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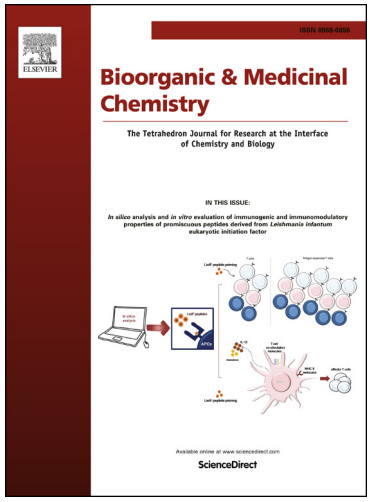

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Structure-based Design, Synthesis, and Biological Evaluation of Imidazo[1,2-*b*]pyridazine-based p38 MAP Kinase Inhibitors

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KEYWORDS: p38 mitogen-activated protein kinase inhibitors, rheumatoid arthritis, structure-based design, imidazo[1,2-*b*]pyridazine derivatives, pyridine *N*-oxide

ABSTRACT

We identified novel potent inhibitors of p38 MAP kinase using structure-based design strategy. X-ray crystallography showed that when p38 MAP kinase is complexed with **TAK-715** (**1**) in a co-crystal structure, Phe169 adopts two conformations, where one interacts with **1** and the other shows no interaction with **1**. Our structure-based design strategy shows that these two conformations converge into one via enhanced protein-ligand hydrophobic interactions. According to the strategy, we focused

on scaffold transformation to identify imidazo[1,2-*b*]pyridazine derivatives as potent inhibitors of p38 MAP kinase. Among the herein described and evaluated compounds, *N*-oxide **16** exhibited potent inhibition of p38 MAP kinase and LPS-induced TNF-α production in human monocytic THP-1 cells, and significant *in vivo* efficacy in rat collagen-induced arthritis models. In this article, we report the discovery of potent, selective and orally bioavailable imidazo[1,2-*b*]pyridazine-based p38 MAP kinase inhibitors with pyridine *N*-oxide group.

1. Introduction

The p38 mitogen-activated protein (MAP) kinase, a serine/threonine kinase, is widely expressed in endothelial, immune, and inflammatory cells; it is a key component in the cascade leading to the activation of pro-inflammatory cytokines, such as TNF-α and IL-1β, in immune cells.[1-4] The p38 MAP kinase is activated by inflammatory cytokines or extracellular stressors such as heat, mechanical wear, osmotic shock, and ultraviolet light.[5] Recent analysis of synovial tissue, extracted from patients with rheumatoid arthritis (RA), suggests that the p38 MAP kinase is overproduced within the inflamed tissue.[6-7] Inhibition of p38 MAP kinase is, therefore, a promising therapeutic strategy for the treatment of cytokine-driven disorders such as RA, inflammatory bowel disease (IBD), and chronic obstructive pulmonary disease (COPD).[8-9, 5]

Whereas many p38 MAP kinase inhibitors have proceeded to clinical studies, there has been limited progress in the clinical development of these compounds.[10-11] However, this remains an area of intense interest,[12-18] and many compounds are in clinical development.

The p38 MAP kinase inhibitors reported to date can be divided into three types based on the chemical structure and interaction with p38 MAP kinase (Figure 1).[19] All three types occupy the ATP-binding site. Both teardrop binder such as **SB203580** and linear binder such as **Scio-469** and **VX-745** shows ATP competitiveness.[20-24] Another type, extended binder such as **BIRB-796**[25], shows ATP non-

competitiveness due to induction of DFG-out conformation by binding to allosteric site of the ATP pocket.[25]

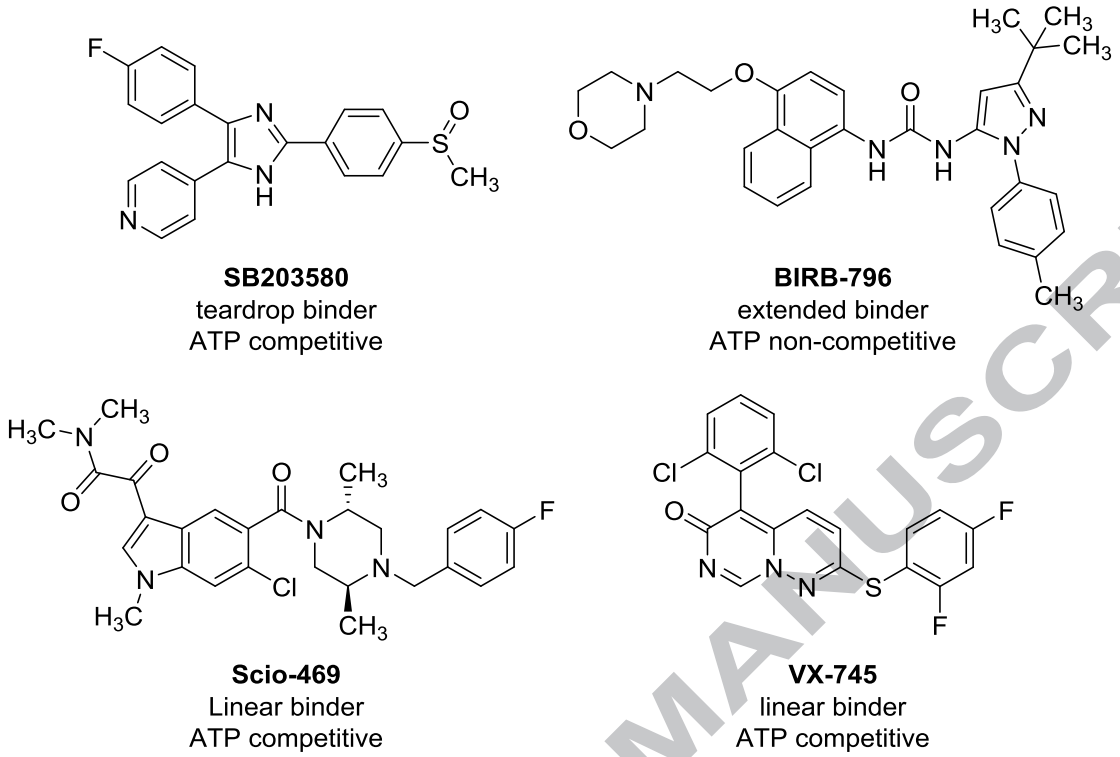
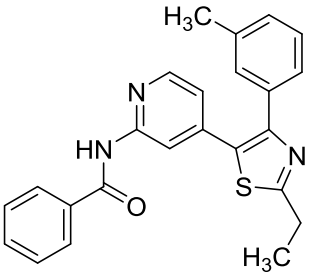


Fig. 1. Classification of representative p38 MAP kinse inhibitors.

We previously reported that the pyridinylthiazole derivatives, such as ***N*-{4-[2-ethyl-4-(3-methylphenyl)-1,3-thiazol-5-yl]-2-pyridyl}benzamide** (**1**, **TAK-715**), are potent inhibitors of p38 MAP kinase, which was classified in teardrop binder (Figure 2).[26] In our further effort toward discovering alternative classes of p38 MAP kinase inhibitors that could be developed as backups to **1**, we investigated novel core structures instead of the thiazole ring of **1**. In this report, we describe rational design using structure-based design, synthesis, SAR, and biological assessment of the derivatives of imidazo[1,2-*b*]pyridazine.



1 (TAK-715)
teardrop binder
ATP competitive

Fig. 2. Chemical Structure of **1**

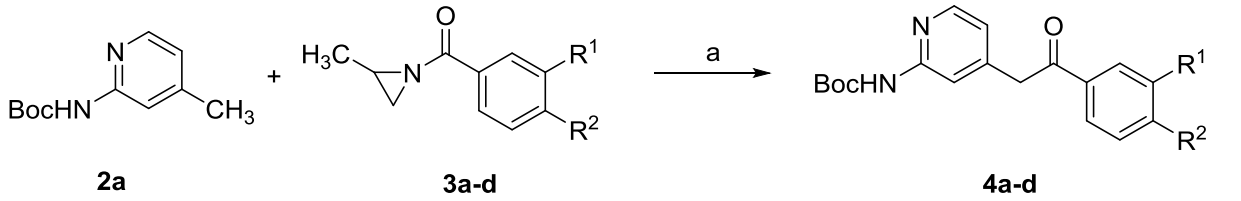
2. Chemistry

The synthesis of imidazo[1,2-*b*]pyridazine analogs **8a-o** and **10a-b** followed a general route as outlined in scheme 1. To the lithium salt of **2a-b** in THF were added the appropriate propylenimines **3a-d** to provide the corresponding ketones **4a-d** and **4f**.[26-27] The Weinreb amide **3e**, which was used instead of the propyleneimines **3a-d**, also led to the formation of ketone **4e**. Bromination of **4a-f** using bromine in acetic acid afforded α-bromoketones **5a-f,** which were treated with 3-aminopyridazine in EtOH to give no desired product. It is likely that α-bromoketone alkylated the N1 of 3-aminopyridazine because of the relatively high nucleophilicity of the N1 of 3-aminopyridazine. To decrease the nucleophilicity of the N1, we used 3-amino-6-chloropyridazine instead of 3-aminopyridazine, successfully achieving the cyclization to imidazo[1,2-*b*]pyridazines **6a-f**. De-chlorination of **6a-f** under hydrogenesis condition led to the key intermediates **7a-f**. Importantly, Et_3N was necessary for HCl scavenging, even though its presence diminished the catalytic ability of Pd/C. The subsequent amidation with the appropriate acyl chloride produced **8a-o** and **9a-b**. Compounds **10a-b** were prepared by de-chlorination of **9a-b** under the hydrogenesis condition.

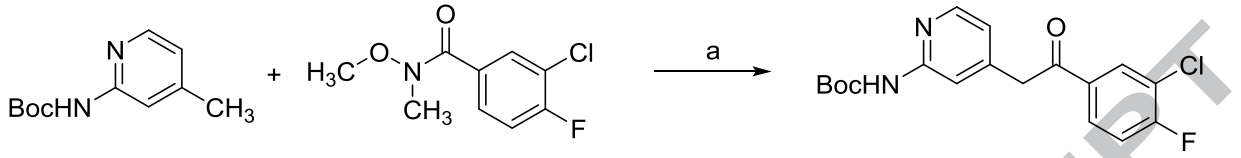
N-Oxides **15a-b** were synthesized as shown in scheme 2. Reductive de-chlorination of **11** generated **12a**. Oxidation of **12a-b** led to **13a-b,** followed by hydrolysis to give carboxylic acids **14a-b**. Condensation between **14a-b** and **7d** afforded *N*-oxides **15a-b**.

As shown in scheme 3, sulfate **16** was prepared using treatment with one equivalent of sulfonic acid in acetic acid and ethanol. Acetic acid as a solvent was the most effective at dissolving **15a** in a small volume of solvent.

