Bioorganic & Medicinal Chemistry Letters 20 (2010) 1724–1727

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

# 2-Amino-aryl-7-aryl-benzoxazoles as potent, selective and orally available JAK2 inhibitors

Marc Gerspacher *, Pascal Furet, Carole Pissot-Soldermann, Christoph Gaul, Philipp Holzer, Eric Vangrevelinghe, Marc Lang, Dirk Erdmann, Thomas Radimerski, Catherine H. Regnier, Patrick Chene, Alain De Pover, Francesco Hofmann, Fabienne Baffert, Thomas Buhl, Reiner Aichholz, Francesca Blasco, Ralf Endres, Jörg Trappe, Peter Drueckes

*Novartis Institutes for BioMedical Research, Novartis Pharma AG, CH-4002 Basel, Switzerland*

ARTICLE INFO

*Article history:*
Received 18 December 2009
Revised 6 January 2010
Accepted 7 January 2010
Available online 21 January 2010

*Keywords:*
Janus kinase
JAK1
JAK2
JAK3
Tyk2
Kinase inhibitors
JAK2 inhibitor

ABSTRACT

A series of novel benzoxazole derivatives has been designed and shown to exhibit attractive JAK2 inhibitory profiles in biochemical and cellular assays, capable of delivering compounds with favorable PK properties in rats. Synthesis and structure–activity relationship data are also provided.



The four Janus kinases (JAK1, JAK2, JAK3, TYK2) are intracellular protein tyrosine kinases that have important roles in the mediation of cytokine receptor signaling.[1] The discovery of an acquired activating point mutation in the pseudokinase domain of JAK2 in a large proportion of patients suffering from chronic myeloproliferative neoplasms (cMPNs) has drawn a lot of attention to this kinase during recent years. For the first time there is a molecular understanding of the underlying disease mechanism and, equally of importance, the mutated JAK2 kinase represents a druggable target for therapeutic intervention. The JAK2 valine 617 to phenylalanine mutation ($JAK2^{V617F}$) is found in nearly every patient with polycythemia vera (PV) and in approximately every second patient suffering from essential thrombocythemia (ET) and myelofibrosis (PMF).[2] Patients typically present an increased hematocrit (PV), increased platelet counts (ET), splenomegaly (PV, PMF), and gradual loss of bone marrow function (PMF). It is hoped that targeting $JAK2^{V617F}$ with small molecule inhibitors will alleviate these symptoms.[3] Here, we report the discovery of benzoxazole derivatives as potent, selective as well as orally available JAK2 inhibitors.

Based on the structure of a pyrrolopyrimidine derivative **1**[4] which was identified as an inhibitor of JAK2 in a focused screen, application of several cycles of scaffold morphing, and lead optimization led to the discovery of a series of novel 2-amino-aryl-7-aryl-benzoxazoles, exhibiting surprisingly favorable JAK2 inhibitory and selectivity profiles (Fig. 1). A derivatisation program aiming at variations of the X and Y aryl/heteroaryl residues quickly showed that a variety of residues is tolerated for potency in JAK2 inhibition (Table 1). This was considered an advantage as it opened up the possibility to simultaneously optimize potency, selectivity and pharmacokinetic properties. Initially, the trimethoxy-phenyl residue at position 2 was kept constant to study the influence of

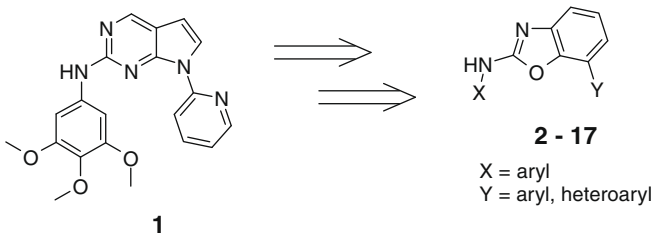
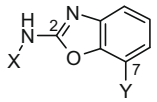


