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Discovery of a series of novel 5*H*-pyrrolo[2,3-*b*]pyrazine-2-phenyl ethers, as potent JAK3 kinase inhibitors

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ABSTRACT

We report the discovery of a novel series of ATP-competitive Janus kinase 3 (JAK3) inhibitors based on the 5*H*-pyrrolo[2,3-*b*]pyrazine scaffold. The initial leads in this series, compounds **1a** and **1h**, showed promising potencies, but a lack of selectivity against other isoforms in the JAK family. Computational and crystallographic analysis suggested that the phenyl ether moiety possessed a favorable vector to achieve selectivity. Exploration of this vector resulted in the identification of **12b** and **12d**, as potent JAK3 inhibitors, demonstrating improved JAK family and kinase selectivity.

The Janus kinases (JAKs) are a family of four non-receptor tyrosine kinases whose central role is to respond to cytokine or growth factor receptor activation. Signals from more than 38 cytokines are transduced through the four JAK family members and seven signal transducers and activators of transcription proteins (STATs). The members of the JAK family include the closely related isoforms JAK1, JAK2, JAK3 and tyrosine kinase 2 (Tyk2). JAK family kinases are essential for the signaling pathways of various cytokines implicated in the pathogenesis of autoimmune and inflammatory diseases.[1,2] Selective JAK inhibitors may be useful as therapeutic agents in the areas of oncology, organ transplantation, and autoimmune diseases.[3–5] This expectation has been realized in the two recent FDA approvals of ruxolitinib, a drug for the treatment of myelofibrosis,[5] and tofacitinib (CP690, 550), a pan-JAK inhibitor that shows efficacy and acceptable safety for the treatment of rheumatoid arthritis (RA)(Fig. 1).[6–9]

While JAK1, JAK2, and Tyk2 are ubiquitously expressed in vertebrates, JAK3 is mainly limited to hematopoietic cells.[4] Therefore, selective targeting of JAK3 may offer therapeutic benefit while minimizing potential liabilities associated with inhibition of broader JAK signaling.[10] Recently we have disclosed a new inhibitor scaffold which demonstrated promising selectivity for JAK3 over JAK1.[11] Herein we report a new phenyl ether-containing series of JAK3 selective inhibitors derived from our previous work through a structure based drug design strategy.

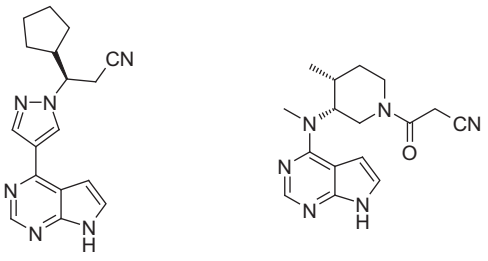

Figure 1. Structures of FDA approved JAK inhibitors.

Initially, we were attracted to the 5*H*-pyrrolo[2,3-*b*]pyrazine-2-phenyl ethers **1a** and **1h** based on their promising JAK3 potency in our enzyme assay (Fig. 2). However, these compounds were limited by their modest JAK family and kinome selectivity.[12] To overcome this selectivity challenge, we developed a strategy targeting a cysteine residue (Cys909) rare to JAK3. Only 10 out of 518 protein kinases possess a cysteine at this position, which is occupied by serine in both JAK2 and JAK1. We hypothesized that hydrophobic groups placed in this region of the binding pocket should experi-

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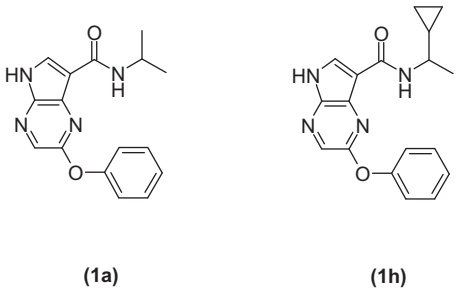


Figure 2. Structures of initial phenyl ethers.