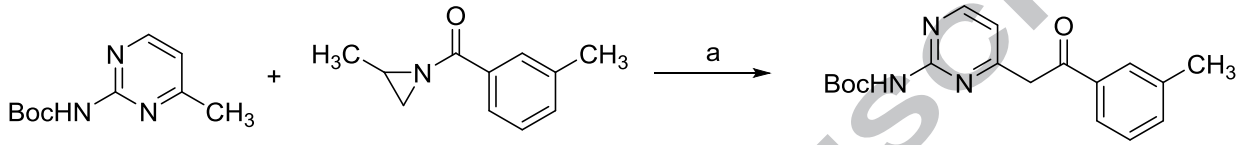
Scheme 1. Synthesis of compounds **8a-o** and **10a-b**[a]



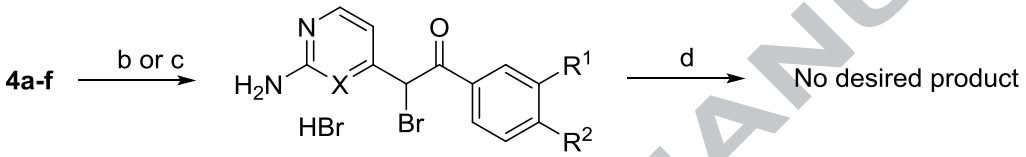
2a **3a-d** **4a-d**



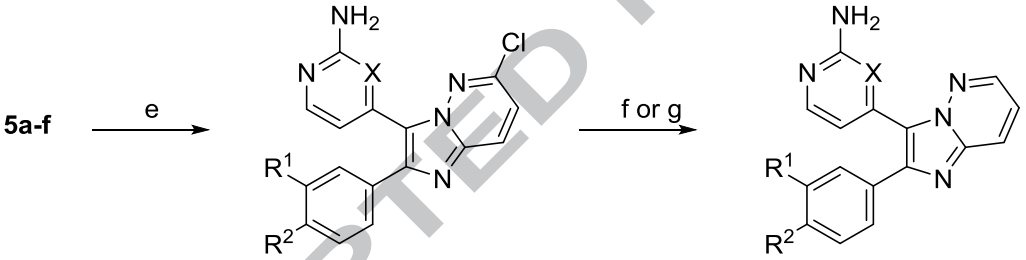
2a **3e** **4e**



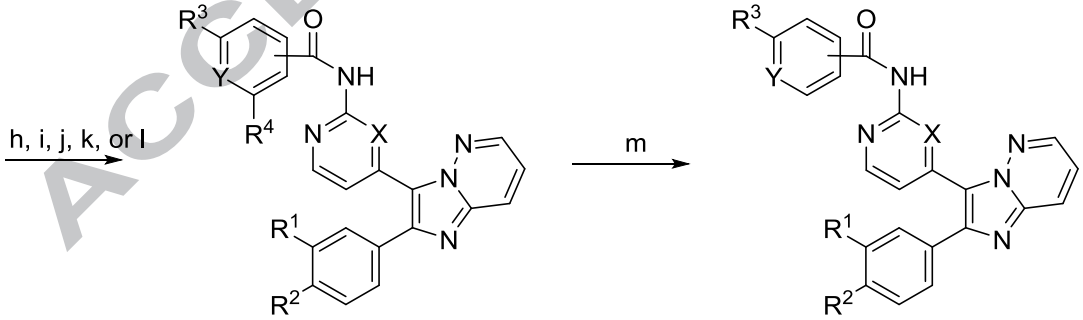
2b **3a** **4f**



5a-f



6a-f **7a-f**

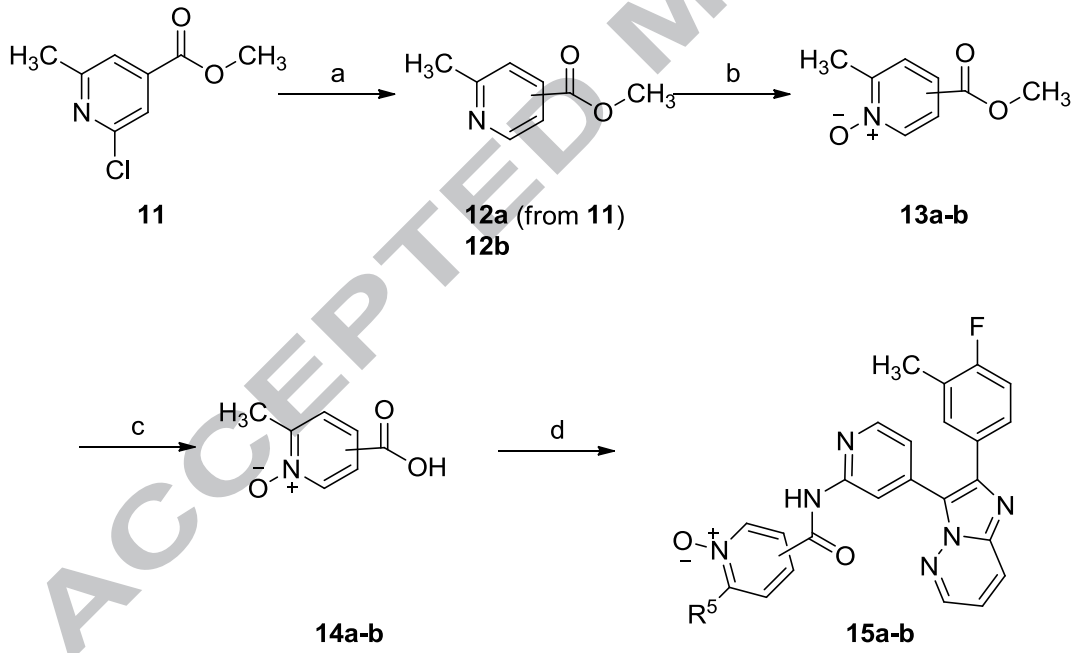


8a-o (R^4=H)
9a-b (R^4=Cl)

10a-b

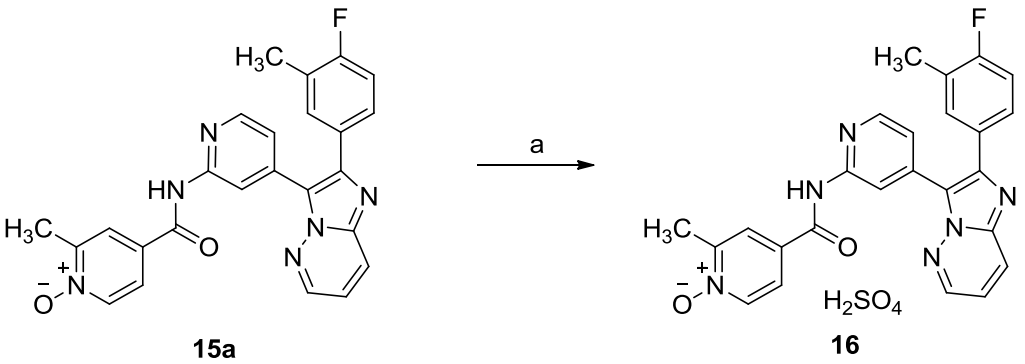
[a] Reagents and conditions: (a) i) ^{n}BuLi, THF, −78 ºC then at 0 ºC; ii) **3a-e**, −78 ºC; (b) Br_2, AcOH, 80 ºC for **5a-e**; (c) Br_2, AcONa, AcOH, rt for **5f**; (d) 3-aminopyridazine, EtOH, 80 ºC; (e) 3-amino-6-chloropyridazine, EtOH, 80 ºC; (f) H_2 (0.5 MPa), Pd/C, AcOH, 50 ºC for **7a**; (g) H_2 (0.1 MPa), Pd/C, Et_3N, DMF, rt for **7b-f**; (h) i) acyl chloride derivative, Et_3N, DCM, rt; ii) NH_3/EtOH, rt for **8a-j**, **8m**, **9b**; (i) i) acyl chloride derivative, pyridine, DMA, rt; ii) NH_3/EtOH, THF, rt for **8k-l**; (j) i) acyl chloride derivative, Et_3N, THF, rt; ii) NH_3/EtOH, THF, rt for **8n**; (k) i) 6-methylpyridine-3-carboxylic acid, $(COCl)_2$, Et_3N, DMF, THF, rt; ii) Et_3N, THF, rt; iii) NH_3/EtOH, THF, rt for **8m**; (l) i) 2-chloro-6-methylpyridine-4-carboxylic acid, $POCl_3$, 50 ºC; ii) Et_3N, THF, rt; iii) NH_3/EtOH, THF, rt for **9a**; (m) H_2 (0.5 MPa), Pd/C, Et_3N, DMF, 50 ºC for **10a-b**.

Scheme 2. Synthesis of compounds **15a-b**[a]



[a] Reagents and conditions: (a) H_2 (0.1 MPa), Pd/C, Et_3N, DMF, rt for **12a**; (b) H_2O_2 urea complex, AcOH, rt; (c) 8 N NaOH (aq.), THF, MeOH, 70 °C; (d) **7d**, HATU, *i*Pr_2EtN, pyridine, 70 ºC.

Scheme 3. Synthesis of sulfate **16**[a]



15a **16**

[a] Reagents and conditions: (a) H_2SO_4, EtOH, AcOH, 100 °C.

3. Results and discussion

Based on a 2.00-Å resolution of the co-crystal structure of **1** bound to p38 MAP kinase, the nitrogen atom of the pyridine ring, and the hydrogen atom of the amide NH group of **1**, form typical hydrogen-bond interactions with the NH and carbonyl oxygen on the main chain of Met109, respectively (Figure 3).[28] Additionally, the nitrogen atom of the thiazole ring forms a hydrogen bond with the NH_2 group on the side chain of Lys53, and the oxygen atom of the amide carbonyl group interacts with the NH on the main chain of Ala172. Interestingly, the side chain of Phe169 adopts two conformations, where one has hydrophobic interactions with ethyl thiazole of **1**, and the other shows no interaction with **1**. We, therefore, hypothesized that enhancing the hydrophobic interactions of the inhibitor with the side chain of Phe169 would improve the inhibition of p38. To enhance the hydrophobic interactions, we initially transformed ethyl thiazole of **1** into several fused rings and discovered that imidazo[1,2-*b*]pyridazine derivative **8a** strongly inhibited p38 (Figure 4, Table 1).

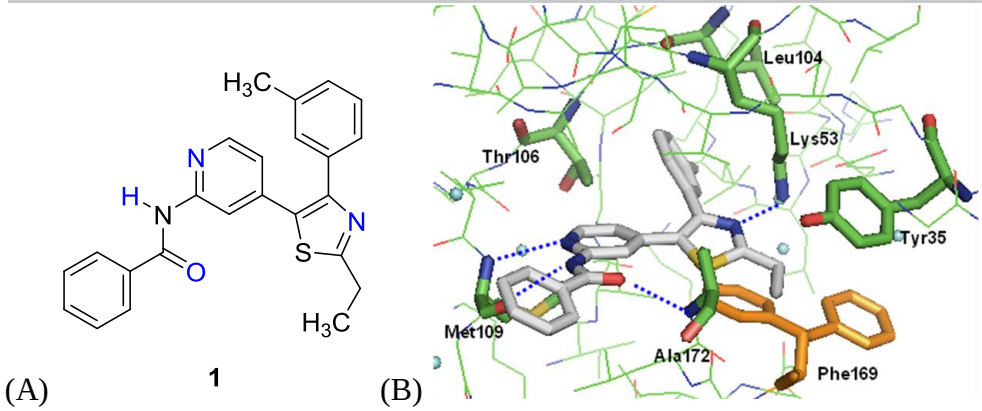


Fig. 3. (A) Structure of inhibitor **1**. The atoms involved in the hydrogen-bond interactions with p38 MAP kinase are shown in blue. (B) X-ray co-crystal structure of **1** bound with p38 MAP kinase (PDB code 6ANL). The blue dashed lines show hydrogen-bond interactions.

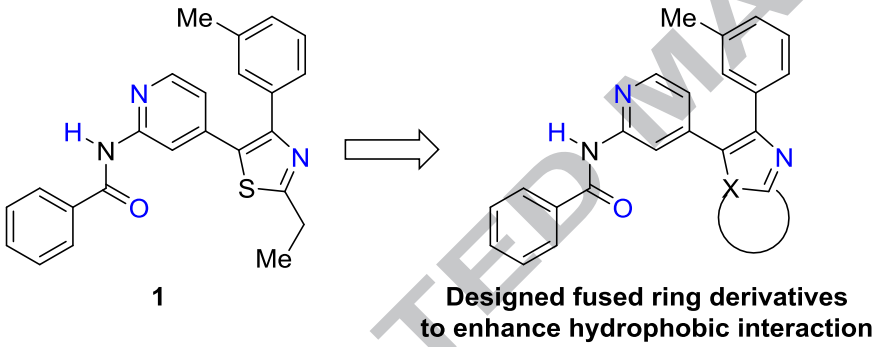
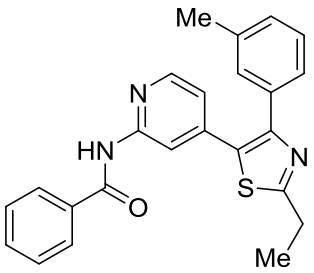


Fig. 4. Design concept of p38 MAP kinase inhibitors. The atoms involving hydrogen-bond interactions with p38 MAP kinase are shown in blue.

The inhibitory potencies (IC_{50}) of the synthesized compounds were estimated using a p38 MAP kinase assay and an assay measuring LPS-induced production of TNF-α in human monocytic THP-1 cells (Table 1). The pyridine **8a** and pyrimidine **8f** analogs showed stronger inhibition of p38 MAP kinase than did **1**. Pyridine **8a** inhibited the LPS-induced production of TNF-α in THP-1 cells with an IC_{50} value of 58 nM, while the inhibitory activity of pyrimidine **8f** was nearly equal to that of **1**. We,

therefore, aimed to synthesize pyridine analogs that would show fewer differences between enzyme and cellular functions. Hence, we focused on optimizing the side chains. Based on previous studies[26-27], we examined the effects of the substituents on phenyl groups. Replacing the methyl group in **8a** with a chloro group at the meta-position of **8b** led to similar activity against p38 MAP kinase. Compound **8c**, possessing a chloro group at the para-position, showed reduced inhibitory activity. Introduction of the smaller fluoro group at the para-position produced **8d**, which was a more potent inhibitor than **8a**. This result is consistent with the co-crystal structure of **1,** in which the space near the para-position on the phenyl group is narrow. Additionally compound **8e** was found to be more potent than **8g**.

