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Discovery of 3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one derivatives as novel JAK inhibitors

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ABSTRACT

Because Janus kinases (JAKs) play a crucial role in cytokine-mediated signal transduction, JAKs are an attractive target for the treatment of organ transplant rejection and autoimmune diseases such as rheumatoid arthritis (RA). To identify JAK inhibitors, we focused on the 1*H*-pyrrolo[2,3-*b*]pyridine derivative **3**, which exhibited moderate JAK3 and JAK1 inhibitory activities. Optimization of **3** identified the tricyclic imidazo-pyrrolopyridinone derivative **19**, which exhibited potent JAK3 and JAK1 inhibitory activities (IC_{50} = 1.1 nM, 1.5 nM, respectively) with favorable metabolic stability.

1. Introduction

Organ transplant rejection is commonly treated with immunosuppressive drugs such as calcineurin inhibitors and mycophenolate mofetil (MMF). Calcineurin inhibitors such as tacrolimus and cyclosporin A inhibit the phosphatase activity of calcineurin and the translocation of nuclear factor of activated T-cells (NF-AT) into the nucleus.[1] MMF inhibits inosine monophosphate dehydrogenase (IMPDH), which plays a crucial role in the proliferation of immune cells.[2] Although these drugs help prevent organ transplant rejection, the ubiquitous expression of their molecular targets results in a range of adverse effects, including nephrotoxicity and neurotoxicity (tacrolimus and cyclosporin A) and gastro-intestinal toxicity (MMF).[3] Drugs with targets that have limited expression might therefore provide immunomodulation with reduced adverse effects.

Given the unique role of Janus kinases (JAKs) in the immune system, they have emerged as potential targets for the treatment of transplant rejection and autoimmune diseases. JAKs are cytoplasmic protein tyrosine kinases that consist of four groups (JAK1, JAK2, JAK3 and TYK2) and play key roles in various forms of cytokine-mediated signal transduction.[4] The binding of cytokines to their corresponding receptors activates JAKs, which then phosphorylate signal transducers and activators of transcription (STAT) to promote cytokine-responsive gene expression.[5] JAK3 and JAK1 are involved in the signaling pathways of common γ-chain (γc) cytokines such as interleukin (IL)-2, -4, -7, -9, -15 and -21, which play important roles in T-cell differentiation, proliferation and survival. In contrast, JAK2 mediates signaling via hematopoietic cytokines such as erythropoietin (EPO). In addition, JAK1, JAK2, and TYK2 are also involved in the signaling pathways of IL-6.

A number of JAK inhibitors have been reported to date.[6] In preclinical studies, tofacitinib (**1**, CP-690,550, Fig. 1) was originally described as a selective JAK3 inhibitor and then as a pan-JAK inhibitor (in-house data; JAK IC_{50} = 0.8 nM, 3.1 nM, 3.7 nM for JAK3, JAK2, JAK1) that demonstrated efficacy in a transplant rejection model in nonhuman primates.[7] **1** also demonstrated efficacy in the treatment of various autoimmune diseases and was recently approved for the treatment of rheumatoid arthritis (RA). Further, the selective JAK1 and JAK2 inhibitor baricitinib (**2**, INCB028050) also demonstrated clinical efficacy in the treatment of RA, suggesting that blocking the signal transaction of the IL-6 by inhibiting of JAK1 and JAK2 contribute to the treatment of RA.[8]

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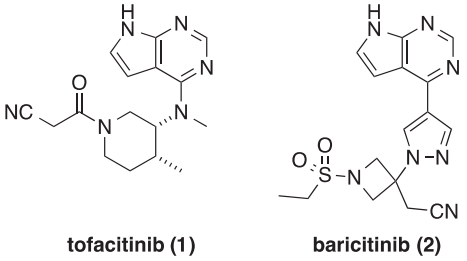


Figure 1. Structures of tofacitinib (**1**) and baricitinib (**2**).

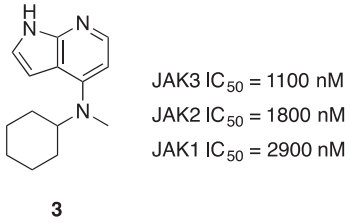


Figure 2. Structure of lead compound **3**.

Although the selectivity of **1** and **2** differs within the JAK family, both compounds exhibit efficacy against various autoimmune diseases in clinical and preclinical studies, which suggests that JAK inhibitors might be effective drugs for immunomodulation. In transplant rejection, blocking the signal transduction of IL-2 by inhibiting JAK3 might be advantageous as its expression is limited to hematopoietic cells such as T-cells and B-cells, and might therefore have a reduced risk of adverse effects. We screened compounds in JAK3 inhibition and IL-2-induced T-cell proliferation assays. As IL-2-induced signaling was associated with not only JAK3 but also JAK1, we also focused on JAK1 inhibitory activity. In addition, JAK2 inhibitory activity was evaluated because JAK2 inhibition may be related to adverse hematopoietic effects such as anemia.

In our initial investigation, 1*H*-pyrrolo[2,3-*b*]pyridine derivative **3** was identified as the lead compound with moderate JAK inhibitory activity (JAK IC_{50} = 1100 nM, 1800 nM and 2900 nM for JAK3, JAK2 and JAK1, respectively) (Fig. 2).[9] After further optimization, we discovered the tricyclic imidazo-pyrrolopyridinone derivative **19** with potent JAK3 and JAK1 inhibitory activity and favorable metabolic stability. Here, we report the design, synthesis, and structure–activity relationship (SAR) of novel tricyclic JAK inhibitors.

2. Chemistry

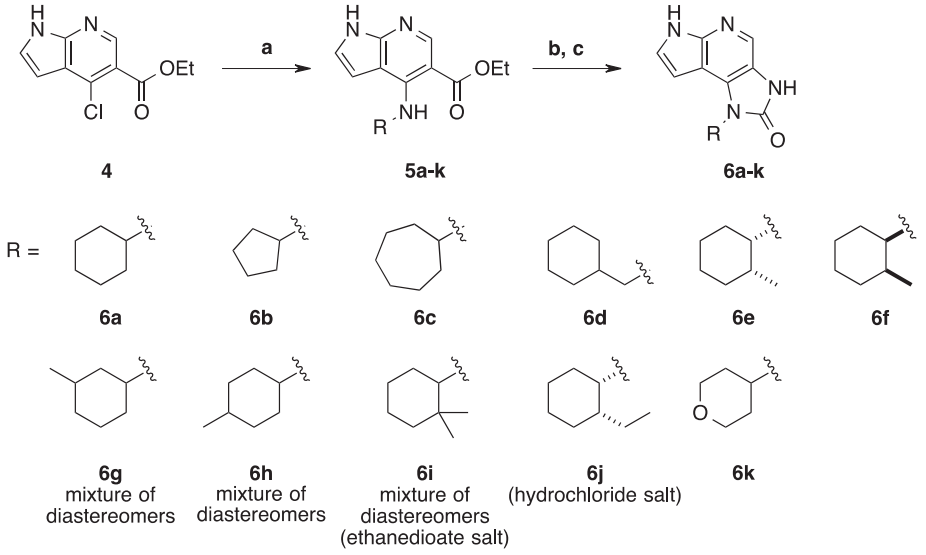
Imidazo-pyrrolopyridinone compounds **6a**–**k** were synthesized as shown in Scheme 1. S_NAr displacement of chloride in pyrrolopyridine **4** with several kinds of amines gave **5a**–**k**. Hydrolysis of ethyl ester gave carboxylic acid, which was then converted into desired tricyclic compounds **6a**–**k** by Curtius rearrangement with diphenylphosphoryl azide (DPPA).

Compounds **10a**–**c** and **11** were synthesized as shown in Scheme 2. S_NAr displacement of chloride in compound **4** gave **7a**–**c**. Hydrolysis of ethyl ester gave carboxylic acid, which was converted into tricyclic compounds **8a**–**c** by treatment with DPPA. Removal of *t*-butoxycarbonyl (Boc) group under an acidic condition gave **9a**–**c**. Mesylation of the amino group of **9a**–**c** gave **10a**–**c**. Condensation of **9b** with cyanoacetic acid gave **11**.

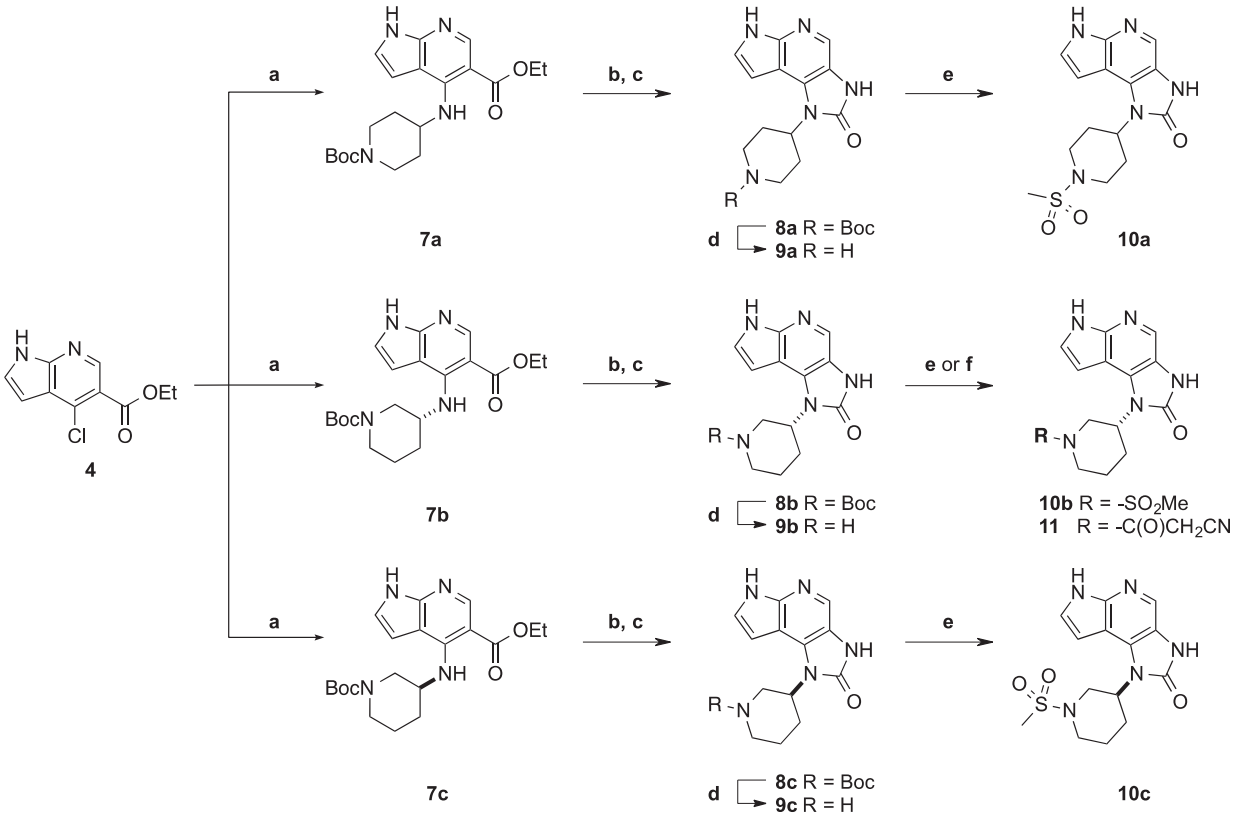
(±)-*cis*-3-Amino-4-methylpiperidine **15** was prepared from pyridine **12** as shown in Scheme 3. Treatment of **12** with benzyl bromide (BnBr) gave pyridinium salt **13**, followed by hydrogenation in the presence of platinum oxide to give a racemic mixture of *cis*-piperidine **14**. Deprotection of Boc group with trifluoroacetic acid (TFA) gave the desired aminopiperidine **15**.

Compound **19** was synthesized as shown in Scheme 4. S_NAr displacement of chloride in compound **4** with aminopiperidine **15** gave **16**. Hydrolysis of ethyl ester gave carboxylic acid, which was converted to compound **17**. Removal of benzyl (Bn) group by hydrogenolysis gave **18**, which was followed by condensation with cyanoacetic acid to give the desired compound **19**.

