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Article

Identification of a Potent Janus Kinase 3 Inhibitor with High Selectivity within the Janus Kinase Family[†]

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We describe a synthetic approach toward the rapid modification of phenyl-indolyl maleimides and the discovery of potent Jak3 inhibitor **1** with high selectivity within the Jak kinase family. We provide a rationale for this unprecedented selectivity based on the X-ray crystal structure of an analogue of **1** bound to the ATP-binding site of Jak3. While equally potent compared to the Pfizer pan Jak inhibitor CP-690,550 (**2**) in an enzymatic Jak3 assay, compound **1** was found to be 20-fold less potent in cellular assays measuring cytokine-triggered signaling through cytokine receptors containing the common γ chain (γC). Contrary to compound **1**, compound **2** inhibited Jak1 in addition to Jak3. Permeability and cellular concentrations of compounds **1** and **2** were similar. As Jak3 always cooperates with Jak1 for signaling, we speculate that specific inhibition of Jak3 is not sufficient to efficiently block γC cytokine signal transduction required for strong immunosuppression.

Introduction

Janus kinase 3 (Jak3) is essential for signal transduction of cytokines utilizing the common γ chain (CD132/γC) and its absence results in immunodeficiency. Thus, it has been assumed that specific inhibition of Jak3 kinase function would have therapeutic potential as a selective and safe immunosuppressive principle.[1] However, signaling through γC cytokines leading to the phosphorylation of the adaptor protein STAT5[a] also requires Jak1 in addition to Jak3.[2] The relative contributions of the kinase function of Jak1 and Jak3 are not fully understood. The Pfizer Jak inhibitor CP-690,550[3] (**2**, see Figure 1) proved to be active in preclinical models[4] and is currently being clinically assessed in multiple indications.[5] Although originally described as being selective for Jak3,[3] **2** also potently inhibits other Jak kinases including Jak1.[6] Thus, the effects observed with **2** cannot be assigned solely to the specific inhibition of Jak3 kinase function and do not necessarily validate the assumption that specific inhibition of Jak3 kinase function will efficiently block signal transduction through γC-receptors leading to immunosuppression.

Setting out to explore the effects of specific Jak3 inhibition on signal transduction, we identified maleimide **1** exhibiting very high selectivity within the Jak kinase family. Here we describe its synthesis via late stage modification of a preformed maleimide. Furthermore, on the basis of the cocrystal structure of a related analogue with Jak3, we provide a rationale for the selectivity of aryl-indolyl maleimides within the Jak kinases. Surprisingly, compared to the pan Jak inhibitor **2**, compound **1** was found to be significantly less potent in cellular assays measuring STAT5 phosphorylation despite similar permeability and cellular uptake for both compounds.

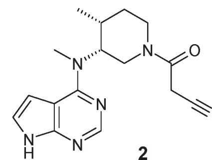

Figure 1. Structure of CP-690,550 = **2**.

Chemistry

Commercially available phenylacetic acid **2** was converted into the corresponding amide **3** (Scheme 1). Reaction with indolyl-oxo-acetic acid ester **4** led to the versatile intermediate **5** allowing for the late stage modification of phenyl-indolyl maleimides. Treatment of **5** with amines **6** furnished compounds **7**−**10**. Coupling of **7** with acid chloride **11** followed by deprotection gave Jak3 inhibitor **1**.

Results and Discussion

The maleimides **1**, **5**, and **7**−**10** were tested in enzymatic assays measuring Jak kinase activity. Compound **1** was highly potent on Jak3 (IC_{50} = 8.0 nM) and showed an unprecedented selectivity within the Jak kinases (IC_{50} > 1000 nM on Jak1, Jak2, and Tyk2).[7] The selectivity for Jak3 seems to be general as indicated by compounds **7**−**10**, which were also most active on Jak3. Compound **5** was inactive on all Jak kinases, indicating the importance of the substituent of the phenyl group.

The X-ray structure of the related maleimide **12**[8] cocrystallized with the Jak3 kinase domain furnished a rationale for

[†]Structure deposited in the RCSB Protein Data Bank under PDB ID: 3PJC.

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[a]Abbreviations: CDI, carbonyldiimidazole; DBU, diazabicycloundecene; DMAP, 4-dimethylaminopyridine; UPLC, ultra performance liquid chromatography; STAT5, signal transducer and activator of transcription 5; PKC, protein kinase C; GSK3, glycogen synthase kinase 3; TCR, T-cell receptor.

