Bioorganic & Medicinal Chemistry Letters 23 (2013) 3592–3598

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Novel triazolo-pyrrolopyridines as inhibitors of Janus kinase 1

Christopher A. Hurley [a,*], Wade S. Blair [g], Richard J. Bull [a], Christine Chang [g], Peter H. Crackett [a], Gauri Deshmukh [d], Hazel J. Dyke [a], Rina Fong [f], Nico Ghilardi [e], Paul Gibbons [c], Peter R. Hewitt [a], Adam Johnson [g], Tony Johnson [a], Jane R. Kenny [d], Pawan Bir Kohli [g], Janusz J. Kulagowski [a], Marya Liimatta [g], Patrick J. Lupardus [f], Robert J. Maxey [a], Rohan Mendonca [c], Raman Narukulla [a], Rebecca Pulk [c], Savita Ubhayakar [d], Anne van Abbema [g], Stuart I. Ward [a], Bohdan Waszkowycz [b], Mark Zak [c]

[a] Department of Medicinal Chemistry, Argenta, 8/9 Spire Green Centre, Harlow, Essex CM19 5TR, United Kingdom
[b] Department of Computer Aided Drug Design, Argenta, 8/9 Spire Green Centre, Harlow, Essex CM19 5TR, United Kingdom
[c] Department of Discovery Chemistry, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, United States
[d] Department of Drug Metabolism and Pharmacokinetics, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, United States
[e] Department of Immunology, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, United States
[f] Department of Structural Biology, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, United States
[g] Department of Biochemical and Cellular Pharmacology, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, United States

ARTICLE INFO

Article history:
Received 22 February 2013
Revised 5 April 2013
Accepted 6 April 2013
Available online 17 April 2013

Keywords:
Janus kinase
JAK1
JAK2
JAK1 inhibitor

ABSTRACT

The identification of a novel fused triazolo-pyrrolopyridine scaffold, optimized derivatives of which display nanomolar inhibition of Janus kinase 1, is described. Prototypical example **3** demonstrated lower cell potency shift, better permeability in cells and higher oral exposure in rat than the corresponding, previously reported, imidazo-pyrrolopyridine analogue **2**. Examples **6**, **7** and **18** were subsequently identified from an optimization campaign and demonstrated modest selectivity over JAK2, moderate to good oral bioavailability in rat with overall pharmacokinetic profiles comparable to that reported for an approved pan-JAK inhibitor (tofacitinib).

Cytokine signaling pathways mediate a broad range of biological functions, including many aspects of inflammation and immunity.[1] Types I and II cytokine receptors lack kinase activity and instead transmit their signals through the receptor-associated Janus kinases (JAK1, JAK2, JAK3, and TYK2).[2,3] When stimulated, cytokine receptor complexes activate specific combinations of JAKs in well-defined patterns,[4] leading to further activation of signal transducer and activator of transcription (STAT) proteins residing in the cytoplasm. Upon JAK-mediated tyrosine phosphorylation, the STATs dimerise and are translocated to the nucleus where they regulate transcription of specific target genes.[5,6] Because of the importance of the JAK/STAT pathways in cytokine signaling, targeting of JAK kinases is envisioned to be useful in the treatment of a variety of diseases including rheumatoid arthritis (RA),[7] myeloproliferative disorders[8] and cancer.[9]

Current evidence suggests that immuno-relevant cytokines (such as IL-6 and the γ_c cytokines) play a pivotal role in RA disease pathogenesis.[10,11] The approved pan-JAK inhibitor tofacitinib (**1**, Fig. 1) has undergone extensive evaluation for RA and has demonstrated efficacy in various clinical trials, likely due to its suppression of the IL-6 and γ_c cytokine pathways.[12] Additionally, a humanized monoclonal antibody (tocilizumab) targeting the IL-6 pathway has been approved for the treatment of moderate to severe RA.[13] Although IL-6 activates JAK1, JAK2, and TYK2, knockout studies in mice have demonstrated that JAK1 plays a particularly important role in signal transduction.[14] Additionally, JAK1 has been shown to play a critical and potentially dominant role in the transduction of γ_c cytokine signaling.[15] Finally, inhibition of JAK2 is associated with anemia,[16] thus limiting its suppression may be beneficial. We, therefore, desired to develop potent JAK1 inhibitors with reduced inhibition of JAK2 to maximize anti-RA effects while limiting the potential for anemia.