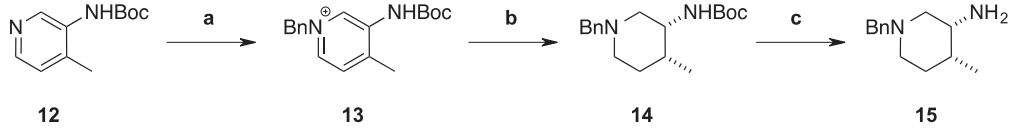
Compound **26** was synthesized as shown in Scheme 5. Protection of pyrrolopyridine NH with 2-(trimethylsilyl)ethoxymethyl (SEM) group gave **20**, which was followed by S_NAr displacement to give **21**. Hydrolysis of the ethyl ester gave carboxylic acid, which was converted to give compound **22**. Methylation of NH gave **23**, followed by deprotection of the SEM group by TFA and following treatment of ethylenediamine to remove *N*-hydroxymethyl intermediate



Scheme 1. Synthesis of **6a**–**k**. Reagents and conditions: (a) amine, *n*-BuOH, microwave, 150 °C or amine, Et_3N, NMP, microwave, 180 °C; (b) LiOH·H_2O, 1,4-dioxane, H_2O, reflux or 2 M NaOH aq, EtOH, THF, reflux; (c) DPPA, Et_3N, DMF (or DMI), 1,4-dioxane, 120 °C for 2 h.



Scheme 2. Synthesis of **10a–c**, **11**. Reagents and conditions: (a) amine, *n*-BuOH, microwave, 150 °C for 1 h or amine, DIPEA, *n*-BuOH, microwave, 150 °C for 1 h; (b) LiOH·H_2O, 1,4-dioxane, H_2O, reflux or 2 M NaOH aq EtOH, reflux; (c) DPPA, Et_3N, DMF, 1,4-dioxane, 120 °C for 2 h; (d) 4 M HCl (1,4-dioxane solution), 1,4-dioxane, rt for 2 h; (e) MsCl, Et_3N, DMI, 0 °C for 20 min; (f) cyanoacetic acid, WSCD·HCl, HOBt, DIPEA, DMF, rt–50 °C for 2 h.



Scheme 3. Preparation of **15**. Reagents and conditions: (a) benzyl bromide, acetone, reflux for 3 h; (b) H_2 (3 atm), PtO_2, EtOH, 40 °C for 4 h; (c) TFA, CH_2Cl_2, rt for 30 min.

to give **24**. The Bn group was removed with palladium hydroxide and ammonium formate to give **25**. Condensation with cyanoacetic acid gave the desired compound **26**.

3. Results and discussion

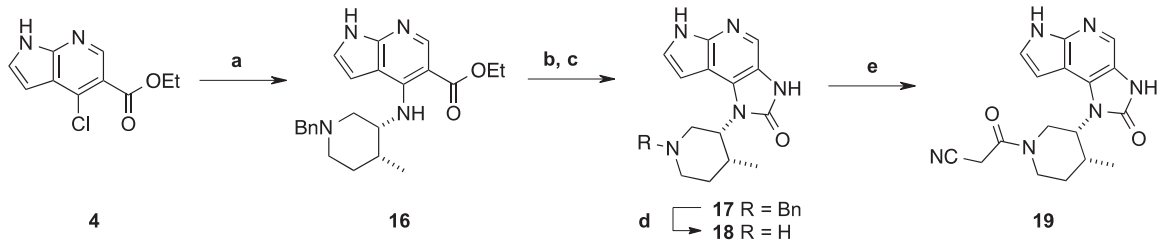
3.1. In vitro structure–activity relationships

We evaluated the inhibitory activity of newly synthesized compounds on human JAK1, JAK2 and JAK3 enzyme. An IL-2-induced rat T-cell proliferation assay was utilized to assess cellular potency that reflects JAK3 and JAK1 inhibitory activity. We also evaluated their metabolic stability in rat liver microsomes.

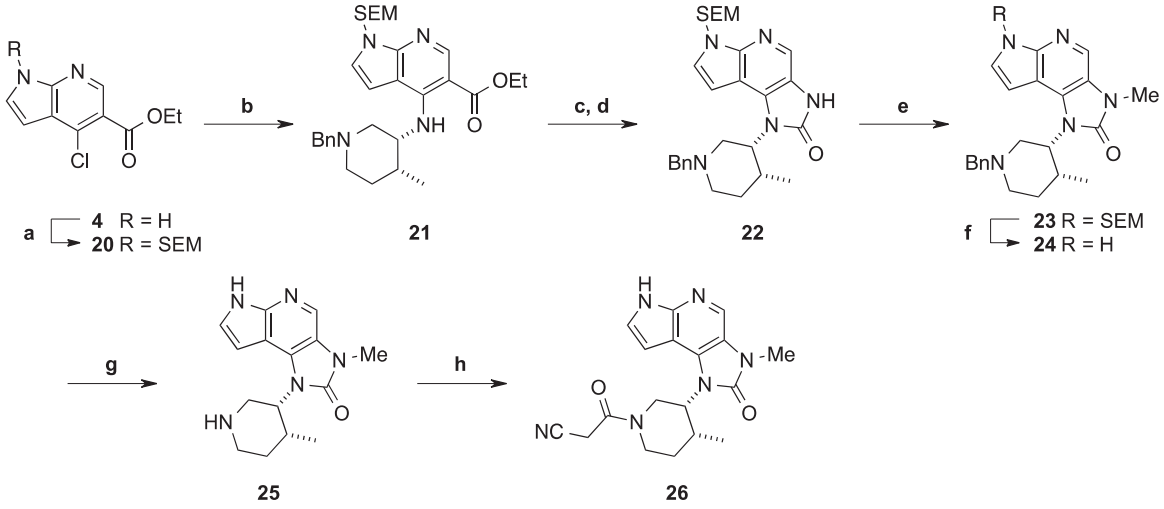
In our JAK inhibitor program, 1*H*-pyrrolo[2,3-*b*]pyridine derivative **3** was selected as the lead compound due to its moderate inhibition of JAKs despite its low molecular weight.[9] Compound **3** exhibited weaker JAK3 and JAK1 inhibitory activity than **1**. In addition, **3** exhibited higher lipophilicity (*C*log*P* = 5.3) than **1** (*C*log*P* = 1.8). Because highly lipophilic compounds tend to exhibit a poor PK profile due to low metabolic stability, optimization of **3** was required to enhance potency and reduce lipophilicity.

To design more potent JAK3 and JAK1 inhibitors, we utilized the reported X-ray co-crystal structure of human JAK3 with **1** due to the structural similarity of JAKs.[10] The co-crystal structure revealed that **1** binds to the ATP-binding site of JAK3, which consists of a hinge region, hydrophobic cavity and glycine-rich loop (Fig. 3a). As we previously reported, the pyrrolopyridine scaffold of lead compound **3** was predicted to interact with the hinge region in a manner similar to **1**.[9] As compound **3** has a free rotatable bond between the pyrrolopyridine scaffold and cyclohexane ring, the cyclohexane ring was placed at a proper position to access this hydrophobic pocket effectively. While our docking study revealed that Type I cyclization (Fig. 4) appeared not to access this hydrophobic pocket, Type II cyclization (Fig. 4) might effectively interact with this area (data not shown).

Based on this hypothesis, we designed tricyclic imidazopyrrolopyridinone **6a** with restricted conformation and performed a molecular docking study with human JAK3 (Fig. 3b).[11] This docking study predicted that the pyrrolopyridine scaffold of **6a** formed two hydrogen bonds with the hinge residues Glu903 and Leu905 in the similar binding mode to that of **1** and that the cyclohexane ring occupied the hydrophobic pocket. In fact, compound **6a** dramatically increased potency for JAK3 (IC_{50} = 54 nM) and JAK1 (IC_{50} = 79 nM) with reduced lipophilicity (*C*log*P* = 2.6), which suggests that rigidifying the cyclohexane ring increases inhibition of JAKs (Table 1).[12–15] Although compound **6a** had increased JAK3 and JAK1 potency, **6a** was still approximately 70-fold weaker than **1** in a JAK3 inhibitory assay. We set an initial goal of an in vitro



Scheme 4. Synthesis of **19**. Reagents and conditions: (a) amine **15**, Et_3N, NMP, microwave, 180 °C for 1 h; (b) LiOH·H_2O, 1,4-dioxane, H_2O, reflux for 10 h; (c) DPPA, Et_3N, DMF, 1,4-dioxane, 120 °C for 2 h; (d) H_2 (4 atm), $Pd(OH)_2$, EtOH, 30 °C for 2 days; (e) cyanoacetic acid, WSCD·HCl, HOBt, DIPEA, DMF, rt–50 °C for 3 h.



Scheme 5. Synthesis of **26**. Reagents and conditions: (a) SEMCl, NaH, DMF, 0 °C–rt for 1.5 h (b) amine **15**, DIPEA, NMP, 180 °C for 2 h; (c) 3 M NaOH aq, EtOH, reflux for 2 h; (d) DPPA, Et_3N, DMF, 1,4-dioxane, 120 °C for 2 h; (e) MeI, NaH, DMF, 0 °C–rt for 20 min; (f) (i) TFA, CH_2Cl_2, rt for 2 h; (ii) ethylenediamine, MeOH, $CHCl_3$, 50 °C for 1 h; (g) ammonium formate, $Pd(OH)_2$, MeOH, reflux for 30 min; (h) cyanoacetic acid, WSCD·HCl, HOBt, DIPEA, DMF, CH_2Cl_2, rt for 1.5 h.

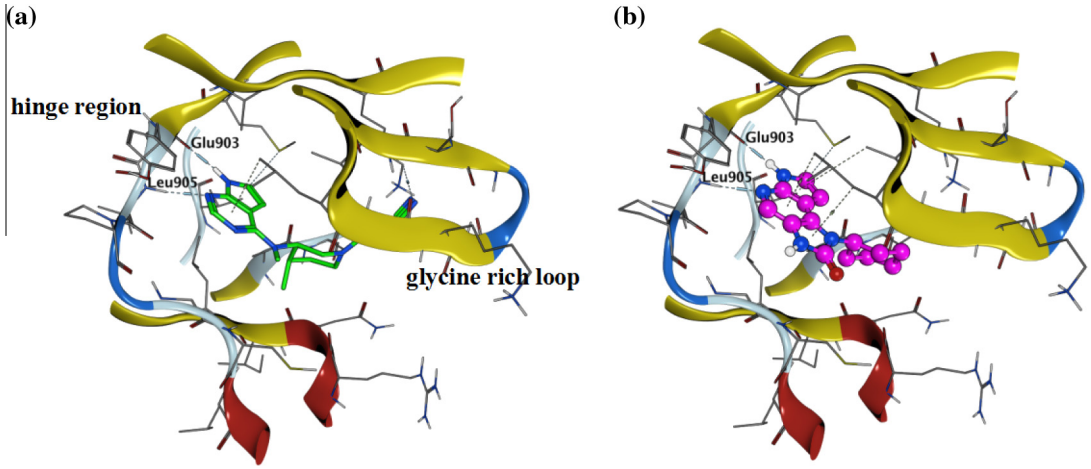


Figure 3. (a) Co-crystal structure of **1** with human JAK3.[10] (b) Predicted binding mode of **6a** to human JAK3.

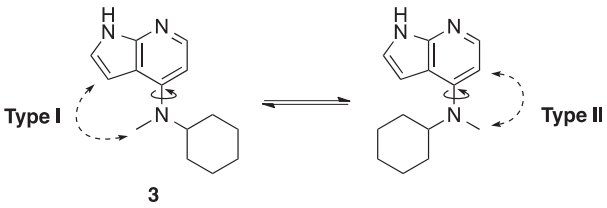


Figure 4. Cyclization strategy of **3**.

profile of JAK3 IC_{50} <10 nM, T-cell proliferation IC_{50} <50 nM, and in vitro CL_{int} <400 mL/min/kg. We therefore conducted further optimization of tricyclic derivatives using compound **6a**.

We first investigated the SARs around the cyclohexane ring of **6a** as summarized in Table 2. Substituting the cyclohexyl group of **6a** with the cyclopentyl group slightly decreased JAK3 inhibitory activity (**6b**, IC_{50} = 180 nM). Although **6c** with cycloheptyl group increased the JAK3 inhibitory activity by 2-fold (IC_{50} = 22 nM), this compound displayed high in vitro clearance in rat liver

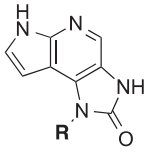
Table 1
Enhancement of potency by restriction of conformation

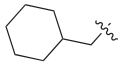
3 **6a**

Compound	JAK3 IC_{50}[a] (nM)	JAK2 IC_{50}[a] (nM)	JAK1 IC_{50}[a] (nM)	T-cell IC_{50}[b] (nM)
3	1100	1800	2900	NT[c]
6a	54	43	79	160

[a] Inhibition IC_{50}. IC_{50} values are shown as the mean of duplicate experiments.
[b] IL-2-induced rat T-cell proliferation IC_{50}. IC_{50} values are shown as the mean of duplicate experiments.
[c] NT = not tested.