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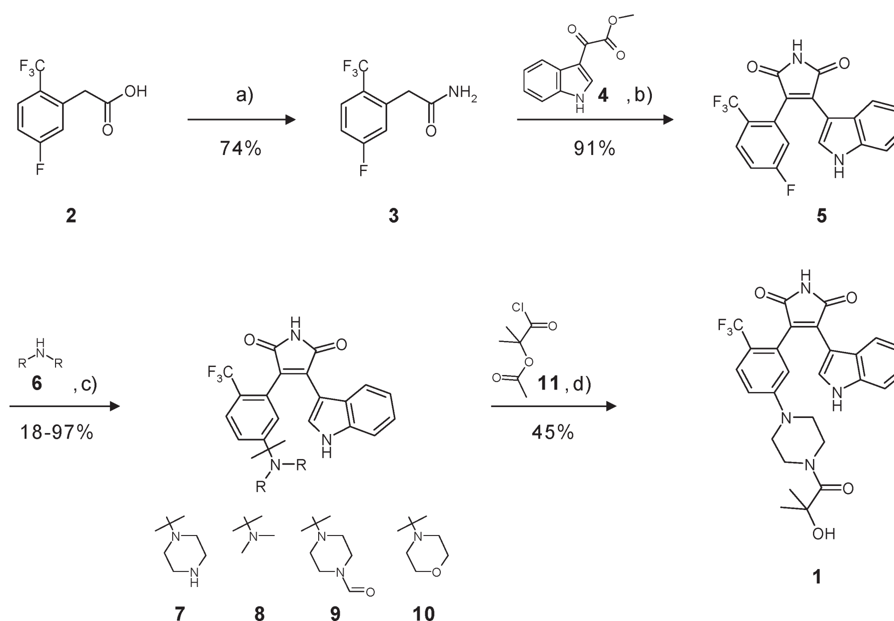
Scheme 1. Synthesis of Phenyl-indolyl Maleimides[a]



[a] Reagents and conditions: (a) (1) CDI, DMF, 25 °C, 0.5 h, (2) NH_3 (7M) in MeOH, 25 °C, 1 h; (b) (1) tBuOK, THF, 10 °C, 2 h, (2) DBU, DMF, 85 °C, 0.5 h; (c) DMSO, microwave, 170 °C, 1 h; (d) (1) DMAP, CH_2Cl_2, 25 °C, 2 h, (2) MeONa, MeOH, 25 °C, 0.5 h.

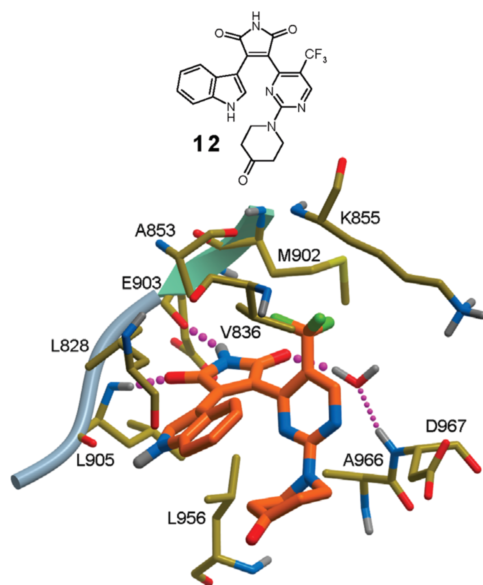


Figure 2. Detailed view of the X-ray crystal structure of compound **12** (carbons in orange) bound to the ATP-binding site of Jak3 (2.5 Å resolution). Hydrogen bonds between the ligand and the protein are represented by dashed magenta lines.

the selectivity of compounds **1** and **7**−**10** within the Jak kinase family (Figure 2, see Table 1 for activity of **12** in enzymatic Jak assays).[9] The maleimide ring binds to the hinge part of the kinase through two hydrogen bonds with adjacent backbone amides (Glu903, Leu905) and makes hydrophobic contacts with the side chains of Ala853 and Leu956. Additional favorable hydrophobic contacts are observed between the indole of **12** and the side chains of Leu828 and Val836. The trifluoromethyl group optimally fits into a small pocket delimited by the side chains of Val836, Ala853, Lys855, and Met902. Most important for the observed selectivity is a water-mediated H-bond between an oxygen atom of the maleimide and the NH of the backbone amide of the catalytic Asp967. In recently