We previously reported the identification and preparation of novel fused imidazo-pyrrolopyridine tricyclic JAK inhibitors, such as compound **2** (Fig. 1), that exhibited modest selectivity for JAK1 over the JAK2 isoform.[17] However, some members of this structural class were compromised by low in vitro permeability in MDCK cells and inadequate oral exposure in rat pharmacokinetic studies. In an effort to circumvent the problems faced with compounds such as **2**, we undertook an exploration to find suitable imidazo-pyrrolopyridine scaffold replacements. One restriction

* Corresponding author. Tel.: +44 1279 645 645; fax: +44 1279 645 646.
E-mail address: Chris.Hurley@glpg.com (C.A. Hurley).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.04.018

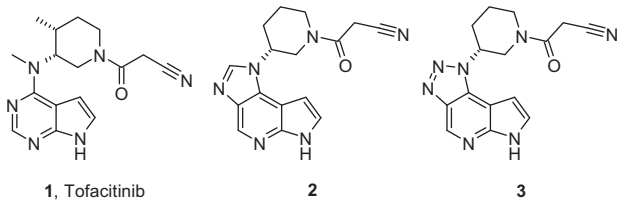

Figure 1. Structures of pan-JAK inhibitor tofacitinib **1**, imidazo-pyrrolopyridine **2** and triazolo-pyrrolopyridine **3**.

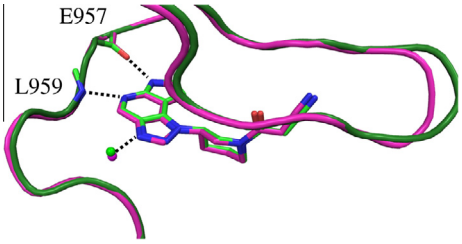

Figure 2. Comparison of the X-ray crystal structures of imidazo-pyrrolopyridine **2** (magenta: PDB code 4E6D) in complex with a JAK1-like JAK2 triple mutant[17] and triazolo-pyrrolopyridine **3** (green: PDB code 4I5C) in complex with JAK1. Hydrogen bonds to the ligand are depicted with black dashed lines. Backbone hinge atoms contacting **3** are highlighted (E957 and L959). Notable crystallographic waters are denoted as spheres. The resolutions of the X-rays are 2.3 and 2.1 Å, respectively.

we applied was the need to readily transfer SAR to any new series identified to expedite progression of the new scaffolds. Therefore we limited our search to scaffolds which maintained the topology of the existing core (compound **2**). This search resulted in the identification of an alternative series of fused triazolo-pyrrolopyridine tricycles (e.g., **3**). When investigated in the JAK1 biochemical assay, compound **3** exhibited similar, albeit slightly weaker, potency compared to **2** (Table 1). Since the measured pK_a of **3** was lower than the pK_a of compound **2**, we were concerned that this reduced basicity would be detrimental to the hydrogen bonding interactions with the hinge residues (JAK1 residues E957 and L959). Consequently, we obtained an X-ray co-crystal structure of **3** bound to JAK1 and compared it to an X-ray co-crystal structure of **2** bound to a JAK1-like JAK2 triple mutant (Fig. 2).[17] We were pleased to find that both the topology and the binding mode remained consistent between the two scaffolds, indicating that major SAR differences between the series would be unlikely.

