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Development of new pyrrolopyrimidine-based inhibitors of Janus kinase 3 (JAK3)

Michael P. Clark,[b,*] Kelly M. George,[a] Roger G. Bookland,[a] Jack Chen,[a] Steven K. Laughlin,[a] Kumar D. Thakur,[a] Wenlin Lee,[a] Jan R. Davis,[a] Ed J. Cabrera,[a] Todd A. Brugel,[a] John C. VanRens,[a] Matthew J. Laufersweiler,[a] Jennifer A. Maier,[a] Mark P. Sabat,[a] Adam Golebiowski,[a] Vijay Easwaran,[a] Mark E. Webster,[a] Biswanath De[a] and George Zhang[a]

[a]*Procter and Gamble Pharmaceuticals, Health Care Research Center, 8700 Mason-Montgomery Road, Mason, OH 45040, USA*
[b]*Metastatix, Inc., 1860 Montreal Road, Tucker, GA 30084, USA*

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Abstract—A new class of bicyclic pyrrolopyrimidine-based Janus kinase 3 (JAK-3) inhibitors are described. Many of these inhibitors showed low nanomolar activity against JAK-3.

Janus kinases (JAK), including JAK1, JAK2, Tyk2, and JAK3, are a small family of cytoplasmic protein tyrosine kinases which play pivotal roles in the initiation of cytokine-triggered signaling events by activators of transcription (STAT) proteins via tyrosine phosphorylation.[1–3] Upon phosphorylation, the STAT proteins dimerize and translocate to the nucleus where they can then induce transcription of the corresponding cytokine-responsive genes. This association with relevant cytokine signaling pathways makes JAK3 an important target for therapeutic intervention in the treatment of autoimmune disorders, inflammatory diseases, and organ transplant rejection. An important feature of JAK3 is that it specifically associates with the common cytokine receptor gamma chain (γc) which is a shared component of the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15. Unlike the other JAK family members, that are more widely expressed, JAK3 expression seems to be mainly limited to hematopoietic cells. The unique interaction with the cytokine receptor gamma chain, along with its limited expression, makes JAK3 an attractive therapeutic target relative to the other JAK family members.

In the relatively young field of Janus kinase inhibition, Pfizer has already progressed their JAK3 inhibitor, **CP-690,550**, into Phase II clinical trials for acute rejection in kidney transplant patients[4] (Fig. 1). Other JAK3 inhibitors have been reported, such as the non-selective (within JAK family) but very potent tetracyclic pyridone **2**, reported by Merck.[5] Aventis has published an oxindole inhibitor, **3**, that is one of the only published JAK3 inhibitors that shows good enzymatic selectivity vs JAK2.[6] However, this oxindole inhibitor also shows very strong inhibition vs a panel of CDK kinases.

Our search for a new structural class of JAK3 inhibitors resulted in the identification of two novel classes of compounds, a previously published trisubstituted pyrimidine class[7] and a new potent pyrrolopyrimidine class of compounds, exemplified by **4**.

Synthesis of the bicyclic pyrrolopyrimidine **4** was accomplished in seven steps from readily available dichloropyrimidine carboxaldehyde **5** (Scheme 1). Displacement of the first chloride with sarcosine

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* Corresponding author. Tel.: +1 404 863 4275; e-mail: mclark@metastatix.com

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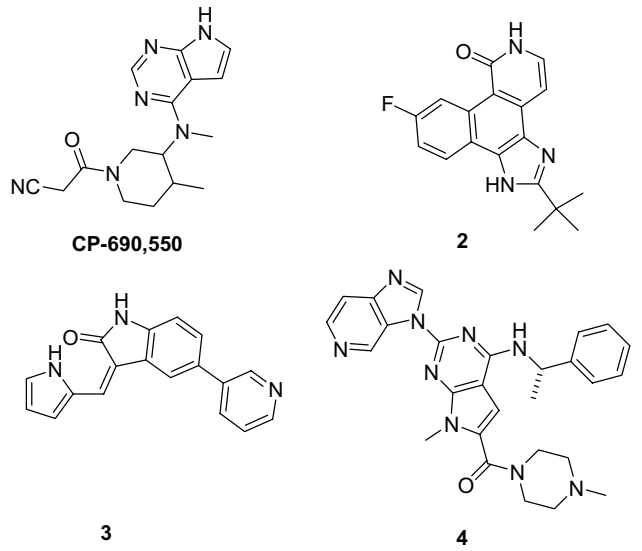
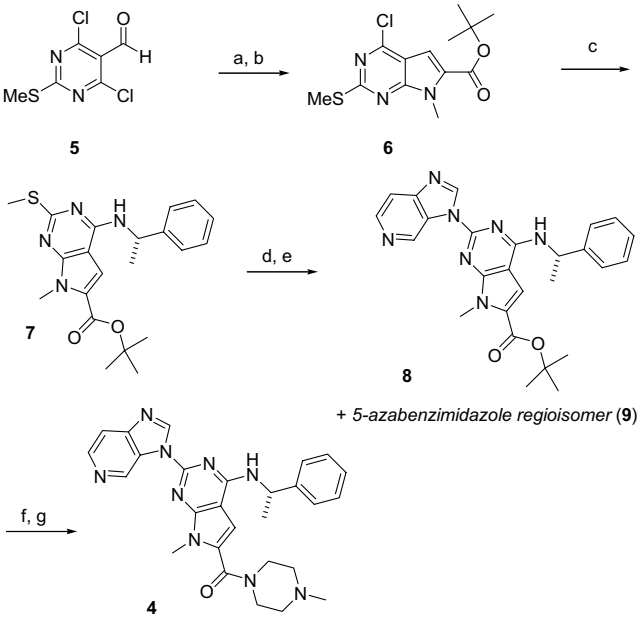