Table 2
SARs of cycloalkyl groups



Compound	R	JAK3 IC_{50}[a] (nM)	JAK2 IC_{50}[a] (nM)	JAK1 IC_{50}[a] (nM)	T-cell IC_{50}[b] (nM)	In vitro CL_{int}[c] (mL/min/kg)	*C*log*P*[d]
1	—	0.8	3.1	3.7	23	—	1.8
6a		54	43	79	160	153	2.6
6b		180	97	110	NT[e]	NT[e]	2.0
6c		22	23	30	74	272	3.1
6d		60	88	1000	860	802	3.1

[a] Inhibition IC_{50}. IC_{50} values are shown as the mean of duplicate experiments.
[b] IL-2-induced rat T-cell proliferation IC_{50}. IC_{50} values are shown as the mean of duplicate experiments.
[c] In vitro metabolism with rat liver microsomes in the presence of the NADPH generating system.
[d] *C*log*P* values are calculated by ACD/Labs (version 12.01, Advanced Chemistry Development, Toronto, Canada).
[e] NT = not tested.

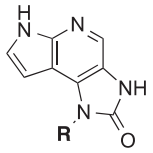
microsomes due to increased lipophilicity (*C*log*P* = 3.1). Although the cyclohexylmethyl analog **6d** retained JAK3 inhibitory activity (IC_{50} = 60 nM), it exhibited reduced activity in a cell-based assay. This was possibly due to a decrease in the potency of JAK1 inhibition (IC_{50} = 1000 nM), which indicates that inhibition of IL-2-induced T-cell proliferation was associated with not only the inhibition of JAK3 but also JAK1 for **6a** (IC_{50} = 79 nM) and **6d** (IC_{50} = 1000 nM).

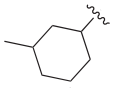
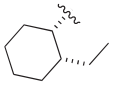
These results suggest that hydrophobic cycloalkyl moieties are responsible for interactions with JAK3 and JAK1. As compound **6a** appears to possess favorable metabolic stability, **6a** was selected as the template for further SAR exploration.

We then examined several substituted cyclohexyl groups (Table 3). Incorporation of a methyl group at the 2-position of the cyclohexane ring of **6a** enhanced the potency of JAK3 and JAK1 inhibition (**6e**, **6f**). In particular, the (1*S*,2*R*)-isomer **6e** was significantly more potent than the (1*R*,2*S*)-isomer **6f**. However, the 3-methylcyclohexyl analog **6g** and 4-methylcyclohexyl analog **6h** did not improve JAK3 inhibitory activity, and the 2,2-dimethylcyclohexyl moiety **6i** did not enhance potency. Considering that Pfizer reported that the methyl group at the 2-position of the piperidine ring of **1** binds the lipophilic pocket of JAK3, this methyl group of the cyclohexane ring might also interact with this pocket.[10] While the introduction of an ethyl group at the 2-position also increased activity (**6j**, JAK3 IC_{50} = 3.0 nM), its metabolic stability was lower than that of **6e** due to increased lipophilicity (*C*log*P* = 3.6). As compound **6e** satisfied our criteria for compound selection (JAK3 IC_{50} <10 nM, T-cell IC_{50} <50 nM, and in vitro CL_{int} <400 mL/min/kg), the profile of **6e** was studied further in an in vitro assay.

Organ transplant rejection is generally treated by the combination of calcineurin inhibitors and MMF. The JAK inhibitors

Table 3
SARs of substituted cyclohexyl groups



Compound	R	JAK3 IC_{50}[a] (nM)	JAK2 IC_{50}[a] (nM)	JAK1 IC_{50}[a] (nM)	T-cell IC_{50}[b] (nM)	In vitro CL_{int}[c] (mL/min/kg)	Clog*P*[d]
6a		54	43	79	160	153	2.6
6e		3.0	4.4	14	32	300	3.1
6f		27	17	28	110	NT[e]	3.1
6g[f]		21	23	430	1000	NT[e]	3.1
6h[f]		42	23	24	120	NT[e]	3.1
6i[f,g]		69	85	90	110	NT[e]	3.5
6j[h]		3.0	3.6	18	36	>1000	3.6

[a] Inhibition IC_{50}. IC_{50} values are the mean of duplicate experiments.
[b] IL-2-induced rat T-cell proliferation IC_{50}. IC_{50} values are the mean of duplicate experiments.
[c] In vitro metabolism with rat liver microsomes in the presence of the NADPH generating system.
[d] Clog*P* values calculated by ACD/Labs (version 12.01, Advanced Chemistry Development, Toronto, Canada).
[e] NT = not tested.
[f] Mixture of diastereomers.
[g] Ethanedioate salt.
[h] Hydrochloride salt.

Table 4
Properties of **6e**

Compound	CYP3A4 reversible inhibition[a] % remaining	CYP3A4 TDI[a] % remaining
6e	95	49

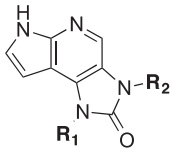
[a] Calculated as percentage of difference in the rate of CYP3A4-mediated conversion of midazolam to 1′-hydroxymidazolam without pre-incubation (reversible inhibition) and with pre-incubation (TDI) in the presence of **6e**. A result of 100% remaining indicates no measurable reversible or time-depend inhibition of CYP3A4.

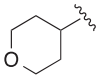
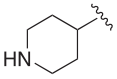
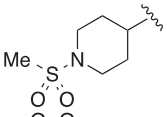
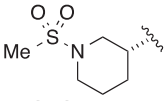
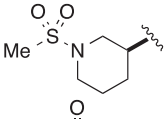
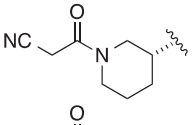
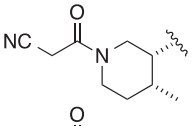
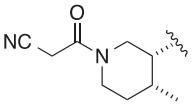
investigated in the present study might also be used in combination with these drugs in clinical treatment. Therefore, reducing the risk of drug–drug interaction (DDI) is essential for the treatment of organ transplant rejection. For example, as tacrolimus is mainly metabolized by CYP3A4,[16] its inactivation by concomitant drugs might lead to DDI. We evaluated both the CYP3A4 reversible and time-dependent inhibition (TDI) of compound **6e** (Table 4).

Although compound **6e** exhibited weak reversible inhibition of CYP3A4, **6e** exhibited CYP3A4 TDI that possibly resulted from the formation of an active metabolite capable of inhibiting CYP3A4. Our in vitro metabolite identification study on compound **6e** revealed that oxidation of the cyclohexane ring was a major metabolic route. We hypothesized that reducing the oxidative metabolism of the cyclohexane ring might reduce the risk of TDI. We therefore examined the reduction of lipophilicity by replacing the cyclohexane ring with a polar heterocycle (Table 5).

Conversion of the cyclohexane ring to a tetrahydropyran ring (**6k**) improved metabolic stability and TDI, which suggests that reduced lipophilicity (Clog*P* = 1.0) following the introduction of a polar heterocycle effectively minimized CYP3A4 TDI. In contrast, a decrease in JAK3 inhibitory activity (IC_{50} = 270 nM) was observed. Introduction of a piperidine ring resulted in a further decrease in potency (**9a**, JAK3 IC_{50} = 1200 nM). As piperidine NH appeared to decrease inhibitory activity against JAKs, its modification was investigated. The sulfonamide derivative **10a** exhibited a slight increase in the potency of JAK 3 inhibition (IC_{50} = 710 nM) compared to **9a**. The 3-piperidinyl analogs **10b** and **10c** exhibited a further increase in JAK3 inhibitory activity. The potency of JAK3 inhibition exerted by the (*R*)-isomer **10b** (IC_{50} = 63 nM) was 7-fold more than that of the corresponding (*S*)-isomer **10c** (IC_{50} = 460 nM), which suggests that a specific interaction with JAK3 might occur due to the different stereochemistry of the piperidine ring. The reported co-crystal structure of human JAK3 suggested that the methylsulfonamide **10b** was located near the

Table 5
SARs of heterocycles



Compound	R_1	R_2	JAK3 IC_{50}[a] (nM)	JAK2 IC_{50}[a] (nM)	JAK1 IC_{50}[a] (nM)	T-cell IC_{50}[b] (nM)	In vitro CL_{int}[c] (mL/min/kg)	CYP3A4 TDI[e] % remaining	Clog*P*[f]
6e		H	3.0	4.4	14	32	300	49	3.1
6k		H	270	500	520	1400	No depletion[d]	90	1.0
9a		H	1200	1300	NT	2800	NT[g]	NT[g]	1.2
10a		H	710	140	140	NT[g]	NT[g]	NT[g]	0.90
10b		H	63	82	230	1700	NT[g]	NT[g]	1.0
10c		H	460	850	NT[g]	NT[g]	NT[g]	NT[g]	1.0
11		H	28	17	20	310	NT[g]	NT[g]	0.11
19 (racemate)		H	1.1	2.6	1.5	36	No Depletion[d]	86	0.65
26 (racemate)		Me	5.0	7.3	29	158	NT[g]	NT[g]	0.71

[a] Inhibition IC_{50}. IC_{50} values are the mean of duplicate experiments.
[b] IL-2-induced rat T-cell proliferation IC_{50}. IC_{50} values are the mean of duplicate experiments.
[c] In vitro metabolism with rat liver microsomes in the presence of NADPH generating system.
[d] Stable against rat liver microsomal metabolism.
[e] Calculated as percentage of difference in the rate of CYP3A4-mediated conversion of midazolam to 1′-hydroxymidazolam with pre-incubation in the presence of test compound.
[f] Clog*P* values calculated by ACD/Labs (version 12.01, Advanced Chemistry Development, Toronto, Canada).
[g] NT = not tested.

glycine-rich loop and therefore interacted with this glycine-rich loop. Given that cyanoacetamide, which is a characteristic of the structure of **1**, formed polar interaction with this glycine-rich loop of JAK3,[10] cyanoacetamide was incorporated into compound **10b**. Conversion of sulfonamide to cyanoacetamide gave **11** with enhanced JAK3 inhibitory activity (IC_{50} = 28 nM). As the introduction of a methyl group into the 6-membered ring system improved potency, we introduced a methyl group to **11**. The resulting **19** exhibited potent inhibition of JAK3 (IC_{50} = 1.1 nM) and JAK1 (IC_{50} = 1.5 nM) and possessed improved metabolic stability and reduced TDI. Methylation of imidazolone NH gave **26** with reduced JAK3 inhibitory activity (IC_{50} = 5.0 nM) and, in particular, JAK1 inhibitory activity (IC_{50} = 29 nM).

