Identification of Imidazo[1,2-*b*]pyridazine Derivatives as Potent, Selective, and Orally Active Tyk2 JH2 Inhibitors

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Supporting Information

ABSTRACT: In sharp contrast to a previously reported series of 6-anilino imidazopyridazine based Tyk2 JH2 ligands, 6-((2-oxo-*N*1-substituted-1,2-dihydropyridin-3-yl)amino)imidazo[1,2-*b*]pyridazine analogs were found to display dramatically improved metabolic stability. The *N*1-substituent on 2-oxo-1,2-dihydropyridine ring can be a variety of alkyl, aryl, and heteroaryl groups, but among them, 2-pyridyl provided much enhanced Caco-2 permeability, attributed to its ability to form intramolecular hydrogen bonds. Further structure–activity relationship studies at the C3 position led to the identification of highly potent and selective Tyk2 JH2 inhibitor **6**, which proved to be highly effective in inhibiting IFN γ production in a rat pharmacodynamics model and fully efficacious in a rat adjuvant arthritis model.

KEYWORDS: Tyk2 JH2 inhibitor(s), Tyk2 JH2 ligand(s), Tyk2 pseudokinase inhibitor(s), Tyk2 pseudokinase ligand(s)

Tyk2 JH2 K_i = 0.086 nM
IFN α IC_{50} = 25 nM
hWB IC_{50} = 81 nM
effective in inhibiting IFN γ production in rats
efficacious in a rat adjuvant arthritis model

Tyrosine kinase 2 (Tyk2), a member of the Janus kinase (JAK) family of nonreceptor tyrosine kinases, regulates the phosphorylation of Signal Transducer and Activation of Transcription (STAT) proteins downstream of the receptors for the p40-containing cytokines IL-12 and IL-23 as well as Type I interferons such as IFN α and IFN β , resulting in the activation of STAT-dependent transcription and functional responses specific for these receptors.^{1–3} These receptor signaling pathways play key roles in the pathogenesis of autoimmune and inflammatory diseases. IL-12 and IL-23 were found at high levels in lesional skin of psoriatic patients,^{4–6} and the expression of these cytokines were shown to decrease after various treatments that provide symptomatic relief in psoriasis.^{7,8} Elevated serum IFN α levels were observed in Systemic Lupus Erythematosus (SLE) patients,⁹ and the levels correlated to both disease activity and severity.¹⁰ Inhibition of both IL-12 and IL-23 by targeting their common p40 subunit with ustekinumab (Stelara) proved to be clinically effective for the treatment of psoriasis^{11,12} and Crohn's disease,^{13,14} and ustekinumab was approved by FDA for the treatment of these diseases. Targeting IFN α as a potential therapeutic solution to SLE was also validated by the Phase IIB results from anifrolumab, a human monoclonal antibody that binds to and blocks the receptor for Type I interferons.^{15,16} Meanwhile, Tyk2 deficient mice were reported to be resistant to collagen-induced arthritis (CIA) and experimental autoimmune encephalomyelitis (EAE).^{17,18} Thus, Tyk2 has been rationalized as a promising target for developing orally active

therapeutic agents for autoimmune and inflammatory disorders.¹⁹

As the Janus kinase family is named after the two faced Roman god Janus, the structures of Tyk2 and other Jak family members feature dual kinase domains proximal to each other, a catalytic kinase domain and a pseudokinase domain, also called Jak homology 1 (JH1) and Jak homology 2 (JH2), respectively. The Tyk2 JH2 is capable of binding adenosine triphosphate (ATP), but it is catalytically incompetent.²⁰ However, Tyk2 JH2 has been shown to play an important regulatory role in Tyk2 function.²¹ Tyk2 JH1 inhibitors such as **1**²² and **2**²³ (Figure 1) have been reported. Due to the high

Figure 1. Literature Tyk2 JH1 inhibitors **1** and **2**.

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degree of homology among the JH1 of all Jak family members, it is not surprising that **1** and **2** display only moderate Tyk2 selectivity, as they also show significant activities against Jak1–3.

In order to target the Tyk2-dependent signaling pathway more selectively, we focused on Tyk2 JH2 due to its unique structural difference in the binding pocket compared to JH1 and have recently disclosed the identification of Tyk2 JH2 ligand **3** (Figure 2) through a chemogenomic approach.²⁴ This

Figure 2. Tyk2 JH2 inhibitors **3**–**6**.