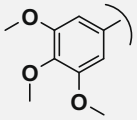
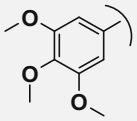
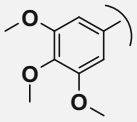
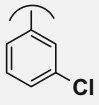
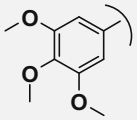
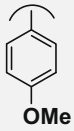
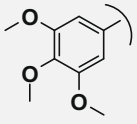
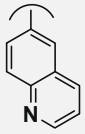
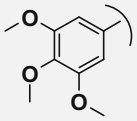
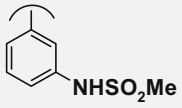
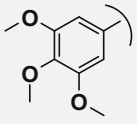
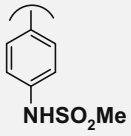
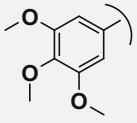
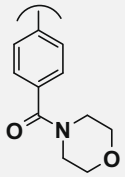
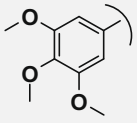
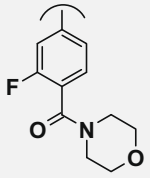
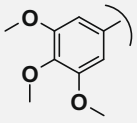
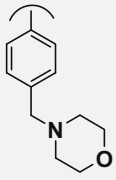
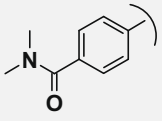
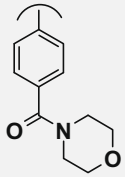

**Figure 1.** Discovery of benzoxazoles.

* Corresponding author. Tel.: +41 61 696 76 36; fax: +41 61 696 62 46.
*E-mail address:* marc.gerspacher@novartis.com (M. Gerspacher).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.01.069

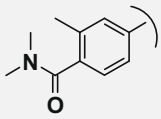
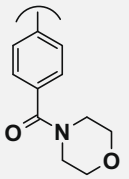
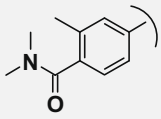
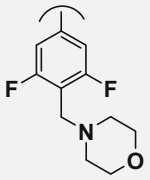
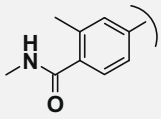
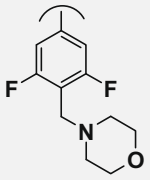
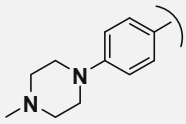
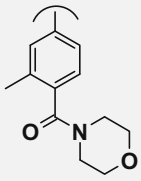
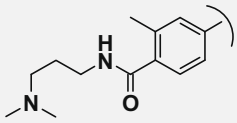
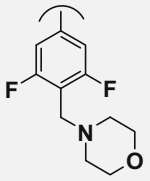
**Table 1**
Inhibition of JAK family kinases[5] and inhibition of cell proliferation in SET-2$^{V617F}$ cells[6] for compounds **2–17**



| Compd | X | Y | JAK2 inhibition $IC_{50}$ (μM) | JAK1 inhibition $IC_{50}$ (μM) | JAK3 inhibition $IC_{50}$ (μM) | TYK2 inhibition $IC_{50}$ (μM) | Inhibition of SET-2$^{V617F}$ proliferation (cellular assay), $GI_{50}$ (μM) |
|---|---|---|---|---|---|---|---|
| **2** |  |  | 0.11 | 0.76 | 0.45 | 0.64 | — |
| **3** |  |  | 0.015 | 0.71 | >10 | 3.4 | — |
| **4** |  |  | 0.44 | 5.3 | >10 | >10 | — |
| **5** |  |  | 0.84 | 8.9 | >10 | >10 | — |
| **6** |  |  | 0.008 | 0.21 | 0.67 | 1.4 | 0.50 |
| **7** |  |  | 0.15 | 0.68 | 2.0 | 3.6 | — |
| **8** |  |  | 0.005 | 0.047 | 0.13 | 0.15 | 0.13 |
| **9** |  |  | 0.008 | 0.0275 | 0.4 | 0.11 | 0.16 |
| **10** |  |  | <0.003 | 0.022 | — | 0.063 | 0.15 |
| **11** |  |  | 0.014 | 0.082 | 0.30 | 0.31 | 0.42 |
| **12** |  |  | 0.039 | 0.18 | 0.62 | 0.73 | — |

