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Structural and Thermodynamic Characterization of the TYK2 and JAK3 Kinase Domains in Complex with CP-690550 and CMP-6

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Janus kinases (JAKs) are critical regulators of cytokine pathways and attractive targets of therapeutic value in both inflammatory and myeloproliferative diseases. Although the crystal structures of active JAK1 and JAK2 kinase domains have been reported recently with the clinical compound CP-690550, the structures of both TYK2 and JAK3 with CP-690550 have remained outstanding. Here, we report the crystal structures of TYK2, a first in class structure, and JAK3 in complex with PAN-JAK inhibitors CP-690550 ((3R,4R)-3-[4-methyl-3-[*N*-methyl-*N*-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino]piperidin-1-yl]-3-oxopropionitrile) and CMP-6 (tetracyclic pyridone 2-t-butyl-9-fluoro-3,6-dihydro-7H-benz[h]-imidaz[4,5-f]isoquinoline-7-one), both of which bind in the ATP-binding cavities of both JAK isozymes in orientations similar to that observed in crystal structures of JAK1 and JAK2. Additionally, a complete thermodynamic characterization of JAK/CP-690550 complex formation was completed by isothermal titration calorimetry, indicating the critical role of the nitrile group from the CP-690550 compound. Finally, computational analysis using WaterMap further highlights the critical positioning of the CP-690550 nitrile group in the displacement of an unfavorable water molecule beneath the glycine-rich loop. Taken together, the data emphasize the outstanding properties of the kinome-selective JAK inhibitor CP-690550, as well as the challenges in obtaining JAK isozyme-selective inhibitors due to the overall structural and sequence similarities between the TYK2, JAK1, JAK2 and JAK3 isozymes. Nevertheless, subtle amino acid variations of residues lining the ligand-binding cavity of the JAK enzymes, as well as the global positioning of the glycine-rich loop, might provide the initial clues to obtaining JAK-isozyme selective inhibitors.

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Keywords: TYK2; JAK3; isozymes; crystal structure; inflammation

Introduction

Janus kinase (JAK) family members are relatively large (120–130 kDa) receptor-associated protein tyrosine kinases involved in the JAK/signal transducers and activators of transcription (STAT) signaling pathway. Four JAK family members (JAK1, JAK2, JAK3 and TYK2) have been identified in mammals; JAK1, JAK2 and TYK2 are ubiquitously expressed, whereas JAK3 expression is restricted to

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Abbreviations used: JAK, Jason kinase; STAT, signal transducers and activators of transcription; ITC, isothermal titration calorimetry; TCEP, Tris 2-carboxymethyl phosphine.

the hematopoietic system.[1–4] In cells, JAK3 interacts with the gamma common chain of the cytokine receptors for interleukin-2 (IL-2), IL-4, IL-7, IL-9, IL-15 and IL-21. Interleukin binding to the γc cytokine receptor results in JAK3/JAK1 heterodimerization and JAK activation through autophosphorylation.[5] The activated JAK complex subsequently activates the STAT protein (STAT5 for IL-2 stimulation), resulting in STAT dimerization and translocation to the nucleus, leading to gene expression. Mutations in TYK2, JAK3, STAT1 and STAT5b result in human immunodeficiencies,[6–8] underscoring the critical role of the JAK/STAT pathways in many biological systems. More specifically, JAK3 mutations have been linked to severe combined immunodeficiency in humans,[9–11] and JAK3 knockout mice have defects in T, B and NK cell development and function.[12] Thus, JAK3 offers applications as a target in the treatment of inflammation, allergy, autoimmune diseases and organ transplant rejection.[13] Like JAK3, TYK2 deficiencies lead to distinct clinical disorders, particularly autosomal recessive hyperimmunoglobulin E syndrome.[14,15] TYK2 interacts with IL-12 and IL-23, leading to the activation of STAT proteins by a mechanism similar to that described for JAK3. Association of TYK2 with the cytokine receptor occurs through heterodimerization of TYK2 with either JAK2 (IL-12 and IL-23) or JAK1 (type 1 IFNs), but not JAK3. Identification of novel compounds that display high-level selectivity for TYK2 is of critical interest for indications including Crohn's disease, rheumatoid arthritis and systemic lupus erythematosus.[16–19] Understanding the structural information for the TYK2–inhibitor complex will aid in discerning the discrepancies between TYK2 and JAK1/2/3, and enable inhibitor optimization.