Figure 1. JAK3 inhibitors.

tert-butyl ester, followed by base induced cyclization of the resulting aminoaldehyde, gave the core bicyclic pyrrolopyrimidine ring system **6**. Oxidation of the thiomethyl group with *m*-CPBA, followed by reaction of the resulting sulfoxide with 5-azabenzimidazole under basic conditions at elevated temperature, yielded a mixture of 5-azabenzimidazole and 6-azabenzimidazole analogs, typically favoring the 5-azabenzimidazole regioisomer. Separation of the two regioisomers was accomplished via meticulous silica gel chromatography to yield the desired 6-azabenzimidazole analog. Standard manipulations of the ester functionality provided final amides, such as pyrrolopyrimidine **4**.



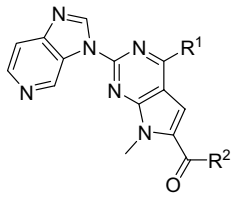
Scheme 1. Reagents and conditions: (a) sarcosine-*tert*-butyl ester-HCl, Et_3N, THF, 75%; (b) NaH, DMF, 0 °C, 58%; (c) (*S*)-α-methyl benzylamine, $^iPr_2NEt$, DMF, 90 °C, 86%; (d) *m*-CPBA, CH_2Cl_2, 0 °C; (e) 5-azabenzimidazole, K_2CO_3, DMF, 140 °C, 74%; (f) CF_3CO_2H, CH_2Cl_2; (g) 1-methylpiperazine, EDAC, HOBt, $^iPr_2NEt$, THF, 68% —2 steps.

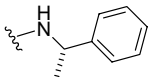
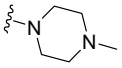
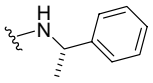
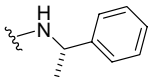
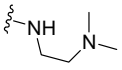
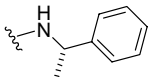
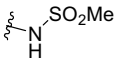
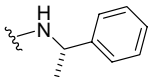
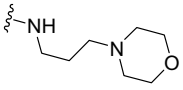
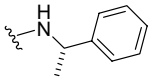
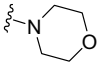
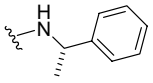
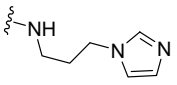
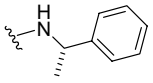
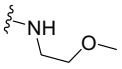
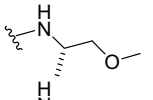
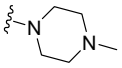
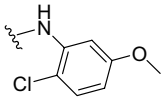
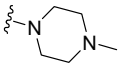
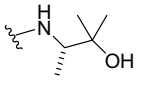
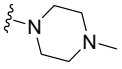
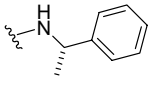
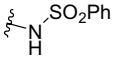
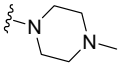
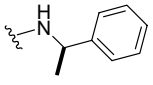
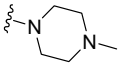
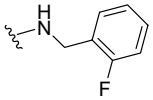
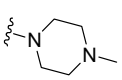
Table 1 summarizes a survey of bicyclic pyrrolopyrimidine analogs. All compounds were tested for inhibition of JAK3 and JAK2 using the Kinase-Glo Luminescent Assay.[8] We found a remarkable difference in potency between the 6-azabenzimidazole and the corresponding 5-azabenzimidazole regioisomers (data not shown). The 6-azabenzimidazole analogs typically showed a 4-fold to 15-fold increase in potency (both JAK3 and JAK2) relative to the 5-azabenzimidazoles. In most cases, the most potent analogs in this series show either equipotency between JAK3 and JAK2 (compounds **12**, **13**, and **15**) or in some cases, a stronger potency for JAK2 than JAK3 (compounds **4**, **10**, **11**, **14**, and **16**). Interestingly, the Pfizer inhibitor **CP-690,550**, reported to possess 20:1 selectivity favoring JAK3 over JAK2,[1] shows virtually no selectivity in either the Kinase-Glo assay (Table 1), or in a separate radiometric filter binding assay.[9] While the analogs substituted at the 4-position with a (*S*)-α-methylbenzylamino substituent showed stronger potency, it comes at the expense of selectivity versus JAK2. Analogs that incorporate an alkylamino substituent at the 4-position of the pyrrolopyrimidine core show hints of JAK3:JAK2 enzymatic selectivity, exemplified by **17** and **19–21**. Analysis of the 2-amido substituent SAR shows that it has a less significant role in potency, and provides a good opportunity for fine tuning selectivity, along with other properties, such as solubility.