Table 1. Inhibitory activities of imidazo[1,2-*b*]pyridazine derivatives against p38 MAP kinase and production of TNF-αin human THP-1 cells.



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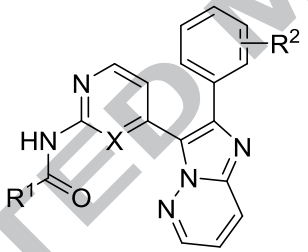


8a-n, 10b

Cmpd.	X	R^1	R^2	p38 MAPK IC_{50} [a] (nM)	Production of TNF-α IC_{50} [a] (nM)
1				240 (170 - 350)	240 (110 - 540)
8a	CH	Ph	3-Me	73 (36 - 150)	58 (35 - 94)
8f	N	Ph	3-Me	56 (50 - 63)	260 (140 - 510)

8b	CH	Ph	3-Cl	34 (28 - 41)	88 (40 - 190)
8c	CH	Ph	4-Cl	290 (200 - 440)	130 (69 - 250)
8d	CH	Ph	3-Me, 4-F	9.4 (8.4 - 10)	52 (34 - 81)
8e	CH	3-CF_3-Ph	3-Me, 4-F	11 (10-13)	13 (8.5-19)
8g	CH	3-CF_3-Ph	3-Cl, 4-F	210 (160-280)	>10000
8h	CH	2-CH_3-Ph	3-Me, 4-F	84 (74 - 95)	1300 (610 - 2800)
8i	CH	3-CH_3-Ph	3-Me, 4-F	11 (9.0 - 13)	19 (13 - 29)
8j	CH	4-CH_3-Ph	3-Me, 4-F	23 (20 - 27)	1000 (110 - 9900)
8k	CH	3-F-Ph	3-Me, 4-F	12 (10 - 13)	18 (14 - 24)
8l	CH	3-Cl-Ph	3-Me, 4-F	14 (8.5 - 22)	14 (7.8 - 24)
8m	CH	2-Py	3-Me, 4-F	23 (19 - 28)	360 (220 - 610)
10b	CH	3-Py	3-Me, 4-F	2.2 (0.58 – 8.0)	3.5 (2.2 – 5.5)
8n	CH	4-Py	3-Me, 4-F	5.4 (3.1 – 9.3)	16 (9.0 - 29)

[a] IC_{50} values shown are the mean values of quadruple measurements. Numbers in parentheses represent 95% confidence intervals.

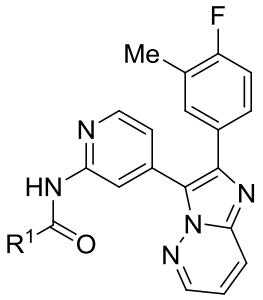
We further investigated the effects of the amide side chain linked at the 2-position on the pyridine ring. A methyl group, as an electron-donating substituent, was introduced to the *ortho*-, *meta*-, or *para*-position (**8h-j**) on the phenyl group of the benzoyl moiety. Compared with **8d**, the *ortho*-methyl **8h**

showed decreased potency; the *meta*- and *para*-methyl analogs **8i** and **8j** exhibited tolerable inhibitory activity, inhibiting the p38 MAP kinase with IC_{50} values of 11 and 23 nM, respectively. Introduction of an electron-withdrawing substituent, such as a fluoro or a chloro group (**8k**, **8l**), at a *meta*-position on the phenyl group of the benzoyl moiety, produced such potent inhibitors as **8i**. To control the lipophilicity of inhibitors, we replaced the phenyl ring with the pyridine ring. The 2-pyridyl **8m** showed decreased inhibitory activity against p38, while the 3-pyridyl **10b** or 4-pyridyl group **8n** displayed more potent inhibitory activity against p38 than did **8d**. Additionally, in the human THP-1 cells, **10b** and **8n** were more effective than **8d** at inhibiting the production of TNF-α

Non-substituted pyridine derivatives can inhibit CYPs by chelating the heme iron of CYP enzymes.[26, 29, 30] As shown in Table 2, compounds **10b** and **8n** showed potent inhibition of CYP3A4. We, therefore, introduced the methyl group into the adjacent position on the nitrogen of the pyridine ring. Surprisingly, compound **8o** showed increased inhibition of CYP3A4; compound **10a** showed reduced inhibition of CYP3A4 while maintaining potent inhibitory activity against p38 and production of TNF-α Although compound **10a** showed tolerable oxidative metabolic stability in rat microsomes, there was a distinct discrepancy in rat and human oxidative metabolic stability. Interestingly, compound **10a** had greater oxidative metabolic stability compared with that of **8n**. These results suggest that the methyl group plays a role in preventing oxidative metabolism to give the pyridine *N*-oxide derivative. In order to confirm that the main metabolism of **10a** was oxidation of pyridine, we examined the *in vitro* metabolism of **10a** in human liver microsomes and detected MS3 as a main metabolite at the rate of 17.8% (Table 3). On the basis of the LC/UV/MS analysis for **10a** and the metabolite, MS3 was predicted *N*-oxide analog (see experimental section, Figure 5). The pyridine *N*-oxide **15a**, which was indeed synthesized, exhibited a dramatically improved stability in human liver microsomes and reduced the discrepancy between rat and human metabolic stabilities compared with 10a, with inhibitory activity as potent as that of compound **10a**. The structure of **15a** was confirmed to be identical to that of MS3. Although the oxygen atom of the *N*-oxide **15a** can coordinate with the heme ion, **15a** showed tolerable

inhibition of CYP3A4. Therefore, the *N*-oxide analog **15a** was selected for further investigation as a drug candidate.

Table 2. DMPK profiles and inhibitory activities of imidazo[1,2-*b*]pyridazine derivatives against p38 MAP kinase and production of TNF-αin human THP-1 cells.



Cmpd.	R^1	p38 IC_{50} [a] (nM)	TNF-α IC_{50} [a] (nM)	Solubility pH = 6.8 (μg/mL)	CYP3A4 inhibition [b] (% inhibition)	Stability in liver microsomes Rat / human (μL/min/mg)
10b	3-Py	2.2 (0.58 – 8.0)	3.5 (2.2 – 5.5)	0.92	61	71/105
8n	4-Py	5.4 (3.1 – 9.3)	16 (9.0 - 29)	0.75	85	75/177
8o	6-Me-3-Py	1.8 (0.63 – 5.1)	1.2 (0.93 – 1.6)	0.38	86	53/104
10a	2-Me-4-Py	4.4 (1.8 - 11)	15 (9.0 – 26)	0.15	42	65/132
15b	6-Me-3-Py	2.7 (2.4 – 3.0)	16 (8.9 – 28)	2.4	55	23/89

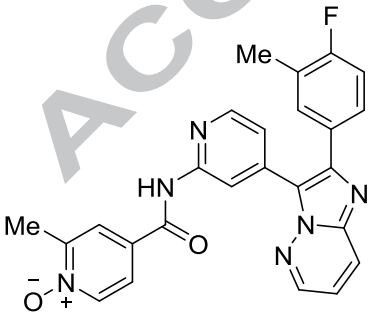
	N-oxide					
15a	2-Me-4-Py *N*-oxide	5.4 (4.6 – 6.4)	18 (10 – 33)	0.91	38	16/32

[a] IC_{50} values shown are the mean values of quadruple measurements. Numbers in parentheses represent 95% confidence intervals. [b] Compound concentration is 10 μM.

Table 3. HPLC and LC/MS^n data of **10a** and its metabolites after incubation with human liver microsomes.

Metabolite	Retention time (min)	Ratio [a] (%)	$[M+H]^+$ (*m/z*)	Major fragment ions
10a	33.4	72.5	439	303, 321, **411**, 421
MS3	23.0	17.8	455	303, 321, 409, **438**

[a] Residual and production ratios after 30 min incubation with human liver microsomes.



MS3 (15a)

Fig. 5. Predicted structure of the main metabolite MS3 after 30 min incubation with human liver microsomes.

To understand the binding mode of the novel class of p38 MAP kinase inhibitors, **15a** was co-crystallized with p38 MAP kinase. The determined 1.6-Å-resolution X-ray structure of the complex revealed that **15a** binds to the hinge region of p38 MAP kinase, which adopts the single Phe169 conformation (Figure 6). In the complex, the interactions observed in the co-crystal structure of **1**, bound with p38 MAP kinase, were expectedly conserved. Additionally, the solvent-exposed oxygen atom of the pyridine-*N*-oxide interacts with the side chain of His174. The 4-fluoro-3-methyl phenyl ring fits well into the shallow inner hydrophobic pocket defined by the residues Thr106 and Leu104. Finally, the imidazo[1,2-*b*]pyridazine moiety interacts with the side chains of Tyr35, Val38, and Phe169. This interaction, strengthened by the scaffold transformation, contributes to increased inhibition of p38, as was shown in the SAR study. In a kinase panel study to probe potential off-target liabilities, **15a** exhibited more than 100 fold selectivity over 40 kinases among 42 diverse kinases and showed several fold selectivity against Her4 and CK1δ(data not shown).

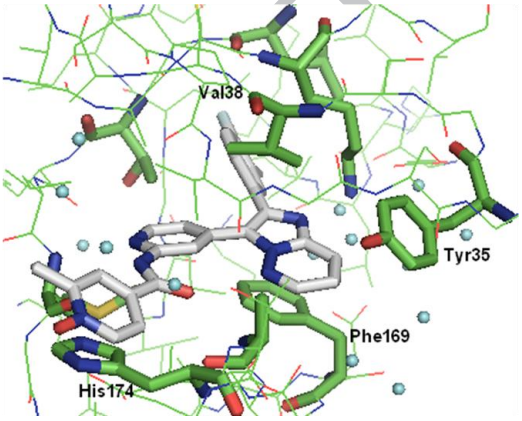


Fig. 6. X-ray crystal structure of the complex of **15a** bound with p38 MAP kinase (PDB code 5WJJ).

The pharmacokinetic properties of **15a** and the sulfate **16**, in rats, are shown in Table 4. Since the solubility of **15a** was low with the value of 0.91 μg/mL, the sulfate **16** was prepared. However **16** showed no improvement in solubility (data not shown). After exploration of administration medium, usage of corn oil as media of **16** fortunately led to significant increases in rat Cmax and AUC compared with those of **15a** in 0.5% methyl cellulose suspension. Absorption and exposure of the sulfate **16** were favorable in rats at oral doses of 3 mg/kg and were sufficient for further study.

Table 4. Pharmacokinetic properties of **15a** and **16**[a] in rats[b].

animal	cmpd	Cmax, po	Tmax, po	AUCpo	MRT, po	Vd	CL	*F*
		(ng/mL)	(h)	(ng·h/mL)	(h)	(mL/kg)	(mL/h/kg)	(%)
Rat[c]	**15a**	75	2.00	703	5.32	1380	600	13.5
Rat[d]	**16**	190	1.67	2130	5.63	1380[e]	600[e]	42.6

[a] H_2SO_4 salt of **15a**. [b] Mean values of measurements conducted in three animals. [c] i.v. 0.2 mg/kg, p.o. 3.0 mg/kg in 0.5% methyl cellulose suspension. [d] p.o. 3.0 mg/kg in corn oil suspension. [e] Because the main body of **16** and **15a** is the same, Vd and CL of **15a** are applied for **16**.

Compound **16** was evaluated in a pseudoestablished model of collagen-induced arthritis (CIA) in rats (Figure 7). The rats were administered compound **16** orally, with b.i.d dosing of 0.1, 0.3, 1, 3, and 10 mg/kg for 11 days; swelling of the paw was measured on day 14. Compound **16** showed dose-

dependent efficacy in achieving a marked reduction in paw swelling at a dose of 10 mg/kg. Statistically significant reduction in paw swelling was also observed at doses of 1, 3, and 10 mg/kg.