Tyk2 JH2 ligand does not bind to Tyk2 JH1 and exhibits high selectivity over other kinases including other Jak family members. Moreover, **3** is effective in blocking the activation of Tyk2 JH1. 6-Anilino imidazopyridazines (IZP) **4** represents another chemotype of Tyk2 JH2 ligands that we have preliminarily reported most recently.²⁵ The structure–activity relationship (SAR) for this series was investigated, but the extremely poor metabolic stability remained a formidable issue. For example, after 10 min of incubation of **4** in human, rat, and mouse liver microsomes, the remaining **4** was found to be only 11%, 14%, and 1%, respectively. Previous effort to address the metabolic stability issue led to **5**, which displayed much improved liver microsomal stability with 99%, 76%, and 44% recoveries in human, rat, and mouse, respectively. However, unfortunately, it showed very limited permeability, indicated by its low Caco-2 value of 34 nm/s, and subsequently very limited exposure in vivo. Now, we would like to report our modification of the 6-anilino IZP series into 6-(2-oxo-N1-substituted-1,2-dihydropyridin-3-yl)amino IZP, represented by **6**. Tyk2 JH2 inhibitor **6** not only dramatically improved the metabolic stability but it also proved to be orally bioavailable, highly effective in inhibiting IFN γ in rat, and fully efficacious in a rat adjuvant arthritis (AA) model at a low dose (5 mg/kg, bid).

The much improved liver microsomal stability for compound **5**, compared to **4**, is most likely due to its much reduced cLogP of 1.99, calculated by ChemBioDraw Ultra 14.0, versus 3.80 for **4**. It is well-known that decreasing cLogP can improve metabolic stability.²⁶ The limited permeability observed for **5** may be understandable considering that the molecule carries five H-bond donors (three NH and two OH groups). Searching for a moiety that can effectively reduce cLogP without introducing too much polarity, we wondered if

the anilino group in **4** could be replaced with 2-oxo-N1-substituted-1,2-dihydropyridin-3-ylamino functionality. Thus, **6a**, the calculated cLogP of which is 0.66, was synthesized. The compound turned out to be approximately 6-fold less potent against Tyk2 JH2 compared to **4** based on their K_i determinations (Table 1). It was also less potent by about 6-

Table 1. SAR Observed for R¹ with **6a**–**m**

compd	R ¹	Tyk2 JH2 K_i (nM)	IFN α IC_{50} (nM)	hWB IC_{50} (nM)
4	NA	0.13	96	ND
6a	Me	0.74	614	ND
6b	cPr	0.33	131	817
6c	NC-	0.20	90	268
6d		0.07	111	811
6e		0.34	94	398
6f		0.14	140	380
6g		0.29	148	346
6h		0.22	70	371
6i		0.15	99	236
6j		0.12	61	313
6k		0.38	235	464
6l		0.091	147	247
6m		0.48	135	340

fold than **4** in the IFN α stimulated luciferase reporter assay in Kit225 T cells. The human whole blood (hWB) activity for **6a** was not obtained due to its poor solubility in this assay. However, to our satisfaction, **6a** displayed significantly improved liver microsomal stability. After 10 min of incubation in human, rat, and mouse liver microsomes, the remaining **6a** was found to be 56%, 63%, and 10%, respectively, versus only 11%, 14%, and 1% for **4** (The liver microsomal stability and Caco-2 data for all analogs are arranged in Table S1 in the Supporting Information). More encouragingly, when the N-methyl on the pyridone ring in **6a** was substituted with cyclopropyl, the liver microsomal stability for **6b** was further improved, with the recoveries now being 88%, 71%, and 39%, respectively. Its Tyk2 JH2 binding affinity and cellular activity were also improved to be comparable to that for **4**. Furthermore, Tyk2 JH2 ligand **6b** was found to be active in

hWB assay with its IC_{50} being determined to be 817 nM. Use of an aryl group such as *para*-cyanophenyl on the pyridone resulted in **6c**, which improved the metabolic stability further to the incubation recoveries of 92%, 77%, and 71% from human, rat, and mouse liver microsomes, respectively. Analog **6c** displayed an hWB IC_{50} of 268 nM, 3-fold more active than what was observed for **6b**, although its Tyk2 JH2 affinity and cellular functional activity were only slightly better.

