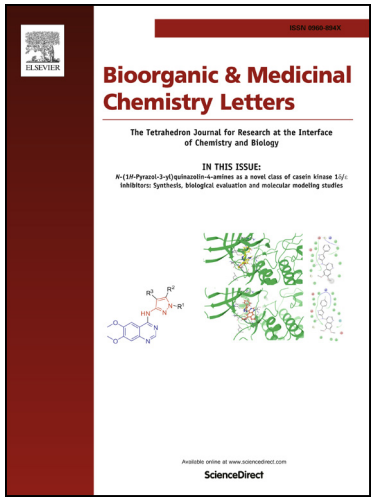

Identification of an imidazopyridine scaffold to generate potent and selective TYK2 inhibitors that demonstrate activity in an in vivo psoriasis model

Jun Liang, Anne Van Abbema, Mercedesz Balazs, Kathy Barrett, Leo Berezhkovsky, Wade S. Blair, Christine Chang, Donnie Delarosa, Jason DeVoss, Jim Driscoll, Charles Eigenbrot, Simon Goodacre, Nico Ghilardi, Calum MacLeod, Adam Johnson, Pawan Bir Kohli, Yingjie Lai, Zhonghua Lin, Priscilla Mantik, Kapil Menghrajani, Hieu Nguyen, Ivan Peng, Amy Sambrone, Steven Shia, Jan Smith, Sue Sohn, Vickie Tsui, Mark Ultsch, Karen Williams, Lawren C. Wu, Wenqian Yang, Birong Zhang, Steven Magnuson

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Identification of an imidazopyridine scaffold to generate potent and selective TYK2 inhibitors that demonstrate activity in an in vivo psoriasis model

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Abstract:

Herein we report identification of an imidazopyridine class of potent and selective TYK2 inhibitors, exemplified by prototype **6**, through constraint of the rotatable amide bond connecting the pyridine and aryl rings of compound **1**. Further optimization led to generation of compound **30** that potently inhibits the TYK2 enzyme and the IL-23 pathway in cells, exhibits selectivity against cellular JAK2 activity, and has good pharmacokinetic properties. In mice, compound **30** demonstrated dose-dependent reduction of IL-17 production in a PK/PD model as well as in an imiquimod-induced psoriasis model. In this efficacy model, the IL-17 decrease was accompanied by a reduction of ear thickness indicating the potential of TYK2 inhibition as a therapeutic approach for psoriasis patients.

Keywords:

TYK2

kinase

psoriasis

imidazopyridine

IL-23

Tyrosine Kinase 2 (TYK2) is a member of the Janus kinase (JAK) family. It associates with the cytoplasmic tails of numerous cytokine receptors and induces intracellular signaling, through activation of signal transducers and activators of transcription (STAT) proteins, to regulate gene expression and transcription.[1,2] IL-12 and IL-23 are two TYK2 dependent cytokines of particular interest, because antibodies against IL-12 and IL-23 have proven therapeutic utility in several diseases. In the case of the IL-12 pathway, phosphorylation of STAT4 leads to expression of IFNγ[3,4] while in the IL-23 pathway, phosphorylation of STAT3 leads to increased IL-17 levels (Figure 1).[5,6] The IL-12 and IL-23 pathways have been implicated in diseases of the immune system including psoriasis and inflammatory bowel diseases (IBD).[7-12] These chronic inflammatory disorders of epithelial cells triggered by an overly activated immune system, including Th1and Th17 immune responses, require new and safe therapies.

The other members of the Janus Kinase family (JAK1, JAK2, and JAK3) are highly homologous to TYK2.[13] As we set forth to find tool compounds suitable for in vivo studies, we were particularly aware of the need for selectivity against JAK2, as this related kinase is required for the signaling of several hematopoietic growth factors, including the erythropoietin (EPO) receptor that is responsible for the production of red blood cells and the thrombopoeitin (TPO) receptor that is required for platelet formation.[14] Complete inhibition of the kinase activity of JAK2 will lead to thrombocytopenia. We set forth to identify TYK2 tool compounds with a range of selectivity against JAK2 and set a minimum of 10-fold selectivity against JAK2 based on testing in relevant cell based assays.[15]

Previously, we have described the lead identification of cyclopropyl analog **1**[16] and its subsequent optimization to fluorocyclopropane **2**.[17] During the course of those studies we

followed IL-12 pathway activity, by monitoring IL-12 mediated STAT4 phosphorylation in a cell assay and IFNγ production in a pharmacokinetics/pharmacodynamics (PK/PD) animal model. Around the time of completing these efforts, we modified our assay cascade to evaluate activity in the IL-23 pathway, measuring IL-23-mediated STAT3 phosphorylation in cells[15] and IL-17 production in a PK/PD animal model. As measured in this IL-23-mediated cell assay, compound **2** had an EC_{50} of 1.2 μM, around 5-fold less potent than it was in our IL-12-dependent cell assay (Figure 1). This erosion in cell-based potency coincided with a decrease in the cellular selectivity relative to the JAK2-based cellular readout (measuring EPO-mediated STAT5 phosphorylation). There are several possible reasons why a compound might be less potent in the IL-23-dependent assay as compared with the IL-12-driven assay such as the use of different cell types (Th17 memory vs NK92) or different requirements of TYK2 and JAK2 inhibition to fully block the IL-23 or IL-12 pathways.[15] Nevertheless, improving potency against TYK2 (and thereby improving activity in the IL-23-mediated cell assay) became a priority for the project team.