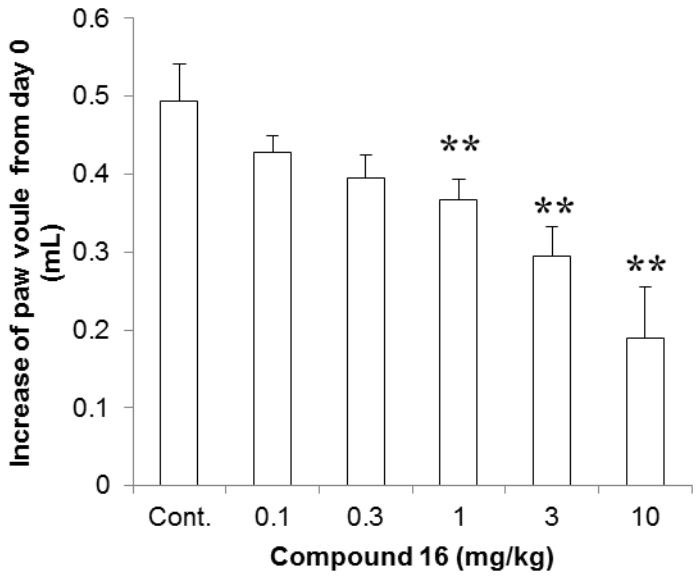


Fig. 7. Anti-inflammatory effects of **16** in the model of collagen-induced arthritis in the rat (po, 11 days treatment). **; p≤0.01 vs. control by Dunnett's test.

4. Conclusion

On the basis of X-ray crystallography of the complex of **1** with p38 MAP kinase, we focused on the scaffold transformation of 2-ethylthiazole of **1**. The designed imidazo[1,2-*b*]pyridazine derivatives were identified as potent inhibitors of p38 MAP kinase. The extensive SAR analysis of imidazo[1,2-*b*]pyridazine derivatives led to compound **10a,** which strongly inhibited p38 and the production of TNF-αin human THP-1 cells. During the course of optimizing the DMPK profile, the *in vitro* structural analysis of the metabolites of compound **10a** resulted in the discovery of pyridine *N*-oxide **15a** which exhibited a dramatically improved metabolic stability in human liver microsomes. Sulfate **16** also

potently inhibited p38 and production of TNF-α and showed an excellent DMPK profile and *in vivo* efficacy.

5. Experimental Section

5.1. Chemistry

Melting points were determined using a Yanagimoto or a Büchi melting point apparatus B-545; the values are presented uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini-200 (200 MHz), Varian Ultra-300 (300 MHz), or Bruker DPX-300 (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C) spectrometer and are reported in parts per million (δ) relative to tetramethylsilane (TMS, δ 0.0 ppm). Data are reported as follows: chemical shifts, integration, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; br, broad signal; brs, broad singlet; m, multiplet (denotes complex pattern)), and coupling constants (*J*, Hz). Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers and used without further purification. The yields were unoptimized. Column chromatography was performed using Merck silica gel 60 (70–230 mesh). Thin-layer chromatography (TLC) was performed on Merck silica gel plates 60F254. LC-MS analysis was performed on a Shiseido CAPCELL PACK C-18 UG120 S-3 column (1.5 mmϕ× 35 mm) in a Waters Alliance 2795 or an Agilent 1100 LC system equipped with a Waters 2487 absorbance detector and a Micromass ZQ2000 mass spectrometer. Analytes were eluted using a linear gradient of water (0.05% TFA)/acetonitrile (0.04% TFA) from 90:10 to 0:100 over 4 min at a flow rate of 0.5 mL/min. UV detection was at 220 nm. Preparative HPLC was performed on a Shiseido CAPCELL PACK C-18 UG120 S-5 column (20 mmϕ × 50 mm), eluting at 25 mL/min with a gradient of water (0.1% TFA)/acetonitrile (0.1% TFA). UV detection was at 220 nm. The purity of all compounds used in biological studies was determined to be ≥ 95% by elemental analysis. Elemental analyses were performed by Takeda Analytical Research Laboratories, Ltd. Experimentally determined hydrogen,

carbon, and nitrogen composition by elemental analysis was within ±0.4% of the expected value, implying a purity of ≥95%. All experiments using animals were reviewed and approved by the Internal Animal Care and Use Committee of Takeda Pharmaceutical Research Division.

The 2-*tert*-butoxycarbonylamino-4-methylpyridine (**2a**), *N*-(3-methylbenzoyl)propyleneimine (**3a**), and *N*-(3-chlorobenzoyl)propyleneimine (**3b**) were prepared according to a previous report.[11, 12]

2-*tert*-Butoxycarbonylamino-4-methylpyrimidine (2b). Di(*tert*-butyl) dicarbamate (12 mL, 50 mmol) was added dropwise to a solution of 4-methylpyrimidine-2-amine (5.0 g, 46 mmol) in *tert*-butanol over 1 h, and the solution was stirred at room temperature for 14 h. The solvent was evaporated and the residue was purified by silica gel column chromatography (hexane/ethyl acetate= 1/1) to give a solid precipitate. The solid precipitate was crystallized from isopropyl ether and hexane to give **2b** as a white solid (6.7 g, 70%), and this compound was used in the next reaction. Mp: 87-88 °C.

***N*-(4-Chlorobenzoyl)propyleneimine (3c).** A solution of propyleneimine (12 mL, 0.15 mol) in tetrahydrofuran (160 mL) was added to a 1 N aqueous solution of sodium hydroxide. To this mixture was added 4-chlorobenzoyl chloride (25 g, 0.143 mol) dropwise at 0 °C. The mixture was stirred for 30 min then extracted with ethyl acetate. The extract was dried, and the solvent was distilled to give **3c** as an oil (25 g, 89%). ^{1}H NMR ($CDCl_3$) δ 1.39 (3H, d, J = 5.5 Hz), 2.15 (1H, d, J = 2.9 Hz), 2.51-2.66 (2H, m), 7.39-7.47 (2H, m), 7.93-8.01 (2H, m).

***N*-(4-Fluoro-3-methylbenzoyl)propyleneimine (3d).** This compound was prepared from 4-fluoro-3-methylbenzoyl chloride as described in the synthesis of **3c**, as a colorless oil (91%). ^{1}H NMR ($CDCl_3$) δ 1.39 (3H, d, J = 5.4 Hz), 2.14 (1H, d, J = 3.4 Hz), 2.33 (3H, s), 2.51-2.61 (2H, m), 7.06 (1H, t, J = 8.8 Hz), 7.81-7.90 (2H, m).

3-Chloro-4-fluoro-*N*-methoxy-*N*-methylbenzamide (3e). To a solution of 3-chloro-4-fluorobenzoyl chloride (25.0 g, 0.130 mol) in diethylether (40 mL) was dropwise added a solution of *N*, *O*-dimethylhydroxylamine (13.3 g, 0.136 mol) and a 2N aqueous solution of sodium hydroxide (136 mL,

0.272 mol) in diethylether (80 mL) at 0 °C. The mixture was stirred at room temperature for 15 h. The organic layer was separated and the aqueous layer was extracted with diethylether. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated to give **3e** as a pale yellow oil (27.6 g, 98%). This compound was used in the next reaction. ^{1}H NMR ($CDCl_3$) δ 3.37 (3H, s), 3.55 (3H, s), 7.17 (1H, t, *J* = 8.7 Hz), 7.61-7.68 (1H, m), 7.82 (1H, dd, *J* = 2.2, 7.1 Hz).

2-(2-*tert*-Butoxycarbonylaminopyridin-4-yl)-1-(3-methylphenyl)ethanone (4a). To a solution of **2a** (146 g, 0.700 mol) in anhydrous tetrahydrofuran (1300 mL) was dropwise added a solution of 1.6 M n-butyl lithium in hexane (875 mL, 1.40 mol) at -78 °C. Then, the mixture was stirred at 0 °C for 30 min and cooled to −78 °C. To the mixture was dropwise added a solution of **3a** (123 g, 0.700 mol) in anhydrous tetrahydrofuran (130 mL). The mixture was stirred at −78 °C for 1 h and at room temperature for 1 h. To the mixture was added brine (1300 mL), and the organic layer was separated. The aqueous layer was extracted with ethyl acetate and combined organic layer was dried and evaporated. The residue was crystallized from ethyl acetate to give **4a** (185 g, 81%) as a yellow solid. Mp: 144–146 °C. ^{1}H NMR ($CDCl_3$) δ 1.53 (9H, s), 2.42 (3H, s), 4.28 (2H, s), 6.87 (1H, d, *J* = 5.1 Hz), 7.32-7.43 (2H, m), 7.75-7.83 (2H, m), 7.92 (1H, s), 8.06 (1H, brs), 8.21 (1H, d, *J* = 5.1 Hz).

2-(2-*tert*-Butoxycarbonylaminopyridin-4-yl)-1-(3-chlorophenyl)ethanone (4b). This compound was prepared from **3b** as described in the synthesis of **4a** as a solid**,** and used in the next reaction. Mp: 152-153 °C (ethyl acetate/isopropyl ether). ^{1}H NMR ($CDCl_3$) δ 1.53 (9H, s), 4.26 (2H, s), 6.85 (1H, dd, *J* = 5.2, 1.8 Hz), 7.43 (1H, dd, *J* = 8.0, 7.7 Hz), 7.56 (1H, ddd, *J* = 8.0, 2.2, 1.1 Hz), 7.86 (1H, ddd, *J* = 7.7, 1.7, 1.1 Hz), 7.91 (1H, s), 7.95 (1H, brs), 7.97 (1H, dd, *J* = 2.2, 1.7 Hz), 8.22 (1H, dd, *J* = 5.2, 0.6 Hz).

2-(2-*tert*-Butoxycarbonylaminopyridin-4-yl)-1-(4-chlorophenyl)ethanone (4c). This compound was prepared from **3c** as described in the synthesis of **4a** as a solid (81%), and used in the next reaction. Mp: 155–156 °C. ^{1}H NMR ($CDCl_3$) δ 1.53 (9H, s), 4.25 (2H, s), 6.84 (1H, dd, *J* = 1.5, 5.1 Hz), 7.44 (2H, d, *J* = 8.9 Hz), 7.89-7.90 (3H, m), 8.12 (1H, brs), 8.20 (1H, dd, *J* = 0.7, 5.1 Hz).

2-(2-*tert*-Butoxycarbonylaminopyridin-4-yl)-1-(4-fluoro-3-methylphenyl)ethanone (4d). This compound was prepared from **3d** as described in the synthesis of **4a** as a white solid (85%), and used in the next reaction. Mp: 143–144 °C. ^{1}H NMR ($CDCl_3$) δ 1.53 (9H, s), 2.33 (3H, d, *J* = 1.7 Hz), 4.24 (2H, s), 6.86 (1H, dd, *J* = 1.3, 5.1 Hz), 7.08 (1H, t, *J* = 8.9 Hz), 7.79-7.89 (2H, m), 7.92 (1H, s), 8.11 (1H, s), 8.21 (1H, d, *J* = 5.1 Hz).

2-(2-*tert*-Butoxycarbonylaminopyridin-4-yl)-1-(3-chloro-4-fluorophenyl)ethanone (4e). To a solution of **2a** (1.91 g, 9.19 mmol) in tetrahydrofuran (20 mL) was dropwise added a solution of 1.6 M n-butyl lithium in hexane (12.1 mL) at −78 °C over 10 min. Then, the mixture was stirred at 0 °C for 30 min and cooled to −78 °C. To the mixture was added a solution of **3e** (2.00 g, 9.19 mmol) in tetrahydrofuran (2 mL) dropwise over 10 min. The mixture was stirred at room temperature for 1 h. To the mixture was added a saturated aqueous solution of sodium hydrogen carbonate, and the organic layer was separated. The aqueous layer was neutralized with 1N hydrogen chloride and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was washed with diisopropylether and dried to give **4a** (2.67 g, 80%) as a white solid. This compound was used in the next reaction. Mp: 141–143 °C. ^{1}H NMR ($CDCl_3$) δ 1.53 (9H, s), 4.24 (2H, s), 6.84 (1H, dd, *J* = 1.5, 5.1 Hz), 7.20-7.28 (1H, m), 7.82-7.93 (3H, m), 8.07 (1H, dd, *J* = 2.3, 7.2 Hz), 8.21 (1H, d, *J* = 5.1 Hz).

2-(2-*tert*-Butoxycarbonylaminopyrimidin-4-yl)-1-(3-methylphenyl)ethanone (4f). This compound was prepared from **2b** as described in the synthesis of **4a** as a solid, and used in the next reaction. Mp: 194–195 °C.