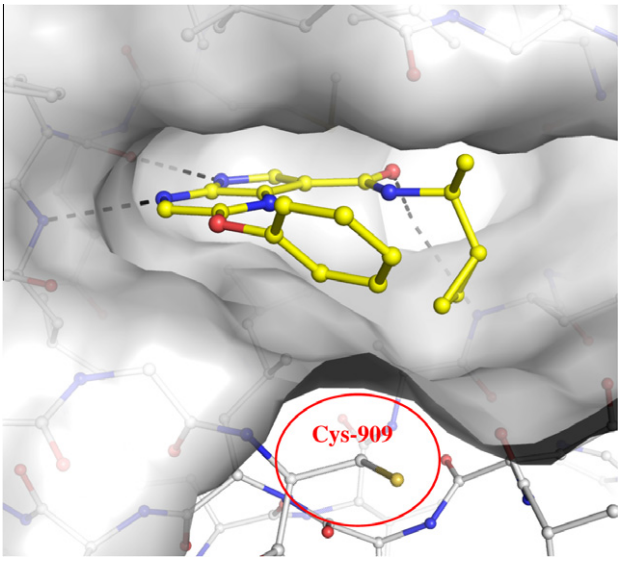


Figure 3. Compound **1h** bound to JAK3 non-phosphorylated kinase domain (1.85 Å resolution, PDB accesion number 416Q).[13] The enantiomer bound to the protein was clearly identified.

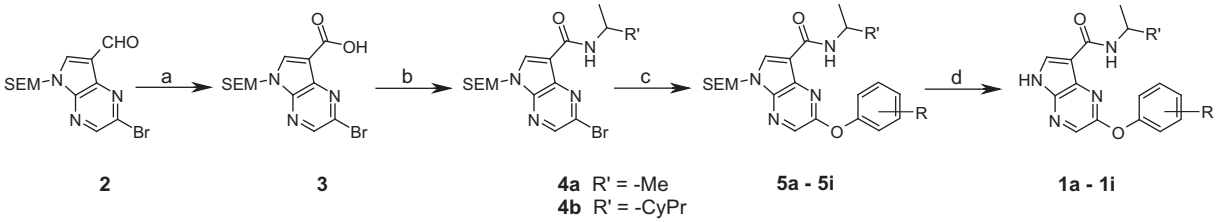
ence fewer unfavorable interactions with cysteine in JAK3 than serine (and its water network) in other JAK family members, thus leading to improved JAK family selectivity. The X-ray structure of the phenyl ether **1h** bound to JAK3 confirmed that the phenyl ring does indeed offer attractive vectors to reach Cys-909 (Fig. 3). Thus, further computational and structure-based design resulted in the syntheses of **12a, 12b**, and **12d**, which demonstrated potent JAK3 inhibition as well as JAK family and kinase selectivity.

Our first efforts to explore this series focused on meta-substitution which appeared to be the most attractive vector on the phenyl ring to reach Cys 909. Thus, we designed and synthesized a series of phenyl ethers around parent compounds **1a** and **1h** to optimize selectivity for JAK3 over JAK1/2.

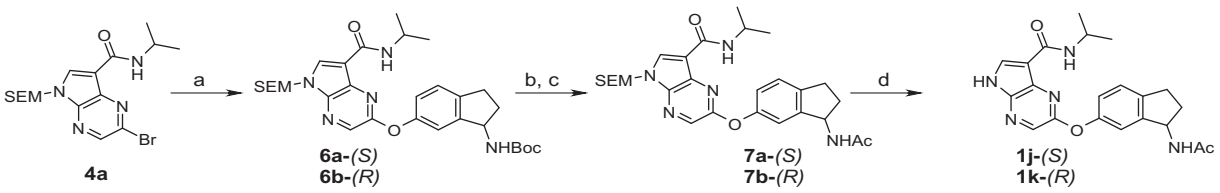
The syntheses of these compounds were achieved starting from the bromo-aldehyde compound **2**.[14] Oxidation of the aldehyde **2** provided the corresponding carboxylic acid **3**. Amidation of **3** with iso-propylamine or 2-cyclopropyl-ethylamine afforded compounds **4a** and **4b** in good yield. These bromo-amide compounds were crucial intermediates for the preparation of our first series of compounds. Buchwald cross coupling[15] of the bromo-compounds **4a** and **4b** with the corresponding phenol gave the phenyl-ethers **5a**–**5i**. Subsequent removal of the trimethylsilanyl-ethoxymethyl group (SEM) provided the final products **1a**–**1i** in this series (Scheme 1). For phenyl ethers **1j** and **1k** a similar approach was followed using the *N*-(*tert*-butoxycarbonyl)(Boc) protected intermediates **6a** and **6b** (Scheme 2).