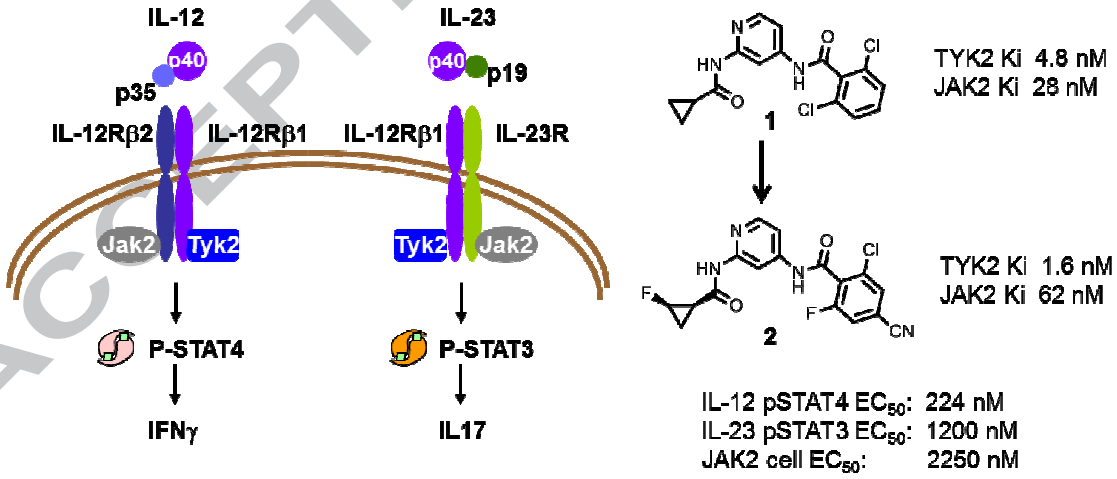


Figure 1: Comparison of the effects of compound **2** on IL-12 mediated STAT4 phosphorylation and IL-23-mediated STAT3 phosphorylation.

To improve TYK2 potency, we evaluated constraint of the amide bond between the aryl and pyridyl rings through construction of another ring.[18] When we considered the bound confirmation of amides **1** and **2** in TYK2 (and those of other amide crystal structures from the same series), constraining the amide to carbon 5 of the pyridine ring, which sit in-plane with each other, appeared to be a promising idea (Figure 2, cyclization direction "a"). However, we were mindful of the fact that the alternate amide rotamer could also be constrained to carbon 3 of the pyridine ring (cyclization direction "b").

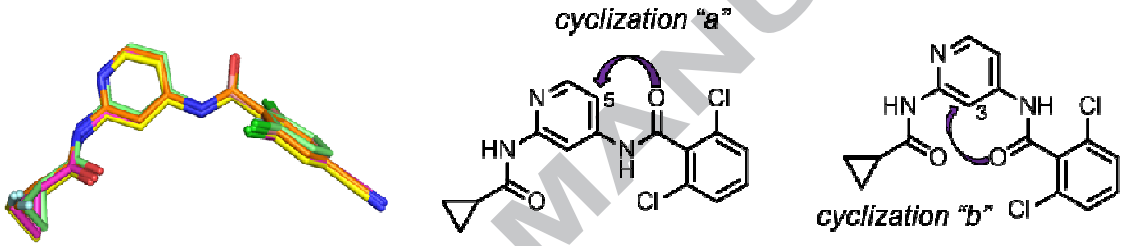


Figure 2: Possible cyclization directions of different amide rotamers.

We proceeded by making of a number of constrained analogs of amide **1** to determine which cyclization strategy might present a path forward to improved potency (Table 1).[19] Comparison of isoquinolones **3** and **4** illustrated two very important trends. First, cyclization direction "b" (isoquinoline **4**) afforded better potency than cyclization direction "a" (isoquinoline **3**). More importantly, isoquinoline **4** has much better JAK2 selectivity (8-fold) than isoquinoline **3** (0.4-fold). These differences are even more pronounced comparing the 5,6-ring system for imidazopyridines **5** and **6**. Imidazopyridine **6** is 2000-fold more potent against TYK2 and 90-fold more JAK2 selective than imidazopyridine **5**. The loss of potency for 5-member ring **5** vs 6-member ring **3** can be attributed to a compacting of the smaller ring system requiring an

undesired shift in the positioning of the dichlorophyenyl ring. Conversely, the 20-fold improvement in TYK2 potency for the smaller ring system (imidazopyridine **6** vs isoquinoline **4**) can be easily understood as the 5-member ring system relieves a steric clash with the amide carbonyl of isoquinoline **4**, and installs a favorable intramolecular hydrogen bond between the imidazole NH and the amide carbonyl. We obtained a crystal structure of this prototype constrained imidazopyridine **6** in TYK2 and compared it to the TYK2 co-crystal structure of amide **1** (Figure 3a). The aforementioned intramolecular hydrogen bond is apparent from the co-crystal structure. Furthermore, we noted that the constrained analog sits deeper in the binding pocket, packing more closely with Ile960 than amide **1**. This improved van der Waals interaction may also contribute to the potency of imidazopyridine **6**. The hinge backbone does shift slightly to accommodate the ligand **6**, but productive hydrogen bond distances with the hinge are maintained. We also compared the TYK2 co-crystal structure of analog **6** to its JAK2 co-crystal structure (Figure 3b). Here, too, we see that analog **6** sits deeper in the TYK2 binding pocket, creating more favorable interactions with Ile980 than it does with the corresponding residue, Val911, of JAK2. The significant improvement in biochemical selectivity over JAK2 for this constrained core is a definite advantage over the unconstrained amide core.