(*continued on next page*)

**Table 1** (*continued*)

| Compd | X | Y | JAK2 inhibition $IC_{50}$ (μM) | JAK1 inhibition $IC_{50}$ (μM) | JAK3 inhibition $IC_{50}$ (μM) | TYK2 inhibition $IC_{50}$ (μM) | Inhibition of SET-$2^{V617F}$ proliferation (cellular assay), $GI_{50}$ (μM) |
|---|---|---|---|---|---|---|---|
| **13** |  |  | 0.008 | 0.13 | – | – | 0.49 |
| **14** |  |  | 0.005 | 0.14 | 0.16 | 0.20 | 0.20 |
| **15** |  |  | 0.005 | 0.048 | 0.19 | 0.12 | 0.26 |
| **16** |  |  | 0.008 | 0.067 | 0.17 | 0.3 | 0.20 |
| **17** |  |  | 0.005 | 0.035 | 0.22 | 0.097 | 0.41 |

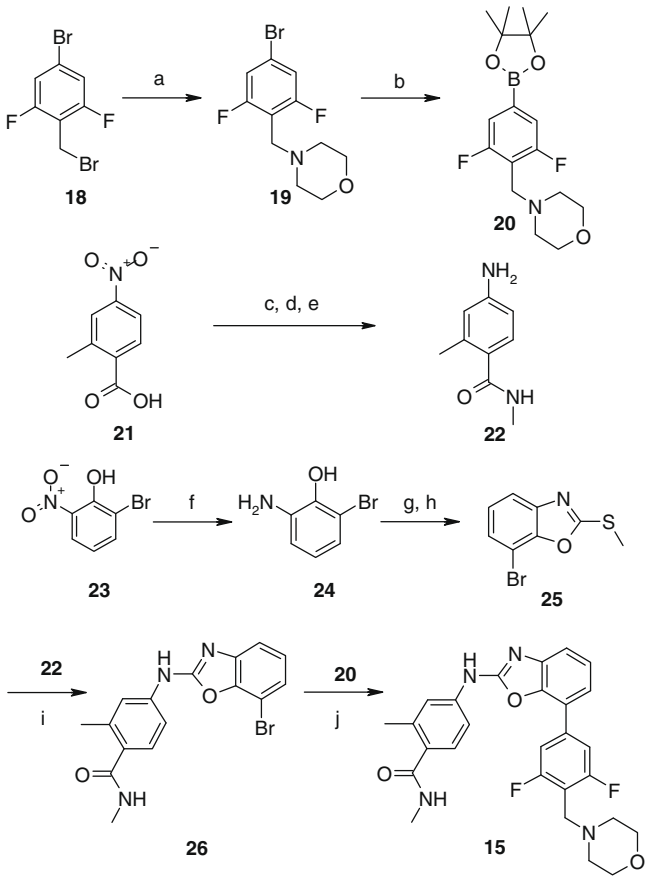
various aryl moieties at position 7. A simple phenyl residue (**3**) leads to a fairly potent but insoluble and very lipophilic JAK2 inhibitor, whereas addition of *meta*-chloro (**4**) or *para*-methoxy groups (**5**) leads to a decrease in activity. In order to make compounds more soluble, polar residues were introduced that are best tolerated at the para position as the comparison of compounds **7** and **8** illustrates (additional examples: **6**, **9**–**11**). Compound **8** also shows very good inhibition of SET-$2^{V617F}$ cell proliferation. Replacement of the trimethoxy-phenyl group with benzamide or piperazine-phenyl residues also leads to potent and cellularly active compounds that can be combined with a variety of 7-aryl moieties (compounds **12**–**17**). It is noteworthy that the introduction of a methyl group ortho to the carboxamide residue leads to an increase in potency (**12** compared to **13**). The introduction of 1 (or 2) fluorine atoms to the 7-aryl residue leads to better JAK2 inhibitory activity (**9** vs **10**). While in general there is relatively favorable selectivity of the compounds for JAK2 versus the other JAKs, there is less of an influence of the substitution pattern on the selectivity factors per se. Apparently, a substitution pattern that leads to an increase in JAK2 inhibition also leads to increased inhibition of the other JAKs.

