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Scaffold hopping towards potent and selective JAK3 inhibitors: Discovery of novel C-5 substituted pyrrolopyrazines

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ABSTRACT

The discovery of a novel series of pyrrolopyrazines as JAK inhibitors with comparable enzyme and cellular activity to tofacitinib is described. The series was identified using a scaffold hopping approach aided by structure based drug design using principles of intramolecular hydrogen bonding for conformational restriction and targeting specific pockets for modulating kinase activity.

The Janus protein tyrosine kinases (JAK1, JAK2, JAK3, and TYK2) mediate intracellular signaling of numerous cytokines and thereby play critical and cooperative roles in a variety of biological processes, including hematopoiesis and the regulation of immune and inflammatory responses.[1,2] When stimulated, cytokine receptor complexes activate specific combinations of JAKs in well-defined patterns,[3] 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.[4,5] 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)[6,7] myeloproliferative disorders[8] and cancer.[9] This expectation has been realized in the two recent FDA approvals of ruxolitinib, a drug for the treatment of myelofibrosis[10] and tofacitinib (**1**), a potent JAK 1/2/3 inhibitor with reduced activity against TYK2 that shows efficacy and acceptable safety for the treatment of rheumatoid arthritis[11–14] and psoriasis.[15,16] Tofacitinib (**1**) also revealed side effects that could relate to inhibition of JAK2 and/or JAK1.[17,13] Inhibition of JAK2 is associated with anemia,[18] thus limiting its suppression may be beneficial. While JAK1, JAK2, and Tyk2 are ubiquitously expressed in vertebrates, JAK3 is mainly limited to hematopoietic cells.[19] Therefore, selective targeting of JAK3 may offer therapeutic benefit while minimizing potential liabilities associated with inhibition of broader JAK signaling.[20] Multiple research groups have therefore investigated different medicinal chemistry strategies for modulating JAK family selectivity.[21] We mainly focused our research on selective JAK3 inhibitors for inflammatory indications.

As part of our lead finding efforts,[22] we were searching for a scaffold able to modulate JAK3 selectivity with good physicochemical properties and kinome selectivity. Thus, we envisioned that we can reach our goal by designing a scaffold that maintains a similar 5,6 bicyclic heteroaromatic core hinge binder as tofacitinib[23] but with a locked conformation of the saturated six membered ring (Fig. 1). The piperidine ring of tofacitinib offers good vectors for access the Gly loop (amino acids 828–836) for activity and kinome selectivity (Fig. 2A).[23] The piperidine ring of tofacitinib (**1**) can be

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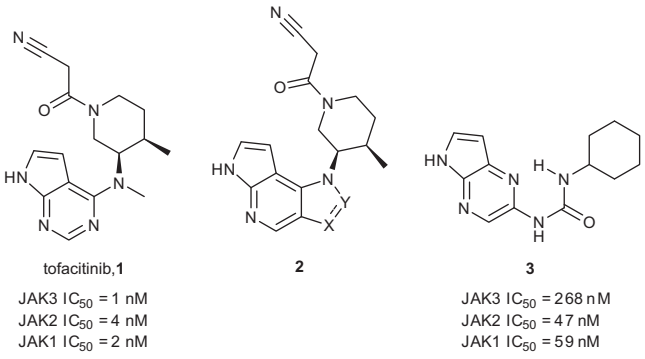

Figure 1. Structures of tofacitinib and conformationally restricted JAK inhibitors.

