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Discovery of potent and efficacious pyrrolopyridazines as dual JAK1/3 inhibitors

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ABSTRACT

A series of potent dual JAK1/3 inhibitors have been developed from a moderately selective JAK3 inhibitor. Substitution at the C6 position of the pyrrolopyridazine core with aryl groups provided exceptional biochemical potency against JAK1 and JAK3 while maintaining good selectivity against JAK2 and Tyk2. Translation to in vivo efficacy was observed in a murine model of chronic inflammation. X-ray co-crystal structure determination confirmed the presumed inhibitor binding orientation in JAK3. Efforts to reduce hERG channel inhibition will be described.

The therapeutic blockade of the Janus kinase (JAK) family of non-receptor tyrosine kinases has shown promise for a variety of human diseases. These kinases (JAK1, JAK2, JAK3, and Tyk2) are phosphorylated in response to cytokine receptor activation, leading to gene transcription and signal propagation.[1] In the context of inflammatory diseases, JAK1, JAK3, and Tyk2 have been implicated as targets for therapeutic intervention and a variety of JAK inhibitors have completed or are currently being studied in clinical trials.[2] Tofacitinib (pan-JAK1/JAK2/JAK3 inhibitor) was approved in 2012 for rheumatoid arthritis (RA) and represents the first small molecule kinase inhibitor to reach the market in this autoimmune disease.[3] Ruxolitinib (JAK1/JAK2 inhibitor) has been approved for the treatment of myelofibrosis targeting a gain of function mutation (V617F) in JAK2.[4] Selective JAK1 inhibition with filgotinib[5] and ABT-494[6] have been reported to be efficacious in RA patients and are advancing into Phase 3 clinical trials. An NDA for the JAK1/2 dual inhibitor, baricitinib, has recently been filed with the FDA for the treatment of RA.[7]

Previously, we have described pyrrolo[1,2-*b*]pyridazine-3-carboxamides as JAK inhibitors.[8] The focus of that effort was to optimize JAK3 inhibition while sparing JAK2-mediated side effects such as anemia.[9] Compound **1** (Table 1) was identified as a potent JAK3 inhibitor (IC_{50} 5 nM) with moderate JAK family enzyme selectivity.[10] In cell-based functional assays measuring EPO-induced JAK2 signaling vs. IL-2-induced JAK1/3 signaling, **1** was 130-fold selective over JAK2. In human whole blood, **1** had an IC_{50} of 210 nM (JAK1/3 dependent IL-2 induced IFN-γ production).[11] Moderate oral exposure in rodents was observed for **1**. In addition, **1** was weakly bound to serum proteins (52% free).

Structurally, **1** represented an excellent starting point for further modification on the pyrrole ring (C5–C7) with the goal of improving potency and PK. Given the established binding mode of this inhibitor class, substitution at C7 was anticipated to negatively impact the hinge (Leu905) binding due to sterics and was not pursued. C5 substitution was not tolerated (JAK3 IC_{50} > 500 - nM, data not shown). However, the C6 phenyl analog (**2**) improved JAK family inhibition with the most notable improvement in biochemical potency against JAK1 (48-fold). Cellular selectivity against JAK2 was maintained as was the whole blood potency.[12] In mice, **2** was orally bioavailable (F% 67) and provided sustained serum exposures at or above the mouse whole blood (JAK1/3 dependent IL-15 induced phospho-STAT5 production, IC_{50} 270 nM) for 24 h with a single 10 mg/kg dose.

The dual JAK1/3 inhibition profile of **2** was particularly attractive. JAK3 was initially targeted by many in the field but emerging data implicates JAK1 in the pathogenesis of inflammatory diseases such as RA.[13] Key inflammatory cytokines that signal through a

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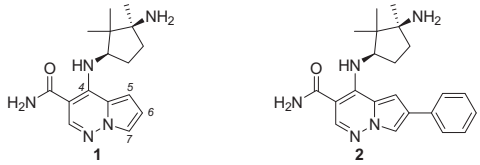
Table 1
Lead compound JAK profiles.



Ex	JAK3 IC_{50}, nM	JAK1 IC_{50}, nM	TYK2 IC_{50}, nM	JAK2 IC_{50}, nM	EPO/IL2 IC_{50} ratio	hWB IFNγ IC_{50}, nM	PK[a]
1	5	19	97	62	130	210	Rat F% 24 Cl > 100%[b]
2	0.9	0.4	7.5	9.0	300	200	Mouse F% 67 Cl 32%[c,d]

[a] Clearance is reported as percent hepatic blood flow.
[b] Rat total CL 145 mL/min/kg.
[c] Mouse total CL 29 mL/min/kg.
[d] C24 230 nM.