The preparation of the benzoxazole derivatives is relatively straightforward, convergent and allows introduction of the various substitution patterns in the last two steps. The synthesis is exemplified with the preparation of **15** as shown in Scheme 1. Starting from commercially available 5-bromo-2-bromomethyl-1,3-difluoro-benzene (**18**), the morpholine derivative **19** was prepared followed by a reaction of the latter with bis(pinacolato)-diboron to produce the boronic acid derivative **20**. The aniline building block **22** was prepared in a three steps reaction sequence starting from commercially available 2-methyl-4-nitro benzoic acid (**21**) as outlined in the Scheme. The nitro group of 2-bromo-6-nitro-phenol (**23**, commercially available) was reduced and the resulting hydroxyl-aniline derivative **24** was reacted with potassium ethyl xanthogenate, followed by methylation of the thiol residue to produce 7-bromo-2-methylsulfanyl-benzoxazole (**25**). Activation of the sulfur leaving group of **25** with *m*CPBA, followed by reaction with **22** led to 4-(7-bromo-benzoxazol-2-ylamino)-2,*N*-dimethyl-benzamide (**26**). Suzuki coupling of **26** with **20** led to the final product 4-[7-(3,5-difluoro-4-morpholin-4-yl-methyl-phenyl)-benzoxazol-2-ylamino]-2,*N*-dimethyl-benzamide (**15**).

**Table 2**
Pharmacokinetic properties of **15** in rats[a]

| Parameter | Mean ± SD |
|---|---|
| CL (mL $min^{-1}$ $kg^{-1}$) | 15 ± 4 |
| $V_{SS}$ (L/kg) | 2.3 ± 0.4 |
| $t_{1/2}$ term (h) | 3.2 ± 1.7 |
| AUC, iv d.n. (nmol h $L^{-1}$) | 2391 ± 562 |
| AUC, po d.n. (nmol h $L^{-1}$) | 2435 ± 695 |
| $T_{max}$ (h) | 3.5 ± 1.9 |
| $C_{max}$ d.n. (nM) | 242 ± 33 |
| BAV (%) | 102 ± 29 |

d.n.: dose normalized to 1 mg/kg.
[a] Mean of four animals.




**Scheme 1.** Preparation of **15**. Reagents and conditions: (a) morpholine, $Et_3N$, DMF; (b) bis-(pinacolato)-diboron, $Pd(dppf)Cl_2$, $CH_2Cl_2$, KOAc, DMA, 80 °C; (c) $(COCl)_2$, DMF (cat.), $CH_2Cl_2$; (d) $MeNH_2$ (2 M in THF), $CH_2Cl_2$; (e) $H_2$, Pd/C, MeOH, THF; (f) Ra-Ni, MeOH, THF; (g) potassium ethyl xanthogenate, EtOH; (h) MeI, $K_2CO_3$, DMF; (i) *m*CPBA, $CH_2Cl_2$, rf; (j) $Pd(PPh_3)_4$, $K_3PO_4$, 1,2-dimethoxyethane, 100 °C.

Based on its most attractive broader overall in vitro and physicochemical profile, **15** was selected for pharmacokinetic property assessment.[7] As shown in Table 2, **15** has a low clearance and low volume of distribution at steady state ($V_{SS}$) and a terminal $t_{1/2}$ of 3.2 h after iv administration to rats. After po administration, **15** exhibits a high $C_{max}$, a moderately late $T_{max}$ and a very high oral bioavailability. Profiling of **15** in a variety of animal models is still ongoing and will be published in due course.

In summary, compounds from a series of novel benzoxazole derivatives display attractive JAK2 inhibitory profiles in biochemical and cellular assays. In addition, a selected example (**15**) was demonstrated to exhibit favorable PK properties in rats.

