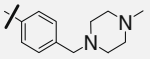
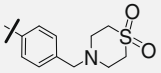
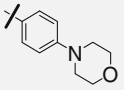
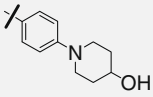
selectivity, Table 2 ranks compounds by decreasing inhibition of the Baf3TEL-JAK3 cell line. Comparative data is included for CP-690,550, a JAK inhibitor currently in clinical trials for transplant rejection.[12] Both 3- and 4-substituted anilines are tolerated and appear to afford a similar degree of selectivity over JAK3. While biochemical selectivity and potency values for the sulfonamides were promising as compared to compound **12**, they were not as profound in measures of corresponding cellular selectivity, suggesting possible non-JAK3-related activity in the Baf3TEL-JAK3 and CTLL-2-driven assays.

Despite slightly reduced cellular selectivity we selected compound **21** for further investigation. Preliminary pharmacokinetic profiling of compound **21** in male Sprague Dawley rats (21 mg/kg) indicated a low C_{max} (0.27 μM) and a low absolute oral bioavailability of 5.4%. The total blood clearance after IV administration was determined to be 36.6 mL/min/kg. As this value is only 66% of the nominal value for hepatic blood flow in the rat (i.e., 55.2 mL/min/kg),[13] it is unlikely that hepatic first pass elimination is the only contributor to the low oral bioavailability. Another potential factor limiting the oral bioavailability of compound **21** may be poor absorption (vide infra).

As compound **21** displayed low oral bioavailability we chose to investigate other phenyl substitutions. From our initial screening hit panel, carboxamides attached to the 4-phenyl ring (Table 1, compounds **3**, **5**, and **9**) also were active. In combination with the carboxamide as an H-bond donor, we chose a nitrile group as a lipophilic probe with the potential to act as an H-bond acceptor. A series of compounds with this functionality allowed us to investigate the same binding pocket that the phenol of compound **12** and the sulfonamide of compound **21** occupy. We accessed this series in an analogous manner to that shown in Scheme 1.[5] Table 3 illustrates the progression of compounds from this series. Reversal of the carboxamide orientation (compound **25**) or removal of the carbonyl itself (compound **26**) did not significantly change binding versus the *meta*-substituted example (compound **24**). The preferred regiochemical substitution placed the cyanomethylamide in the *para*-position (**27** and **28**). Substitution *ortho* to the cyanomethylamide of compound **28** (compounds **29** and **30**) or methylation on this group (compounds **31** and **32**) reduced potency toward JAK2. Finally we modified the pyrimidine ring at C5 to explore the effect of this substitution, as there appears to be available space in the JAK2 ATP binding site (Fig. 1). The

Table 2
SAR of the *N*-phenylmethanesulfonamide series



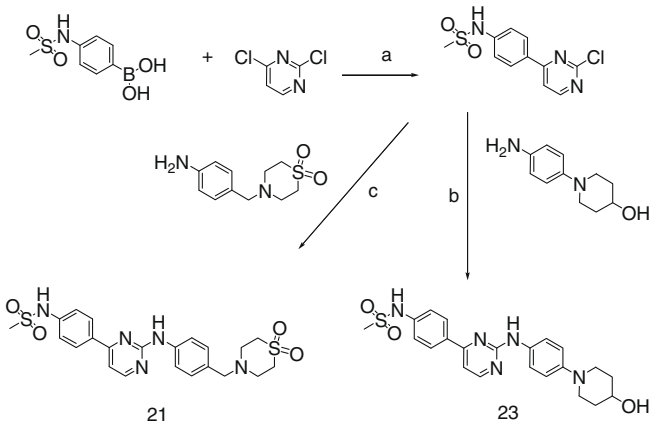
Compd	R	JAK2 IC_{50} (nM)	JAK3 IC_{50} (nM)	Baf3TEL-JAK2 IC_{50} (nM)	Baf3TEL-JAK3 IC_{50} (nM)	CTLL-2 IC_{50} (nM)
CP-690,550	NA	33	5	1574	570	132
12	NA	3	62	380	2553	<160
13		6	110	234	240	569
14		11	538	580	730	735
15		8	930*	812	1730	2663
16		12	636	771	1944	6764
17		9	>1000*	896	1944	1669
18		16	1619	892	2196	5607
19		14	960*	941	3143	5177
20		18	>1000*	1002	3433	1552
21		2	587	802	3557	2220
22		4	351	396	3945	489
23		6	149	933	6551	1487

* Data is from a 3-point screen (5, 1, and 0.2 μM).