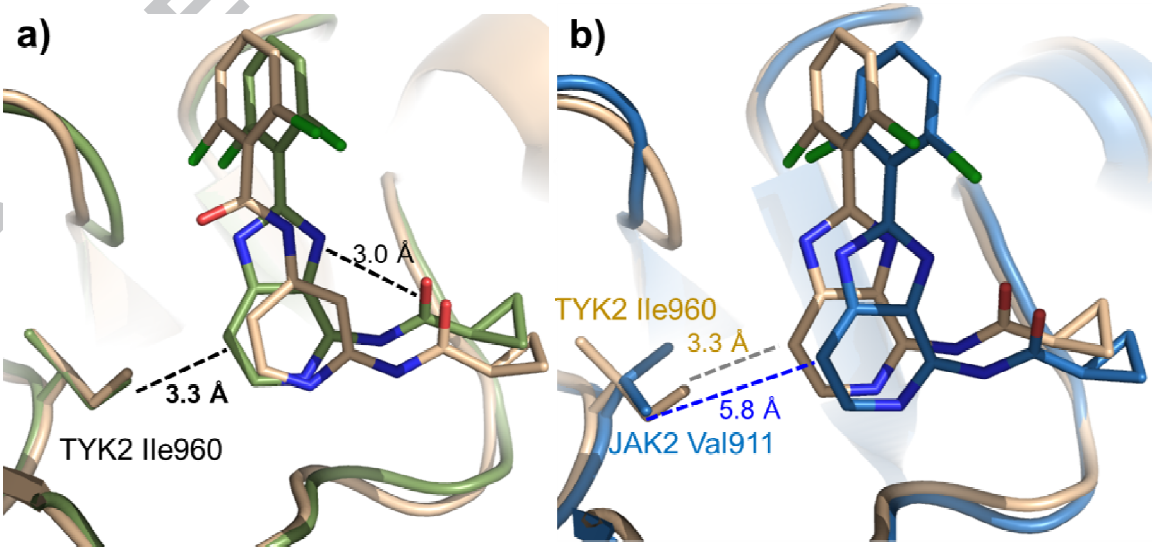
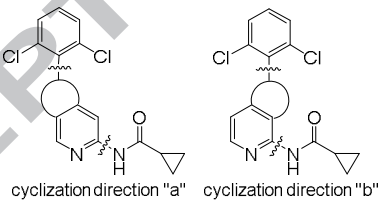
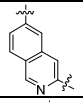
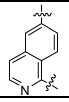


Figure 3: a) overlay of compound **1** (beige, PDB code 4GIH) and compound **6** (green, PDB code 5WAL) in TYK2; b) overlay of compound **6** in TYK2 (beige) and JAK2 (blue, PDB code 5WEV).

With the pronounced preference for cyclization direction "b" for 5,6-bicyclo systems, we continued our exploration of other heterocycles (Table 1). Analogs that are not able to form a productive intramolecular hydrogen bond with the amide carbonyl (**7**-**11**) all have decreased TYK2 potency relative to imidazopyridine **6**. Conversely, analogs capable of productive hydrogen bonds, either through sulfur (**12** and **13**) or through CH (**14**) were all very potent against TYK2 with varying levels of biochemical selectivity against JAK2.[20] Indeed, thiazolopyridine **13**, and pyrazolopyridine **14** both represented new cores as starting points for further optimization.[21,22] For the purpose of this report, we will focus on optimization of the imidazopyrdine **6**.

Table 1. Analogs with different constrained heterocycles in place of the pyridine amide



Ex	Constrained Heterocycle	Cyclization Direction	[a]TYK2 K_i (μM)	[a]JAK2 K_i (μM) (Fold Selectivity)[b]
3		a	0.042	0.016 (0.4 fold)
4		b	0.017	0.15 (8.3 fold)
5		a	3.5	3.2 (0.9 fold)

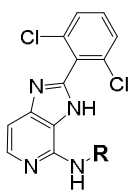
6		b	0.0017	0.15 (88 fold)
7		b	0.013	0.13 (10 fold)
8		b	0.019	2.1 (111 fold)
9		b	0.0088	0.90 (103 fold)
10		b	0.020	0.59 (29 fold)
11		b	0.031	0.37 (12 fold)
12		b	0.0008	0.0066 (7.5 fold)
13		b	0.0005	0.026 (52 fold)
14		b	0.0013	0.19 (142 fold)

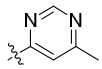
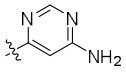
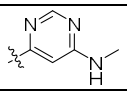
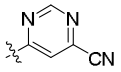
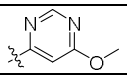
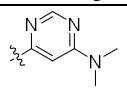
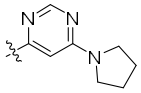
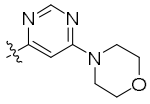
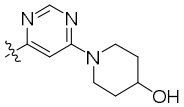
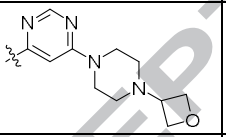
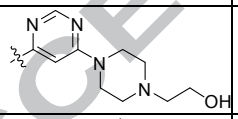
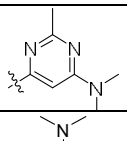
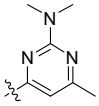
[a] Biochemical assays. Arithmetic mean of at least 3 separate determinations ($n \geq 3$). On average, the coefficients of variation were less than 0.3 times the mean for these assays. [b]Fold selectivity for TYK2 inhibition vs. JAK2 inhibition as measured by JAK2 $K_i \div$ TYK2 K_i.