3.2. Docking study to human JAK3 and WaterMap analysis

The tricyclic derivative **19** exhibited a significant increase in JAK3 and JAK1 inhibitory activity as well as cellular potency compared to **3**. To elucidate the mechanism behind this increase in potency, we performed a molecular docking study of **19** with human JAK3.[11] The predicted binding mode of **19** was similar to that of **1** (Fig. 5).[10] Our docking study revealed that the pyrrolopyridine scaffold of **19** might form two hydrogen bonds with hinge residues Glu903 and Leu905 (Fig. 5a). Moreover the 3-piperidine ring of **19** might occupy the same space that 3-piperidine ring of **1** occupied. These findings suggest that the restricted conformation of **19** mimics the co-crystal binding conformation reported for **1** and results in enhanced potency. In addition, the cyano group of **19** appeared to extend toward the glycine-rich loop, which

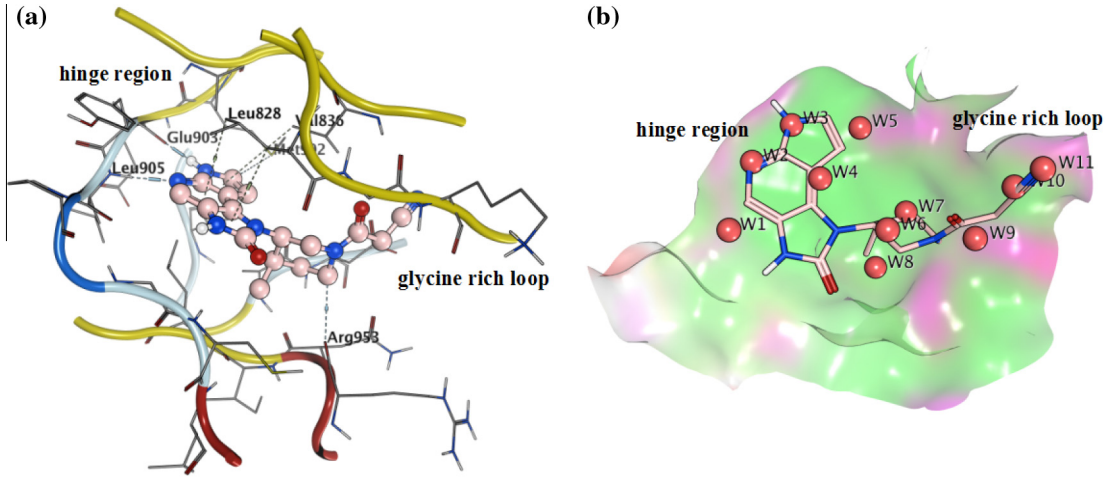


Figure 5. (a) Predicted binding mode of **19** to human JAK3. (b) **19** with unfavorable water molecules determined by WaterMap ($\Delta G \geqslant 2.0$ kcal/mol).

suggests that the introduction of cyanoacetamide contributed to the further increase in potency.

We then performed computational analysis using WaterMap,[17] which is a recently developed protocol that combines molecular dynamics, solvent clustering, and statistical thermodynamics to assess the enthalpy, entropy, and free energy (ΔG) of water 'hydration site'. The ΔG was computed for the **1**-bound human JAK3 structure (PDB code 3LXK). WaterMap analysis revealed that displacement of the unfavorable water molecule ($\Delta G \geqslant 2.0$ kcal/mol) by introduction of the methyl group to the cyclohexane ring (Fig. 5b, W8) and cyanoacetamide (W9, W10, W11) might contribute to the binding affinity of **19**.

4. Conclusions

We designed tricyclic imidazo-pyrrolopyridinone compounds as a novel scaffold for JAK inhibitor with restricted conformation to obtain an effective interaction with the hydrophobic pocket. The tricyclic derivatives identified in the present study, such as compound **6e**, demonstrated a significant increase in JAK3 and JAK1 inhibitory activities. However, compound **6e** exhibited CYP3A4 TDI, which was probably due to the low metabolic stability. To address this problem, reduction of lipophilicity by substituting the cyclohexane ring with heterocycles was examined. This approach led to the identification of compound **19**, which exhibited potent JAK3 and JAK1 inhibitory activities with favorable metabolic stability and reduced CYP3A4 TDI. As compound **19** also exhibited potent JAK2 inhibitory activity ($IC_{50} = 2.6$ nM), the degree of selectivity for JAK3 and JAK1 over JAK2 that is required to avoid potential adverse hematopoietic effects should be investigated. Further results will be reported in due course.

5. Experimental section

5.1. Chemistry

^{1}H NMR spectra were recorded on a Bruker Avance 400 (400 MHz) or AV400M (400 MHz) spectrometer. Chemical shifts are expressed in δ values (ppm) using tetramethylsilane as the internal standard (NMR peak description: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad peak). Mass spectra (MS) were recorded using Agilent 1100. Elemental analyses were performed using a Yanaco MT-6 (C, H, N), Elementar Vario EL III (C, H, X), and Dionex ICS-3000 (S, halogene) and were within ±0.4% of theoretical values. Column chromatography was carried out on silica gel (Kieselgel 60). Unless otherwise noted, all reagents and solvents were obtained from commercial suppliers and used without further purification. The following abbreviations are used: DIPEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DMI, 1,3-dimethyl-2-imidazolidinone; DMSO, dimethylsulfoxide; DPPA, diphenylphosphoryl azide; EtOAc, ethyl acetate; EtOH, ethanol; Et_2O, diethyl ether; HOBt, 1-hydroxybenzotriazole; IPE, diisopropyl ether; MeOH, methanol; NMP, *N*-methylpyrrolidone; SEM, 2-(trimethylsilyl)ethoxymethyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran; and WSCD, 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide.

5.1.1. Ethyl 4-(cyclohexylamino)-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylate (5a)

A mixture of ethyl 4-chloro-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylate (**4**) (24 mg, 0.11 mmol) and cyclohexanamine (61 μL, 0.53 mmol) in *n*-BuOH (120 μL) was heated in a microwave reactor at 150 °C for 1 h. After cooling to room temperature, the reaction mixture was concentrated in vacuo and the residue was purified by column chromatography on silica gel to give the product (17 mg, 56%). ^{1}H NMR (DMSO-d_6) δ 1.32 (t, $J = 7.1$ Hz, 3H), 1.33–1.77 (m, 8H), 1.99–2.08 (m, 2H), 3.95–4.08 (m, 1H), 4.26 (q, $J = 7.1$ Hz, 2H), 6.55 (d, $J = 3.5$ Hz, 1H), 7.18 (d, $J = 3.5$ Hz, 1H), 8.54 (s, 1H), 8.84–8.88 (m, 1H), 11.67 (br s, 1H); MS (ESI) *m*/*z* 288 $[M+H]^+$.

5.1.2. Ethyl 4-(cyclopentylamino)-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylate (5b)

A mixture of **4** (112 mg, 0.50 mmol), cyclopentanamine (99 μL, 1.0 mmol), and Et_3N (209 μL, 1.5 mmol) in NMP (3.0 mL) was heated in a microwave reactor at 180 °C for 1 h. After cooling to room temperature, to the reaction mixture was added water and the solids were collected by filtration and dried in vacuo to give the product (136 mg, quantitative yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.31 (t, $J = 7.2$ Hz, 3H), 1.58–1.78 (m 6H), 1.99–2.11 (m, 2H), 4.25 (q, $J = 7.0$ Hz, 2H), 4.46–4.55 (m, 1H), 6.62–6.70 (m, 1H), 7.16–7.19 (m, 1H), 8.53 (s, 1H), 8.83 (d, $J = 7.4$ Hz, 1H), 11.66 (br s, 1H); MS (ESI) *m*/*z* 274 $[M+H]^+$.

5.1.3. Ethyl 4-(cycloheptylamino)-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylate (5c)

A mixture of **4** (112 mg, 0.50 mmol), cycloheptanamine (76 μL, 0.60 mmol), and Et_3N (209 μL, 1.5 mmol) in NMP (3.0 mL) was heated in a microwave reactor at 180 °C for 1 h. After cooling to room temperature, the reaction mixture was quenched with water,

extracted with EtOAc, dried over $MgSO_4$, and evaporated in vacuo. The crude mixture was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 100:0 to 90:10) to give the product (135 mg, 90%). ^{1}H NMR (DMSO-d_6) δ 1.31 (t, J = 7.2 Hz, 3H), 1.98–2.10 (m, 2H), 1.43–1.72 (m, 10H), 4.17–4.30 (m, 3H), 6.57–6.61 (m, 1H), 7.16–7.20 (m, 1H), 8.54 (s, 1H), 8.89 (d, J = 8.1 Hz, 1H), 11.67 (br s, 1H); MS (ESI) m/z 302 $[M+H]^+$.

5.1.4. Ethyl 4-[(cyclohexylmethyl)amino]-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylate (5d)

Compound **5d** was prepared in 62% yield as a white solid by a method similar to that described for **5c**. ^{1}H NMR (DMSO-d_6) δ 1.01–1.33 (m, 5H), 1.31 (t, J = 7.1 Hz, 3H), 1.61–1.86 (m, 6H), 3.52 (t, J = 6.1 Hz, 2H), 4.26 (q, J = 7.1 Hz, 2H), 6.66 (d, J = 3.6 Hz, 1H), 7.15 (d, J = 3.6 Hz, 1H), 8.53 (s, 1H), 8.81 (t, J = 5.4 Hz, 1H), 11.67 (br s, 1H); MS (ESI) m/z 302 $[M+H]^+$.

5.1.5. Ethyl 4-{[(1*S*,2*R*)-2-methylcyclohexyl]amino}-1*H*-pyrrolo-[2,3-*b*]pyridine-5-carboxylate (5e)

Compound **5e** was prepared in 53% yield as a white solid by a method similar to that described for **5a**. ^{1}H NMR (DMSO-d_6) δ 0.91 (d, J = 6.9 Hz, 3H), 1.32 (t, J = 7.1 Hz, 3H), 1.35–2.16 (m, 9H), 4.23–4.34 (m, 3H), 6.59 (d, J = 3.5 Hz, 1H), 7.17 (d, J = 3.5 Hz, 1H), 8.68 (s, 1H), 9.02–9.06 (m, 1H), 11.66 (br s, 1H); MS (ESI) m/z 302 $[M+H]^+$.

5.1.6. Ethyl 4-{[(1*R*,2*S*)-2-methylcyclohexyl]amino}-1*H*-pyrrolo-[2,3-*b*]pyridine-5-carboxylate (5f)

Compound **5f** was prepared in 36% yield as a white solid by a method similar to that described for **5c**. ^{1}H NMR (DMSO-d_6) δ 0.91 (d, J = 6.9 Hz, 3H), 1.32 (t, J = 7.1 Hz, 3H), 1.35–2.16 (m, 9H), 4.23–4.34 (m, 3H), 6.59 (d, J = 3.5 Hz, 1H), 7.17 (d, J = 3.5 Hz, 1H), 8.68 (s, 1H), 9.02–9.06 (m, 1H), 11.66 (br s, 1H); MS (ESI) m/z 302 $[M+H]^+$.

5.1.7. Ethyl 4-[(3-methylcyclohexyl)amino]-1*H*-pyrrolo[2,3-*b*]-pyridine-5-carboxylate (5g)

Compound **5g** was prepared in 82% yield by a method similar to that described for **5c**. MS (ESI) m/z 302 $[M+H]^+$.

5.1.8. Ethyl 4-[(4-methylcyclohexyl)amino]-1*H*-pyrrolo[2,3-*b*]-pyridine-5-carboxylate (5h)

Compound **5h** was prepared in 44% yield by a method similar to that described for **5c**. ^{1}H NMR (DMSO-d_6) δ 0.88–0.96 (m, 3H), 1.11–1.27 (m, 2H), 1.28–1.35 (m, 4H), 1.38–1.64 (m, 2H), 1.67–1.86 (m, 3H), 2.06–2.14 (m, 1H), 3.82–4.37 (m, 3H), 6.52–6.59 (m, 1H), 7.15–7.21 (m, 1H), 8.51–8.59 (m, 1H), 8.73–9.14 (m, 1H), 11.66 (br s, 1H); MS (ESI) m/z 302 $[M+H]^+$.

5.1.9. Ethyl 4-[(2,2-dimethylcyclohexyl)amino]-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylate (5i)

Compound **5i** was prepared in 86% yield as a white solid by a method similar to that described for **5c**. MS (ESI) m/z 316 $[M+H]^+$.

5.1.10. Ethyl 4-{[(1*S*,2*R*)-2-ethylcyclohexyl]amino}-1*H*-pyrrolo [2,3-*b*]pyridine-5-carboxylate (5j)

Compound **5j** was prepared in 84% yield as a pale yellow powder by a method similar to that described for **5a**. ^{1}H NMR (DMSO-d_6) δ 0.81 (t, J = 7.1 Hz, 3H), 1.21–1.39 (m, 8H), 1.53–1.72 (m, 5H), 1.86–1.94 (m, 1H), 4.36 (q, J = 7.1 Hz, 2H), 4.47–4.51 (m, 1H), 6.84–6.86 (m, 1H), 7.34–7.36 (m, 1H), 8.61 (s, 1H), 9.67–9.72 (m, 1H), 12.44 (br s, 1H); MS (ESI) m/z 316 $[M+H]^+$.

5.1.11. Ethyl 4-(tetrahydro-2*H*-pyran-4-ylamino)-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylate (5k)

Compound **5k** was prepared in quantitative yield as a white solid by a method similar to that described for **5c**. ^{1}H NMR (DMSO-d_6) δ 1.32 (t, J = 7.2 Hz, 3H), 1.42–1.63 (m, 2H), 1.98–2.11 (m, 2H), 3.50–3.65 (m, 2H), 3.81–3.93 (m, 2H), 4.19–4.35 (m, 3H), 6.62 (d, J = 3.8 Hz, 1H), 7.21 (d, J = 3.8 Hz, 1H), 8.56 (s, 1H), 8.86 (d, J = 8.0 Hz, 1H), 11.72 (br s, 1H); MS (ESI) m/z 290 $[M+H]^+$.