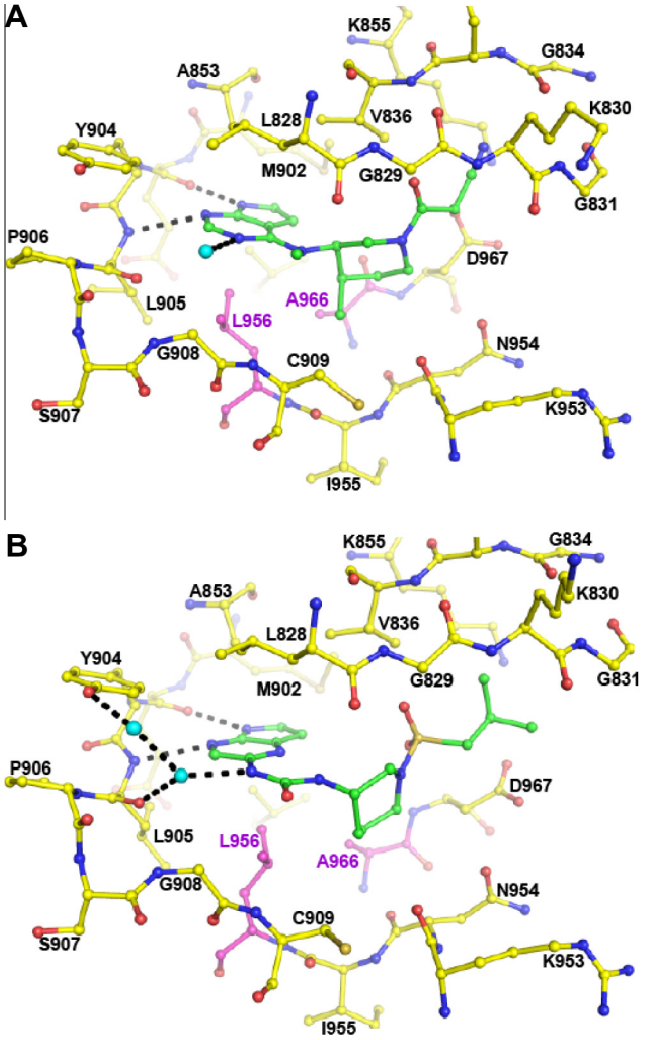

Figure 2. X-ray crystal structures of JAK3 kinase domain in complex with (A) tofacitinib (PDB accession number 3LXK) and (B) **12** (PDB accession number 4QT1).

locked into its bound conformation by tricyclic heterocycles (Fig. 1). This strategy has been extensively studied by multiple research groups[24–27] including ours.[28] We also investigated a related approach utilizing virtual third rings containing intramolecular hydrogen bonds[29] enforcing similar conformations. This de novo approach was first tested with the preparation of compound **3**.[30]

Compound **3** provided an initial starting point with JAK enzymatic activity[31] as a proof of concept encouraging further optimization. Initial SAR studies demonstrated that the 6-membered cycloalkyl amine was the optimal ring size.[30] We explored analogs bearing a variety of polar substitution patterns on the 6-membered ring (Table 1). Substituted 3-amino-piperidines such as *rac*-**7** and *rac*-**9** were superior over the corresponding 4-amino-piperidines **4** and **6**. Interestingly, incorporation of a cyano group at the alpha position of acetamide *rac*-**7** did not improve the enzymatic activity (*rac*-**7** vs *rac*-**8**, Table 1). This data indicated that the piperidine SAR of this new series diverges from tofacitinib. The sulfonamide group appeared to specifically increase JAK3 activity. The (*S*)-3-aminosulfonamide enantiomer was found to be optimal (**10**) based on activity, selectivity and modeling expectations which were later confirmed by protein crystallography. The JAK3/2 selectivity comparison of closely related piperidines bearing a sulfonamide (*rac*-**9**), an acetamide (*rac*-**7**), and an alkyl group (*rac*-**5**) suggested that larger groups are favorable at this position for JAK3 selectivity over JAK2. This observation is in line with closely related inhibitors and structural studies of JAK3 and JAK2 proteins.[32] Further extension of the sulfonamide group with alkyl groups such as isobutyl (**12**) or neopentyl (**13**) provided a boost in activity and JAK family selectivity up to 10 fold.[33]

We obtained a crystal structure of **12** bound to JAK3 (2.4 Å resolution, PDB accession code 4QT1, Fig. 2B). The structure showed the pyrrolopyrazine at the ATP binding site making a hinge (amino acids 903–906) interaction similarly to tofacitinib. The urea and piperidine group also adopted the anticipated conformation with the sulfonamide group filling similar space as the cyanoacetamide of tofacitinib. The isobutyl group was accommodated under this region hence the significant activity improvement observed with this type of substitution pattern.

Once the binding mode of **12** was confirmed by crystallography, we then sought to increase activity and selectivity by filling the piperidine group pocket shaped by Ala 966 and Leu 956 (Fig. 2). This pocket is enlarged in the tofacitinib-bound JAK3 crystal structure, where the L956 side chain is shifted towards the hinge region and the A966–D967 peptide bond is flipped. The installation of an equatorial methyl group at the 5 position of the piperidine ring of **12** was expected to be tolerated based on molecular modeling (compound **14**, Table 1). This small modification also showed an improvement in kinome selectivity (Fig. 3). While compound **12** showed binding to 49 out of 442 kinases (95% of competition at 10 μM), compound **14** bearing an equatorial methyl group showed affinity to 33 out of 442 kinases with a significant reduction in binding to the CMGC group of kinases. The introduction of a small group at this position for modulating kinome selectivity could be further capitalized among this or other classes of JAK kinase inhibitors.

As part of our SAR studies, we then turned our attention to the urea linker. Installation of a methyl group at the urea linker resulted in a moderate loss of JAK activity (compound **15**, Scheme 1); however, the crystallographic data suggested that co-planar groups with the pyrrolopyrazine core should be tolerated. Following this hypothesis and with the objective to further restrict the conformation of the linker, the corresponding pyridyl linker was prepared. We were delighted to see that pyridyl linker **16** had comparable activity to the initial urea linker compound **10**. The corresponding pyrazine **17** and pyrimidine **18** were also tolerated. Pyrimidine analog **18** also showed a slightly different JAK family selectivity profile.

The SAR studies developed on the sulfonamide region for the urea linkers were then successfully translated to the pyridyl type linker (Table 2). Pyridine linker compounds also achieved single digit nanomolar JAK3 enzyme activity with isobutyl (**20**), neopentyl (**21**), and CH_2CN (**22**) groups. In order to assess the JAK family