5-methylpyrimidine (compound **33**) retained JAK2 potency but showed lower functional selectivity, relative to compound **28**, measured in the CTLL-2 assay. In contrast, the 5-methoxypyrimidine (compound **34**) reduced potency toward JAK2 by 10-fold, indicating a size limit for substitution at this position.

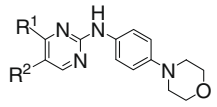
Further modification of compound **28** allowed us to investigate the effect of replacement of the morpholinoaniline moiety. Table 4 shows analogues of compound **28** wherein some compounds bearing a more basic amine group (compounds **36** and **37**) are 10-fold less potent against JAK2. This is in contrast to data from the earlier series (Table 2, compound **17**) where the different electronics of the sulfonamide series may be a contributing factor. Attachment of electron-withdrawing groups *ortho* to the morpholine group (compounds **35 and 38)** also cost biochemical or JAK2-driven cellular potency. Likewise, analogues of compound **28** with other *N*-phenyl substituent's did not afford an increase in JAK2 biochemical potency. Therefore, we considered the profile of compound **28** to be sufficiently optimized to proceed into pharmacokinetic studies.

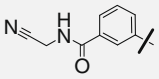
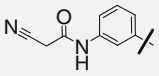
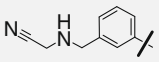
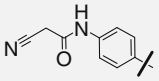
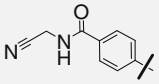
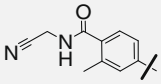
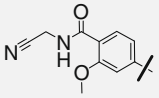
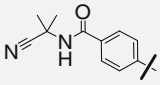
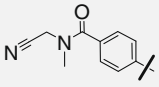
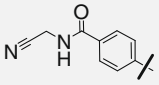
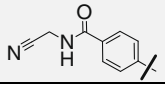
We first assessed the permeability of compounds **28** and **21** across Caco-2 cell monolayers, as a model of human intestinal permeability (Table 5).[14] Compound **21** displayed directional non-uniform permeability across the monolayer, suggestive of efflux. This finding may in part explain the measured absolute oral bioavailability for compound **21** of only 5.4%. In contrast, compound **28** displayed similar apparent permeability values in both directions, indicating the compound was not a substrate for efflux. The pharmacokinetic properties of compound **28**, following both IV and oral



Scheme 1. (a) Toluene/*n*-PrOH, $Pd(PPh_3)_4$, 2 M aq Na_2CO_3, 100 °C (61%); (b) $TsOH{\cdot}H_2O$, dioxane, reflux (38%); (c) DME, $Pd_2(dba)_3$, K_3PO_4, (*o*-biphenyl)$P(t\text{-}Bu)_2$, 100 °C (42%).

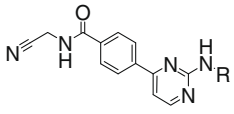
Table 3
SAR of the nitrile carboxamide series



Compd	R^1	R^2	JAK2 IC_{50} (nM)	JAK3 IC_{50} (nM)	Baf3TEL-JAK2 IC_{50} (nM)	Baf3TEL-JAK3 IC_{50} (nM)	CTLL-2 IC_{50} (nM)
24		H	545	>5000*	>20,000	>20,000	>20,000
25		H	303	1760	10,347	>20,000	18,891
26		H	132	>1000*	9824	>20,000	8031
27		H	10	66	720	4205	915
28		H	18	155	798	2425	1249
29		H	65	>1000*	2631	7567	2043
30		H	30	9	205	275	245
31		H	549	3910	3725	4250	ND
32		H	298	1418	6340	16,880	4170
33		Me	8	85	433	5959	240
34		OMe	186	2310	8505	>20,000	6160

* Data is from a 3-point screen (5, 1 and 0.2 μM); ND—not determined.