5.1.12. 1-Cyclohexyl-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]-pyridin-2(1*H*)-one (6a)

To a solution of **5a** (150 mg, 0.52 mmol) in 1,4-dioxane (2.1 mL) and water (1.2 mL) was added $LiOH{\cdot}H_2O$ (88 mg, 2.1 mmol) and the mixture was refluxed for 3 h. After cooling to room temperature, to the reaction mixture was added 1 M HCl aqueous solution and the solids were collected by filtration and dried in vacuo to give the crude product (83 mg) as a white solid. To a mixture of the obtained crude product (77 mg, 0.30 mmol), Et_3N (75 μL, 0.53 mmol), 1,4-dioxane (1.5 mL) and DMF (0.39 mL) was added DPPA (96 μL, 0.45 mmol) and the mixture was stirred under a nitrogen atmosphere at 120 °C for 2 h. After cooling to room temperature, water and $CHCl_3$ were added, and the organic layer was separated. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 10:1) to give the product (30 mg, 24% from **5a**) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 1.26–1.54 (m, 3H), 1.65–1.96 (m, 5H), 2.14–2.31 (m, 2H), 4.31–4.43 (m, 1H), 6.60 (m, 1H), 7.45 (t, J = 3.0 Hz, 1H), 7.92 (s, 1H), 10.88 (br s, 1H), 11.60 (br s, 1H); MS (ESI) m/z 257 $[M+H]^+$; HRMS (ESI) m/z Calcd for $C_{14}H_{17}N_4O$ ($[M+H]^+$): 257.1402, Found: 257.1400.

5.1.13. 1-Cyclopentyl-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]-pyridin-2(1*H*)-one (6b)

Compound **6b** was prepared from **5b** in 35% yield as a white solid by a method similar to that described for **6a**. ^{1}H NMR (DMSO-d_6) δ 1.68–1.79 (m, 2H), 1.91–2.02 (m, 4H), 2.11–2.02 (m, 2H), 4.86–4.97 (m, 1H), 6.53 (dd, J = 1.9, 3.5 Hz, 1H), 7.42–7.45 (m, 1H), 7.92 (s, 1H), 10.89 (br s, 1H), 11.58 (br s, 1H); MS (ESI) m/z 243 $[M+H]^+$; HRMS (ESI) m/z Calcd for $C_{13}H_{15}N_4O$ ($[M+H]^+$): 243.1246, Found: 243.1243.

5.1.14. 1-Cycloheptyl-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]-pyridin-2(1*H*)-one (6c)

Compound **6c** was prepared from **5c** in 74% yield as a white solid by a method similar to that described for **6a**. ^{1}H NMR (DMSO-d_6) δ 1.52–2.37 (m, 12H), 4.47–4.61 (m 1H), 6.52–6.58 (m, 1H), 7.41–7.47 (m, 1H), 7.92 (s, 1H), 10.86 (br s, 1H), 11.58 (br s, 1H); MS (ESI) m/z 271 $[M+H]^+$; HRMS (ESI) m/z Calcd for $C_{15}H_{19}N_4O$ ($[M+H]^+$): 271.1559, Found: 271.1559.

5.1.15. 1-(Cyclohexylmethyl)-3,6-dihydroimidazo[4,5-*d*]pyrrolo [2,3-*b*]pyridin-2(1*H*)-one (6d)

Compound **6d** was prepared from **5d** in 53% yield as a white solid by a method similar to that described for **6a**. ^{1}H NMR (DMSO-d_6) δ 1.05–1.15 (m, 5H), 1.58–1.86 (m, 6H), 3.82 (d, J = 7.2 Hz, 2H), 6.51–6.53 (m, 1H), 7.42 (t, J = 2.9 Hz, 1H), 7.92 (s, 1H), 10.86 (br s, 1H), 11.56 (br s, 1H); MS (ESI) m/z 271 $[M+H]^+$; HRMS (ESI) m/z Calcd for $C_{15}H_{19}N_4O$ ($[M+H]^+$): 271.1559, Found: 271.1561.

5.1.16. 1-[(1*S*,2*R*)-2-Methylcyclohexyl]-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one (6e)

To a solution of **5e** (4.70 g, 15.6 mmol) in EtOH (38 mL) and THF (10 mL) was added 2 M NaOH aqueous solution (17 mL, 54.6 mmol) and the mixture was refluxed for 5 h. After cooling to room temperature, to the reaction mixture was 3 M HCl aqueous solution and the solids were collected by filtration and dried in vacuo to give the crude product (4.26 g) as a white solid. To a mixture of the obtained crude product (4.00 g, 14.6 mmol), Et_3N

(3.3 mL, 23.4 mmol) and 1,4-dioxane (40 mL) was added DPPA (3.8 mL, 17.6 mmol) and the mixture was stirred under a nitrogen atmosphere at 120 °C for 3 h. After cooling to room temperature, water and $CHCl_3$/MeOH = 4:1 solution were added, and the organic layer was separated. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was suspended in hot 2-propanol. After cooling to room temperature, the precipitate was filtered and washed with EtOAc to give the product (2.10 g, 53% from **5e**) as a white powder. ^{1}H NMR (DMSO-d_6) δ 0.94 (d, J = 7.1 Hz, 3H), 1.38–1.84 (m, 7H), 2.32 (m, 1H), 2.91–3.02 (m, 1H), 4.39–4.48 (m, 1H), 6.47–6.48 (m, 1H), 7.41–7.44 (m, 1H), 7.89 (s, 1H), 10.71 (br s, 1H), 11.56 (br s, 1H); MS (ESI) m/z 271 $[M+H]^+$; Anal. Calcd for $C_{15}H_{18}N_4O$: C, 66.64; H, 6.71; N, 20.73. Found: C, 66.48; H, 6.64; N, 20.68.

5.1.17. 1-[(1*R*,2*S*)-2-Methylcyclohexyl]-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one (6f)

Compound **6f** was prepared from **5f** in 90% yield as a yellow powder by a method similar to that described for **6e**. ^{1}H NMR (DMSO-d_6) δ 0.94 (d, J = 7.1 Hz, 3H), 1.38–1.84 (m, 7H), 2.32 (m, 1H), 2.91–3.02 (m, 1H), 4.39–4.48 (m, 1H), 6.47–6.48 (m, 1H), 7.41–7.44 (m, 1H), 7.89 (s, 1H), 10.71 (br s, 1H), 11.56 (br s, 1H); MS (ESI) m/z 271 $[M+Na]^+$; HRMS (ESI) m/z Calcd for $C_{15}H_{19}N_4O$ ($[M+H]^+$): 271.1559, Found: 271.1567.

5.1.18. 1-(3-Methylcyclohexyl)-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one (6g)

Compound **6g** was prepared from **5g** in 24% yield as a white solid by a method similar to that described for **6a**. ^{1}H NMR (DMSO-d_6) δ 0.96 (d, J = 6.4 Hz, 3/2H), 1.14 (d, J = 7.2 Hz, 3/2H), 1.44–2.54 (m, 9H), 4.37–4.66 (m, 1H), 6.55–6.58 (m, 1H), 7.44–7.46 (m, 1H), 7.91–7.92 (m, 1H), 10.88 (br s, 1H), 11.60 (br s, 1H); MS (ESI) m/z 271 $[M+H]^+$; HRMS (ESI) m/z Calcd for $C_{15}H_{19}N_4O$ ($[M+H]^+$): 271.1559, Found: 271.1563.

5.1.19. 1-(4-Methylcyclohexyl)-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one (6h)

Compound **6h** was prepared from **5h** in 50% yield as a white solid by a method similar to that described for **6a**. ^{1}H NMR (DMSO-d_6) δ 0.96 (d, J = 6.4 Hz, 3/2H), 1.12 (d, J = 7.2 Hz, 3/2H), 1.16–1.24 (m, 1H), 1.53–2.06 (m, 6H), 2.22–2.51 (m, 1H), 3.30–3.37 (m, 1H), 4.27–4.41 (m, 1H), 6.58–6.62 (m, 1H), 7.43–7.47 (m, 1H), 7.91–7.92 (m, 1H), 10.87–10.89 (m, 1H), 11.61 (br s, 1H); MS (ESI) m/z 271 $[M+H]^+$; HRMS (ESI) m/z Calcd for $C_{15}H_{19}N_4O$ ($[M+H]^+$): 271.1559, Found: 271.1563.

5.1.20. 1-(2,2-Dimethylcyclohexyl)-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one ethanedioate (1:1) (6i)

To a solution of **5i** (158 mg, 0.50 mmol) in 1,4-dioxane (4.0 mL) and water (2.0 mL) was added $LiOH{\cdot}H_2O$ (105 mg, 2.5 mmol) and the mixture was refluxed for 12 h. After cooling to room temperature, to the reaction mixture was added 1 M HCl aqueous solution. The reaction mixture was extracted with $CHCl_3$/MeOH = 10:1 solution, dried over $MgSO_4$ and evaporated in vacuo to give a crude product as a pale brown solid. To a mixture of the obtained crude product (144 mg, 0.50 mmol), Et_3N (139 μL, 1.0 mmol), 1,4-dioxane (3.0 mL) and DMI (3.0 mL) was added DPPA (162 μL, 0.75 mmol) and the mixture was stirred under a nitrogen atmosphere at 120 °C for 2 h. After cooling to room temperature, water and $CHCl_3$ were added, and the organic layer was separated. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by recrystallization from $CHCl_3$ and EtOAc to give a brown solid. The solid was dissolved in EtOH. To the solution was added oxalic acid (12 mg, 0.13 mmol) in EtOH and the mixture was stirred for 30 min at room temperature. The precipitate was filtered to give the product (37.2 mg, 20% from **5i**) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 0.84 (s, 3H), 1.09 (s, 3H), 1.31–1.93 (m, 7H), 2.82–3.08 (m, 1H), 4.08–4.22 (m, 1H), 6.64–6.70 (m, 1H), 7.37–7.43 (m, 1H), 7.89 (s, 1H), 10.77 (br s, 1H), 11.56 (br s, 1H); MS (ESI) m/z 285 $[M+H-C_2H_2O_2]^+$; HRMS (ESI) m/z calculated for $C_{16}H_{21}N_4O$ ($[M+H]^+$): 285.1715, Found: 285.1717.

5.1.21. 1-[(1*S*,2*R*)-2-Ethylcyclohexyl]-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one hydrochloride (1:1) (6j)

To a solution of **5j** (220 mg, 0.70 mmol) in EtOH (2.0 mL) was added 2 M NaOH (1.40 mL) and the mixture was stirred at 110 °C for 3 h. After cooling to 4 °C, to the reaction mixture was added 1 M HCl aqueous. The reaction mixture was extracted with $CHCl_3$/MeOH = 4:1 solution, dried over $MgSO_4$ and evaporated in vacuo to give a crude product (185 mg) as a white powder. To a solution of the obtained crude product (185 mg), Et_3N (0.27 mL, 1.9 mmol) and 1,4-dioxane (1.9 mL) was added DPPA (0.28 mL, 1.3 mmol) and the mixture was stirred under a nitrogen atmosphere at 120 °C for 2 h. The mixture was cooled to room temperature and the precipitate was filtrated and washed with small amount of 1,4-dioxane to give a crude product (75 mg) as a white solid. The solid was dissolved in 2-propanol (1.5 mL) and 1 M HCl aqueous (0.47 mL, 0.47 mmol). To the mixture was added EtOAc and the mixture was stirred. The precipitate was filtrated and washed with acetonitrile to give the product (68 mg, 30% from **5j**) as a white powder. ^{1}H NMR (DMSO-d_6) δ 0.71 (t, J = 7.4 Hz, 3H), 1.35–1.99 (m, 8H), 2.86–2.89 (m, 1H), 3.49 (m, 2H), 4.55–4.56 (m, 1H), 6.71–6.72 (m, 1H), 7.60–7.62 (m, 1H), 8.08 (s, 1H), 11.35 (br s, 1H), 12.26 (br s, 1H); MS (ESI) m/z 285 $[M+H]^+$; HRMS (ESI) m/z Calcd for $C_{16}H_{21}N_4O$ ($[M+H]^+$): 285.1715, Found: 285.1711.