Table 1. Enzymatic Activities[a]

compd	Jak1	Jak2	Jak3	Tyk2
1	1017 ± 117	2550 ± 326	8.0 ± 2	8055 ± 589
5	> 10000	> 10000	> 10000	> 10000
7	737 ± 137	4700 ± 326	133 ± 45	> 10000
8	4700 ± 173	9150 ± 50	423 ± 20	> 10000
9	754 ± 166	1444 ± 455	36 ± 8	5040 ± 682
10	6500 ± 1305	> 10000	460 ± 154	> 10000
12	253 ± 66	330 ± 68	27 ± 7	4333 ± 841
2	6.1 ± 2	12 ± 2	8.0 ± 2	176 ± 25

[a] The final ATP concentration used in the assays corresponds to the individually determined K_m ATP for the respective enzyme. IC_{50} values are reported in nM as the average ± SEM of ≥3 experiments.

reported structures of Jak1 and Jak2, this backbone amide adopts a different conformation which would not favor the water-mediated interaction with the maleimide ring observed in Jak3.[10] This different conformation of both the Jak1 and the Jak2 structure can be explained by the presence of a glycine adjacent to the conserved Asp967. Because Tyk2 also has a glycine in this position, Jak3 is the only Jak kinase with an alanine (Ala966) adjacent to Asp967. The side chain of Ala966 restrains the backbone amide into a conformation favorable to a water-mediated interaction, with the maleimide ring explaining the observed selectivity.

Maleimide **1** was tested against a panel of 40 kinases and found to only inhibit Pkc and Gsk3β with IC_{50} values below 1000 nM. The Pfizer Jak inhibitor **2** was also tested and found to be highly selective against non-Jak kinases. However, within the Jak kinase family, compound **2** was unselective, inhibiting Jak1, Jak2, and Jak3, with IC_{50} values between 6.1 and 13 nM (Table 1). Compounds **2** and **1** were equally potent on Jak3 (IC_{50} = 8 nM). Compound **1** was tested in cellular assays measuring STAT5 phosphorylation triggered by cytokine stimulation (IL-2 in CTLL cells; IL-15 in MO7 cells) and found to be > 20-fold less potent compared to **2** (Table 2).

The weak activity of compound **1** in these cellular assays was not caused solely by a poor permeability of **1**, as permeation through artificial membranes and flux through Caco2

Table 2. Cellular Activity[a]

compd	pSTAT5/IL2 (CTLL cells)	pSTAT5/IL15 (MO7 cells)	TCR/CD28[8] (Jurkat cells)
1	1294 ± 259	525 ± 182	689 ± 125
2	48 ± 2	24 ± 1	> 5000

[a] IC_{50} values are reported in nM as the average ±SEM of ≥3 experiments.

Table 3. Compound Amounts in Whole Cell Extracts

cell type	analyte in growth medium [μM]	compd **2** whole cell extract [pmol/50000 cells]	compd **1** whole cell extract [pmol/50000 cells]
Jurkat	0.33	1.04 ± 0.08	2.4 ± 0.38
	1.0	3.0 ± 0.08	7.88 ± 1.85
	3.3	8.09 ± 0.58	23.48 ± 2.91
	10.0	20.7 ± 2.24	55.58 ± 6.91
CTLL	0.33	1.19 ± 0.17	3.12 ± 1.51
	1.0	3.05 ± 0.73	10.97 ± 1.92
	3.3	10.41 ± 0.31	31.27 ± 4.71
	10.0	22.14 ± 4.23	64.33 ± 5.02

cells were only moderately inferior for compound **1** compared to **2** (compound **1**: Log PAMPA[11] = −5.5, PappA-B_{Caco} = 3×10^6 cm/s; 2: Log PAMPA = −5.2, PappA-B_{Caco} = 10×10^6 cm/s). In addition to Jak3, compound **1** inhibits Pkcα, Pkcθ, and GSK3β, with IC_{50} values of 13, 68, and 3 nM, respectively.[12] To probe its ability to block kinase function in cells, compound **1** was tested in a reporter gene assay measuring TCR/CD28-mediated T cell activation[12] (Jurkat cells) which is dependent on Pkcα and Pkcθ (but not on Jak kinases or Gsk3β).[13] Compound **1** showed moderate activity in this cellular assay (IC_{50} = 689 nM, Table 2), which is in good agreement with its enzymatic Pkc activity.[14] As expected, the pan Jak inhibitor **2** was inactive (Table 2). Next, we measured amounts of compounds **1** and **2** in whole cell extracts of both CTLL cells (IL-2 pSTAT5 assay) and Jurkat cells (T-cell activation assay). We found that compound amounts associated with whole cells (the sum of membrane-associated amount and intracellular amount) were independent of the cell type and similar for compounds **1** and **2** (Table 3). However, cytosolic concentrations which might be even more relevant for the inhibition of Jak3 in the cell could not be determined. Nevertheless, our data suggest that compound **1** is present in cells and can block intracellular processes triggered by PKC. Thus, because Jak3 and Jak1 always cooperate for signaling through γC-receptors, the inferior cellular potency of the selective Jak3 inhibitor **1** compared to the pan Jak inhibitor **2** could indicate that selective inhibition of Jak3 is not sufficient to achieve strong immunosuppressive effects.