We then utilized a TF-1 cellular assay[10] to further measure the responses through either the JAK3 (IL-4 induced proliferation) or the JAK2 (IL-3 induced proliferation) signaling pathways, respectively (Table 2). The Pfizer benchmark, **CP-690,550**, shows a 10:1 selectivity in favor of JAK3 in the TF-1 cellular assays. Our pyrrolopyrimidine analogs typically showed moderate selectivity in the 2- to 5-fold range in favor of JAK3. The most promising analogs screened in the TF-1 cell proliferation assay, pyrrolopyrimidines **18** and **19** show a 4-fold and 10-fold selectivity in favor of JAK-3, respectively. Once again, the 4-alkylamino substituted analogs generally showed improved selectivity versus JAK2 than the 4-benzylamino analogs.

We have reported a novel series of bicyclic pyrrolopyrimidine Janus Kinase inhibitors. Many of the pyrrolopyrimidine inhibitors showed strong potency in the JAK3 enzymatic assay below the 100 nM level. Several of these analogs showed promising selectivity for JAK3 relative to JAK2. One of the pyrrolopyrimidines, **19**, showed comparable potency and selectivity in the TF-1 cell proliferation assay to the Pfizer benchmark **CP-690,550**. Further optimization of this novel scaffold may be reported in future publications.

Table 1. JAK3 and JAK2 data for bicyclic pyrrolopyrimidine analogs



Compound	R^1	R^2	JAK3 IC_{50}[a] (nM)	JAK2 IC_{50}[a] (nM)
CP-690,550	—	—	13	11
4			22	11
10			18	6
11			34	19
12			42	55
13			46	42
14			49	21
15			67	53
16			77	25
17			126	222
18			142	132
19			142	209
20			143	359
21			200	323
22			430	298
23			3050	1860

[a] S.D. for enzyme assays were typically ±30% of the mean or less.

Table 2. Inhibition of proliferation of TF-1 cells, induced by either IL-3 or IL-4, by pyrrolopyrimidine analogs

Compound	Inhibition of IL-4 induced TF-1 cell proliferation IC_{50}[a] (nM)	Inhibition of IL-3 induced TF-1 cell proliferation IC_{50}[b] (nM)
CP-690,550	80[b]	800[b]
4	80	90
10	450	740
11	460	1950
13	910	2790
14	670	1400
17	1160	4790
18	490	2200
19	140	1420

[a] S.D. for enzyme assays were typically ±25% of the mean or less.
[b] Mean, $n > 10$.

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8. Promega's Kinase-GloTM Luminescent Kinase Assay is a homogeneous non-radioactive method of determining the activity of purified kinases by quantifying the amount of ATP remaining in solution following a kinase reaction. The assay procedure involves addition of a single reagent (Kinase-Glo® Reagent) directly to a completed kinase reaction. This addition results in the generation of a luminescent signal correlated with the amount of ATP present and inversely proportional to the amount of kinase activity.
9. Kinase Profiler™ Assays (radiometric filter binding assays) were run by Upstate Cell Signaling Solutions (JAK3 $IC_{50} = 5$ nM , JAK2 $IC_{50} = 6$ nM).
10. All compounds were tested using an assay developed using TF-1 (1, 2) cells (CRL-2003 from ATCC). These cells are unique because they respond to multiple cytokines. They proliferate in response to GM-CSF, IL-3 (3), and IL-4. They were maintained as recommended by the ATCC. IL-3 (Human recombinant Sigma #I 1646) or IL-4 (Human recombinant. Sigma #I 4269) at 1 nM was used to stimulate the cells. Proliferation of the TF-1 cells was used to measure the responses through JAK2 (IL-3) or JAK3 (IL-4) and the inhibition of such response by specific compounds. Five replicates per compound were seeded at a concentration of 5000 cells/well in a 100-μl volume. Five different concentrations of experimental compounds (five replicates each) were used to determine their EC_{50}. After 4 days of incubation, cell proliferation was measured during the last 18 h of incubation, using an ELISA (BrdU, chemiluminescence) kit (Roche Diagnostics, Indianapolis, IN). The relative luminescence units (RLU) were measured using a Packard Fusion instrument (Perkin-Elmer). The RLU were used to calculate EC_{50}s (Prism4 software).