From the initial SAR on these compounds, the (*S*)-α-amino indano-ether **1k** stood out based on its good JAK3 enzyme potency and promising JAK family selectivity (Table 1). In an attempt to improve both potency and kinase selectivity, we modeled **1h** into the binding site of JAK3 and looked for the best vectors from the iso-propyl amide to optimize the filling of back pockets of the protein. This led to the cyclopropyl-methyl amides **12a** and **12b** with a significant potency boost while maintaining good selectivity compared to **1j** and **1k**. The crystal structure of **12a** bound to JAK3 (Fig. 4) showed the anticipated lipophilic interaction with Cys909 and a novel backbone interaction of the inhibitor with Leu828. We rationalized that this intermolecular H-bond provides a rigidifying element for selectivity and the cyclopropyl improves potency due to space filling of a back pocket.

Next and based on previous knowledge[11] we decided to prepare larger bis-amide analogs in order to better fill the upper-back pocket of the protein. For this exercise, we also used the bromo-aldehyde compound **2** as starting material (Scheme 3). Buchwald cross coupling[15] of the bromo-compound **2** with the corresponding

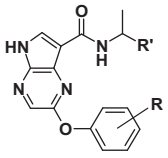


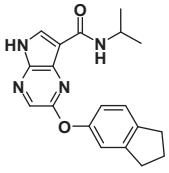
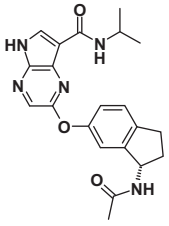
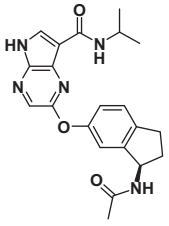
Scheme 1. Reagents and conditions: (a) sulfamic acid, $NaClO_2$, KH_2PO_4, dioxane/water, 94%; (b) *i*-$PrNH_2$ or 2-cyclopropyl-ethylamine, HATU, DMF, 80–94%; (c) $Pd(OAc)_2$, K_3PO_4, 2-di-*t*-butylphosphino-2′-(*N*,*N*-dimethylamino)-biphenyl, Ar-OH, toluene 140 °C, 20–60%; (d) AcOH/HCl, at 65 °C, then EDA/MeOH/H_2O, 20–65%.



Scheme 2. Reagents and conditions: (a) (*R*)-*t*-butyl 6-hydroxy-2,3-dihydro-1*H*-inden-1-ylcarbamate, $Pd(OAc)_2$, K_3PO_4, 2-di-*t*-butylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl, toluene 140 °C, 53%; (b) AcCl, MeOH, quantitative yield; (c) Ac_2O, pyridine, DCM, 59%; (d) AcOH/HCl, at 65 °C, then EDA/MeOH/H_2O, 65%.