Conclusion

We have described the discovery of the phenyl-indolyl maleimide **1**, which is a potent Jak3 inhibitor with high selectivity within the Jak kinase family. Surprisingly, **1** was found to be significantly less potent in cellular STAT5 phosphorylation assays compared to the pan Jak inhibitor **2** which is equally potent on Jak1 and Jak3. Cellular permeability and compound amounts associated with whole cells were found to be similar for compounds **1** and **2**. In addition, compound **1** (which also inhibits PKC) was as potent as expected in a cellular assay dependent on PKC. Thus, our data suggest that selective inhibition of Jak-3 is not sufficient to efficiently block immunologically relevant pathways mediated by γC cytokines. Further work to better understand the individual roles of Jak kinases in STAT phosphorylation is in progress.[15]

Experimental Section

General. All reactions were carried out under an atmosphere of dry argon. Commercially available absolute solvents were used. The NMR spectra were recorded on a Bruker Avance DPX 400 spectrometer at room temperature. The HR-MS spectra were obtained on Finnigan MAT 900 S or Bruker Daltronics 9.4T APEX-III FT-MS mass spectrometers. LC/MS was performed on a Waters Acquity UPLC linked to a Waters ZQ 2000 mass spectrometer using a Waters BEH C18 1.7 μm 2.1 mm × 50 mm column (flow = 0.7 mL/min; detection 240−350 nM DAD; solvent A, water + 0.1% formic acid; solvent B, acetonitrile; method for compound **7**, t = 0 min, 95% A, 5% B; t = 1 min 90% A, 10% B; t = 4.0 min 10% A, 90% B; t = 4.1 min 100% B; t = 4.5 min, 100% B; method for compounds **1**, **8**, **9**, **10**, **12**: t = 0 min, 80% A, 20% B; t = 1 min 75% A, 25% B; t = 4.2 min 5% A, 95% B; t = 4.3 min 100% B; t = 4.5 min, 100% B). The purity of all test compounds was > 95%.

2-(5-Fluoro-2-trifluoromethyl-phenyl)-acetamide (3). CDI (18.8 g, 110 mmol) was added to a solution of (5-fluoro-2-trifluoromethyl-phenyl)-acetic acid (**2**) in DMF (60 mL). After stirring for 0.5 h at 25 °C, NH_3 (60 mL of a 7 M solution in MeOH) was added and stirring continued for 1 h. Water was added (200 mL) and the precipitate was filtered off, washed with water, and dried to give **3** (16.5 g, 74%), which was used without further purification. ^1H NMR (400 MHz, DMSO-d_6) δ = 7.75 (m, 1 H), 7.50 (s, 1 H), 7.30 (m, 2 H), 7.00 (s, 1 H), 3.65 (s, 2 H). MS/ESI 222 $(M + 1)^+$.

3-(5-Fluoro-2-trifluoromethyl-phenyl)-4-(1*H*-indol-3-yl)-pyrrole-2,5-dione (5). At 0 °C, tBuOK (370 mL 1 M in THF) was added to a solution of **3** (16.5 g, 74.6 mmol) and **4** (18.2 g, 85.1 mmol) in THF (350 mL). The solution was stirred for 2 h at 10 °C, HCl (125 mL 4N) was added, the mixture was extracted with ethyl acetate, and the organic phase was dried with Na_2SO_4. The solvent was removed and the residue dried. DBU (17 g) was added to a solution of this residue in DMF (50 mL) and stirred for 0.5 h at 85 °C. The mixture was cooled to 25 °C, diluted with ethyl acetate, and the pH adjusted to 1 with HCl (4N). The organic phase was separated, dried with Na_2SO_4, the solvent removed, and the residue purified by chromatography (SiO_2, cyclohexane/acetone gradient) to give **5** (25.6 g, 91%) as a yellow powder. ^1H NMR (400 MHz, DMSO-d_6) δ = 12.0 (s, 1 H), 11.2 (s, 1 H), 8.02 (m, 1 H), 7.96 (m, 1 H), 7.54 (m, 1 H), 7.42 (m, 2 H), 7.06 (m, 1 H), 6.74 (m, 1 H), 6.45 (m, 1 H). MS/ESI 375 $(M + 1)^+$.