With the imidazopyridine core selected for further profiling, we explored alternatives to the cyclopropyl amide to further enhance TYK2 potency. Initial SAR was largely consistent with SAR observed in the open amide series,[16] such that pyrimidine **15** was identified with submicromolar biochemical potency and good JAK2 selectivity (Table 2). We next explored a number of different substitutions on the pyrimidine focusing primarily on the C6-position, which we felt, based on crystal structures, would allow us to explore a range of substitutions to tune both selectivity and physicochemical properties. Functional groups of varying sizes were tolerated (analogs **16**-**26**), many with sub-nanomolar TYK2 biochemical potency, but with variable JAK2 selectivity. Small substituents (**16**-**21**) afforded good JAK2 selectivity, while

increasing size of the substituent, particularly those with more polar atoms, led to erosion of JAK2 biochemical selectivity. With this series of analogs we were also able to generate meaningful cellular SAR and selectivity data using our IL-23-mediated cell-based assay and the corresponding JAK2 (EPO-mediated) cell assay. Relative to amide **2** (IL-23-mediated cell IC_{50} = 1.2 μM), we observed that the enhanced biochemical potency of the constrained compounds such as amine **18** gave a corresponding improvement in cell potency (0.044 μM). In general there was a reasonable correlation between TYK2 biochemical potency and IL-23-mediated cell activity[15] with occasional outliers such as methyl ether **20**, in which poor solubility may be limiting its cellular potency readout. When assessing cellular JAK2 selectivity, it was clear that for most analogs there was erosion in the fold-selectivity observed relative to biochemical selectivity. The reduced cellular selectivity could potentially be attributed to a number of factors including the use of different cell types in the assays, translation of kinase-domain constructs used in the biochemical assay to the full length enzymes in the cell assays, and poor compound solubility. Nevertheless, we were happy to see that analogs such as **16**, **18**, **19** and **21** displayed good IL-23-mediated cell potency and reasonable cellular selectivity (> 10-fold). We also explored SAR at the 2-position of the pyrimidine, albeit not as extensively. While small substitutions were tolerated, they usually led to a loss of TYK2 biochemical potency (*cf* **15** and **27**; **21** and **28**; **16** and **29**). This loss of TYK2 potency can possibly be attributed to disruption of the intramolecular hydrogen bond between the nitrogen on the pyrimidine and the NH of the imidazole as a result of the 2-substitution.

Table 2. Heterocycle replacement of cyclopropyl amide

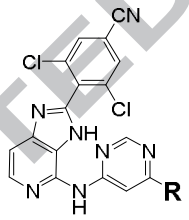


Ex	Constrained Heterocycle	[a]TYK2 K_i (μM)	[a]JAK2 K_i (μM) (Fold Sel.)[b]	IL-23-pSTAT3[c] EC_{50} (μM)	EPO-pSTAT5[d] EC_{50} (μM) (Fold Sel.)[e]
15		0.0006	0.095 (158 fold)		
16		0.0004	0.021 (52 fold)	0.17	1.9 (11 fold)
17		0.0003	0.014 (47 fold)	0.26	1.3 (5.0 fold)
18		0.0005	0.026 (52 fold)	0.044	0.64 (16 fold)
19		0.0007	0.093 (133 fold)	0.035	7.7 (220 fold)
20		0.0007	0.11 (157 fold)	3.6	6.5 (1.8 fold)
21		0.0006	0.034 (56 fold)	0.14	2.1 (15 fold)
22		0.0005	0.016 (32 fold)	0.52	1.0 (1.9 fold)
23		0.0006	0.0065 (11 fold)	0.20	0.46 (2.3 fold)
24		0.0010	0.0054 (5.4 fold)	0.36	1.1 (3.1 fold)
25		0.0012	0.014 (12 fold)	0.059	0.82 (14 fold)
26		0.0012	0.010 (8.3 fold)	0.14	1.1 (7.8 fold)
27		0.0051	0.44 (86 fold)	9.4	10 (1.1 fold)
28		0.0032	0.043 (13 fold)	n.d.	n.d.
29		0.0099	0.49 (49 fold)	n.d.	n.d.

[a] Biochemical assays. Arithmetic mean of at least 3 separate determinations (n ≥ 3). On average, the coefficients of variation were less than 0.3 times the mean for these assays. [b]Fold selectivity for TYK2 inhibition vs. JAK2 inhibition as measured by JAK2 K_i ÷ TYK2 K_i. [c] Cell-based assay of TYK2 inhibition. Arithmetic mean of at least 3 separate runs (n ≥ 3). On average, the coefficients of variation were less than 0.5 times the mean for cell-based assays. [d]Cell-based assay of JAK2 inhibition. Arithmetic mean of at least 3 separate runs (n≥ 3). [e]Fold selectivity: EPO pSTAT5 EC_{50} ÷ IL-12 pSTAT4 EC_{50}.

Previously, we had shown in the original open amide series that placement of a cyano group at the 4-position of the dichlorophenyl ring, as shown in amide **2**, was tolerated from a potency and selectivity point of view and, more importantly, led to improved in vivo clearance in rodents.[17] We took some of our favored analogs from Table 2 and made the cyano versions of them (Table 3). Of these analogs, only 6-methyl pyrimidine **30** had appropriate potency and selectivity to advance to further selectivity and pharmacokinetic profiling.