2-(2-Aminopyridin-4-yl)-2-bromo-1-(3-methylphenyl)ethanone hydrobromide (5a). Bromine (29.2 mL, 0.566 mol) was added to a solution of **4a** (185 g, 0.566 mol) in acetic acid (400 mL), and the mixture was stirred at 80 °C for 2 h. The mixture was concentrated and the residue was crystallized from acetonitrile-ethyl acetate to give **5a** (171 g, 78%) as a solid. ^{1}H NMR (DMSO-d_6) δ 2.41 (3H, s),

3.41 (1H, brs), 6.98 (1H, d, *J* = 6.8 Hz), 7.12 (1H, s), 7.20 (1H, s), 7.43-7.58 (2H, m), 7.87-8.00 (3H, m), 8.17 (2H, brs).

2-(2-Aminopyridin-4-yl)-2-bromo-1-(3-chlorophenyl)ethanone hydrobromide (5b). This compound was prepared from **4b** as described in the synthesis of **5a** as a solid, and used in the next reaction. ^{1}H NMR (DMSO-d_6) δ 3.42 (3H, s), 6.98 (1H, dd, *J* = 6.9, 1.7 Hz), 7.12 (1H, s), 7.20 (1H, s), 7.64 (1H, t, *J* = 8.0 Hz), 7.80 (1H, d, *J* = 8.0 Hz), 7.96 (1H, d, *J* = 6.9 Hz), 8.05 (1H, d, *J* = 8.0 Hz), 8.15 (1H, s).

2-(2-Aminopyridin-4-yl)-2-bromo-1-(4-chlorophenyl)ethanone hydrobromide (5c). This compound was prepared from **4c** as described in the synthesis of **5a** as a white solid (87%), and used in the next reaction. ^{1}H NMR (DMSO-d_6) δ 6.97 (1H, d, *J* =6.6 Hz), 7.12 (1H, dd, *J* =2.4, 3.4 Hz), 7.21 (1H, d, *J* =3.4 Hz), 7.69 (2H, d, *J* = 8.6 Hz), 7.97 (1H, dd, *J* = 2.4, 6.6 Hz), 8.12 (2H, d, *J* =8.6 Hz), 8.19 (2H, brs), 13.34 (1H, brs).

2-(2-Aminopyridin-4-yl)-2-bromo-1-(4-fluoro-3-methylphenyl)ethanone hydrobromide (5d). This compound was prepared from **4d** as described in the synthesis of **5a** as a solid (90%), and used in the next reaction. ^{1}H NMR (DMSO-d_6) δ 2.33 (3H, s), 6.98 (1H, dd, *J* =1.6, 6.7 Hz), 7.11 (1H, s), 7.21 (1H, s), 7.36 (1H, t, *J* = 9.0 Hz), 7.97 (1H, d, *J* = 6.7 Hz), 8.00-8.07 (1H, m), 8.10 (1H, dd, *J* =1.1, 7.9 Hz), 8.19 (2H, brs), 13.35 (1H, brs).

2-(2-Aminopyridin-4-yl)-2-bromo-1-(3-chloro-4-fluorophenyl)ethanone hydrobromide (5e). Bromine (0.367 mL, 7.16 mmol) was added to a solution of **4e** (2.61 g, 7.16 mmol) in acetic acid (15 mL), and the mixture was stirred at 70 °C for 3 h. The mixture was cooled to room temperature and the precipitate was collected on a filter, washed with ethyl acetate, and dried to give **5a** (2.66 g, 88%) as a white solid. This compound was used in the next reaction. Mp: 231–233 °C. ^{1}H NMR (DMSO-d_6) δ 6.96 (1H, dd, *J* =1.6, 6.6 Hz), 7.10 (1H, s), 7.16 (1H, d, *J* = 1.1 Hz), 7.68 (1H, t, *J* = 8.9 Hz), 7.96 (1H, d, *J* = 6.6 Hz), 8.00-8.17 (3H, m), 8.36 (1H, dd, *J* = 2.3, 7.0 Hz).

2-(2-Aminopyrimidin-4-yl)-2-bromo-1-(3-methylphenyl)ethanone hydrobromide (5f). Bromine (0.16 mL, 3.1 mmol) was added to a solution of **4f** (1.00 g, 3.1 mmol) and sodium acetate (0.50 g, 6.11 mmol) in acetic acid (10 mL) at room temperature. The mixture was stirred at room temperature for 2 h and concentrated. A saturated aqueous solution of sodium hydrogen carbonate was added to the residue and the mixture was extracted with ethyl acetate. The organic layer was dried and evaporated to give **5e** as an oil. This compound was used in the next reaction.

4-[6-Chloro-2-(3-methylphenyl)imidazo[1, 2-*b*]pyridazin-3-yl]pyridin-2-amine (6a). 3-Amino-6-chloropyridazine (6.71 g, 51.8 mmol) and **5a** (20.0 g, 51.8 mmol) were stirred at 80 °C for 14 h in ethanol (200 mL). The mixture was cooled to room temperature, and the solvent was removed by evaporation under reduced pressure. A saturated aqueous sodium hydrogen carbonate was added to the residue. The precipitated solid was collected by filtration, washed with water, and dried. The obtained solid was dissolved in tetrahydrofuran, filtered, and the solvent was removed by evaporation under reduced pressure. The obtained solid was recrystallized from ethyl acetate to give **6a** (10.5 g, 60%) as a solid. Mp: 247–248 °C. MS (ESI+): 336 (M+H). ^{1}H-NMR ($CDCl_3$) δ 2.36 (3H, s), 4.51 (2H, brs), 6.80 (1H, dd, *J* = 1.4, 0.8 Hz), 6.83 (1H, ddd, *J* = 5.2, 4.1, 0.8 Hz), 7.11 (1H, d, *J* = 9.3 Hz), 7.14-7.24 (2H, m), 7.36 (1H, d, *J* = 7.4 Hz), 7.58 (1H, s), 7.94 (1H, d, *J* = 9.3 Hz), 8.14 (1H, d, *J* = 5.2 Hz). Anal. Calcd for $C_{18}H_{14}ClN_5$+0.75H_2O: C, 61.89; H, 4.47; N, 20.05. Found: C, 61.75; H, 4.39; N, 20.34.

4-[6-Chloro-2-(3-chlorophenyl)imidazo[1, 2-*b*]pyridazin-3-yl]pyridin-2-amine (6b). This compound was prepared from **5b** as described in the synthesis of **6a** as a solid (43%). Mp: 268–269 °C (tetrahydrofuran). MS (ESI+): 356 (M+H). ^{1}H NMR (DMSO-d_6) δ 6.19 (2H, brs), 6.57 (1H, dd, *J* = 5.2, 1.4 Hz), 6.60 (1H, dd, *J* = 1.4, 0.8 Hz), 7.40-7.44 (2H, m), 7.47 (1H, d, *J* = 9.3 Hz), 7.52-7.59 (1H, m), 7.68-7.72 (1H, m), 8.06 (1H, dd, *J* = 5.2, 0.8 Hz), 8.31 (1H, d, *J* = 9.3 Hz). Anal. Calcd for $C_{17}H_{11}Cl_2N_5$: C, 57.13; H, 3.11; N, 19.66. Found: C, 57.13; H, 3.08; N, 19.46.

4-[6-Chloro-2-(4-chlorophenyl)imidazo[1, 2-*b*]pyridazin-3-yl]pyridin-2-amine (6c). This compound was prepared from **5c** as described in the synthesis of **6a** as a solid (63%). Mp: 183–184 °C.

MS (ESI+): 356 (M+H). ^{1}H NMR (DMSO-d_6) δ 6.17 (2H, brs), 6.57 (1H, dd, *J* = 5.2, 1.2 Hz), 6.59 (1H, s), 7.46 (1H, d, *J* = 9.5 Hz), 7.47 (2H, d, *J* = 8.7 Hz), 7.65 (2H, d, *J* = 8.7 Hz), 8.04 (1H, d, *J* = 5.4 Hz), 8.29 (1H, dd, *J* = 9.5 Hz).

4-[6-Chloro-2-(4-fluoro-3-methylphenyl)imidazo[1, 2-*b*]pyridazin-3-yl]pyridin-2-amine (6d). This compound was prepared from **5d** as described in the synthesis of **6a** as a solid (71%). Mp: 217–218 °C. MS (ESI+): 354 (M+H). ^{1}H NMR (DMSO-d_6) δ 2.24 (3H, d, *J* = 1.5 Hz), 6.21 (2H, brs), 6.58 (1H, dd, *J* = 5.3, 1.3 Hz), 6.63 (1H, s), 7.12-7.20 (1H, m), 7.34-7.42 (1H, m), 7.46 (1H, d, *J* = 9.5 Hz), 7.67 (1H, dd, *J* = 7.5, 1.5 Hz), 8.04 (1H, dd, *J* = 5.3, 0.4 Hz), 8.29 (1H, d, *J* = 9.5 Hz). Anal. Calcd for $C_{18}H_{13}ClFN_5$+3.5H_2O: C, 51.87; H, 4.84; N, 16.80. Found: C, 51.85; H, 3.41; N, 18.42.

4-[6-Chloro-2-(3-chloro-4-fluorophenyl)imidazo[1, 2-*b*]pyridazin-3-yl]pyridin-2-amine (6e). This compound was prepared from **5e** as described in the synthesis of **6a** as a pale yellow solid (50%). Mp: 237–239 °C. MS (ESI+): 374 (M+H). ^{1}H NMR (DMSO-d_6) δ 6.19 (2H, s), 6.54-6.63 (2H, m), 7.42-7.52 (2H, m), 7.54-7.63 (1H, m), 7.82 (1H, dd, *J* = 2.2, 7.3 Hz), 8.07 (1H, d, *J* = 5.1 Hz), 8.31 (1H, d, *J* = 9.4 Hz).

4-[6-Chloro-2-(3-methylphenyl)imidazo[1, 2-*b*]pyridazin-3-yl]pyrimidin-2-amine (6f). This compound was prepared from **5f** as described in the synthesis of **6a** as a solid (22%). Mp: 237–238 °C (ethyl acetate/hexane). MS (ESI+): 337 (M+H). ^{1}H-NMR ($CDCl_3$) δ 2.38 (3H, s), 5.10 (2H, s), 7.08 (1H, d, *J* = 5.1 Hz), 7.14 (1H, d, *J* = 9.5 Hz), 7.16-7.20 (1H, m), 7.24 (1H, t, *J* = 7.5 Hz), 7.40-7.50 (1H, m), 7.61-7.65 (1H, m), 7.96 (1H, d, *J* = 9.5 Hz), 8.42 (1H, d, *J* = 5.1 Hz). Anal. Calcd for $C_{17}H_{13}ClN_6$: C, 60.63; H, 3.89; N, 24.95. Found: C, 60.28; H, 3.97; N, 24.32.

4-[2-(3-Methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-amine (7a). 4-[6-Chloro-2-(3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridine-2-amine (5.00 g, 14.9 mmol) and 10% palladium carbon powder (50% water containing product) (1.00 g) were stirred in acetic acid under hydrogen atmosphere at 50 °C for 3 h under the hydrogen pressure of 0.5 MPa. The reaction mixture was cooled

to room temperature and filtered, and the solvent was removed by evaporation under reduced pressure. A saturated aqueous solution of sodium hydrogen carbonate was added to the residue, and the precipitated solid was collected by filtration. The solid was washed with water and dried. The obtained solid was recrystallized from ethanol to give **7a** (2.43 g, 54%) as a yellow solid. Mp: 188–189 °C. MS (ESI+): 302 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.37 (3H, s), 4.50 (2H, brs), 6.83 (1H, dd, *J* = 1.4, 0.8 Hz), 6.87 (1H, dd, *J* = 5.2, 1.4 Hz), 7.10 (1H, dd, *J* = 9.3, 4.4 Hz), 7.13-7.19 (1H, m), 7.22 (1H, t, *J* = 7.1 Hz), 7.37-7.42 (1H, m), 7.60-7.63 (1H, m), 8.01 (1H, dd, *J* = 9.3, 1.6 Hz), 8.14 (1H, dd, *J* = 5.2, 0.8 Hz), 8.33 (1H, dd, *J* = 4.4, 1.6 Hz). Anal. Calcd for $C_{18}H_{15}N_5$+0.5H_2O: C, 69.66; H, 5.20; N, 22.57. Found: C, 69.72; H, 5.18; N, 22.40.