Table 1
Enzyme potencies[b] for compounds prepared according to Schemes 1 and 2



Compound	R	R'	IC_{50} (μM)			Selectivity	
			JAK3	JAK2	JAK1	JAK2/JAK3	JAK1/JAK3
1a	–H	–Me	0.21 (0.06)[c]	0.25 (0.06)	0.84 (0.05)	1.2	3.9
1b	*m*-Me	–Me	0.28 (0.07)	0.41 (0.10)	2.15 (0.15)	1.4	7.5
1c	*m*-Et	–Me	0.47 (0.06)	1.21 (0.10)	4.16 (0.19)	2.6	8.8
1d	3,5-di-OMe	–Me	0.40 (0.09)	1.00 (0.29)	8.22 (0.80)	2.5	20.2
1e	*m*-CN	–Me	0.97 (0.36)	1.25 (0.02)	3.19 (0.30)	1.3	3.3
1f	*p*-CN	–Me	0.42 (0.04)	0.90 (0.15)	3.47 (0.71)	2.1	8.2
1g	*o*-Me	–Me	1.38 (0.47)	2.32 (0.41)	5.09 (0.17)	1.7	3.7
1h	–H	–CyPr	0.03 (0.004)	0.07 (0.008)	0.22 (0.05)	2.1	6.5
1i			0.37 (0.06)	0.58 (0.12)	3.32 (0.09)	1.5	8.8
1j[a]			0.34 (0.05)	1.71 (0.10)	4.39 (0.87)	4.9	12.6
1k[a]			0.14 (0.04)	1.01 (0.10)	6.30 (0.51)	7.1	43.9

[a] These compounds were prepared according with the Scheme 2.
[b] Inhibition of phosphorylation of a biotinylated synthetic peptide catalyzed by JAK1-3.[11] All enzyme reactions were run at adenosine triphosphate (ATP) concentrations of 1.5 μM. Km's of these enzymes for ATP under our experimental conditions were determined to be 1.5 μM (JAK3), 6 μM (JAK2), and 20 μM (JAK1).
[c] Mean [SEM(standard error of the mean)], $n \geqslant 3$.

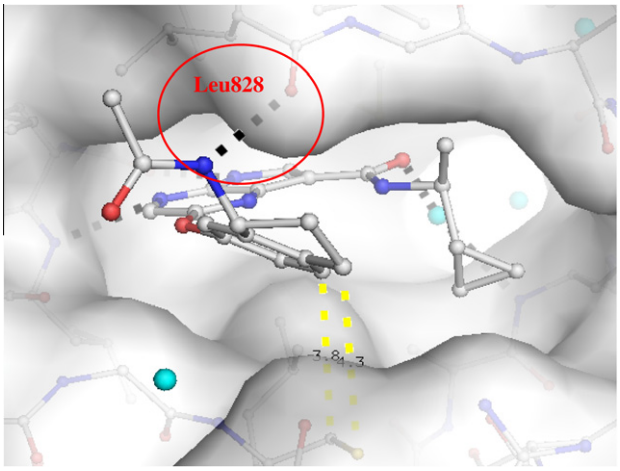
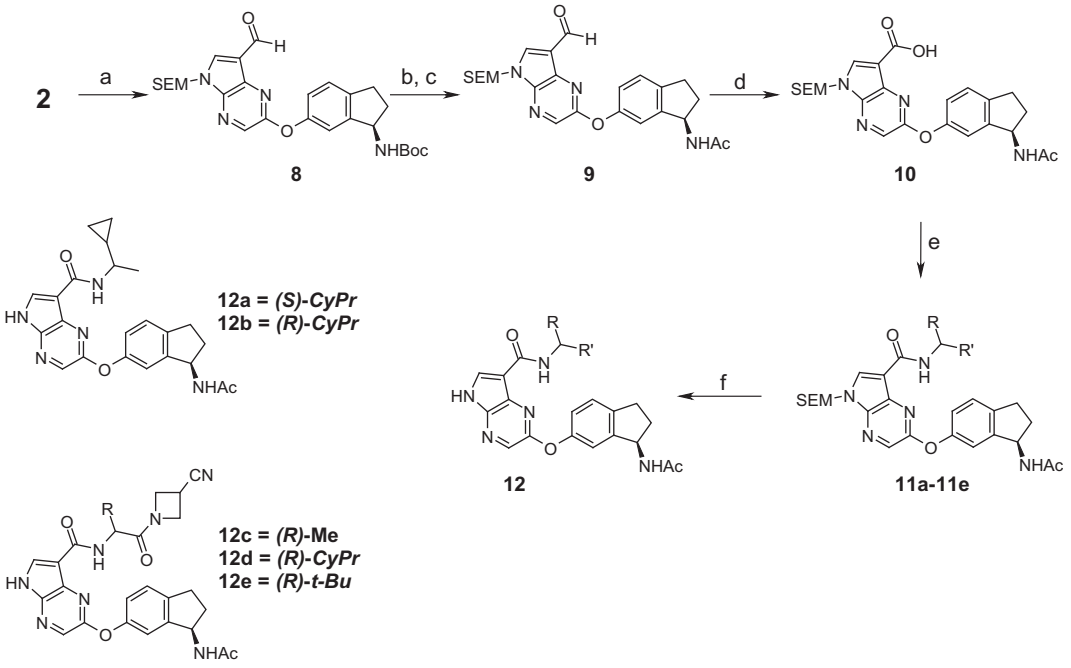