Table 4
SAR of the *N*-cyanomethylbenzamide series



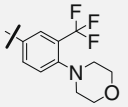
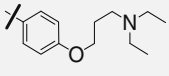
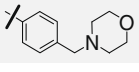
Compd	R	JAK2 IC_{50} (nM)	JAK3 IC_{50} (nM)	Baf3TEL-JAK2 IC_{50} (nM)	Baf3TEL-JAK3 IC_{50} (nM)	CTLL-2 IC_{50} (nM)
35		374	2020	1237	1709	3993
36		166	362	840	1105	3675
37		163	1450	2805	9505	5070

Table 4 (*continued*)

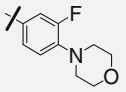
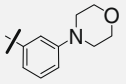
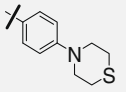
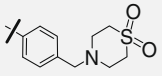
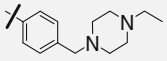
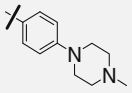
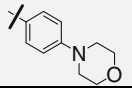
Compd	R	JAK2 IC_{50} (nM)	JAK3 IC_{50} (nM)	Baf3TEL-JAK2 IC_{50} (nM)	Baf3TEL-JAK3 IC_{50} (nM)	CTLL-2 IC_{50} (nM)
38		116	274	1143	3280	1265
39		113	1876	2565	7450	3770
40		69	364	2205	6245	1510
41		52	364	2045	9975	7895
42		47	152	423	1230	1852
43		41	192	525	2050	1832
28		18	155	798	2425	1249

Table 5
Apparent permeability (P_{app}) values

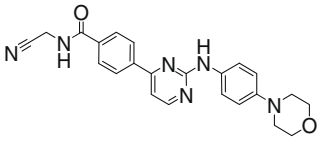
Compound	P_{app} $A-B$ ($\times 10^6$ cm/s)	P_{app} $B-A$ ($\times 10^6$ cm/s)	Ratio
Mannitol	0.7	1.1	1.5
Propranolol	38.7	48.8	1.3
21	4.6	56.4	12.3
28	30.4	27.3	0.9

administration to male Sprague Dawley rats (2.5 and 18 mg/kg, respectively) were assessed. After oral dosing, compound **28** exhibited high plasma concentrations (C_{max} = 40.4 μM; T_{max} = 4 h), with quantitative absolute oral bioavailability and an apparent half life of 2.4 h. The high oral bioavailability, can likely be partly ascribed to the low blood clearance of compound **28** (6.3 mL/min/kg) and therefore low susceptibility to hepatic first pass metabolism.

Kinase profiling demonstrated, that in addition to its JAK2 activity, compound **28** is also an inhibitor of JAK1 (IC_{50} = 11 nM). Potent inhibition of both JAK1 and JAK2 is a common feature of other JAK2 inhibitors currently in clinical development.[15] Compound **28** displayed little activity against a broader panel of other kinases with only 2 of >150 kinases showing an IC_{50} < 100 nM.[15b] Furthermore, we investigated inhibition of the clinically relevant mutant JAK2 enzyme (V617F) in biochemical assays and cellular screens (Table 6). Compound **28** was equipotent on the mutant JAK2 enzyme and inhibited proliferation of cell lines dependant on wild type JAK2 (BaF3 wt) or JAK2V617F (SET-2 and HEL 92.1.7) but did not affect a cell line lacking such a dependence (K562).

Compound **28** (CYT387) possesses excellent pharmaceutical and pharmacokinetic properties. In addition CYT387 induced a profound reversal of symptoms in a murine model of MPDs involving transplantation of JAK2V617F-transduced bone marrow[16] and inhibited the growth of erythropoietin-independent erythroid colonies from PV patients.[15a] The data obtained from these and other preclinical studies supports progression of CYT387 into the clinic for the treatment of diseases associated with aberrant JAK activity, such as MPDs.