Table 3. Imidazopyridines with nitrile on the phenyl ring

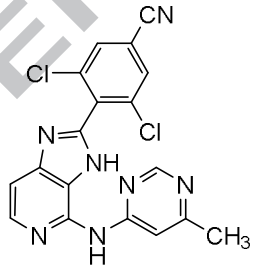


Ex	R	compare to analog from Table 3	[a]TYK2 K_i (μM)	[a]JAK2 K_i (μM) (Fold Sel.)[b]	IL-23-pSTAT3[c] EC_{50} (μM)	EPO-pSTAT5[d] EC_{50} (μM) (Fold Sel.)[e]
30	$-CH_3$	16	0.0007	0.022 (31 fold)	0.066	1.4 (21 fold)
31	$-NH_2$	17	0.0006	0.023 (38 fold)	0.45	1.6 (3.5 fold)
32	$-NHCH_3$	18	0.0006	0.036 (60 fold)	0.072	0.62 (8.6 fold)

[a] Biochemical assays. Arithmetic mean of at least 3 separate determinations (n ≥ 3). On average, the coefficients of variation were less than 0.3 times the mean for these assays. [b]Fold selectivity for TYK2 inhibition vs. JAK2 inhibition as measured by JAK2 K_i ÷ TYK2 K_i. [c] Cell-based assay of TYK2 inhibition. Arithmetic mean of at least 3 separate runs (n ≥ 3). On average, the coefficients of variation were less than 0.5 times the mean for cell-based

assays. [d]Cell-based assay of JAK2 inhibition. Arithmetic mean of at least 3 separate runs (n≥ 3). [e]Fold selectivity: EPO pSTAT5 EC_{50} ÷ IL-12 pSTAT4 EC_{50}.

Regarding the other JAK family kinases, compound **30** has a JAK1 K_i of 24 nM and JAK3 K_i of 58 nM, indicating good TYK2 selectivity (35-fold for JAK1 and 83-fold for JAK3). Compound **30** was further profiled, at a concentration of 70 nM (100x the TYK2 K_i), against a panel of 58 kinases that were known to be relevant to JAK family kinase inhibitors, where it inhibited only two kinases by >50%. Full inhibitor titration curves were measured for these kinases with CDK2 K_i = 89 nM (126-fold selective for TYK2) and CDK9 K_i = 108 nM (154-fold selective). Compound **30** was also tested, at a concentration of 10 μM, against a panel of 40 pharmacologically important and safety-related receptors and channels, where it only showed appreciable activity against NK1 (67% inhibition). Physicochemical and DMPK properties of compound **30** are shown in Figure 4. The compound is highly permeable, and has moderate stability in liver microsomes. Stability in hepatocytes is good and this correlates well with in vivo clearance. In mice, the compound is cleared quickly, but appreciable exposure can be obtained at higher doses enabling in vivo PD and efficacy evaluation.



MW	396
TPSA	103
LogD	2.6
LLE	6.7

In vivo Cl(mL/min/kg)
Rat - 5.1; Dog 3.6

MDCK Permeability

A:B	12.7 10E-06 cm/s
B:A/A:B	1.1

Plasma Protein Binding

Mouse	96.6%
Rat	96.1%
Human	97.2%

In vitro Cl (mL/min/kg)
Liver microsomes:
mouse - 59; rat - 20; human - 11
Hepatocytes:
mouse - 31; rat - 11; human - 14

Figure 4: Physicochemical and DMPK properties of compound **30**

We progressed compound **30** for testing in our IL-23-induced PK/PD model (Figure 5).[23] In this model, pathway activation was achieved by intravenous dosing of IL-23 and IL-1B, followed 3 h later by collection of plasma for measurement of IL-17F (measured by cytokine ELISA) and drug concentration. The positive control, anti-p40 antibody, was dosed intraperitoneally 60 minutes prior to cytokine stimulation, while compound **30** was dosed orally 30 minutes before cytokine injection. Statistically significant reduction of plasma IL-17F was observed at the 100 and 70 mg/kg doses (Figure 5a), but at 30 mg/kg inter animal variability was evident and the reduction of IL-17F was not statistically different from vehicle. The corresponding plasma free drug concentrations (Table 4) reveal that strong inhibition of the pathway (87% at 100 mg/kg and 72% at 70 mg/kg) occurred when free drug concentrations at Cmax were 39-fold and 23-fold over the TYK2-dependent IL-23 cell assay IC_{50} (66 nM). Notably, at each of these doses there was also at least 1-fold coverage of the JAK2-related cell IC_{50} (1400 nM). At 30 mg/kg, where there was not statistically significant reduction of IL-17F, plasma exposure indicated 13-fold coverage of the TYK2-related cell IC_{50} but only 0.6-fold coverage of the JAK2 cell IC_{50} at Cmax. It is curious that the 30 mg/kg dose was not active, given the strong coverage to TYK2. One possible explanation is that some level of JAK2 inhibition, which is also coupled to the IL-23 receptor, along with strong coverage of TYK2, is required to strongly inhibit IL-17F production in this assay.[24] It is also worth noting that both IL-23 and IL-1B are used to stimulate the pathway and so potentially TYK inhibition alone may not be sufficient to inhibit this model.