Figure 4. Compound **12a** bound to JAK3 non-phosphorylated kinase domain (2.35 Å resolution, PDB accesion number 3ZEP).[16]

phenol gave the (*R*)-Boc-aminoindanephenyl ether **8**. Cleavage of the *t*-butoxycarbonyl (Boc) group on compound **8** followed by acetylation gave the aldehyde **9** in good yields. Pinnick oxidation of this compound provided the corresponding carboxylic acid **10** in excellent yields. Finally, amidation of **10** using regular peptide coupling conditions with the corresponding amine afforded compounds **11** in good yields. Further SEM-deprotection provided the final amides **12c**–**12e** in acceptable yields (Scheme 3). Comparison of the methyl, cyclopropyl, and *t*-butyl analogs in this subseries showed that cyclopropyl compound **12d** had the best potency for JAK3 while maintaining selectivity over JAK1 (Table 2). We also selected this compound for a KinomeScan profile (Fig. 5). Compound **12d** showed exceptional kinase selectivity over a panel of 451 kinases (394 wild-type).

Selected examples were further tested in cellular assays of target modulation. These assays measured inhibition of phosphorylation of downstream STATs, in peripheral blood mononuclear cells (PBMCs) (Table 3). These analogs experienced a pronounced shift in potency in the cellular context. This was likely a consequence



Scheme 3. Reagents and conditions: (a) (*R*)-*t*-butyl 6-hydroxy-2,3-dihydro-1*H*-inden-1-ylcarbamate, $Pd(OAc)_2$, K_3PO_4, 2-di-*t*-butylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl, toluene 90 or 140 °C/sealed tube, 37%; (b) AcCl, MeOH, quantitative; (c) Ac_2O, pyridine, DCM, 62%; (d) sulfamic acid, $NaClO_2$, KH_2PO_4, dioxane/water, 75%; (e) R-NH_2, HATU, DMF, 22–71%; (f) TFA, EDA, DCM or TBAF, EDA, THF, 18–69%.

Table 2
Enzyme potencies[a] for compounds prepared according to Scheme 3

Compound	IC_{50} (μM)			Selectivity	
	JAK3	JAK2	JAK1	JAK2/JAK3	JAK1/JAK3
12a	0.022 (0.009)[b]	0.084 (0.028)	0.863 (0.082)	3.7	38.3
12b	0.014 (0.002)	0.194 (0.034)	2.076 (0.466)	13.2	140.4
12c	0.112 (0.050)	1.35 (0.436)	11.6 (1.663)	12.0	103.1
12d	0.005 (0.002)	0.039 (0.013)	0.262 (0.079)	7.1	47.4
12e	0.057 (0.017)	0.446 (0.046)	0.863 (0.082)	7.7	42.2
Tofacitinib	0.002 (0.6)	0.004 (0.7)	0.002 (0.0001)	1.8	0.7

[a] Inhibition of phosphorylation of a biotinylated synthetic peptide catalyzed by JAK1-3.[11] All enzyme reactions were run at adenosine triphosphate (ATP) concentrations of 1.5 μM. Km's of these enzymes for ATP under our experimental conditions were determined to be 1.5 μM (JAK3), 6 μM (JAK2), and 20 μM (JAK1).
[b] Mean (SEM (standard error of the mean)), $n \geqslant 3$.