4-[2-(3-Chlorophenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-amine (7b). 4-[6-Chloro-2-(3-chlorophenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridine-2-amine (5.62 g, 15.8 mmol), 10% palladium carbon powder (50% water containing product) (0.56 g), and triethylamine (4.4 mL, 31.6 mmol) were stirred in *N*, *N*-dimethylformamide (200 mL) under hydrogen atmosphere, at room temperature, for 1 h. The mixture was filtered, and the solvent was removed by evaporation under reduced pressure. A saturated aqueous solution of sodium hydrogen carbonate was added to the residue, and the precipitated solid was collected by filtration. The solid was washed with water and dried to give **7b** (4.83 g, 95%) as an off-white solid. MS (ESI+): 322 (M+H). ^{1}H NMR (DMSO-d_6) δ 6.13 (2H, brs), 6.59 (1H, dd, *J* = 5.3, 1.3 Hz), 6.65 (1H, s), 7.35 (1H, dd, *J* = 9.2, 4.3 Hz), 7.39-7.45 (2H, m), 7.56-7.62 (1H, m), 7.71-7.76 (1H, m), 8.04 (1H, dd, *J* = 5.3, 0.6 Hz), 8.23 (1H, dd, *J* = 9.2, 1.7 Hz), 8.55 (1H, dd, *J* = 4.3, 1.7 Hz).

4-[2-(4-Chlorophenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-amine (7c). This compound was prepared from **6c** as described in the synthesis of **7b** as a solid (61%). Mp: 252–253 °C (ethyl acetate). MS (ESI+): 322 (M+H). ^{1}H NMR (DMSO-d_6) δ 6.10 (2H, brs), 6.57 (1H, dd, *J* = 5.2, 1.7 Hz), 6.63 (1H, s), 7.32 (1H, dd, *J* = 9.1, 4.4 Hz), 7.46 (2H, d, *J* = 8.8 Hz), 7.67 (2H, d, *J* = 8.8 Hz), 8.01 (1H, d, *J*

= 5.2 Hz), 8.21 (1H, dd, *J* = 9.1, 1.7 Hz), 8.53 (1H, dd, *J* = 4.4, 1.7 Hz). Anal. Calcd for $C_{17}H_{12}ClN_5$+0.4H_2O: C, 62.07; H, 3.92; N, 21.29. Found: C, 62.16; H, 4.20; N, 21.41.

4-[2-(4-Fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-amine (7d). This compound was prepared from **6d** as described in the synthesis of **7b** as a pale yellow solid (44%). Mp: 179–180 °C. MS (ESI+): 320 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.29 (3H, d, *J* = 1.9 Hz), 4.52 (2H, brs), 6.82 (1H, dd, *J* = 1.6, 0.6 Hz), 6.85 (1H, dd, *J* = 5.4, 1.6 Hz), 6.96 (1H, dd, *J* = 9.3, 8.5 Hz), 7.11 (1H, dd, *J* = 9.2, 4.4 Hz), 7.35-7.41 (1H, m), 7.63 (1H, dd, *J* = 8.0, 2.1 Hz), 8.00 (1H, dd, *J* = 9.2, 1.7 Hz), 8.15 (1H, dd, *J* = 5.4, 0.6 Hz), 8.34 (1H, dd, *J* = 4.4, 1.7 Hz). Anal. Calcd for $C_{18}H_{14}FN_5$: C, 67.70; H, 4.42; N, 21.93. Found: C, 67.67; H, 4.43; N, 21.85.

4-[2-(3-Chloro-4-fluorophenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-amine (7e). This compound was prepared from **6e** as described in the synthesis of **7b** as a pale yellow solid (50%). Mp: 201–203 °C. MS (ESI+): 340 (M+H). ^{1}H NMR (DMSO-d_6) δ 6.59 (2H, brs), 6.68 (1H, dd, *J* = 1.5, 5.7 Hz), 6.81 (1H, s), 7.38 (1H, dd, *J* = 4.5, 9.1 Hz), 7.48 (1H, t, *J* = 8.9 Hz), 7.57-7.64 (1H, m), 7.85 (1H, dd, *J* = 1.9, 7.2 Hz), 8.03 (1H, d, *J* = 5.3 Hz), 8.26 (1H, dd, *J* = 1.7, 9.3 Hz), 8.59 (1H, dd, *J* = 4.5, 1.5 Hz).

4-[2-(3-Methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyrimidin-2-amine (7f). This compound was prepared from **6f** as described in the synthesis of **7b** as a pale yellow solid (90%). Mp: 250–251 °C. MS (ESI+): 303 (M+H). ^{1}H NMR (DMSO-d_6) δ 2.31 (3H, s), 6.09 (2H, brs), 6.56 (1H, dd, *J* = 5.2, 1.7 Hz), 6.66 (1H, s), 7.16 (1H, d, *J* = 7.4 Hz), 7.26 (1H, dd, *J* = 7.7, 7.4 Hz), 7.32 (1H, dd, *J* = 9.4, 4.4 Hz), 7.38 (1H, d, *J* = 7.7 Hz), 7.59 (1H, s), 8.01 (1H, d, *J* = 5.2 Hz), 8.21 (1H, dd, *J* = 9.1, 1.7 Hz), 8.53 (1H, dd, *J* = 4.4, 1.7 Hz).

***N*-{4-[2-(3-Methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}benzamide (8a).** To a solution of **7a** (150 mg, 0.498 mmol) in dichloromethane (8 mL) were added triethylamine (208 μL, 1.49 mmol) and benzoyl chloride (173 μL, 1.49 mmol). The mixture was stirred at room temperature

for 30 min, and a saturated aqueous solution of sodium hydrogen carbonate was added to the reaction mixture. An ammonia-ethanol solution (2.0 M, 10 mL) was added to the resultant mixture, and the mixture was stirred at room temperature for 30 min. The solvent was removed by evaporation under reduced pressure, and the obtained residue was purified by silica gel chromatography (ethyl acetate: hexane = 3: 7– 8: 2) and recrystallized from ethyl acetate to give **8a** (145 mg, 72%) as a solid. Mp: 178–180 °C. MS (ESI+): 406 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.37 (3H, s), 7.12-7.19 (3H, m), 7.23 (1H, t, *J* = 7.5 Hz), 7.40 (1H, d, *J* = 7.5 Hz), 7.47-7.60 (3H, m), 7.63 (1H, s), 7.93-7.96 (2H, m), 8.05 (1H, dd, *J* = 9.1, 1.6 Hz), 8.26 (1H, dd, *J* = 5.3, 0.8 Hz), 8.41 (1H, dd, *J* = 4.4, 1.6 Hz), 8.88 (1H, s), 8.92 (1H, d, *J* = 0.8 Hz). ^{13}C NMR ($CDCl_3$) δ 21.4, 77.2, 114.6, 117.7, 121.1, 122.3, 125.5, 126.0, 127.3, 128.4, 128.9, 129.4, 132.3, 133.3, 134.3, 138.4, 139.4, 139.5, 143.1, 145.2, 148.1, 152.1, 165.8 (s). Anal. Calcd for $C_{25}H_{19}N_5O$: C, 74.06; H, 4.72; N, 17.27. Found: C, 73.81; H, 4.71; N, 17.24.

***N*-{4-[2-(3-Chlorophenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}benzamide (8b).** This compound was prepared from **7b** as described in the synthesis of **8a** as a pale yellow solid (53%). Mp: 213–214 °C (ethanol). MS (ESI+): 426 (M+H). ^{1}H NMR ($CDCl_3$) δ 7.13-7.20 (2H, m), 7.24-7.36 (2H, m), 7.47-7.62 (4H, m), 7.78 (1H, dd, *J* = 1.9, 1.7 Hz), 7.90-7.96 (2H, m), 8.04 (1H, dd, *J* = 9.1, 1.7 Hz), 8.35 (1H, dd, *J* = 5.2, 0.6 Hz), 8.41 (1H, dd, *J* = 4.4, 1.7 Hz), 8.71 (1H, brs), 8.85 (1H, dd, *J* = 1.4, 0.8 Hz). ^{13}C NMR ($CDCl_3$) δ 77.2, 114.7, 118.1, 120.9, 122.8, 125.7, 126.8, 127.3, 128.6, 128.7, 128.9, 129.8, 132.3, 134.2, 134.7, 135.3, 138.9, 139.6, 143.3, 143.4, 148.3, 152.2, 165.8 (s). Anal. Calcd for $C_{24}H_{16}ClN_5O$+0.2H_2O: C, 67.12; H, 3.85; N, 16.31. Found: C, 67.38; H, 3.80; N, 16.19.

***N*-{4-[2-(4-Chlorophenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}benzamide (8c).** This compound was prepared from **7c** as described in the synthesis of **8a** as an off-white solid (74%). Mp: 218–219 °C (ethanol). MS (ESI+): 426 (M+H). ^{1}H NMR ($CDCl_3$) δ 7.13-7.20 (2H, m), 7.36 (2H, d, *J* = 8.7 Hz), 7.48-7.60 (3H, m), 7.65 (2H, d, *J* = 8.7 Hz), 7.91-7.98 (2H, m), 8.05 (1H, dd, *J* = 9.2, 1.7 Hz), 8.36 (1H, dd, *J* = 5.1, 0.8 Hz), 8.41 (1H, dd, *J* = 4.4, 1.7 Hz), 8.72 (1H, s), 8.85 (1H, dd, *J* = 1.4, 0.8 Hz). ^{13}C NMR ($CDCl_3$) δ 77.3, 114.7, 118.0, 121.0, 122.5, 125.6, 127.3, 128.9, 128.9, 130.0, 132.0,

132.4, 134.2, 134.6, 139.1, 139.6, 143.3, 143.7, 148.3, 152.2, 165.8 (s). Anal. Calcd for $C_{24}H_{16}ClN_5O$: C, 67.69; H, 3.79; N, 16.44. Found: C, 67.74; H, 3.81; N, 16.51.

***N*-{4-[2-(4-Fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}benzamide (8d).** This compound was prepared from **7d** as described in the synthesis of **8a** as a pale yellow solid (83%). Mp: 189–190 °C (ethanol). MS (ESI+): 424 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.29 (3H, d, *J* = 1.9 Hz), 6.96 (1H, dd, *J* = 9.3, 8.8 Hz), 7.14 (1H, dd, *J* = 9.1, 4.4 Hz), 7.17 (1H, dd, *J* = 5.2, 1.4 Hz), 7.35-7.41 (1H, m), 7.47-7.66 (4H, m), 7.90-7.95 (2H, m), 8.03 (1H, dd, *J* = 9.1, 1.7 Hz), 8.33 (1H, dd, *J* = 5.2, 0.8 Hz), 8.40 (1H, dd, *J* = 4.4, 1.7 Hz), 8.69 (1H, brs), 8.86 (1H, dd, *J* = 1.4, 0.8 Hz). ^{13}C NMR ($CDCl_3$) δ 14.6 (d, *J* = 3.30 Hz), 77.3, 114.7 (s), 115.2 (d, *J* = 23.1 Hz), 117.9, 120.9, 122.2, 125.3, 125.5, 127.3 (s), 127.9 (d, *J* = 8.3 Hz), 128.9 (s), 129.2 (d, *J* = 3.9 Hz), 131.9 (d, *J* = 5.5 Hz), 132.3, 134.2 (s), 139.4 (d, *J* = 17.6 Hz), 143.2, 144.3, 148.2, 152.2 (s), 161.6 (d, *J* = 247.0 Hz), 165.8 (s). Anal. Calcd for $C_{25}H_{18}FN_5O$: C, 70.91; H, 4.28; N, 16.54. Found: C, 70.77; H, 4.33; N, 16.53.