3-(1*H*-Indol-3-yl)-4-(5-piperazin-1-yl-2-trifluoromethyl-phenyl)-pyrrole-2,5-dione (7). A mixture of **5** (1.0 g, 2.67 mmol), piperazine (1.0 g, 11.6 mmol), and DMSO (5 mL) was heated in a microwave oven for 1 h at 150 °C. The mixture was diluted with ethyl acetate and *tert*-butyl-methylether and the precipitate filtered off. Compound **7** (1.15 g, 97%) was isolated as a yellow powder. ^1H NMR (400 MHz, DMSO-d_6) δ = 11.85 (s, 1 H), 11.2 (broad m, 1 H), 7.95 (s, 1 H), 7.60 (m, 1 H), 7.28 (m, 1 H), 7.08 (m, 2 H), 6.90 (m, 1 H), 6.71 (m, 2 H), 3.08 (m, 4 H), 2.68 (m, 4 H). The signal for the NH of the piperazine could not be detected most likely due to line broadening. HR-MS $[M + 1]^+$ observed = 441.1534, estimated = 441.1533.

3-{5-[4-(2-Hydroxy-2-methyl-propionyl)-piperazin-1-yl]-2-trifluoromethyl-phenyl}-4-(1*H*-indol-3-yl)-pyrrole-2,5-dione (1). Compound **11** (0.83 g, 5.0 mmol) was slowly added to a suspension of **7** (0.881 g, 2.0 mmol) and *N*,*N*-dimethylpyridine-4-amine (0.204 g, 2.0 mmol) in THF (20 mL) and the mixture stirred for 2 h at 25 °C. The mixture was diluted with ethyl acetate, washed with HCl and brine, and dried with Na_2SO_4. The solvent was removed and the residue subjected to chromatography (SiO_2, cyclohexane/acetone gradient) to give **1** (0.50 g, 45%) as a yellow powder. ^1H NMR (400 MHz, DMSO-d_6) δ = 11.87 (s, 1 H), 11.08 (s, 1 H), 7.95 (s, 1 H), 7.62 (m, 1 H), 7.38 (m, 1 H), 7.14 (m,

1 H), 7.05 (m, 1 H), 6.95 (s, 1 H), 6.72 (m, 1 H), 6.65 (m, 1 H), 5.43 (s, 1 H), 3.95 (m, 2 H), 3.50 (m, 2 H), 3.20 (m, 4 H), 1.30 (2s, 6 H). HR-MS M + 1^+ observed = 527.1904, estimated = 527.1901.

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Supporting Information Available: Analytical data for compounds and **8**, **9**, **10**, and **12**, details of enzymatic and cellular assays as well as procedures for the measurement of compound amounts in whole cells. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) O'Shea, J. J.; Pesu, M.; Borie, D. C.; Changelian, P. S A new modality for immunosuppression: targetting the JAK/STAT pathway. *Nature Rev. Drug Discovery* **2004**, *3*, 555–564. (b) Pesu, M.; Laurence, A.; Kishore, N.; Zwillich, S. H.; Chan, G.; O'Shea, J. J. Therapeutic targeting of Janus kinases. *Immunol. Rev*. **2008**, *223*, 132–142.

(2) (a) Imada, K.; Leonard, W. J. The Jak-STAT pathway. *Mol Immunol*. **2000**, *37*, 1–11. (b) Gaffen, S. L. Signaling domains of the interleukin 2 receptor. *Cytokine* **2001**, *14*, 63–77. (c) Benczik, M.; Gaffen, S. L. The interleukin (IL)-2 family cytokines: survival and proliferation signaling pathways in T lymphocytes. *Immunol. Invest*. **2004**, *33*, 109–142. (d) Rodig, S. J.; Meraz, M. A.; White, J. M.; Lampe, P. A.; Riley, J. K.; Arthur, C. D.; King, K. L.; Sheehan, K. C. F.; Yin, L.; Pennica, D.; Johnson, E. M., Jr.; Schreiber, R. D. Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. *Cell* **1998**, *93*, 373–383.