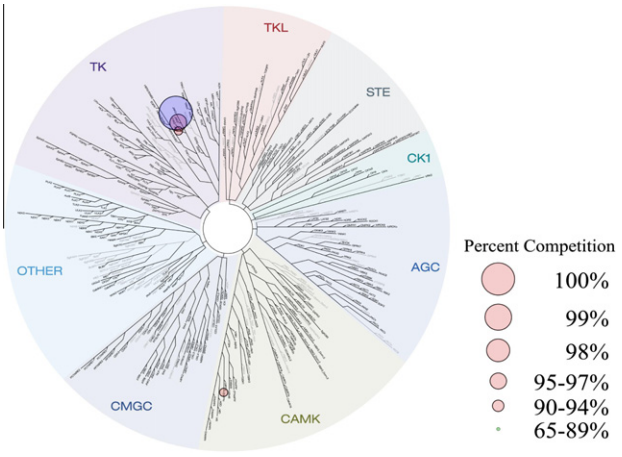


Figure 5. Kinomescan dendrogram of **12d** versus 451 kinases (394 wild-type) at 1 μM. JAK3 is shaded in blue.

of both an expected potency shift moving to a high concentration of ATP in the cellular environment, and the unoptimized physical chemical properties of these compounds. The relationship between

Table 3
Cellular potencies for selected compounds

Compound	PBMC IC_{50}[a] (μM)		
	JAK1/3 (IL-2)[b]	JAK2 (GM-CSF)[b]	JAK1/2 (IFNγ)[c]
12a	0.70	3.61	14.40
12b	1.75	16.22	>30
12d	7.41	23.30	>30
Tofacitinib	0.03	0.19	0.17

[a] IC_{50} values are the average of at least two experiments, except for **12d** ($n = 1$).
[b] Inhibition of phosphorylation of STAT5a in PBMCs.
[c] Inhibition of phosphorylation of STAT1 in PBMCs. The IL-2 readouts are gated to CD3-expressing T-cells; the GM-CSF and IFN-γ readouts are gated to CD14-expressing monocytes.

measured enzyme and cellular selectivity ratios was not easily defined. All tested compounds, including tofacitinib, showed selectivity to inhibit an IL-2 stimulated STAT5a phosphorylation (a JAK3/1-dependent process) versus GM-CSF and IFNγ-stimulated phosphorylations (JAK2 and JAK1/2-dependent processes). Compared to tofacitinib, analogs from our series appeared to show improved selectivity inhibiting IL-2 signaling versus IFNγ signaling; however, improvements versus GM-CSF signaling were mod-

est or non-existent. Our compounds have improved selectivity against JAK3 versus JAK2, but they also have improved selectivity against JAK2 versus JAK1. Cellular selectivities between IL-2 and GM-CSF signaling likely depend on both JAK2/3 and JAK2/1 selectivities. Therefore, the observed modest cellular selectivities for our compounds inhibiting IL-2 signaling versus GM-CSF signaling may be due to the diminished relative potencies against JAK1, and highlight the complexity of JAK/STAT signaling.

In summary, we discovered a series of phenyl ethers as potent JAK3 inhibitors with good, JAK family and kinase selectivity. We demonstrated by a structure-based approach that phenyl ethers are good scaffolds for achieving JAK family selectivity. The expected lipophilic interaction with Cys909 and a novel polar backbone interaction of the inhibitor with the Gly-loop may explain the excellent kinase selectivity observed within this series.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.03.015.

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