Compound **3** displayed dramatically improved apical to basolateral MDCK permeability compared to **2**. This improved permeability likely contributes to the observed reduction in the cell potency shift and superior cell potency. Consistent with the imidazo-pyrrolopyridine scaffold, the triazole **3** demonstrated intrinsic but modest selectivity over JAK2 in both the biochemical and cell-based assays. Having shown a dramatic in vitro cell permeability advantage, we decided to explore the SAR of this new chemotype with the aim of improving selectivity against the JAK2 isoform while ensuring favorable JAK1 potency in cells (Table 2). Synthetic intermediate **4** exhibited modest biochemical potency and this prompted us to prepare the less stereo-chemically complex 4-piperidine isomer **5**. Pleasingly, this compound demonstrated single digit nanomolar JAK1 potency, suggesting a preference for the 4-piperidine ring. Compound **5** also displayed comparable potency in the cell-based assay to **3**. However, analogues **4** and **5** suffered from poor metabolic stability in human liver microsomes. We believed that the sub-optimal metabolic profile was due to these weakly basic compounds possessing relatively high lipophilicity (*c* Log *D*). Therefore, in an attempt to improve microsomal stability, we prepared compounds **6**, **7** and **8** that contained substituents on the piperidine nitrogen designed to reduce lipophilicity (Log *D*). The inclusion of a cyanoethyl moiety (**6**) led to elevated metabolic stability along with enhanced biochemical potency and better JAK1 isoform selectivity in cells. Compound **7** had similar selectivity and human microsomal stability profile to compound **6**, but was slightly less potent. The introduction of the pyridine (**8**) was disfavored and we made no further attempts to include heterocyclic systems within this scaffold.

We were keen to identify additional structural features to improve selectivity against JAK2. Previous investigations within our laboratories demonstrated that substitution on the piperidine nitrogen with suitable α-amino amide groups afforded enhanced selectivity against JAK2.[17] Accordingly, the proline analogue **9** was prepared and exhibited 5.9-fold selectivity over the JAK2 enzyme. However, the relatively weak biochemical JAK1 potency (121 nM) was insufficient to warrant further characterization. An alternative approach we had previously discovered involved the introduction of a 4-methyl substituent on the piperidine ring.[17] When applied to this system, compounds **10** and **11** demonstrated improved selectivity over the JAK2 isoform in the biochemical assay. Biochemically, the cyanoethyl moiety was strongly disfavored (compare **6** with **10**) highlighting that this modification was incompatible with the previously established SAR. Conversely, compound **11** demonstrated adequate potency and selectivity in the cell-based assay accompanied by robust microsomal stability to justify further progression.

During the course of our investigations, routine screening of late stage intermediates meant that compound **12** was assessed for activity and exhibited preferential potency for JAK1 over JAK2. We have previously reported that hydrogen bonding elements within this region of the imidazole series can lead to enhanced selectivity profiles.[17] Therefore, we examined a number of additional analogues incorporating H-bond donor groups to probe this region for beneficial selectivity enhancements (compounds **13**–**24**). Fluorinated piperidines compounds **14** and **15** were designed to reduce the basicity of the piperidine nitrogen (ACD cpK_a: **12** = 9.7; **15** = 7.7)[18] and the *trans*-analogue (**15**) was

Table 1
Physicochemical and in vitro data for compounds **2** and **3**

Ex	JAK1 K_i[a,b] (nM)	JAK2 K_i[a,b] (nM)	JAK1 cell EC_{50}[a,c] (nM)	JAK1 cell selectivity[d]	JAK1 cell potency shift[e]	MDCK A:B P_{app}[f] ($\times 10^{-6}$ cm/s)	Log *D*[g]	pK_a (measured)
2	0.4	1.2	180	4.8	450	0.3	0.39	4.34
3	1.5	3.8	114	6.8	76	3.7	0.60	1.96

[a] Arithmetic mean of at least three separate determinations.
[b] Biochemical potency.
[c] pSTAT3-IL6 JAK1 driven TF-1 cell-based assay.
[d] Cell-based selectivity for JAK1 over JAK2 (pSTAT5-EPO EC_{50}/pSTAT3-IL6 EC_{50}).
[e] Fold reduction in cell potency relative to biochemical potency (pSTAT3-IL6 EC_{50}/JAK1 K_i).
[f] Apparent permeability in MDCK transwell culture (arithmetic mean of at least two separate determinations), A:B apical to basolateral.
[g] Measured at pH 7.2.