***N*-{4-[2-(4-Fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}-3-trifluoromethylbenzamide (8e).** This compound was prepared from **7d** as described in the synthesis of **8a** as a white solid (82%). Mp: 228–229 °C (hexane/ethyl acetate). MS (ESI+): 492 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.29 (3H, d, *J* = 1.9 Hz), 6.94-7.02 (1H, m), 7.16 (1H, dd, *J* = 4.5, 9.2 Hz), 7.22-7.28 (1H, m), 7.35-7.43 (1H, m), 7.61-7.71 (2H, m), 7.85 (1H, d, *J* = 8.0 Hz), 8.05 (1H, dd, *J* = 1.6, 9.2 Hz), 8.13 (1H, d, *J* = 7.6 Hz), 8.22 (1H, s), 8.36 (1H, d, *J* = 5.3 Hz), 8.41 (1H, dd, *J* = 1.6, 4.5 Hz), 8.75 (1H, s), 8.86 (1H, s). ^{13}C NMR ($CDCl_3$) δ 14.6 (d, *J* = 3.8 Hz), 77.2, 114.7 (s), 115.2 (d, *J* = 23.1 Hz), 117.9, 121.2, 121.8, 122.0 (s), 124.4 (q, *J* = 3.7 Hz), 125.3, 125.4, 125.6 (s), 127.9 (d, *J* = 8.2 Hz), 128.9 (q, *J* = 3.1 Hz), 129.2 (d, *J* = 3.8 Hz), 129.5, 130.4 (s), 131.5 (q, *J* = 33.3 Hz), 131.9 (d, *J* = 5.5 Hz), 135.0 (s), 139.5 (d, *J* = 11.0 Hz), 143.2, 144.4, 148.2, 151.8 (s), 161.7 (d, *J* = 247.0 Hz), 164.3 (s). Anal. Calcd for $C_{26}H_{17}F_4N_5O$: C, 63.54; H, 3.49; N, 14.25. Found: C, 63.42; H, 3.50; N, 14.23.

***N*-{4-[2-(3-Methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyrimidin-2-yl}benzamide (8f).** This compound was prepared from **7f** as described in the synthesis of **8a** as a pale yellow solid (45%). Mp:

200–201 °C (ethanol). MS (ESI+): 407 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.39 (3H, s), 7.19-7.31 (3H, m), 7.44-7.53 (3H, m), 7.54-7.61 (1H, m), 7.64 (1H, d, *J* = 5.2 Hz), 7.66 (1H, s), 7.87-7.93 (2H, m), 8.08 (1H, dd, *J* = 9.3, 1.6 Hz), 8.46 (1H, dd, *J* = 4.4, 1.7 Hz), 8.61 (1H, brs), 8.81 (1H, d, *J* = 5.2 Hz). ^{13}C NMR ($CDCl_3$) δ 21.5, 77.2, 116.9, 118.5, 121.6, 125.8, 126.6, 127.5, 128.1, 128.8, 129.6, 129.8, 132.4, 133.5, 134.3, 138.1, 140.0, 143.3, 148.0, 157.3, 157.8, 159.0, 164.8 (s). Anal. Calcd for $C_{24}H_{18}N_6O$+0.2H_2O: C, 70.30; H, 4.52; N, 20.50. Found: C, 70.34; H, 4.61; N, 20.20.

***N*-{4-[2-(3-Chloro-4-fluorophenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}-3-trifluoromethyl benzamide (8g).** This compound was prepared from **7e** as described in the synthesis of **8a** as a white solid (57%). Mp: 235–237 °C (hexane/ethyl acetate). MS (ESI+): 512 (M+H). ^{1}H NMR (DMSO-d_6) δ 7.34-7.52 (3H, m), 7.55-7.64 (1H, m), 7.72-7.80 (1H, m), 7.87 (1H, dd, *J* = 2.2, 7.3 Hz), 7.94-8.01 (1H, m), 8.29 (2H, dd, *J* = 1.5, 9.2 Hz), 8.37 (1H, s), 8.50 (1H, s), 8.56 (1H, dd, *J* = 0.6, 5.1 Hz), 8.61 (1H, dd, *J* = 1.5, 4.5 Hz), 11.34 (1H, s). ^{13}C NMR (DMSO-d_6) δ 115.9 (s), 117.8 (d, *J* = 21.5 Hz), 119.8 (s), 120.4 (d, *J* = 17.6 Hz), 121.4, 122.7 (s), 125.3 (d, *J* = 3.9 Hz), 126.3, 129.0 (s), 129.1 (d, *J* = 7.7 Hz), 129.6 (d, *J* = 32.5 Hz), 130.2 (d, *J* = 15.4 Hz), 131.8 (d, *J* = 3.9 Hz), 132.7, 135.4, 138.5, 139.6, 141.1, 144.9, 149.1, 153.1, 156.0, 159.3, 165.3 (s). Anal. Calcd for $C_{25}H_{14}ClF_4N_5O$: C, 58.66; H, 2.76; N, 13.68. Found: C, 58.55; H, 2.87; N, 13.59.

***N*-{4-[2-(4-Fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}-2-methylbenzamide (8h).** This compound was prepared from 2-methylbenzoyl chloride as described in the synthesis of **8d** as a solid (65%). Mp: 265–266 °C (ethanol-tetrahydrofuran). MS (ESI+): 438 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.30 (3H, d, *J* = 1.9 Hz), 2.53 (3H, s), 6.95-7.02 (1H, m), 7.15 (1H, dd, *J* = 9.1, 4.4 Hz), 7.19 (1H, dd, *J* = 5.2, 1.7 Hz), 7.25-7.30 (2H, m), 7.36-7.42 (2H, m), 7.55-7.59 (1H, m), 7.63-7.66 (1H, m), 8.04 (1H, dd, *J* = 9.1, 1.7 Hz), 8.27 (1H, dd, *J* = 5.2, 0.8 Hz), 8.41 (1H, dd, *J* = 4.4, 1.7 Hz), 8.43 (1H, s), 8.82-8.86 (1H, m). Anal. Calcd for $C_{26}H_{20}FN_5O$: C, 71.38; H, 4.61; N, 16.01. Found: C, 71.23; H, 4.67; N, 15.89.

***N*-{4-[2-(4-Fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}-3-methylbenzamide (8i).** This compound was prepared from 3-methylbenzoyl chloride as described in the synthesis of **8d** as a white solid (80%). Mp: 203–204 °C (ethanol). MS (ESI+): 438 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.29 (3H, d, *J* = 1.7 Hz), 2.44 (3H, s), 6.92-7.02 (1H, m), 7.15 (1H, dd, *J* = 9.3, 4.4 Hz), 7.18 (1H, dd, *J* = 5.3, 1.5 Hz), 7.35-7.43 (3H, m), 7.64 (1H, dd, *J* = 7.4, 1.7 Hz), 7.68-7.74 (1H, m), 7.75 (1H, s), 8.03 (1H, dd, *J* = 9.3, 1.6 Hz), 8.33 (1H, dd, *J* = 5.3, 0.6 Hz), 8.40 (1H, dd, *J* = 4.4, 1.6 Hz), 8.70 (1H, s), 8.89 (1H, dd, *J* = 1.5, 0.6 Hz). ^{13}C NMR (DMSO-d_6) δ 14.2 (d, *J* = 3.3 Hz), 20.9 (s), 115.1 (d, *J* = 22.5 Hz), 115.3, 118.9, 120.7, 121.7 (s), 124.6 (d, *J* = 17.6 Hz), 125.1, 125.6 (s), 127.4 (d, *J* = 8.2 Hz), 128.4 (d, *J* = 22.0 Hz), 129.5 (d, *J* = 3.3 Hz), 131.4 (d, *J* = 5.5 Hz), 132.5, 133.8, 137.7, 138.3, 138.9, 142.2, 144.0, 148.4, 152.7, 166.1 (s). Anal. Calcd for $C_{26}H_{20}FN_5O$: C, 71.38; H, 4.61; N, 16.01. Found: C, 71.24; H, 4.55; N, 16.02.

***N*-{4-[2-(4-Fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}-4-methylbenzamide (8j).** This compound was prepared from 4-methylbenzoyl chloride as described in the synthesis of **8d** as a white solid (83%). Mp: 212–214 °C (ethanol). MS (ESI+): 438 (M+H). ^{1}H NMR (DMSO-d_6) δ 2.24 (3H, d, *J* = 1.5 Hz), 2.38 (3H, s), 7.11-7.19 (1H, m), 7.27-7.34 (3H, m), 7.35-7.45 (2H, m), 7.70 (1H, dd, *J* = 7.4, 1.7 Hz), 7.93 (2H, d, *J* = 8.3 Hz), 8.25 (1H, dd, *J* = 9.1, 1.5 Hz), 8.48-8.52 (2H, m), 8.58 (1H, dd, *J* = 4.3, 1.7 Hz), 10.84 (1H, s). ^{13}C NMR (DMSO-d_6) δ 14.2 (d, *J* = 3.3 Hz), 21.0, 115.0, 115.3, 118.9, 120.6, 121.7 (s), 124.6 (d, *J* = 17.6 Hz), 125.5 (s), 127.4 (d, *J* = 8.2 Hz), 128.0, 128.9 (s), 129.5 (d, *J* = 3.3 Hz), 131.1 (s), 131.4 (d, *J* = 4.9 Hz), 138.3, 138.9, 142.1, 142.2, 144.0, 148.3, 152.8, 165.9 (s). Anal. Calcd for $C_{26}H_{20}FN_5O$: C, 71.38; H, 4.61; N, 16.01. Found: C, 71.39; H, 4.63; N, 15.93.

***N*-{4-[2-(4-Fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}-3-fluorobenzamide (8k).** To a solution of **7d** (500 mg, 1.57 mmol) and pyridine (0.633 mL, 7.83 mmol) in *N*, *N*-dimethylacetamide (5 mL) was added 3-fluorobenzoyl chloride (745 mg, 4.70 mmol) at room temperature. The mixture was stirred at room temperature for 3 h; then, the mixture was evaporated. To the residue were added ethyl acetate, tetrahydrofuran, and a saturated aqueous solution of sodium

hydrogen carbonate. The organic layer was washed with brine, dried over sodium sulfate, and evaporated. To the residue were added tetrahydrofuran (20 mL) and an ammonia-ethanol solution (2.0 M, 10 mL) at room temperature; the mixture was then stirred at room temperature for 3 h. The solvent was removed by evaporation under reduced pressure, and the obtained residue was purified by silica gel chromatography (ethyl acetate: hexane = 1: 1– 1: 0) and recrystallized from hexane and ethyl acetate to give **8k** (598 mg, 86%) as a white solid. Mp: 206–207 °C. MS (ESI+): 442 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.29 (3H, d, *J* = 1.5 Hz), 6.98 (1H, t, *J* = 8.9 Hz), 7.16 (1H, dd, *J* = 9.2, 4.4 Hz). 7.21 (1H, dd, *J* = 5.1, 1.6 Hz), 7.27-7.32 (1H, m), 7.35-7.42 (1H, m), 7.44-7.54 (1H, m), 7.59-7.74 (3H, m), 8.04 (1H, dd, *J* = 9.2, 1.5 Hz), 8.35 (1H, d, *J* = 5.1 Hz), 8.41 (1H, dd, *J* = 4.4, 1.6 Hz), 8.68 (1H, brs), 8.84 (1H, s). ^{13}C NMR (DMSO-d_6) δ 14.7 (d, *J* = 3.3 Hz), 115.2, 115.5, 115.8, 115.9 (s), 119.4 (d, *J* = 20.9 Hz), 119.4, 121.4, 122.1 (s), 124.7 (d, *J* = 2.8 Hz), 125.1 (d, *J* = 17.6 Hz), 126.1 (s), 127.9 (d, *J* = 8.3 Hz), 130.0 (d, *J* = 3.3 Hz), 131.0 (d, *J* = 7.7 Hz), 131.9 (d, *J* = 5.5 Hz), 136.7 (d, *J* = 6.6 Hz), 138.9, 139.5, 142.8, 144.6, 148.9, 153.0 (s), 161.2 (d, *J* = 245.4 Hz), 162.3 (d, *J* = 244.8 Hz), 165.2 (d, *J* = 2.8 Hz). Anal. Calcd for $C_{25}H_{17}F_2N_5O$: C, 68.02; H, 3.88; N, 15.87. Found: C, 67.87; H, 3.74; N, 15.83.