Table 4. PK data for IL-23-induced PK/PD experiment with compound **30**.

Dose of 30 (mg/kg)	[Free drug] of 30 at T = 3 h[a] (μM)	Fold coverage of TYK2 cell EC_{50}[b]	Fold coverage of JAK2 cell EC_{50}[c]
100	2.6	39 fold	1.9 fold
70	1.5	23 fold	1.1 fold
30	0.86	13 fold	0.61 fold

[a] Collection for plasma analysis 3.5 h after dose of compound **30** – approximately at Cmax. [b]Fold coverage calculated by free drug concentration/IL-23-pSTAT3 EC_{50} of compound **30** (0.066 μM). [c]Fold coverage calculated by free drug concentration/EPO-pSTAT5 EC_{50} of compound **30** (1.4 μM).

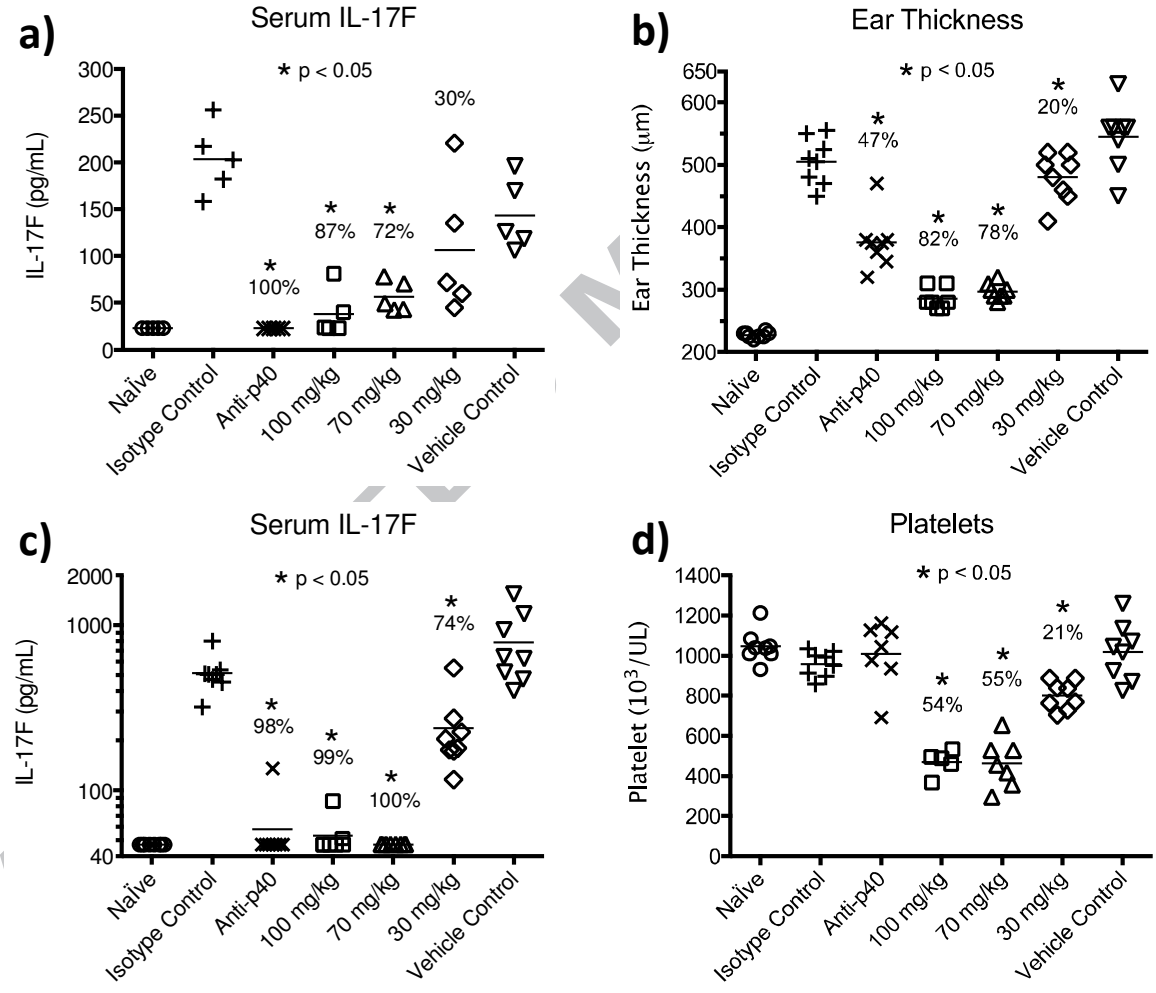


Figure 5: In vivo PD and efficacy data for compound **30**. a) Serum IL-17F levels in IL-23-induced PD assay; b) ear thickness reduction measured on day 5 in imiquimod-induced psoriasis

model; c) serum IL-17F levels measured on day 5 in imiquimod model; d) platelet levels measured on day 5 in imiquimod model