5.1.22. 1-(Tetrahydro-2*H*-pyran-4-yl)-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one (6k)

Compound **6k** was prepared from **5k** in 21% yield as a white solid by a method similar to that described for **6a**. ^{1}H NMR (DMSO-d_6) δ 1.71 (dd, J = 4.4, 12.8 Hz, 2H), 2.44–2.55 (m, 2H), 3.51–3.59 (m, 2H), 4.04 (dd, J = 4.4, 11.6 Hz, 2H), 4.60–4.69 (m, 1H), 6.64 (dd, J = 2.0, 3.6 Hz, 1H), 7.46–7.48 (m, 1H), 7.94 (s, 1H), 10.94 (br s, 1H), 11.62 (br s, 1H); MS (ESI) m/z 259 $[M+H]^+$; HRMS (ESI) m/z Calcd for $C_{13}H_{15}N_4O_2$ ($[M+H]^+$): 259.1195, Found: 259.1200.

5.1.23. Ethyl 4-{[1-(*tert*-butoxycarbonyl)piperidin-4-yl]amino}-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylate (7a)

To a solution of **4** (500 mg, 2.23 mmol) in *n*-BuOH (2.0 mL) were added *tert*-butyl 4-amino-1-piperidinecarboxylate (1.34 g, 6.68 mmol). The mixture was heated in a microwave reactor at 150 °C for 1 h. After cooling to room temperature, to the reaction mixture was added water. The precipitate was filtered and washed with water to give the product (673 mg, 78%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6) δ 1.32 (t, J = 7.2 Hz, 3H), 1.41 (s, 9H), 1.37–1.43 (m, 2H), 2.00–2.05 (m, 2H), 3.32 (m, 2H), 3.79–3.83 (m, 2H), 4.23–4.29 (m, 3H), 6.63–6.64 (m, 1H), 7.20–7.21 (m, 1H), 8.56 (s, 1H), 8.83–8.85 (m, 1H), 11.72 (br s, 1H); MS (ESI) m/z 389 $[M+H]^+$.

5.1.24. Ethyl 4-{[(3*R*)-1-(*tert*-butoxycarbonyl)piperidin-3-yl]amino}-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylate (7b)

To a solution of **4** (400 mg, 1.78 mmol) in *n*-BuOH (2.0 mL) were added *tert*-butyl (3*R*)-3-aminopiperidine-1-carboxylate (712 mg, 3.56 mmol) and DIPEA (0.62 mL, 3.56 mmol). The mixture was heated in a microwave reactor at 150 °C for 1 h. After cooling to room temperature, the reaction mixture was quenched with water, extracted with EtOAc, dried over $MgSO_4$, and evaporated in vacuo. The crude mixture was purified by column chromatography on silica gel (hexane/AcOEt = 60:40 to 25:75) to give the product (525 mg, 76%) as a white powder. ^{1}H NMR (DMSO-d_6) δ 1.00–2.12 (m, 13H), 1.30 (t, J = 7.1 Hz, 3H), 3.17–3.79 (m, 4H), 4.07–4.34 (m, 1H), 4.26

(q, J = 7.1 Hz, 2H), 6.64 (d, J = 3.4 Hz, 1H), 7.21 (d, J = 3.4 Hz, 1H), 8.56 (s, 1H), 8.96 (d, J = 7.8 Hz, 1H), 11.71 (br s, 1H); MS (ESI) m/z 389 $[M+H]^+$.

5.1.25. Ethyl 4-{[(3*S*)-1-(*tert*-butoxycarbonyl)piperidin-3-yl]amino}-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylate (7c)

Compound **7c** was prepared from **4** in 32% yield as a white amorphous solid by a method similar to that described for **7b**. ^{1}H NMR (DMSO-d_6) δ 1.03 (s, 9H), 1.15–1.99 (m, 8H), 1.30 (t, J = 7.2 Hz, 3H), 3.58–3.62 (m, 1H), 4.23–4.27 (m, 2H), 6.62 (m, 1H), 7.20–7.22 (m, 1H), 8.56 (s, 1H), 8.95–8.97 (m, 1H), 11.70 (br s, 1H); MS (ESI) m/z 389 $[M+H]^+$.

5.1.26. *tert*-Butyl 4-(2-oxo-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-1(2*H*)-yl)piperidine-1-carboxylate (8a)

Compound **8a** was prepared from **7a** in 63% yield as a white powder by a method similar to that described for **6e**. ^{1}H NMR (DMSO-d_6) δ 1.47 (s, 9H), 1.74–1.77 (m, 2H), 2.25–2.33 (m, 2H), 2.98 (m, 2H), 4.11 (m, 2H), 4.59–4.66 (m, 1H), 6.45–6.46 (m, 1H), 7.40–7.42 (m, 1H), 7.94 (s, 1H), 10.95 (br s, 1H), 11.63 (br s, 1H); MS (ESI) m/z 358 $[M+H]^+$.

5.1.27. *tert*-Butyl (3*R*)-3-(2-oxo-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-1(2*H*)-yl)piperidine-1-carboxylate (8b)

Compound **8b** was prepared from **7b** in 68% yield as a white powder by a method similar to that described for **6a**. ^{1}H NMR (DMSO-d_6) δ 1.07–2.05 (m, 4H), 1.41 (s, 9H), 2.66–3.03 (m, 1H), 3.25–3.58 (m, 1H), 3.90–4.19 (m, 2H), 4.19–4.49 (m, 1H), 6.54–6.66 (m, 1H), 7.41–7.52 (m, 1H), 7.94 (s, 1H), 10.98 (br s, 1H), 11.65 (br s, 1H); MS (ESI) m/z 380 $[M+Na]^+$.

5.1.28. *tert*-Butyl (3*S*)-3-(2-oxo-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-1(2*H*)-yl)piperidine-1-carboxylate (8c)

Compound **8c** was prepared from **7c** in 66% yield as a white powder by a method similar to that described for **6e**. ^{1}H NMR (DMSO-d_6) δ 1.07–2.05 (m, 4H), 1.41 (s, 9H), 2.66–3.03 (m, 1H), 3.25–3.58 (m, 1H), 3.90–4.19 (m, 2H), 4.19–4.49 (m, 1H), 6.54–6.66 (m, 1H), 7.41–7.52 (m, 1H), 7.94 (s, 1H), 10.98 (br s, 1H), 11.65 (br s, 1H); MS (ESI) m/z 380 $[M+Na]^+$.

5.1.29. 1-(Piperidin-4-yl)-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one dihydrochloride (9a)

To a solution of **8a** (210 mg, 0.588 mmol) in 1,4-dioxane (2.1 mL) was added 4 M HCl in 1,4-dioxane (4.1 mL). The mixture was stirred at room temperature for 2 h. The reaction mixture was evaporated in vacuo to give the product (194 mg, quantitative yield) as a white powder. ^{1}H NMR (DMSO-d_6) δ 2.02–2.05 (m, 2H), 2.63–2.73 (m, 2H), 3.22–3.31 (m, 2H), 3.40–3.44 (m, 2H), 4.77–4.84 (m, 1H), 7.37–7.38 (m, 1H), 7.66–7.68 (m, 1H), 8.15 (s, 1H), 8.85 (br s, 1H), 9.40–9.43 (m, 1H), 11.75 (br s, 1H), 12.54 (br s, 1H); MS (ESI) m/z 258 $[M+H-2HCl]^+$; HRMS (ESI) m/z Calcd for $C_{13}H_{16}N_6O$ ($[M+H-2HCl]^+$): 258.1355, Found: 258.1351.

5.1.30. 1-[(3*R*)-Piperidin-3-yl]-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one dihydrochloride (9b)

Compound **9b** was prepared from **8b** in quantitative yield as a white powder by a method similar to that described for **9a**. ^{1}H NMR (DMSO-d_6) δ 1.83–2.22 (m, 3H), 2.31–2.63 (m, 1H), 2.77–4.50 (m, 4H), 4.94–5.19 (m, 1H), 7.21–7.33 (m, 1H), 7.61–7.72 (m, 1H), 8.15 (s, 1H), 9.s–9.37 (m, 1H), 9.76–10.06 (m, 1H), 11.74 (br s, 1H), 12.48 (br s, 1H); MS (ESI) m/z 258 $[M+H-2HCl]^+$.

5.1.31. 1-[(3*S*)-Piperidin-3-yl]-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one dihydrochloride (9c)

Compound **9c** was prepared from **8c** in quantitative yield as a white powder by a method similar to that described for **9a**. ^{1}H NMR (DMSO-d_6) δ 1.97–2.02 (m, 3H), 2.41–2.46 (m, 1H), 3.00 (m, 1H), 3.33–3.43 (m, 3H), 4.96 (m, 1H), 7.04–7.05 (m, 1H), 7.60–7.61 (m, 1H), 8.08 (s, 1H), 9.09 (m, 1H), 9.50 (br s, 1H), 11.47 (br s, 1H), 12.14 (br s, 1H); MS (ESI) m/z 258 $[M+H-2HCl]^+$.

5.1.32. 1-[1-(Methylsulfonyl)piperidin-4-yl]-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one (10a)

To a solution of **9a** (30 mg, 0.091 mmol) in DMI (1.0 mL) was added Et_3N (38 μL, 0.27 mmol). The mixture was stirred at room temperature for 15 min. The mixture was cooled under ice bath and methanesulfonyl chloride (7.7 μL, 0.10 mmol) was added. The mixture was stirred at the same temperature for 20 min and water was added. The precipitate was filtered and washed with water to give the product (22 mg, 72%) as a white powder. ^{1}H NMR (DMSO-d_6) δ 1.87–1.91 (m, 2H), 2.95 (s, 3H), 2.99–3.20 (m, 4H), 3.73–3.76 (m, 2H), 4.53–4.59 (m, 1H), 6.65–6.66 (m, 1H), 7.47–7.49 (m, 1H), 7.94 (s, 1H), 10.96 (br s, 1H), 11.63 (br s, 1H); MS (ESI) m/z 336 $[M+H]^+$; HRMS (ESI) m/z Calcd for $C_{14}H_{18}N_5O_3S$ ($[M+H]^+$): 336.1130, Found: 336.1129.

5.1.33. 1-[(3*R*)-1-(Methylsulfonyl)piperidin-3-yl]-3,6-dihydroimidazo [4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one (10b)

Compound **10b** was prepared from **9b** in 41% yield as a white solid by a method similar to that described for **10a**. ^{1}H NMR (DMSO-d_6) δ 1.69–1.85 (m, 1H), 1.90–2.03 (m, 2H), 2.39–2.56 (m, 1H), 2.86 (dd, J = 11.3, 11.3 Hz, 1H), 2.94 (s, 3H), 3.42 (dd, J = 11.3, 11.3 Hz, 1H), 3.61–3.77 (m, 2H), 4.46–4.59 (m, 1H), 6.59–6.64 (m, 1H), 7.44–7.50 (m, 1H), 7.94 (s, 1H), 11.00 (br s, 1H), 11.66 (br s, 1H); MS (ESI) m/z 336 $[M+H]^+$; HRMS (ESI) m/z Calcd for $C_{14}H_{18}N_5O_3S$ ($[M+H]^+$): 336.1130, Found: 336.1136.

5.1.34. 1-[(3*S*)-1-(Methylsulfonyl)piperidin-3-yl]-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one (10c)

Compound **10c** was prepared from **9c** in 43% yield as a white powder by a method similar to that described for **10a**. ^{1}H NMR (DMSO-d_6) δ 1.23 (m, 1H), 1.79 (m, 1H), 1.95–1.98 (m, 4H), 2.85–2.88 (m, 1H), 2.94 (s, 3H), 3.65–3.73 (m, 1H), 4.50–4.54 (m, 1H), 6.55–6.67 (m, 1H), 7.49–7.51 (m, 1H), 7.97 (s, 1H), 11.12 (br s, 1H), 11.78 (br s, 1H); MS (ESI) m/z 336 $[M+H]^+$; HRMS (ESI) m/z Calcd for $C_{14}H_{18}N_5O_3S$ ($[M+H]^+$): 336.1130, Found: 336.1135.

