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2,7-Pyrrolo[2,1-*f*][1,2,4]triazines as JAK2 inhibitors: Modification of target structure to minimize reactive metabolite formation

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ABSTRACT

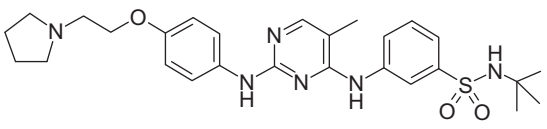
The JAK2/STAT pathway has important roles in hematopoiesis. With the discovery of the JAK2 V617F mutation and its presence in many patients with myeloproliferative neoplasms, research in the JAK2 inhibitor arena has dramatically increased. We report a novel series of potent JAK2 inhibitors containing a 2,7-pyrrolotriazine core. To minimize potential drug-induced toxicity, targets were analyzed for the ability to form a glutathione adduct. Glutathione adduct formation was decreased by modification of the aniline substituent at C2.

Janus kinase 2 (JAK2) is one of four members of the JAK family of non-receptor tyrosine kinases. The JAK family includes JAK1, JAK2, JAK3, and tyrosine kinase 2 (Tyk2).[1] JAKs have a role in transducing signals for many cytokines, interferons and growth factors.[1] JAK2 phosphorylates tyrosine residues on the cytoplasmic side of the cytokine receptor[2,3] leading to subsequent recruitment and phosphorylation of signal transducers and activators of transcription (STATs).[1] Phosphorylated STATs form dimers, migrate to the nucleus, and activate gene transcription.[2] STATs bind to specific gene promoters which regulate proliferation and differentiation.[3] Thus, interrupting this signaling could impede growth in tumor types that rely wholly, or in part, on JAK2 signaling for growth and/or survival.

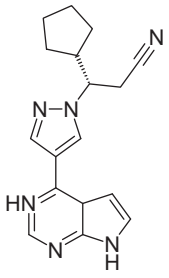
A somatic V617F mutation in the JH2 domain of the JAK2 gene has been linked to incidence of myeloproliferative neoplasms (MPNs), such as polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF).[3] Since the discovery of the V617F mutation, interest in developing JAK2 inhibitors to treat this defined patient population has increased leading to a number of patent applications claiming a variety of chemotypes.[2]

The efficacy of 'PAN' JAK inhibitor CP-690550, in clinical trials for rheumatoid arthritis (RA), is thought to be enhanced by JAK1 and JAK2 inhibition. JAK1 and JAK2 regulate signaling of proinflammatory cytokines such as IL-6.[4] In a study using small molecule JAK2 inhibitor INCB-018424, JAK/STAT activator IL-6 levels were suppressed in the plasma of RA patients.[5]

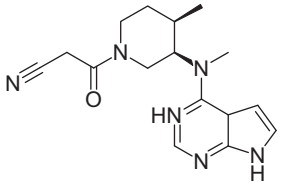
Some JAK inhibitors currently in clinical trials are shown in Figure 1.[4,6]



TG-101348 (SAR 30253)



INCB-018424 (Ruxolitinib)



CP-690550 (Tofacitinib)

Figure 1. JAK inhibitors in clinical trials.[4,6]

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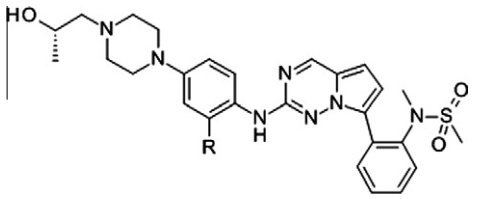
Table 1
JAK2 SAR and % GSH adduct for analogs **1** and **2**



Compd	R	JAK2 IC_{50}[a]	ALK IC_{50}[a]	LMS $t_{1/2}$[b]	% GSH adducts[c]
1	H	1.14 ± 0.046	30	>40,>40,34,35	63
2	OMe	379 ± 61	9	>40,>40,>40,>40	61

[a] Average ± standard deviation (nM) of 3 or more determinations; see Supplementary data for assay conditions.
[b] LMS: Liver microsome stability half life (min) in mouse, rat, dog, human.
[c] Relative to parent.

As part of our efforts to identify new kinase inhibitor scaffolds, we elaborated the SAR around the 2,7-pyrrolo[2,1-*f*][1,2,4]triazine core. This scaffold was conceptualized as a constrained analog of the 2,4-diaminopyrimidine core (e.g., TG-101348), a well-recognized kinase inhibitor scaffold.[7] From this work, orally bioavailable pyrrolotriazine analog **2** was identified as an ALK inhibitor with modest JAK2 activity, and this analog served as a starting point for a medicinal chemistry effort targeting JAK2 inhibitors (Table 1).[8,9]

The human toxicological risks associated with reactive species capable of forming covalent adducts (either through the parent or its metabolite) are difficult to quantitate preclinically and reducing or eliminating this possible liability is generally preferable.[10] One method to assess the propensity of reactive metabolite formation is to incubate samples with liver microsomes in the presence of glutathione (GSH) and analyze for adducts by LC–MS/MS.[11,12] In the presence of glutathione, incubation of compound **2** with liver microsomes formed significant levels of covalent adducts (61% after 30 min incubation).[13] Presumably, CYP induced oxidation of the C2 aniline generated a reactive quinonediimine and/or quinoneimine intermediate that was trapped by GSH (Fig. 2).[14]

Due to this undesired activity associated with the aniline substituent, medicinal chemistry was carried out to maximize inherent JAK2 inhibitory activity, minimize the propensity of glutathione adduct formation and optimize pharmaceutical properties such as liver microsome stability and PK.