(3) Changelian, P. S.; Flanagan, M. E.; Ball, D. J.; Kent, C. R.; Magnuson, K. S.; Martin, W. H.; Rizzuti, B. J.; Sawyer, P. S.; Perry, B. D.; Brissette, W. H.; McCurdy, S. P.; Kudlacz, E. M.; Conklyn, M. J.; Elliott, E. A.; Koslov, E. R.; Fisher, M. B.; Strelevitz, T. J.; Yoon, K.; Whipple, D. A.; Sun, J.; Munchhof, M. J.; Doty, J. L.; Casavant, J. M.; Blumenkopf, T. A.; Hines, M.; Brown, M. F.; Lillie, B. M.; Subramanyam, C.; Chang, S.-P.; Milici, A. J.; Beckius, G. E.; Moyer, J. D.; Su, C.; Woodworth, T. G.; Gaweco, A. S.; Beals, C. R.; Littman, B. H.; Fisher, D. A.; Smith, J. F.; Zagouras, P.; Magna, H. A.; Saltarelli, M. J.; Johnson, K. S.; Nelms, L. F.; Des Etages, S. G.; Hayes, L. S.; Kawabata, T. T.; Finco-Kent, D; Baker, D. L.; Larson, M.; Si, M.-S.; Paniagua, R.; Higgins, J.; Holm, B.; Reitz, B.; Zhou, Y.-J.; Morris, R. E.; O'Shea, J. J.; Borie, D. C. Prevention of Organ Allograft Rejection by a Specific Janus Kinase 3 Inhibitor. *Science* **2003**, *302*, 875–878.

(4) (a) Kudlacz, E.; Conklyn, M.; Andresen, C.; Whitney-Pickett, C.; Changelian, P. The JAK-3 inhibitor CP-690550 is a potent anti-inflammatory agent in a murine model of pulmonary eosinophilia. *Eur. J. Pharmacol*. **2008**, *582*, 154–161. (b) Ming, F.; Xiang, D. Effect of CP-690550 on acute rejection for allogeneic heart transplanting in mice. *Guizhou Yiyao* **2009**, *33*, 299–301. (c) Rousvoal, G.; Si, M.-S.; Lau, M.; Zhang, S.; Berry, G. J.; Flores, M. G.; Changelian, P. S.; Reitz, B. A.; Borie, D. C. Janus kinase 3 inhibition with CP-690,550 prevents allograft vasculopathy. *Transplant Int*. **2006**, *19*, 1014–1021. (d) Borie, D. C.; Larson, M. J.; Flores, M. G.; Campbell, A.; Rousvoal, G.; Zhang, S.; Higgins, J. P.; Ball, D. J.; Kudlacz, E. M.; Brissette, W. H.; Elliott, E. A.; Reitz, B. A.; Changelian, P. S. Combined Use of the JAK3 Inhibitor CP-690,550 with Mycophenolate Mofetil to Prevent Kidney Allograft Rejection in Nonhuman Primates. *Transplantation* **2005**, *80*, 1756–1764. (e) Paniagua, R.; Si, M.-S.; Flores, M. G.; Rousvoal, G.; Zhang, S.; Aalami, O.; Campbell, A.; Changelian, P. S.; Reitz, B. A.; Borie, D. C. Effects of JAK3 Inhibition with CP-690,550 on Immune Cell Populations and Their Functions in Nonhuman Primate Recipients of Kidney Allografts. *Transplantation* **2005**, *80*, 1283–1292. (f) Borie, D. C.; Changelian, P. S.; Larson, M. J.; Si, M.-S.; Paniagua, R.; Higgins, J. P.; Holm, B.; Campbell, A.; Lau, M.; Zhang, S.; Flores, M. G.; Rousvoal, G.; Hawkins, J.; Ball, D. A.; Kudlacz, E. M.; Brissette, W. H.; Elliott, E. A.; Reitz, B. A.; Morris, R. E. Immunosuppression by the JAK3 Inhibitor CP-690,550 Delays Rejection and Significantly Prolongs Kidney Allograft Survival in Nonhuman Primates. *Transplantation* **2005**, *79*, 791–801.