5.1.35. 3-Oxo-3-[(3*R*)-3-(2-oxo-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-1(2*H*)-yl)piperidin-1-yl]propanenitrile (11)

A mixture of **9b** (38 mg, 0.12 mmol), cyanoacetic acid (13 mg, 0.15 mmol), HOBt (23 mg, 0.17 mmol), DIPEA (44 μL, 0.25 mmol), and WSCD·HCl (33 mg, 0.17 mmol) in DMF (1.4 mL) was stirred at room temperature for 1 h and then stirred at 50 °C for 1 h. The mixture was diluted with AcOEt, washed with saturated $NaHCO_3$ aqueous solution and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 15:1 to 10:1) to give the product (2.3 mg, 6%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.15–3.91 (m, 7H), 4.02–4.11 (m, 2H), 4.27–4.58 (m, 2H), 6.60–6.65 and 6.74–6.80 (each m, total 1H), 7.42–7.49 (m, 1H), 7.93 and 7.94 (each s, total 1H), 10.99 (br s, 1H), 11.61 and 11.65 (each br s, total 1H); MS (ESI) m/z 325 $[M+H]^+$; HRMS (ESI) m/z Calcd for $C_{16}H_{17}N_6O_2$ ($[M+H]^+$): 325.1413, Found: 325.1413.

5.1.36. 1-Benzyl-3-[(*tert*-butoxycarbonyl)amino]-4-methylpyridinium bromide (13)

To a solution of *tert*-butyl (4-methylpyridin-3-yl)carbamate (**12**) (12.0 g, 57.62 mmol) in acetone (480 mL) was added benzyl bromide (6.98 mL, 58.77 mmol), followed by refluxing for 3 h. After cooling to room temperature, the resulting solid was collected by filtration and washed with acetone (120 mL) to obtain the product (21.0 g, 96%) as a pale yellow solid. ^{1}H NMR (DMSO-

d_6) δ 1.50 (s, 9H), 2.50 (s, 3H), 5.84 (s, 2H), 7.40–7.52 (m, 5H), 8.00 (dd, J = 6.3 Hz, 1H), 8.23 (dd, J = 6.3, 1.4 Hz, 1H), 9.30 (d, J = 1.1 Hz, 1H), 9.57 (s, 1H); MS (ESI) m/z 299 $[M-Br]^+$.

5.1.37. *tert*-Butyl (±)-*cis*-[1-benzyl-4-methylpiperidin-3-yl] carbamate (14)

To a solution of **13** (1.20 g, 3.16 mmol) in EtOH (48 mL) was added PtO_2 (Adam's catalyst) (36 mg, 0.16 mmol), followed by catalytic reduction at 40 °C under 3 atm of hydrogen gas for 4 h. The reaction mixture was separated by filtration through a Celite pad and the filtrate was concentrated under reduced pressure. The obtained residue was neutralized with a saturated $NaHCO_3$ aqueous solution, and the mixture was then extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and then filtered and concentrated in vacuo. The obtained residue was purified by column chromatography on silica gel silica gel (hexane/EtOAc = 90:10 to 87:13) to obtain the product (664 mg, 69%) as a colorless oil. ^{1}H NMR (DMSO-d_6) δ 0.80 (d, J = 6.7 Hz, 3H), 1.30–1.36 (m, 1H), 1.37 (s, 9H), 1.42–1.55 (m, 1H), 1.56–1.68 (m, 1H), 1.92–2.10 (m, 2H), 2.53–2.70 (m, 2H), 3.38–3.49 (m, 2H), 3.51–3.59 (m, 1H), 6.12–6.23 (m, 1H), 7.20–7.26 (m, 1H), 7.27–7.33 (m, 4H); MS (ESI) m/z 305 $[M+H]^+$.

5.1.38. (±)-*cis*-1-Benzyl-4-methylpiperidin-3-amine (15)

To a solution of **14** (2.00 g, 6.57 mmol) in CH_2Cl_2 (5.0 mL) was added TFA (5.0 mL) and the mixture was stirred at the same temperature for 30 min. The mixture was evaporated in vacuo and the residue was quenched with saturated $NaHCO_3$ aqueous solution, extracted with $CHCl_3$, dried over Na_2SO_4, and evaporated in vacuo to give the product (1.31 g, 98%). ^{1}H NMR (DMSO-d_6) δ 0.85 (d, J = 6.8 Hz, 3H), 1.29–1.47 (m, 2H), 1.47–1.58 (m, 1H), 1.91–2.01 (m, 1H), 2.05–2.14 (m, 1H), 2.43–2.53 (m, 1H), 2.54–2.66 (m, 2H), 2.66–2.71 (m, 1H), 3.33 (br s, 2H), 3.37–3.47 (m, 1H), 7.20–7.26 (m, 1H), 7.27–7.34 (m, 4H); MS (ESI) m/z 205 $[M+H]^+$.

5.1.39. (±)-*cis*-Ethyl 4-{[1-benzyl-4-methylpiperidin-3-yl]amino}-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylate (16)

Compound **16** was prepared from **4** in 95% yield as a white solid by a method similar to that described for **5c**. ^{1}H NMR (DMSO-d_6) δ 0.87 (d, J = 6.8 Hz, 3H), 1.37 (t, J = 7.2 Hz, 3H), 1.52–4.43 (m, 12H), 6.62 (dd, J = 2.0, 3.6 Hz, 1H), 7.09 (dd, J = 2.8, 3.6 Hz, 1H), 7.13–7.35 (m, 5H), 8.56 (s, 1H), 9.33 (d, J = 9.6 Hz, 1H), 11.59 (br s, 1H); MS (ESI) m/z 393 $[M+H]^+$.

5.1.40. (±)-*cis*-1-[1-Benzyl-4-methylpiperidin-3-yl]-3,6-dihydroimidazo [4,5-*d*]pyrrolo[2,3-b]pyridin-2(1*H*)-one (17)

Compound **17** was prepared from **16** in 57% yield as a white solid by a method similar to that described for **6a**. ^{1}H NMR (DMSO-d_6) δ 0.94 (d, J = 7.1 Hz, 3H), 1.58–1.66 (m, 1H), 2.01–2.13 (m, 1H), 2.25–2.35 (m, 1H), 2.36–2.44 (m, 1H), 2.62–2.70 (m, 1H), 2.83–2.91 (m, 1H), 3.48–3.68 (m, 3H), 4.54–4.61 (m, 1H), 6.43–6.47 (m, 1H), 7.19–7.26 (m, 1H), 7.28–7.37 (m, 4H), 7.42–7.47 (m, 1H), 7.58 (s, 1H), 10.77 (br s, 1H), 11.60 (br s, 1H); MS (ESI) m/z 362 $[M+H]^+$.

5.1.41. (±)-*cis*-1-[4-Methylpiperidin-3-yl]-3,6-dihydroimidazo [4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one (18)

To a solution of **17** (8.10 g, 22.4 mmol) in EtOH (486 mL) was added 20% $Pd(OH)_2$ on carbon (6.29 g, 4.48 mmol) at room temperature under nitrogen. Hydrogen gas was purged and stirred for 2 days at 30 °C under 4 atm. The mixture was filtered through a Celite pad. The filtrate was evaporated in vacuo to give the crude product (6.00 g, 99%). The crude residue was used without further purification. ^{1}H NMR (DMSO-d_6) δ 0.85 (d, J = 7.0 Hz, 3H), 1.56–1.74 (m, 1H), 1.78–1.89 (m, 1H), 2.31–2.43 (m, 1H), 2.82–2.92 (m, 1H), 3.07–3.17 (m, 1H), 3.20–3.27 (m, 1H), 3.40–3.49 (m, 1H), 4.32–4.39 (m, 1H), 4.68–4.78 (m, 1H), 6.58–6.64 (m, 1H), 7.46–7.49 (m, 1H), 7.94 (s, 1H), 10.78 (br s, 1H), 11.65 (br s, 1H); MS (ESI) m/z 272 $[M+H]^+$.

5.1.42. (±)-*cis*-3-[4-Methyl-3-(2-oxo-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-1(2*H*)-yl)piperidin-1-yl]-3-oxopropanenitrile (19)

Compound **19** was prepared from **18** in 66% yield as a white solid by a method similar to that described for **11**. ^{1}H NMR (DMSO-d_6) δ 0.96 (d, J = 7.2 Hz, 3/2H), 0.97 (d, J = 7.2 Hz, 3/2H), 1.63–4.65 (m, 10H), 6.51–6.55 (m, 1H), 7.43 (dd, J = 2.4, 3.6 Hz, 1/2H), 7.46 (dd, J = 2.4, 3.6 Hz, 1/2H), 7.906 (s, 1/2H), 7.911 (s, 1/2H), 10.86 (br s, 1H), 11.57 (br s, 1/2H), 11.62 (br s, 1/2H); MS (ESI) m/z 339 $[M+H]^+$; Anal. Calcd for $C_{17}H_{18}N_6O_2 \cdot 0.1H_2O$: C, 60.62; H, 5.39; N, 24.71. Found: C, 59.64; H, 5.27; N, 24.33.

5.1.43. Ethyl 4-chloro-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylate (20)

To a solution of **4** (2.00 g, 8.90 mmol) in DMF (30 mL) was added NaH (60% dispersion in mineral oil, 427 mg, 10.7 mmol) at 0 °C and the mixture was stirred at the same temperature for 1 h. To the solution was added SEMCl (1.72 mL, 9.79 mmol) and the resulting solution was allowed to stir for 30 min at room temperature. The mixture was quenched with saturated $NaHCO_3$ aqueous solution, extracted with EtOAc, washed with brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography on silica gel (hexane/AcOEt = 100:0 to 90:10) to give the product (3.07 g, 97%) as colorless oil. ^{1}H NMR (DMSO-d_6) δ −0.10 (s, 9H), 0.82 (t, J = 8.1 Hz, 2H), 1.36 (t, J = 7.2 Hz, 3H), 3.52 (t, J = 8.0 Hz, 2H), 4.36 (q, J = 7.0 Hz, 2H), 5.67 (s, 2H), 6.75 (d, J = 3.7 Hz, 1H), 7.82 (d, J = 3.6 Hz, 1H), 8.75 (s, 1H); MS (ESI) m/z 355 $[M+H]^+$.

5.1.44. Ethyl (±)-*cis*-4-{[1-benzyl-4-methylpiperidin-3-yl] amino}-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylate (21)

A mixture of **20** (1.05 g, 2.96 mmol), **15** (725 mg, 3.55 mmol), and DIPEA (1.55 mL, 8.88 mmol) in NMP (3.0 mL) was heated at 180 °C for 2 h. After cooling to room temperature, the reaction mixture was quenched with water, extracted with EtOAc, washed with bine, dried over Na_2SO_4, and evaporated in vacuo. The crude mixture was purified by column chromatography on silica gel (hexane/AcOEt = 95:5 to 85:15) to give the product (1.51 g, 98%). ^{1}H NMR (DMSO-d_6) δ −0.11 (s, 9H), 0.79 (t, J = 8.2 Hz, 2H), 0.86 (d, J = 6.7 Hz, 3H), 1.37 (t, J = 7.0 Hz, 3H), 1.50–1.63 (m, 2H), 1.81–1.94 (m, 1H), 2.08–2.17 (m, 1H), 2.23–2.31 (m, 1H), 2.72–2.88 (m, 2H), 3.38–3.60 (m, 4H), 4.28–4.45 (m, 3H), 5.51 (s, 2H), 6.72 (d, J = 3.9 Hz, 1H), 7.11–7.23 (m, 3H), 7.28 (d, J = 3.6 Hz, 1H), 7.30–7.35 (m, 2H), 8.61 (s, 1H), 9.36 (d, J = 9.9 Hz, 1H); MS (ESI) m/z 523 $[M+H]^+$.

5.1.45. (±)-*cis*-1-[1-Benzyl-4-methylpiperidin-3-yl]-6-{[2-(trimethylsilyl)ethoxy]methyl}-3,6-dihydroimidazo[4,5-*d*]pyrrolo [2,3-*b*]pyridin-2(1*H*)-one (22)

Compound **22** was prepared from **21** in 89% yield by a method similar to that described for **6e**. ^{1}H NMR (DMSO-d_6) δ −0.10 (s, 9H), 0.86 (t, J = 8.0 Hz, 2H), 0.93 (d, J = 7.0 Hz, 3H), 1.57–1.65 (m, 1H), 2.02–2.15 (m, 1H), 2.25–2.43 (m, 2H), 2.61–2.69 (m, 1H), 2.84–2.93 (m, 1H), 3.47–3.55 (m, 3H), 3.59–3.64 (m, 2H), 4.54–4.61 (m, 1H), 5.60 (s, 2H), 6.55 (d, J = 3.7 Hz, 1H), 7.19–7.26 (m, 1H), 7.28–7.37 (m, 4H), 7.63 (d, J = 3.7 Hz, 1H), 7.94 (s, 1H), 10.86 (br s, 1H); MS (ESI) m/z 492 $[M+H]^+$.