A cocrystal structure of **6c** bound to Tyk2 JH2 was obtained,²⁷ and the binding mode was revealed to be the same as that for a 6-anilino IZP ligand.²⁵ Namely, **6c** interacts with Tyk2 JH2 protein mainly through two hydrogen bond networks (Figure 3). One occurs at the hinge region involving

Figure 3. Cocrystal structure of **6c** bound to Tyk2 JH2.

hydrogen bonds between the C8 methylamino NH and the carbonyl of Val690 and between N1 of the IZP core and the NH of Val690. The other takes place near the gatekeeper involving hydrogen bonds from the C3 amide carbonyl to the NH of Lys642 and to the carbonyl of Glu688 through a bridging water molecule. The pyridone ring is sandwiched between the p-loop and Pro694, with the cyanophenyl pointing to a solvent exposed area, which may mean a relatively flat SAR for R^1 . It is noted that the cyanophenyl and pyridone rings are almost perpendicular with the dihedral angle between the two rings being 85.1° .

While **6c** looked promising for its activities and metabolic stability, its extremely low aqueous solubility ($<1 \mu\text{g/mL}$ at pH 6.5 and $2 \mu\text{g/mL}$ at pH 1.0) was a concern. Replacement of the cyano in **6c** with a solubilizing group such as carbinol (**6d**) led to significant loss of Caco-2 permeability. As a result, we turned our focus to the exploration of N1-heteroaryl-2-oxo-1,2-dihydropyridin-3-yl analogs. 2'', 3'', and 4''-Pyridyl analogs **6e**, **6f**, and **6g** all provided Tyk2 JH2 enzymatic, cellular, and hWB activities similar to what were obtained for **6c**. The major differentiation among the three compounds came from their Caco-2 permeability, with **6e** (Caco-2 = 169 nm/s) being significantly more permeable than **6f** and **6g** (Caco-2 = 51 and 38 nm/s, respectively). It should be mentioned that the Caco-2 data were found to correlate very well with in vivo exposures for the current series, and a relatively high Caco-2 value was crucial for desired exposure. In spite of knowing that the cyanophenyl and pyridone rings were perpendicular in **6c** in its

cocrystal structure with Tyk2 JH2, we speculated that the 2''-pyridyl in **6e** could be significantly more coplanar to the pyridone ring, due to two potential intramolecular hydrogen bonds: from the pyridyl nitrogen to the proximal hydrogen on the pyridone ring and from pyridone oxygen to the proximal hydrogen on the 2''-pyridyl. Intramolecular hydrogen bonding interactions involving aromatic C–H are known in the literature.²⁸ As a result, the 2''-pyridyl nitrogen and the pyridone oxygen in **6e** would be shielded by the intramolecular hydrogen bonds, and therefore, the molecule would be significantly less polar and more permeable compared to **6f** and **6g**. The speculation was confirmed by single-crystal X-ray analyses of **6e**. Two conformers resulted from rotations of the single bond between the pyridine and pyridone rings were observed, and one of them is shown in Figure 4. The dihedral

Figure 4. Single crystal structure of **6e**.

angles between the two heteroaryl rings is measured to be 34.8° , which is significantly smaller than what (85.1°) was observed between the cyanophenyl and pyridone rings in **6c**, and the speculated intramolecular H-bonds are conceivable. The pyridyl and pyridone rings in the other conformer²⁹ are also more coplanar (dihedral angle = 51.1°), though less significantly, than the cyanophenyl and pyridone rings in **6c**. The afore-discussed intramolecular hydrogen bonding interactions in the second conformer may be less significant, but the more coplanar first conformer can certainly account for the much higher Caco-2 permeability observed for **6e**, considering the two conformers will be in equilibrium in solution. Metabolically, **6e** is also more stable in the rodent liver microsomes than **6f** and **6g**. On the basis of **6e**, three fluorinated 2''-pyridyl analogs **6h**, **6i**, and **6j** were prepared. All three compounds displayed very similar activity results to that for **6e**. Interestingly, only **6h** and **6i** remained highly permeable as **6e**. The Caco-2 of 59 nm/s for **6j** was more like what was found for **6f** and **6g**. It can be rationalized that due to the presence of 6''-F on the pyridine in **6j**, the pyridyl and pyridone rings will be less coplanar and more polar (lack of potential intramolecular hydrogen bonds), compared to **6h** and **6i**. Among the other heteroaryls on the pyridone ring examined, pyrimidyl and pyridazinyl were suitable to provide activities and liver microsomal stability comparable to that for **6e**, but the Caco-2 results for **6k** and **6l** were in the low to moderate range. However, dimethylpyrazole derived analog **6m** was found to be highly permeable (Caco-2 = 142 nm/s) in addition to showing good activities and liver microsomal stability.