(5) (a) Lawendy, N.; Krishnaswami, S.; Wang, R.; Gruben, D.; Cannon, C.; Swan, S.; Chan, G. Effect of CP-690,550, an orally active Janus kinase inhibitor, on renal function in healthy adult volunteers. *J. Clin. Pharmacol*. **2009**, *49*, 423–429. (b) Busque, S.; Leventhal, J.; Brennan, D. C.; Steinberg, S.; Klintmalm, G.; Shah, T.; Mulgaonkar, S.; Bromberg, J. S.; Vincenti, F.; Hariharan, S.; Slakey, D.; Peddi, V. R.; Fisher, R. A.; Lawendy, N.; Wang, C.; Chan, G. Calcineurin-inhibitor-free immunosuppression based on the JAK inhibitor CP-690,550: a pilot study in de novo kidney allograft recipients. *Am. J. Transplant*. **2009**, *9*, 1936–1945. (c) Kremer, J. M.; Bloom, B. J.; Breedveld, F. C.; Coombs, J. H.; Fletcher, M. P.; Gruben, D.; Krishnaswami, S.; Burgos-Vargas, R.; Wilkinson, B.; Zerbini, C. A. F.; Zwillich, S. H. The safety and efficacy of a JAK inhibitor in patients with active rheumatoid arthritis: results of a double-blind, placebo-controlled phase IIa trial of three dosage levels of CP-690,550 versus placebo. *Arthritis Rheum*. **2009**, *60*, 1895–1905. (d) van Gurp, E. A. F. J.; Schoordijk-Verschoor, W.; Klepper, M.; Korevaar, S. S.; Chan, G.; Weimar, W.; Baan, C. C. The Effect of the JAK Inhibitor CP-690,550 on Peripheral Immune Parameters in Stable Kidney Allograft Patients. *Transplantation* **2009**, *87*, 79–86. (e) van Gurp, E.; Weimar, W.; Gaston, R.; Brennan, D.; Mendez, R.; Pirsch, J.; Swan, S.; Pescovitz, M. D.; Ni, G.; Wang, C.; Krishnaswami, S.; Chow, V.; Chan, G. Phase 1 dose-escalation study of CP-690 550 in stable renal allograft recipients: preliminary findings of safety, tolerability, effects on lymphocyte subsets and pharmacokinetics. *Am. J. Transplant*. **2008**, *8*, 1711–1718.

(6) (a) Jiang, J. K.; Jiang; Ghoreschi, K.; Deflorian, F.; Chen, Z.; Perreira, M.; Pesu, M.; Smith, J.; Nguyen, D.-T.; Liu, E. H.; Leister, W.; Costanzi, S.; O'Shea, J. J.; Thomas, C. J. Examining the chirality, conformation and selective kinase inhibition of 3-((3*R*,4*R*)-4-methyl-3-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)-piperidin-1-yl)-3-oxopropanenitrile (CP-690,550). *J. Med. Chem*. **2008**, *51*, 8012–8018. (b) Williams, N. K.; Bamert, R. S.; Patel, O.; Wang, C.; Walden, P. M.; Wilks, A. F.; Fantino, E.; Rossjohn, J.; Lucet, I. S. Dissecting Specificity in the Janus Kinases: The Structures of JAK-Specific Inhibitors Complexed to the JAK1 and JAK2 Protein Tyrosine Kinase Domains. *J. Mol. Biol*. **2009**, *387*, 219–232. (c) Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri, P.; Davis, M. I.; Edeen, P. T.; Faraoni, R.; Floyd, M.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P. A quantitative analysis of kinase inhibitor selectivity. *Nature Biotechnol*. **2008**, *26*, 127–132. (d) Chen, J. J.; Thakur, K. D.; Clark, M. P.; Laughlin, S. K.; George, K. M.; Bookland, R. G.; Davis, J. R.; Cabrera, E. J.; Easwaran, V.; De, B.; Zhang, Y. G. Development of pyrimidine-based inhibitors of Janus tyrosine kinase 3. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 5633–5638.

(7) Very recently a compound has been described as a selective Jak3 inhibitor. However, this compound inhibited all Jak kinases with low nanomolar IC_{50} values and did not substantially differ from the pan Jak inhibitor **2** when directly compared in cellular assays. Data from enzymatic Jak kinase assays allowing for a direct comparison of this compound and **2** have not been provided. Lin, T. H.; Hegen, M.; Quadros, E.; Nickerson-Nutter, C. L.; Appell, K. C.; Cole, A. G.; Shao, Y.; Tam, S.; Ohlmeyer, M.; Wang, B.; Goodwin, D. G.; Kimble, E. F.; Quintero, J.; Gao, M.; Symanowicz, P.; Wrocklage, C.; Lussier, J.; Schelling, S. H.; Hewet, A. G.; Xuan, D.; Krykbaev, R.; Togias, J.; Xu, X.; Harrison, R.; Mansour, T.; Collins, M.; Clark, J. D.; Webb, M. L.; Seidl, K. J. Selective Functional Inhibition of JAK3 Kinase Is Sufficient for Efficacy in Collagen Induced Arthritis in Mice. *Arthritis Rheum*. **2010**, *62*, 2283–2293.