With these positive PK/PD results we proceeded to an efficacy study using an imiquimod-mediated psoriasis model.[25,26] In this model imiquimod, a topical agent that stimulates multiple pathways such as IL-1, IL-23/IL-17, and TLR7, is administered to the ear of a mouse for 5 days, while the contralateral ear is used as the unstimulated control. Compound **30** was dosed orally at 100, 70, and 30 mg/kg BID for those same 5 days as imiquimod treatment, and at the start of the sixth day, ear thickness was measured. Plasma was also collected to assess serum IL-17F levels and other blood parameters. Statistically significant reductions in ear thickness, comparable to the anti-p40 positive control, were observed at 100 and 70 mg/kg, whereas the 30 mg/kg dose gave only a minimal reduction (Figure 5b). At the 30 mg/kg BID dose, free drug trough levels are well below the TYK2 cellular EC_{50} and it is not surprising that this dose is not active in this assay. The efficacy results are consistent with the reductions in serum IL-17F observed in this 5-day model (Figure 5c) where complete reduction was observed at the highest two doses and more modest reduction was attained at the 30 mg/kg dose. While most other serum measurements showed little change relative to the naïve controls, a 50% reduction in platelet levels was observed at the 100 and 70 mg/kg doses (Figure 5d). It is tempting to speculate that this mild thrombocytopenia could potentially be related to combined JAK2 and TYK2 inhbition and the resultant effects on the TPO pathway.[27] However, the relationship between JAK2 and/or TYK2 inhibition and the effects on the IL-23 and TPO pathways needs to be probed further with compounds of different TYK2/JAK2 selectivity profiles, and potentially compounds from other chemical scaffolds.[28] Our efforts in this investigation will be the subject of future publications.

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Supporting Information

Details of the crystallographic methods and sythetic and analytical information for compounds **3**-**32** are available.

References:

1. Leonard WJ, O'Shea JJ. Jaks and STATs: biological implications. *Annual Review of Immunology* 1998;16:293-322.
2. Brierley MM, Fish EN. STATs: multifaceted regulators of transcription. *J. Interferon & Cytokine Research* 2005;23:733-744.
3. Thierfelder WE, van Deursen JM, Yamamoto K, Tripp RA, Sarawar SR, Carson RT, Sangster MY, Vignali DAA, Doherty PC, Grosveld GC, Ihle JN. Requirement for STAT4 in interleukin-12-mediated response of natural killer and T cells. *Nature* 1996;382:171-174.
4. Kaplan MH, SunY-L, Hoey T, Grusby MJ. Impaired IL-12 responses and enhanced development of Th2 cells in STAT4-deficient mice. *Nature* 1996;382:174-177.
5. Parham C, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J, Pflanz S, Zhang R, Singh K, Vega F, To W, Wagner J, O'Farrell A-M, McClanahan T, Zurawski S, Hannum C, Gorman D, Rennick D, Kastlein RA, Maleft RDW, Moore KW. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12β1 and a novel cytokine receptor subunit, IL-23R. *J. Immunol.* 2002;168:5699-5708.
6. Aggarwal S, Ghilardi N, Xie M-H, de Sauvage FJ, Gurney AL. Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. *J. Biol. Chem.* 2003;278:1910-1914.
7. Ouyang W, Kolls JK, ZhengY. The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* 2008;28:454-467.
8. Ahem PP, Izcue A, Maloy KJ, Powrie F. The interleukine-23 axis in intestinal inflammation. *Immunological Reviews* 2008;226;147-159.
9. Cho JH. The genetics and immunopathogenesis of inflammatory bowel disease. *Nature Reviews Immunology* 2008;8:458-466.
10. Duerr RJ, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, Abraham C, Regueiro M, Griffiths A, Dassopoulos T, Bitton A, Yang H, Targan S, Datta LW, Kistner EO, Schumm LP, Lee AT, Gregersen PK, Barmada MM, Rotter JI, Nicolae DL, Cho JH. A genome-wide association study identifies IL-23R as an inflammatory bowel disease gene. *Science* 2006;314:1461-1463.

11. Yawalkar N, Karlen S, Hunger R, Brand CU, Braathen LR. Expression of interleukin-12 is increased in psoriatic skin. *Journal of Investigative Dermatology* 1998;111:1053-1057.
12. Lee E, Trepiccio WL, Oestreicher JL, Pittman D, Wang F, Chamian F, Dhodapkar M, Krueger JG. Increased expression of interleukin 23 p19 and p40 in lesional skin of patients with psoriasis vulgaris. *Journal of Experimental Medicine* 2004;199:125-130.
13. Alicea-Velazquez NL, Boggon TJ. The use of structural biology in Janus kinase targeted drug discovery. *Current Drug Targets* 2011;12:546-555.

14. Won H-H, Park I, Lee E, Kim J-W, Lee D. Comparative analysis of the JAK/STAT signaling through erythropoietin receptor and thrombopoietin receptor using a systems approach. *BMC Bioinformatics* 2009;10(Suppl 1):S53

15. Sohn SJ, Barrett K, van Abbema A, Chang C, Bir Kohli P, Kanda H, Smith J, Lai Y, Zhou A, Zhang B, Yang W, Williams K, MacLeod C, Hurley CA, Kulagowski JJ, Lewin-Koh N, Dengler HS, Johnson AR, Ghilardi N, Zak M, Liang J, Blair WS, Magnuson S, Wu, Lawren C. A restricted role for TYK2 catalytic activity in human cytokine responses revealed by novel TYK2-selective inhibitors. *J. Immunol.* 2013;191:2205-2216.

16. Liang J, Tsui V, van Abbema A, Bao L, Barrett K, Beresini M, Berezhkovskiy L, Blair WS, Chang C, Driscoll J, Eigenbrot C, Ghilardi N, Gibbons P, Halladay J, Johnson A, Bir Kohli P, Lai Y, Liimatta M, Mantik P, Menghrajani K, Murray J, Sambrone A, Xiao Y, Shia S, Shin Y, Smith J, Sohn S, Stanley M, Ultsch M, Zhang B, Wu LC, Magnuson S. Lead Identification of Novel and Selective TYK2 Inhibitors. *Eur. J. Med. Chem.* 2013;67:175-187.