Table 2 presents the SAR results observed for R^2 of the C3 amide side chain with R^1 being fixed to be 2-pyridyl.

Table 2. SAR Observed for R² with 6e, 6n–p, and 6

compd	R ²	Tyk2 JH2 K _i (nM)	IFNα IC ₅₀ (nM)	hWB IC ₅₀ (nM)
6e	cPr	0.34	94	382
6n	iPr	0.13	153	750
6o	cBu	0.26	60	384
6p		0.29	84	285
6		0.086	25	81

Replacement of the *N*-cyclopropyl in 6e with *N*-isopropyl appeared to improve the Tyk2 JH2 binding affinity by 2- or 3-fold, but the cellular and hWB activities for 6n were slightly less potent than that for 6e. The cyclobutyl analog 6o displayed approximately the same potencies as 6e in all three assays. A larger substitution such as 3-hydroxy-2,2-dimethylpropyl was also well tolerated, as 6p was shown to be equipotent to 6e. The best finding for R² was the enantiomeric (1*R*,2*S*)-2-fluorocyclopropyl group, which enhanced the Tyk2 JH2 affinity by 4-fold, as inhibitor 6 exhibited a K_i of 0.086 nM. The enhancement in enzymatic potency led to the same level of improvement in its cellular and hWB activities.

Combining (1*R*,2*S*)-2-fluorocyclopropyl, the best group identified for R², with some of the preferred R¹ from Table 1 prompted the synthesis of 6q–t (Table 3). As expected, these compounds were highly potent inhibitors of Tyk2 JH2, with their K_i values in the range of 0.015 to 0.035 nM. The K_i values appeared to suggest that 6q–t would be somewhat more potent inhibitors than 6, but in the cellular and hWB assays, they behaved very similarly to 6, with IFNα and hWB

Table 3. SAR Observed for R¹ with 6 and 6q–t

compd	R ¹	Tyk2 JH2 K _i (nM)	IFNα IC ₅₀ (nM)	hWB IC ₅₀ (nM)
6		0.086	25	81
6q		0.035	26	85
6r		0.024	12	136
6s		0.015	22	90
6t		0.018	41	63

IC₅₀ values in the ranges of 12 to 41 nM and 63 to 136 nM, respectively. The liver microsomal stability for these compounds were reasonable except for 6q. It was noticed that all the (1*R*,2*S*)-2-fluorocyclopropyl analogs showed reduced Caco-2 permeability to some extent, compared to their corresponding des-fluorocyclopropyl counterparts.

Compounds 6 and 6a–t were synthesized according to Scheme 1, using a previously reported advanced intermediate

Scheme 1. Synthesis of 6, 6a–t^a

^aReagent and conditions: (a) LiOH, MeOH/THF, rt, 2 h, 97%; (b) R¹NH₂, BOP, *N,N*-diisopropylethylamine, rt, 16 h, 55–97%; (c) i. 10a–m, tris(dibenzylideneacetone)dipalladium(0)/xantphos/Cs₂CO₃ or Pd(OAc)₂/BrettPhos/K₂CO₃, 1,4-dioxane, 80–125 °C, 2–3 h, ii. HCl/1,4-dioxane, CH₂Cl₂, rt, 30 min, 6–46% over 2 steps.

⁷²⁵ (Scheme 1). Hydrolysis of 7 provided IZP carboxylic acid 8, which was then converted to carboxamide 9a–e by benzotriazole-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) promoted coupling reactions. Buchwald reactions of 9a–e with aminopyridone 10a–m, catalyzed by tris(dibenzylideneacetone)dipalladium(0)/xantphos or Pd(OAc)₂/BrettPhos, followed by the removal of the *p*-methoxybenzyl (PMB) protection group, gave rise to 6 and 6a–t.