When the methoxy group on compound **2** was replaced with hydrogen, the resulting compound **1** displayed dramatically improved JAK2 potency; however, glutathione adduct formation remained virtually unchanged (63%, Table 1). JAK2 inhibitory activity was tolerant of a variety of C7-aryl groups ranging from methoxyphenyl, methoxypyridyl, *N*-sulfonamidophenyl to benzenesulfonamide (Table 4). However, when less polar groups were substituted for the piperazinylpropanol group appended on the C2-aniline, liver microsomal stability (LMS) was reduced (Table 2). Two strategies were investigated to reduce the reactive metabolite liability while maintaining JAK2 activity and LMS. The initial effort involved incorporating electron withdrawing substituents (Cl and F) on the C2 aniline to reduce its oxidation potential.[15] The second strategy involved replacing the piperazine with piperidine to prevent formation of quinonediimine reactive species.

Target compounds were synthesized by functionalization of 7-bromo-2-methylsulfanyl core **3**. After a Suzuki coupling, thiomethyl intermediate **4** was oxidized to either a sulfoxide **5** or a

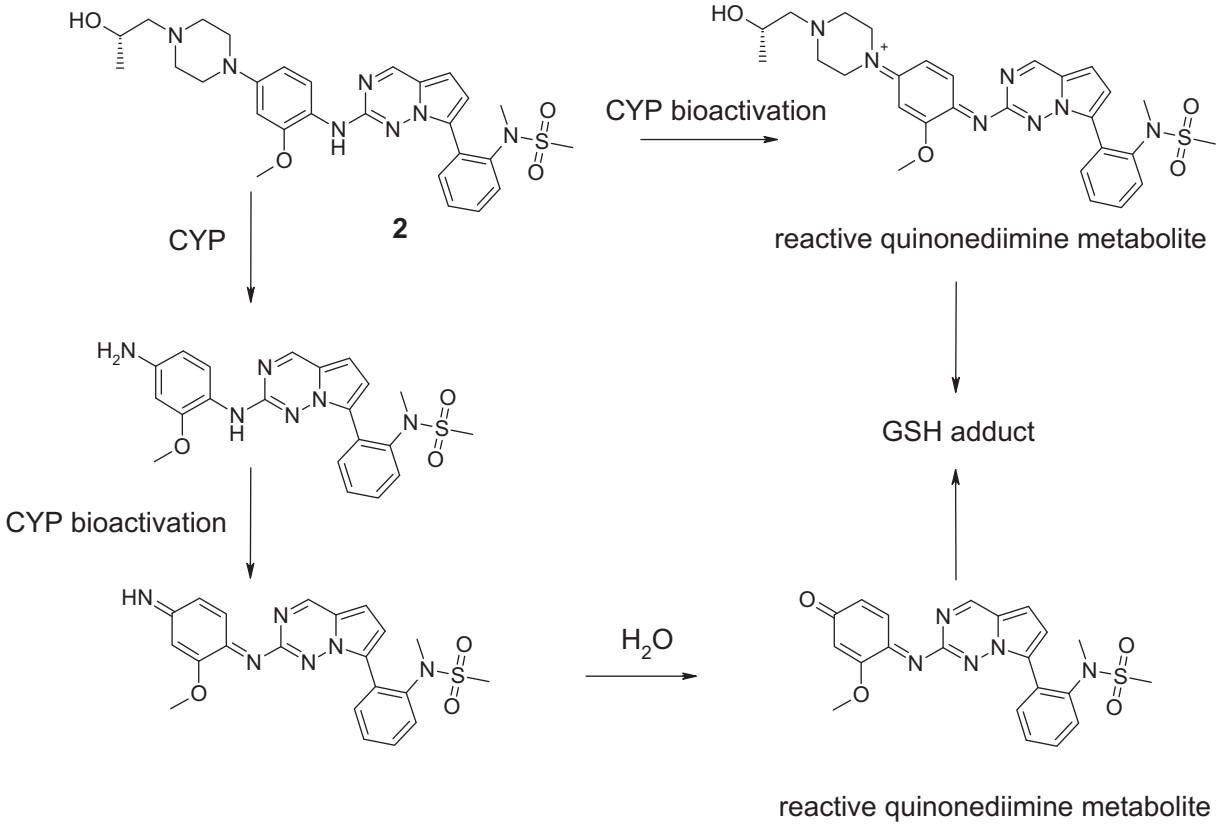


Figure 2. Proposed mechanism to form a reactive quinoneimine metabolite.