Table 6
Compound **28** (CYT387) inhibition of V617F mutant JAK2 enzyme and associated cellular proliferation



Enzyme or cell	IC_{50} (nM)
JAK2V617F (JH1-JH2)	11
BaF3 wt (+IL-3)	1430
SET-2	232
HEL 92.1.7	1804
K562	>20,000

References and notes

1. Rane, S. G.; Reddy, E. P. *Oncogene* **2000**, *19*, 5662.
2. (a) Aringer, M.; Cheng, A.; Nelson, J. W.; Chen, M.; Sudarshan, C.; Zhou, Y. J.; O'Shea, J. J. *Life Sci.* **1999**, *64*, 2173; (b) Schindler, C. W. *J. Clin. Invest.* **2002**, *109*, 1133.
3. Tefferi, A. *Leukemia Lymphoma* **2008**, *49*, 388.
4. Pardanani, A. *Leukemia* **2008**, *22*, 23.
5. Enzyme screening was performed using GST-tagged kinase domain in an AlphaScreen™ assay with [ATP] = 80 μM. Cell-based proliferation assays were run over 72 h with Alamar Blue staining. Dose response measurements were performed using 1:2 serial dilutions 8-point from 20 μM for cell-based assays and 8, 12 or 16-point from 5 μM for enzyme assays. All enzyme and cell data are n = 1 or average of duplicates or average of independent n = 2, except for CP-690,550 and compound **28** where n > 20. Acceptable variances for duplicates or n > 1 are values within one dilution point. For a description of biochemical and cellular assay conditions, along with synthetic examples, see: Burns, C. J.; Donohue, A. C.; Feutrill, J. T.; Nguyen, T. L. T.; Wilks, A. F.; Zeng, J., Phenyl Amino Pyrimidine Compounds and Uses Thereof, WO2008109943, CAN 149:402373.

6. Lacronique, V.; Boureux, A.; Monni, R.; Dumon, S.; Mauchauffe, M.; Mayeux, P.; Gouilleux, F.; Berger, R.; Gisselbrecht, S.; Ghysdael, J.; Bernard, O. A. *Blood* **2000**, *95*, 2076.
7. a The bound ligand and water molecules were removed from the JAK2 crystal structure (PDB code 2B7A).[7b] Docking of the inhibitor into the JAK2 ATP binding site was performed with Cytopia's 'structure based drug design' in-house suite of programs, Chemaphore™ (http://www.cytopia.com.au/chemaphore.html). Fig. 1 was created using Pymol (version 1.2, DeLano Scientific, Palo Alto, California, USA. http://www.pymol.org).; (b) Lucet, I. S.; Fantino, E.; Styles, M.; Bamert, R.; Patel, O.; Broughton, S. E.; Walter, M.; Burns, C. J.; Treutlein, H.; Wilks, A. F.; Rossjohn, J. *Blood* **2006**, *107*, 176.
8. All compounds were characterized by ^{1}H NMR and reverse phase LCMS and their purity determined to be $\geqslant$90% except for compounds **8**, **27**, **30**, **34**, and **42** which were $\geqslant$85%.
9. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
10. Bursavich, M. G.; Lombardi, S.; Gilbert, A. M. *Org. Lett.* **2005**, *7*, 4113.
11. Wolfe, J. P.; Tomori, H.; Sadighi, J. P.; Yin, J.; Buchwald, S. L. *J. Org. Chem.* **2000**, *65*, 1158.
12. Sorbera, L. A.; Serradell, N.; Bolos, J.; Rosa, E.; Bozzo, J. *Drugs Future* **2007**, *32*, 674.
13. Davies, B.; Morris, T. *Pharm. Res.* **1993**, *10*, 1093.
14. Artursson, P.; Palm, K.; Luthman, K. *Adv. Drug Delivery Rev.* **2001**, *46*, 27.
15. (a) Pardanani, A.; Lasho, T.; Smith, G.; Burns, C. J.; Fantino, E.; Tefferi, A. *Leukemia* **2009**, *23*, 1441; (b) Fantino, E.; Burns, C. J.; Stevenson, W. S.; Joffe, M.; Kurek, M.; Dubljevic, V.; Patel, O.; Lucet, I. S.; Rossjohn, J.; Farrugia, M.; Averett, D. R.; Court, N.; Wilks, A. F., submitted for publication.
16. Bumm, T. G. P.; Tyner, J. W.; Deininger, J.; Loriaux, M.; VanDyke, J.; Druker, B. J.; Burns, C. J.; Fantino, E.; Deininger, M. W. N. Abstract of Papers, 50th American Society of Hematology Annual Meeting and Exposition, San Francisco, CA, December 2008; abstract 856.