## References and notes


1. (a) Harpur, A. G.; Andres, A. C.; Ziemiecki, A., et al *Oncogene* **1992**, *7*, 1347; (b) Saharinen, P.; Vihinen, M.; Silvennoinen, O. *Mol. Biol. Cell.* **2003**, *14*, 1448; (c) Yamoka, K.; Saharinen, P.; Pesu, M., et al *Genome Biol.* **2004**, *5*, 253.
2. (a) James, C.; Ugo, V.; Le Couedic, J.-P., et al *Nature* **2005**, *434*, 1144; (b) Levine, R. L.; Wadleigh, M.; Cools, J., et al *Cancer Cell* **2005**, *7*, 673; (c) Valentino, L.; Pierre *J. Biochem. Pharmacol.* **2006**, *71*, 713.
3. (a) Vainchenker, W.; Constaninescu, S. N. *Am. Soc. Hematol. Educ. Program* **2005**, 195; (b) Tefferi, A.; Barosi, G.; Mesa, R. A., et al *Blood* **2006**, *108*, 1497.
4. (a) Choi, H.-S.; Wang, Z.; Richmond, W.; He, X.; Yang, K.; Jiang, T.; Sim, T.; Karanewsky, D.; Gu, X.-J.; Zhou, V.; Liu, Y.; Ohmori, O.; Caldwell, J.; Gray, N.; He, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2173; (b) Choi, H.-S.; Wang, Z.; Richmond, W.; He, X.; Yang, K.; Jiang, T.; Karanewsky, D.; Gu, X.-J.; Zhou, V.; Liu, Y.; Che, J.; Lee, C. C.; Caldwell, J.; Kanazawa, T.; Umemura, I.; Matsuura, N.; Ohmori, O.; Honda, T.; Gray, N.; He, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2689.
5. Detailed description of the enzyme assays: Gerspacher, M.; Furet, P.; Vangrevelinghe, E. PCT Int. Appl. WO2008031594, 2008; *Chem. Abstr.* **2008**, *148*, 379605.
6. Human megakaryoblastic SET-2 cells (number ACC608, DSMZ, Braunschweig, Germany) were cultured in standard RPMI medium supplemented with 10% of fetal calf serum (FCS), 2 mM L-glutamine and 1% (v/v) penicillin/streptomycin. To determine the anti-proliferative activity of candidate JAK2 inhibitors, SET-2 cells were incubated for 72 h with an eight point concentration range of compound and cell proliferation relative to DMSO vehicle control treated cells was measured using the colorimetric WST-1 (catalogue number 1644807, Roche Diagnostics GmbH, Penzberg, Germany) cell viability readout. Of each triplicate treatment the mean was calculated and these data were plotted in XLfit 4 (XLfit four curve fitting software for Microsoft Excel, ID Business Solutions Ltd, Guildford, Surrey, United Kingdom) to determine the respective $GI_{50}$ values.
7. Pharmacokinetic properties were determined in conscious, fed, permanently cannulated female rats. For po administration by gavage, aq solution of **15** in citrate buffer pH 3 was used as a vehicle (2.5 mL/kg), whereas for the iv route **15** was administered into the femoral vein as a solution in NMP (30%) in PEG 200 (0.5 mL/kg). Blood samples (approx. 70 μL) were collected from the femoral artery for 48 h after iv administration and for 24 h after oral dosing. Iv and po administration was in the same animals with a 48 h washout period between administrations (cross-over design). After $CH_3CN$ precipitation of blood samples (50 μL), dried residues were re-dissolved in methanol/water, separated by HPLC on a C18 reversed-phase HPLC column, followed by MS/MS analysis on a triple quadrupole mass analyzer (Finnigan TSQ Quantum). The compound was detected as a fragment of its protonated quasi-molecular ion $[M+H]^+$. A structurally closely related compound was used as analytical internal standard. Quantification of blood levels of the parent compound was based on a seven-level calibration curve (in triplicate) using blank rat blood samples spiked with stock solutions of external and internal standards. Pharmacokinetic parameters were estimated using a non-compartmental approach. AUCs iv and po were calculated using the trapezoidal rule, then extrapolated to infinity using the terminal half-life calculated by log-linear regression from the last three (measurable) blood levels after iv administration.