Throughout the SAR studies, several compounds were selected for pharmacokinetic (PK) studies, and it was found that 6 exhibited the most desired PK profiles. The PK results for 6 in mouse, rat, cyno, and dog are summarized in Table 4. In brief, 6 displayed a low clearance rate of 7.8 mL/min/kg in rat and moderate clearance rates of 16, 17, and 25 mL/min/kg in mouse, cyno, and dog, respectively. It provided the highest oral exposure and bioavailability (114%) in rat among the four

Table 4. PK Profiles for 6 in Mouse, Rat, Cyno, and Dog^{a,b}

species	mouse	rat	cyno	dog
dose (PO) (mg/kg)	10	10	10	10
C _{max} (μM)	15	9.4	1.8	0.93
AUC _{0–24 h} (μM·h)	19	57	11	8.0
CL (mL/min/kg)	16	7.8	17	25
F (%)	86	114	46	50

^aVehicle: 5:5:90 TPGS/EtOH/PEG300. ^bIV dose: 2 mg/kg.

species examined. The bioavailability observed in mouse, cyno, and dog were 86%, 46%, and 50%, respectively.

Tyk2 JH2 inhibitor **6** was then evaluated in a pharmacodynamic (PD) model to inhibit IL-12/IL-18 induced IFN γ production. In this study, **6** was first administered to rats, and after one hour, the rats were challenged with IL-12. Another hour later, they were further challenged with IL-18. Five hours after drug administration, plasma samples were collected and analyzed for IFN γ levels. As shown in Figure 5, **6** was effective in a dose-dependent manner in this model, inhibiting IL-12/IL-18 induced IFN γ production by 45% and 77% at doses of 1 and 10 mg/kg, respectively.

Figure 5. Inhibitor **6** in the IL12 + IL18 induced IFN γ in rats (vehicle: 5:5:90 TPGS/EtOH/PEG300)

Compound **6** was also shown to be highly efficacious in a rat adjuvant arthritis (rat AA) model. In this study, adjuvant was given to rats on day zero for the rats to develop arthritis. Meanwhile, **6** was dosed to the rats, twice a day, from day zero to day 20, during which period the rats' paw volumes were periodically measured. As seen in Figure 6, **6** demonstrated full efficacy to prevent the rats' paw from swelling at bid doses of 5 and 10 mg/kg.

Figure 6. Inhibitor **6** in the rat adjuvant arthritis model (bid dosing; vehicle: 5:5:90 TPGS/EtOH/PEG300).

It should be mentioned that **6** proved to be remarkably selective over other kinases, displaying >10,000-fold selectivity for Tyk2 JH2 over a diverse panel of 230 kinases that include Tyk2 JH1 and other Jak family members. HIPK4 is the only kinase, over which **6** showed a selectivity of only 480-fold. More specifically, **6** inhibited Jak1–3 with IC₅₀ values of >2 μ M and displayed the Jak1–3 dependent cellular activities of >12.5 μ M (IC₅₀ values).

In summary, a 2-oxo-1-substituted-1,2-dihydropyridin-3-ylamino moiety was identified as a viable replacement for the anilino group of a previously reported series of 6-anilino imidazopyridazine based Tyk2 JH2 ligands to dramatically improve metabolic stability. A variety of N1-heteroaryl groups on the 2-oxo-1,2-dihydropyridin-3-yl was found appropriate for Tyk2 JH2 activity, but it was 2-pyridyl that provided the desired Caco-2 permeability due to its ability to form intramolecular hydrogen bonds. Further SAR studies led to the identification of highly potent and selective Tyk2 JH2 inhibitor **6**. Compound **6** displayed good to excellent PK profiles among the species of mouse, rat, cyno, and dog. It was highly effective in a pharmacodynamic (PD) model to inhibit IL-12/IL-18 induced IFN γ production in rat and fully efficacious in a preventive rat adjuvant arthritis (rat AA) model at a dose of 5 mg/kg.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.9b00035.

Experimental procedures and characterization for all final compounds, as well as descriptions of in vitro and in vivo studies (PDF)

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Notes

The authors declare no competing financial interest.

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