(8) Generally, phenyl-indolyl maleimides and corresponding pyrimidyl-indolyl maleimides showed similar activites and selectivities within the Jak kinases. Compound **12** was prepared in analogy to procedures previously disclosed. Albert, R.; Cooke, N. G.; Cottens, S.; Ehrhardt, C.; Evenou, J.-P.; Sedrani, R.; Von Matt, P.; Wagner, J.; Zenke, G. Preparation of indolylmaleimide derivatives as protein kinase c inhibitors. PCT Int. Appl. WO 2002/038561 A1, 2002.

(9) Structure deposited in the RCSB Protein Data Bank under PDB ID: 3PJC.

(10) (a) Williams, N. K.; Bamert, R. S.; Patel, O.; Wang, C.; Walden, P. M.; Wilks, A. F.; Fantino, E.; Rossjohn, J.; Lucet, I. S. Dissecting Specificity in the Janus Kinases: The Structures of JAK-Specific Inhibitors Complexed to the JAK1 and JAK2 Protein Tyrosine Kinase Domains (2009). *J. Mol. Biol*. **2008**, *387*, 219–232. (b) Ledeboer, M. W.; Pierce; Albert, C.; Duffy, J. P.; Gao, H.; Messersmith, D.; Salituro, F. G.; Nanthakumar, S.; Come, J.; Zuccola, H. J.; Swenson, L.; Shlyakter, D.; Mahajan, S.; Hoock, T.; Fan, B.; Tsai, W.-J.; Kolaczkowski, E.; Carrier, S.; Hogan, J. K.; Zessis, R.; Pazhanisamy, S.; Bennani, Y. L. 2-Aminopyrazolo[1,5-a]pyrimidines as potent and selective inhibitors of JAK2. *Bioorg. Med. Chem. Lett*. **2009**, *19*, 6529–6533.

(11) (a) Wohnsland, F.; Faller, B. High-Throughput Permeability pH Profile and High-Throughput Alkane/Water log *P* with Artificial Membranes. *J. Med. Chem*. **2001**, *44*, 923–930. (b) Faller, B. Artificial membrane assays to assess permeability. *Cur. Drug Metab*. **2008**, *9*, 886–892.

(12) Details of assays have been described in the following reference. Wagner, J.; von Matt, P.; Sedrani, R.; Albert, R.; Cooke, N.; Ehrhardt, C.; Geiser, M.; Rummel, G.; Stark, W.; Strauss, A.; Cowan-Jacob, S. W.; Beerli, C.; Weckbecker, G.; Evenou, J.-P.; Zenke, G.; Cottens, S. Discovery of 3-(1*H*-Indol-3-yl)-4-[2-(4-methylpiperazin-1-yl)quinazolin-4-yl]pyrrole-2,5-dione(AEB071), a Potent and Selective Inhibitor of Protein Kinase C Isotypes. *J. Med. Chem.* **2009**, *52*, 6193–6196.

(13) Neither the pan Jak inhibitor **2** nor a highly potent and selective GSK3β inhibitor (compound **11** in the following reference) was active in this assay. Engler, T. A.; Henry, J. R.; Malhotra, S.; Cunningham, B.; Furness, K.; Brozinick, J.; Burkholder, T. P.; Clay, M. P.; Clayton, J.; Diefenbacher, C.; Hawkins, E.; Iversen, P. W.; Li, Y.; Lindstrom, T. D.; Marquart, A. L.; McLean, J.; Mendel, D.; Misener, E.; Briere, D.; O'Toole, J. C.; Porter, W. J.; Queener, S.; Reel, J. K.; Owens, R. A.; Brier, R. A.; Eessalu, T. E.; Wagner, J. R.; Campbell, R. M.; Vaughn, R. Substituted 3-Imidazo[1,2-*a*]pyridin-3-yl-4-(1,2,3,4-tetrahydro-[1,4]diazepino-[6,7,1-*hi*]-indol-7-yl)pyrrole-2,5-diones as Highly Selective and Potent Inhibitors of Glycogen Synthase Kinase-3. *J. Med. Chem.* **2004**, *47*, 3934–3937.

(14) Reference 12 describes PKC inhibitors which were also tested in the TCR/CD28-mediated T cell activation assay. Compounds with enzymatic activities comparable to maleimide 1 showed similar potencies in the cellular assay.

(15) Haan, C.; Rolvering, C.; Raulf, F.; Kapp, M.; Drückes, P.; Thoma, G.; Behrmann, I.; Zerwes, H.-G. unpublished results.