17. Liang J, van Abbema A, Balazs M, Barrett K, Berezhkovsky L, Blair W, Chang C, Delarosa D, DeVoss J, Driscoll J, Eigenbrot C, Ghilardi N, Gibbons P, Halladay J, Johnson, A, Bir Kohli P, Lai Y, Liu Y, Lyssikatos J, Mantik P, Menghrajani K, Murray J, Peng I, Sambrone A, Shia S, Shin Y, Smith J, Sohn S, Tsui V, Ultsch M, Wu LC, Xiao Y, Yang W, Young J, Zhang B, Zhu B-Y, Magnuson S. Lead Optimization of a 4-Aminopyridine Benzamide Scaffold To Identify Potent, Selective, and Orally Bioavailable TYK2 Inhibitors. *J. Med. Chem.* 2013;56:4521-4536.

18. Böhm H-J, Klebe G. What can we learn from molecular recognition in protein-ligand complexes for the design of new drugs? *Angew. Chem., Intl. Ed. Engl.* 1996;35:2588-2614.

19. See supplemental information for synthesis and characterization of all analogs.

20. JAK1 biochemical data was also routinely collected for all compounds. See supplemental. JAK3 biochemical data was spot checked against certain compounds since this series typically showed good JAK3 selectivity. Biochemical assay conditions can be found at: Tsui V, Gibbons P, Ultsch M, Mortara K, Chang C, Blair W, Pulk R, Stanley RM, Starovasnik M, Williams D, Lamers M, Leonard P, Magnuson S, Liang J, Eigenbrot C. A new regulatory switch in a Jak protein kinase. *Proteins: structure, function and bioinformatics* 2011;79:393-401.

21. Blench T, Ellwood C, Goodacre S, Lai Y, Liang J, MacLeod C, Magnuson S; Tsui V, Williams K, Zhang B. Preparation of azabenzothiazole derivatives for use as TYK2 kinase inhibitors. WO2012035039, March 22, 2012.

22. Blench T, Goodacre S, Lai Y, Liang J, MacLeod C, Magnuson, S; Tsui V, Williams K, Zhang B. Preparation of pyrazolopyridines and their use as TYK2 kinase inhibitors. WO2012066061, May 24, 2012.

23. Fauber BP, René O, Deng Y, DeVoss, J, Eidenschenk C, Everett C, Ganguli A, Gobbi A, Hawkins J, Johnson AR, La H, Lesch J, Lockey P, Norman M, Ouyang W, Summerhill S, Wong H. Discovery of 1-[4-[3-fluoro-4-((3*S*,6R)-3-methyl-1,1-dioxo-6-phenyl-[1,2]thianzinan-2-ylmethyl)-phenyl]piperazin-1-yl}-ethanone (GNE-3500): a potent , selective and orally bioavailable retinoic acid receptor-related orphan receptor C (RORc or RORγ) inverse agonist. *J. Med. Chem.* 2015;58:5308-5322.

24. Recently it was reported that highly selective TYK2 inhibitors derived through inhibition of the JH2 pseudokinase domain fully inhibit IFNγ production in an IL-12 PK/PD model: Weinstein D, Wrobleski S, Moslin R, Lin S, Zhang Y, Spergel S, Mertzman M, Tokarski J, Sun H, Chiney M, Elzinga P, Aranibar N, Chimalakonda A, Strnad J, Zupa-Fernandez A, Cheng L, Gillooly K, Mcintyre K, Carter P, Lombardo L, Burke J, Macor J. Discovery of a pseudokinase domain ligand as an allosteric inhibitor of TYK2 for the treatment of autoimmune diseases. Abstracts of Papers, 252nd ACS National Meeting & Exposition, Philadelphia, PA, United States, August 21-25, 2016 (MEDI-272).

25. van der Fits L, Mourits S, Voerman JSA, Kant M, Boon L, Laman JD, Cornelissen F, Mus A-M, Florencia E, Prens E, Lubberts E. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J. Immunol.* 2009;182:5836-5845.

26. Walter A, Schäfer M, Cecconi V, Matter C, Urosevic-Maiwald M, Belloni B, Schönewolf N, Dummer R, Bloch W, Werner S, Beer H-D, Knuth, A, van den Broek M. Aldara activates TLR7-independent immune defence. *Nat. Commun.* 2013;4:1560-1573.

27. Fishley B, Alexander WS. Thrombopoietin signalling in physiology and disease. *Growth Factors* 2004;22:151-155.

28. Moslin R, Gardner D, Santella J, Zhang Y, Duncia JV, Liu C, Lin J, Tokarski JS, Strnad J, Pedicord D, Chen J, Blat Y, Zupa-Fernandez A, Cheng L, Sun H, Chaudhry C, Huang C, D'Arienzo C, Sack JS, Muckelbauer JK, Chang C, Tredup J, Xie D, Aranibar N, Burke JR, Carter PH, Weinstein DS. Identification of imidazo[1,2-b]pyridazine TYK2 pseudokinase ligands as potent and selective allosteric inhibitors of TYK2 signalling. *Med. Chem. Commun.* 2016; DOI: 10.1039/c6md00560h.

Graphic Abstract – Magnuson TYK2 Imidazopyridines