The JAK kinases are unique, multidomain proteins that are characterized by seven homology domains (JH1 – JH7).[20] The JH1 domain comprises the catalytic protein tyrosine kinase, which conserves a high level of sequence identity amongst the four JAK isozymes. The catalytically active kinase domain is responsible for the physiological function of the JAK kinase, and small molecule ATP competitive inhibitors, including CP-690550, have been designed to target the ATP-binding pocket of the JAK kinase. The JH1 domain is critically regulated by the unique pseudokinase domain (JH2) through a mechanism that has yet to be described.[21–24] Although the pseudokinase domain has sequence similarities to the kinase domain, it does not appear to exhibit catalytic activity because the domain lacks the amino acids required for kinase activity. Nonetheless, mutations in the pseudokinase domain of JAK2, particularly the JAK2 $V^{617}F$ mutant, result in myloproliferative disorders and, in particular, the observation of this mutation in patients with the polycythemia vera mutant, result in an autosomal recessive phenotype, suggesting that the JH2 domain plays an integral role in the JAK family.[25,26] The N-terminus of JAKs are comprised of an Src-homology-2 (SH2)-like domain (JH3–JH4), and the FERM domain (JH5–JH7). The JH3 and JH4 domains have been described to play a structural role for the overall stabilization of the large JAK protein, while the FERM domain has been implicated in positively regulating the catalytic activity of the JAK kinases through a direct binding interaction with the kinase domain (JH1 domain).[27–29] A deeper understanding of this mechanism will require the full-length crystal structure of an isolated JAK isozyme, which has yet to be described.

The Pfizer inhibitor CP-690550 is a potent, PAN-JAK inhibitor that exhibits phenomenal selectivity against the human kinome. This compound inhibits JAK3 activity with a reported K_i of 0.2 nM, while also inhibiting JAK2 (K_i 1.0 nM), JAK1 (K_i 0.7 nM) and TYK2 (K_i 4.4 nM; data collected in-house). Remarkably, CP-690550 has demonstrated significant selectivity against a panel of >300 other kinases, which represents more than 50% of the predicted human kinome.[30] This oral inhibitor has shown to be well tolerated and effective over six months in phase II trials for the treatment of patients with moderate-to-severe rheumatoid arthritis and kidney allograft rejection.[31–33] It has become increasingly challenging to design JAK isozyme-selective compounds, which is a result of the significant overall sequence homology amongst the four isozymes. Homology is particularly high within the ATP-binding cavity, indicating that very subtle differences in binding properties of an inhibitor would be required to achieve selectivity discrepancy. A series of structures of the JAK1 and JAK2 kinase domains in complex with several PAN inhibitors, including CP-690550, were recently determined, providing new tools towards the development of potentially selective JAK inhibitors utilizing a structure-based approach.[34] In order to further elucidate the structural determinants driving both potency and selectivity within the JAK family, we have solved the crystal structures of both TYK2 and JAK3 in complex with PAN inhibitors CP-690550 and CMP-6. The structure of the TYK2 isozyme is the first structure thus far described, lending novel structural insights for drug discovery initiatives. We have thermodynamically characterized both CP-690550 and an analogue of this inhibitor, termed CP-690550A, revealing critical contacts between the inhibitor and the JAK kinase domain that might drive inhibitor potency and selectivity. Finally, a WaterMap computational analysis confirms the thermodynamic data, and provides preliminary computational insights into advantageous design of JAK inhibitors into unique chemical space. Together, these data provide a framework for the identification and pursuit of novel inhibitors targeted at the JAK family of receptor tyrosine kinases.

Results

Overall structure of TYK2

Although the crystal structures of JAK1, JAK2 and JAK3 have been elucidated,[34–36] the crystal structure