5.1.46. (±)-*cis*-1-[1-Benzyl-4-methylpiperidin-3-yl]-3-methyl-6-{[2-(trimethylsilyl)ethoxy]methyl}-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one (23)

To a solution of **22** (262 mg, 0.53 mmol) in DMF (5.0 mL) was added NaH (60% dispersion in mineral oil, 26 mg, 0.64 mmol) at 0 °C and the mixture was stirred at the same temperature for 5 min. To the solution was added MeI (40 μL, 0.64 mmol) and the resulting solution was allowed to stir for 15 min at room temperature. The mixture was quenched with water, extracted with CH_2Cl_2, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography on amino functionalized silica gel ($CHCl_3$ = 100:0) to give the product (260 mg, 97%). ^{1}H NMR (DMSO-d_6) δ −0.09 (s, 9H), 0.81 (t, J = 8.0 Hz, 2H), 0.92 (d, J = 7.2 Hz, 3H), 1.58–1.67 (m, 1H), 2.04–2.15 (m, 1H), 2.26–2.44 (m, 2H), 2.62–2.74 (m, 1H), 2.83–2.91 (m, 1H), 3.35 (s, 3H), 3.48–3.68 (m, 5H), 4.57–4.64 (m, 1H), 5.62 (s, 2H), 6.57 (d, J = 3.7 Hz, 1H), 7.19–7.25 (m, 1H), 7.27–7.36 (m, 4H), 7.66 (d, J = 3.7 Hz, 1H), 8.12 (s, 1H); MS (ESI) m/z 506 $[M+H]^+$.

5.1.47. (±)-*cis*-1-[1-Benzyl-4-methylpiperidin-3-yl]-3-methyl-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one (24)

To a solution of **23** (260 mg, 0.51 mmol) in CH_2Cl_2 (2.0 mL) was added TFA (2.0 mL) and the mixture was stirred at room temperature for 2 h. The mixture was evaporated in vacuo and the residue was quenched with saturated $NaHCO_3$ aqueous solution, extracted with $CHCl_3$ and MeOH, dried over Na_2SO_4, and evaporated in vacuo. To the resulting residue in CH_2Cl_2 (2.0 mL) and MeOH (2.0 mL) was added ethylenediamine (69 μL, 1.03 mmol) and the mixture was stirred at 50 °C for 1 h. The mixture was quenched with water, extracted with $CHCl_3$ and MeOH, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography on amino functionalized silica gel ($CHCl_3$/MeOH = 100:0 to 95:5) to give the product (180 mg, 93%). ^{1}H NMR (DMSO-d_6) δ 0.92 (d, J = 7.1 Hz, 3H), 1.58–1.67 (m, 1H), 2.06–2.14 (m, 1H), 2.26–2.44 (m, 2H), 2.62–2.71 (m, 1H), 2.82–2.91 (m, 1H), 3.34 (s, 3H), 3.48–3.69 (m, 3H), 4.57–4.65 (m, 1H), 6.47 (d, J = 3.5 Hz, 1H), 7.19–7.25 (m, 1H), 7.27–7.37 (m, 4H), 7.46–7.50 (m, 1H), 8.07 (m, 1H), 11.65 (br s, 1H); MS (ESI) m/z 376 $[M+H]^+$.

5.1.48. (±)-*cis*-3-Methyl-1-[4-methylpiperidin-3-yl]-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one (25)

To a solution of **24** (180 mg, 0.48 mmol) in MeOH (5.0 mL) were added 20% $Pd(OH)_2$ on carbon (34 mg, 0.048 mmol) and ammonium formate (302 mg, 4.8 mmol) at room temperature. The mixture was refluxed for 30 min. After cooling to room temperature, the mixture was filtered through a Celite pad. The filtrate was partitioned between saturated $NaHCO_3$ aqueous solution and $CHCl_3$. The organic layer was dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography on amino functionalized silica gel ($CHCl_3$/MeOH = 100:0 to 95:5) to give the product (85 mg, 62%). ^{1}H NMR (DMSO-d_6) δ 0.89 (d, J = 7.0 Hz, 3H), 1.53–1.63 (m, 1H), 1.76–1.86 (m, 1H), 2.31–2.47 (m, 2H), 2.61–2.71 (m, 1H), 2.76–2.87 (m, 1H), 2.94–3.02 (m, 1H), 3.38 (s, 3H), 3.77–3.89 (m, 1H), 4.51–4.59 (m, 1H), 6.54–6.58 (m, 1H), 7.45–7.49 (m, 1H), 8.70 (s, 1H), 11.63 (br s, 1H); MS (ESI) m/z 286 $[M+H]^+$.

5.1.49. (±)-*cis*-3-[4-Methyl-3-(3-methyl-2-oxo-3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-1(2*H*)-yl)piperidin-1-yl]-3-oxopropanenitrile (26)

A mixture of **25** (80 mg, 0.28 mmol), cyanoacetic acid (36 mg, 0.42 mmol), HOBt (45 mg, 0.34 mmol), WSCD·HCl (64 mg, 0.34 mmol) and DIPEA (98 μl, 0.56 mmol) in DMF (1.5 mL) and CH_2Cl_2 (1.5 mL) was stirred at room temperature for 1.5 h. The reaction mixture was evaporated in vacuo and the residue was purified by column chromatography on amino functionalized silica gel ($CHCl_3$/MeOH = 100:0 to 95:5) to give the product (75 mg, 76%) as a white powder. ^{1}H NMR (DMSO-d_6) δ 0.95 (d, J = 7.1 Hz, 3/2H), 0.96 (d, J = 7.1 Hz, 3/2H), 1.62–1.76 (m, 1H), 1.85–1.96 (m, 1/2H), 2.01–2.13 (m, 1/2H), 2.93–3.11 (m, 1H), 3.14–3.19 (m, 1H), 3.378 (s, 3/2H), 3.381 (s, 3/2H), 3.47–3.56 (m, 1/2H), 3.80–3.89 (m, 1/2H), 3.95–4.26 (m, 3H), 4.34–4.41 (m, 1/2H), 4.43–4.49 (m, 1/2H), 4.52–4.60 (m, 1/2H), 4.63–4.69 (m, 1/2H), 6.53–6.58 (m, 1H), 7.44–7.57 (m, 1H), 8.10 (s, 1H), 11.63 (br s, 1/2H), 11.67 (br s, 1/2H); MS (ESI) m/z 353 $[M+H]^+$; HRMS (ESI) m/z Calcd for $C_{18}H_{21}N_6O_2$ ($[M+H]^+$): 353.1726, Found: 353.1732.

5.2. Biology

5.2.1. JAKs kinase assay

Human JAK1, JAK2, and JAK3 kinase-domains were purchased from Carna Biosciences, Inc. (Kobe, Japan) and assays performed using streptavidin-coated 96-well plates. Reaction mixture contained 15 mM Tris–HCl (pH 7.5), 0.01% Tween 20, 2 mM DTT, 10 mM $MgCl_2$, 250 nM Biotin-Lyn-Substrate-2 (for JAK1, 2 and 3, Peptide Institute, Inc., Osaka, Japan), and ATP at final concentrations of 200 μM for JAK1, 10 μM for JAK2, and 8 μM for JAK3. Test compound was dissolved in DMSO and the reaction was initiated by adding the kinase domain, followed by incubation at room temperature for 1 h. Kinase activity was measured as the rate of phosphorylation of Biotin-Lyn-Substrate-2 using an HRP-conjugated anti-phosphotyrosine antibody (HRP-PY-20; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and a phosphotyrosine-specific ELISA. Experiments were performed in duplicate for test compounds except for **1**, and the IC_{50} value of each experiment was calculated using linear regression analysis. The assay of **1** was performed in four experiments, and the IC_{50} value was calculated using Sigmoid-Emax non-linear regression analysis.

5.2.2. Rat T-cell proliferation

Splenocytes from male Lewis rats were suspended in RPMI1640 (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum and 50 μM 2-mercaptoethanol at a density of 1.5×10^6 cells/mL. Splenocytes were cultured with Concanavalin A (Sigma) for 24 h at 37 °C to induce IL-2 receptor expression and then incubated with IL-2 (BD Biosciences, San Diego, CA, USA) and test compounds at designated concentrations in 96-well tissue culture plates. After a 3-day incubation, alamarBlue® (Life Technologies, Carlsbad, CA, USA) was added to each test well, followed by a 6-h incubation. Fluorescence intensity was measured at an excitation wavelength of 545 nm and an emission wavelength of 590 nm. Experiments were performed in duplicate for test compounds except for **1**, and the IC_{50} value of each experiment was calculated using linear regression analysis. The assay of **1** was performed in four experiments, and the IC_{50} value was calculated using Sigmoid-Emax non-linear regression analysis.

5.2.3. In vitro liver microsomal stability

To estimate stability against rat hepatic CYPs, test compound (0.2 μM) was incubated with male SD rat liver microsomes (0.2 mg protein/mL) with NADPH (1 mM) and EDTA (0.1 mM) in phosphate buffer (100 mM) at 37 °C. Incubations were conducted for 0 and 30 min. The percentage of compound remaining was determined by LCMS analysis.

5.2.4. CYP3A4 reversible and time-dependent inhibition assays

For the CYP3A4 inhibition assay, midazolam was used as a probe substrate to monitor changes in CYP3A4 activity during exposure to each test compound. Reaction mixtures containing 0.1 mg protein/mL human liver microsomes (HLM), 1 mM NADPH, 0.1 mM EDTA, 100 mM Na^+–K^+ phosphate buffer (pH 7.4) and 5 μM test compounds were prepared and pre-incubated for 0 or 30 min at 37 °C. Reactions were initiated by the addition of

2 μM of midazolam and incubated for 20 min and then terminated by addition of 80% acetonitrile with internal standard. The concentration of 1′-hydroxymidazolam was determined by LC–MS/MS. Residual metabolic activities for reversible (Eq. 1) and time-dependent (Eq. 2) inhibition were calculated using the following equations:

$$\%\ \text{Remaining activity} = A_{c,\,0}/A_{v,\,0} \times 100 \quad (1)$$

$$\%\ \text{Remaining activity} = (A_{c,\,30}/A_{v,\,30})/(A_{c,\,0}/A_{v,\,0}) \times 100 \quad (2)$$

where, $A_{c,\,0}$ denotes activity obtained in the presence of compound and without pre-incubation, $A_{v,\,0}$ denotes activity obtained in the absence of compound and without pre-incubation, $A_{c,\,30}$ denotes activity obtained in the presence of compound and with pre-incubation and $A_{v,\,30}$ denotes activity obtained in the absence of compound and with pre-incubation.

5.3. Molecular modeling

5.3.1. Docking study

Docking calculation was done on the crystal structure of tofacitinib (**1**) bound to JAK3 (PDB code: 3LXK[10]). The protein–ligand complex was prepared with the Protein Preparation Wizard in Maestro (version 9.3, Schrödinger, LLC, New York, NY, 2012.), impref applying the appropriate side-chain protonation states, refine, and structure minimization. Docking grids were generated and defined based on the centroid of tofacitinib in the ATP binding site incorporating hydrogen-bond constraints to the hinge and hydrophobic regions. Ligands were prepared using LigPrep (version 2.5, Schrödinger, LLC, New York, NY, 2012) and ConfGen (version 2.3, Schrödinger, LLC, New York, NY, 2012), energy-minimized conformation of each ligands were used to docking calculation input molecules. Ligand receptor docking was conducted using XP mode in Glide (version 5.8, Schrödinger, LLC, New York, NY, 2012). The top-scoring pose assessed by GlideScore was employed for discussions.

5.3.2. WaterMap

WaterMap (version 1.4, Schrödinger, LLC, New York, NY, 2012) calculation was done on the crystal structure of tofacitinib bound to JAK3 (PDB code: 3LXK), the structure preparation method is as described above. WaterMap was run in the default mode using tofacitinib structure to define the binding site but removed in the MD simulation. ΔG_{pred} of binding and ligand strain energies were calculated using the ab initio form of the displaced-solvent functional as described by Abel et al.[17]

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