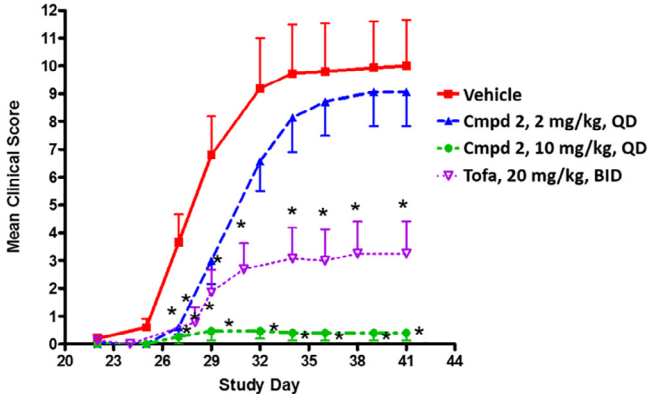


Fig. 1. Mouse CIA efficacy – clinical scores. Values are reported as mean ± SEM, n = 11–15 mice per group. *p < 0.05 vs. vehicle, Mann-Whitney U Test. Vehicle, PEG 300.

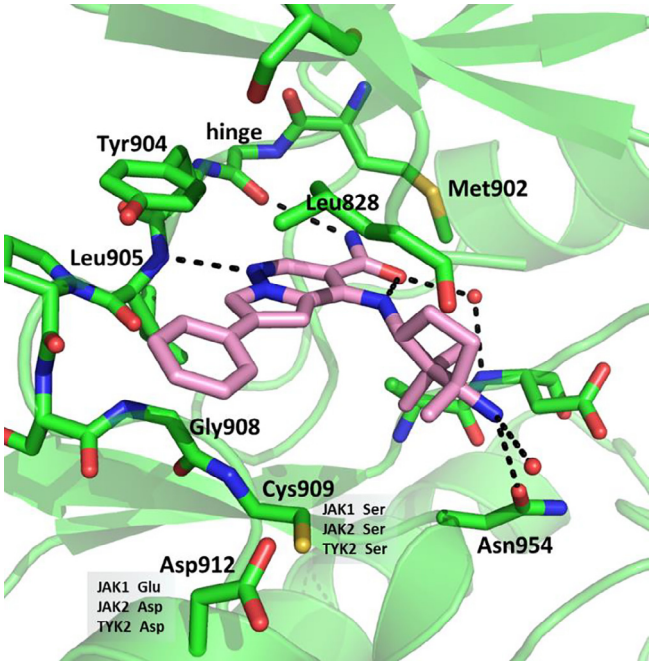


Fig. 2. X-ray co-Crystal structure of **2** bound to the catalytic domain of JAK3 with key residues labeled (PDB ID 5VO6).

JAK1 dependent process include IL-1, IL-6 and IFN-alpha and gamma. Compounds such as **2** would provide a unique opportunity to evaluate the benefit of dual JAK1/3 blockade in animal models of inflammatory diseases.

Initial in life studies in a murine pharmacodynamic model of JAK1/3 signaling (IL-15 induced phospho STAT5 inhibition) provided an EC_{50} of 125 ± 30 nM for **2**.[14] This level of functional potency in combination with the sustained serum drug levels observed in the PK studies prompted further evaluation in a disease model of chronic inflammation. Analog **2** was studied in a pseudo-established mouse collagen-induced arthritis (mouse CIA) model at 2, 10, and 50 mg/kg, once daily oral dosing. Complete suppression of the clinical signs of the disease was achieved at 10 mg/kg (C_{24} 390 nM), Fig. 1. The 2 mg/kg dose was not efficacious, a result consistent with minimal coverage of the mouse EC_{50} over the course of the study (C_{24} 60 nM) while the 50 mg/kg dose was not tolerated in the study. Within the same study, tofacitinib at 20 mg/kg was efficacious albeit with BID dosing. Histological scores (inflammation, bone resorption) were consistent with the visual disease activity scores.

Although this compound was efficacious in this arthritis disease model, safety concerns emerged from the in vitro profiling assays. Cardiac hERG channel inhibition (flux IC_{50} 3.8 μM, patch clamp $IC_{50} \sim 0.3$ μM), and to a lesser extent Ca^{2+} and Na^{+} channel inhibition, were viewed as key liabilities for advancement of this compound.[15] Moderate Cyp3A4 inhibition (IC_{50} 3 μM) was also observed. A small internal kinase panel (16 kinases) indicated good selectivity, however, a broader kinome screen revealed potential issues (30 kinases <30% of control).[16] The focus then turned towards optimizing the cardiac channel safety profile and improving the kinase selectivity while maintaining the whole blood potency.

Structurally, **2** bound to JAK3 in an identical fashion as the previously disclosed C6 unsubstituted inhibitors (Fig. 2). The pyrrolopyridazine N1 forms the critical hinge hydrogen bond with Leu905 while the primary carboxamide NH interacts with the carbonyl of Glu903 which is located deeper in the binding pocket and proximal to the Met902 gatekeeper residue. The cyclopentyl amine engages additional hydrogen bonding interactions with Asn954 and the backbone carbonyl of Arg953. The newly installed C6 phenyl group projects into the extended hinge region of the kinase.

The increased JAK family biochemical potency of **2** relative to **1** may be due to increased lipophilic interactions of the C6 phenyl