***N*-{4-[2-(4-Fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}-3-chlorobenzamide (8l).** This compound was prepared from 3-chlorobenzoyl chloride as described in the synthesis of **8k** as a white solid (69%). Mp: 272–273 °C (hexane-ethyl acetate). MS (ESI+): 458 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.29 (3H, d, *J* = 1.9 Hz), 6.98 (1H, t, *J* = 8.9 Hz), 7.16 (1H, dd, *J* = 9.3, 4.4 Hz). 7.22 (1H, dd, *J* = 5.3, 1.7 Hz), 7.33-7.49 (2H, m), 7.52-7.59 (1H, m), 7.64 (1H, dd, *J* = 7.4, 1.7 Hz), 7.77-7.83 (1H, m), 7.94 (1H, t, *J* = 1.7 Hz), 8.04 (1H, dd, *J* = 9.3, 1.7 Hz), 8.35 (1H, d, *J* = 5.3 Hz), 8.41 (1H, dd, *J* = 4.4, 1.7 Hz), 8.67 (1H, s), 8.84 (1H, s). ^{13}C NMR (DMSO-d_6) δ 14.7 (d, *J* = 2.8 Hz), 115.7 (d, *J* = 22.6 Hz), 115.9, 119.4, 121.5, 122.1 (s), 125.1 (d, *J* = 17.1 Hz), 126.1, 127.3 (s), 127.9 (d, *J* = 8.3 Hz), 128.4 (s), 130.0 (d, *J* = 3.9 Hz), 130.8 (s), 131.9 (d, *J* = 5.5 Hz), 132.3, 133.7, 136.5, 138.9, 139.5, 142.8, 144.6, 148.9, 153.0 (s), 161.2 (d, *J* = 244.8 Hz), 165.2 (s). Anal. Calcd for $C_{25}H_{17}ClFN_5O$: C, 65.58; H, 3.74; N, 15.29. Found: C, 65.40; H, 3.65; N, 15.26.

***N*-{4-[2-(4-Fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}pyridin-2-carboxamide (8m).** This compound was prepared from pyridine-2-carbonyl chloride hydrochloride as described in the synthesis of **8d** as a solid (62%). Mp: 234–235 °C (ethanol-tetrahydrofuran-water). MS (ESI+): 425 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.28 (3H, d, *J* = 1.5 Hz), 6.94-7.00 (1H, m), 7.15 (1H, dd, *J* = 9.2, 4.3 Hz). 7.21 (1H, dd, *J* = 5.2, 1.4 Hz), 7.36-7.43 (1H, m), 7.49-7.53 (1H, m), 7.65 (1H, dd, *J* = 7.4, 1.6 Hz), 7.89-7.93 (1H, m), 8.04 (1H, dd, *J* = 9.2, 1.7 Hz), 8.28 (1H, d, *J* = 7.9 Hz), 8.38-8.45 (2H, m), 8.66 (1H, d, *J* = 4.1 Hz), 8.88 (1H, s), 10.67 (1H, s). ^{13}C NMR ($CDCl_3$) δ 14.6 (d, *J* = 3.3 Hz), 77.2, 114.5 (s), 115.2 (d, *J* = 22.5 Hz), 117.8, 120.8, 122.3, 122.5, 125.2, 125.5, 126.8 (s), 127.8 (d, *J* = 8.2 Hz), 129.2 (d, *J* = 3.8 Hz), 131.9 (d, *J* = 5.5 Hz), 137.6, 139.1, 139.4, 143.1, 144.2 (s), 148.4 (d, *J* = 18.1 Hz), 150.5 (d, *J* = 192.1 Hz), 162.6, 163.2 (s). Anal. Calcd for $C_{24}H_{17}FN_6O$: C, 67.92; H, 4.04; N, 19.80. Found: C, 67.74; H, 3.98; N, 19.77.

***N*-{4-[2-(4-Fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}pyridin-4-carboxamide (8n).** To a solution of **7d** (1.0 g, 3.1 mmol) in tetrahydrofuran (20 mL) were added triethylamine (1.9 g, 19 mmol) and pyridine-4-carbonyl chloride hydrochloride (1.7 g, 9.5 mmol); the mixture was stirred at room temperature for 3 h. To the mixture was added a 5% aqueous solution of sodium hydrogen carbonate, and the mixture was extracted with ethyl acetate and tetrahydrofuran. To the extract was added an ammonia-ethanol solution (2.0 M, 20 mL), and the mixture was stirred at room temperature overnight. The mixture was dried over sodium sulfate, and evaporated. The residue was purified by basic silica gel chromatography (ethyl acetate: hexane = 3: 7– 8: 2) and recrystallized from ethanol and tetrahydrofuran to give **8n** (720 mg, 54%) as a solid. Mp: 219–220 °C (ethanol-tetrahydrofuran). MS (ESI+): 425 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.29 (3H, d, *J* = 1.7 Hz), 6.95-7.01 (1H, m), 7.17 (1H, dd, *J* = 9.1, 4.4 Hz), 7.23-7.27 (1H, m), 7.34-7.41 (1H, m), 7.63 (1H, dd, *J* = 7.5, 1.9 Hz), 7.75-7.79 (2H, m), 8.05 (1H, dd, *J* = 9.1, 1.6 Hz), 8.34 (1H, d, *J* = 5.3 Hz), 8.41 (1H, dd, *J* = 4.4, 1.6 Hz), 8.80-8.86 (4H, m). ^{13}C NMR (DMSO-d_6) δ 14.2 (d, *J* = 2.7 Hz), 115.2 (d, *J* = 22.5 Hz), 115.4, 118.9 (s), 121.3 (d, *J* = 25.8 Hz), 121.8 (s), 124.6 (d, *J* = 18.1 Hz), 125.6 (s), 127.4 (d, *J* = 8.8 Hz),

129.5 (d, *J* = 3.3 Hz), 131.4 (d, *J* = 4.9 Hz), 138.4, 139.0, 141.0, 142.3, 144.1, 148.5, 150.2, 152.2, 164.7 (s). Anal. Calcd for $C_{24}H_{17}FN_6O$: C, 67.92; H, 4.04; N, 19.80. Found: C, 67.56; H, 4.06; N, 19.76.

***N*-{4-[2-(4-Fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}-6-methylpyridin-3-carboxamide (8o).** To a suspension of 6-methylpyridin-3-carboxylic acid (230 mg, 1.7 mmol) in tetrahydrofuran (5 mL) were added oxalyl chloride (210 mg, 1.7 mmol) and a catalytic amount of *N*, *N*-dimethylformamide; then, the mixture was stirred at room temperature for 30 min. The solvent was removed by evaporation under reduced pressure. A suspension of the obtained residue in tetrahydrofuran (1 mL) was added to a solution of **7d** (150 mg, 0.47 mmol) and triethylamine (170 mg, 1.7 mmol) in tetrahydrofuran (3 mL), and the mixture was stirred at room temperature for 3 h. To the mixture was added a 5% aqueous solution of sodium hydrogen carbonate, and the mixture was extracted with ethyl acetate and tetrahydrofuran. To the extract was added an ammonia-ethanol solution (2.0 M, 3 mL), and the mixture was stirred at room temperature overnight. The mixture was dried over sodium sulfate, and evaporated. The residue was purified by basic silica gel chromatography (ethyl acetate: hexane = 3: 7– 8: 2) and recrystallized from ethanol and tetrahydrofuran to give **8o** (25 mg, 12%) as a solid. Mp: 214–215 °C (ethanol-tetrahydrofuran). MS (ESI+): 439 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.29 (3H, d, *J* = 1.3 Hz), 2.66 (3H, s), 6.94-7.02 (1H, m), 7.16 (1H, dd, *J* = 9.1, 4.4 Hz), 7.22 (1H, dd, *J* = 5.1, 1.4 Hz), 7.31 (1H, d, *J* = 8.1 Hz), 7.35-7.41 (1H, m), 7.61-7.66 (1H, m), 8.04 (1H, dd, *J* = 9.1, 1.6 Hz), 8.13 (1H, dd, *J* = 8.1, 2.1 Hz), 8.36 (1H, d, *J* = 5.1 Hz), 8.41 (1H, dd, *J* = 4.4, 1.6 Hz), 8.64 (1H, s), 8.85 (1H, s), 9.06 (1H, d, *J* = 2.1 Hz). Anal. Calcd for $C_{25}H_{19}FN_6O \cdot 0.3H_2O$: C, 67.65; H, 4.45; N, 18.93. Found: C, 67.50; H, 4.36; N, 18.88.

2-Chloro-*N*-{4-[2-(4-fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}-6-methylpyridin-4-carboxamide (9a). A mixture of 2-chloro-6-methylpyridin-4-carboxylic acid (670 mg, 3.9 mmol) and phosphorus oxychloride (15 mL) was stirred at 50 °C for 1 h, and phosphorus oxychloride was removed by evaporation under reduced pressure. A suspension of the residue in tetrahydrofuran (3 mL) was added to a solution of **7d** (460 mg, 1.4 mmol) and triethylamine (790 mg,

7.8 mmol) in tetrahydrofuran (10 mL), and the mixture was stirred at room temperature for 3 h. To the mixture was added a 5% aqueous solution of sodium hydrogen carbonate, and the mixture was extracted with ethyl acetate and tetrahydrofuran. To the extract was added an ammonia-ethanol solution (2.0 M, 10 mL), and the mixture was stirred at room temperature overnight. The mixture was dried over sodium sulfate, and evaporated. The residue was purified by basic silica gel chromatography (ethyl acetate:hexane = 3: 7– 8: 2) and recrystallized from ethanol and tetrahydrofuran to give **9a** (630 mg, 92%) as a solid. Mp: 298–300 °C (ethanol-tetrahydrofuran). MS (ESI+): 473 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.30 (3H, d, *J* = 1.9 Hz), 2.64 (3H, s), 6.95-7.02 (1H, m), 7.17 (1H, dd, *J* = 9.2, 4.5 Hz), 7.25-7.29 (1H, m), 7.34-7.40 (1H, m), 7.53 (1H, d, *J* = 0.8 Hz), 7.60-7.65 (2H, m), 8.05 (1H, dd, *J* = 9.2, 1.7 Hz), 8.35 (1H, dd, *J* = 5.3, 0.8 Hz), 8.41 (1H, dd, *J* = 4.5, 1.7 Hz), 8.71 (1H, s), 8.80 (1H, s).

2-Chloro-*N*-{4-[2-(4-fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}pyridin-3-carboxamide (9b). This compound was prepared from 2-chloropyridine-3-carbonyl chloride as described in the synthesis of **8d** as a solid (73%). MS (ESI+): 459 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.30 (3H, d, *J* = 1.1 Hz), 6.96-7.02 (1H, m), 7.17 (1H, dd, *J* = 9.2, 4.5 Hz). 7.23-7.31 (1H, m), 7.36-7.43 (2H, m), 7.64 (1H, dd, *J* = 7.4, 1.6 Hz), 8.05 (1H, dd, *J* = 9.2, 1.5 Hz), 8.17 (1H, dd, *J* = 7.6, 2.0 Hz), 8.30 (1H, d, *J* = 5.3 Hz), 8.42 (1H, dd, *J* = 4.5, 1.5 Hz), 8.54 (1H, dd, *J* = 4.8, 2.0 Hz), 8.81 (1H, s), 9.09 (1H, s).

***N*-{4-[2-(4-Fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}-2-methylpyridin-4-carboxamide (10a).** A suspension of **9a** (9.0 g, 19 mmol), 10% palladium carbon powder (50% water containing product) (1.8 g), and triethylamine (3.9 g, 38 mmol) in *N*, *N*-dimethylformamide (45 mL) was stirred under hydrogen atmosphere of 0.5 MPa at 50 °C for 8 h. The mixture was cooled to room temperature and filtered, and the solvent was removed by evaporation under reduced pressure. A saturated aqueous solution of sodium hydrogen carbonate was added to the residue, and the mixture was extracted with ethyl acetate and tetrahydrofuran. The organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was purified by basic silica gel chromatography (ethyl acetate: hexane = 3: 7–8: 2) and recrystallized from ethanol to give **10a** (4.4 g, 52%) as a solid. Mp:

200–202 °C (ethanol). MS (ESI+): 439 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.30 (3H, d, *J* = 1.5 Hz), 2.68 (3H, s), 6.95-7.02 (1H, m), 7.17 (1H, dd, *J* = 9.1, 4.4 Hz), 7.23-7.28 (1H, m), 7.35-7.41 (1H, m), 7.53-7.56 (1H, m), 7.61-7.66 (2H, m), 8.05 (1H, dd, *J* = 9.1, 1.6 Hz), 8.36 (1H, d, *J* = 5.3 Hz), 8.41 (1H, dd, *J* = 4.4, 1.6 Hz), 8.66-8.73 (2H, m), 8.84 (1H, s). ^{13}C NMR (DMSO-d_6) δ 14.7 (d, *J* = 3.3 Hz), 24.6, 115.5, 115.8, 119.4, 119.4, 121.6, 122.0 (s), 125.1 (d, *J* = 17.6 Hz), 126.1 (s), 128.0 (d, *J* = 8.3 Hz), 130.0 (d, *J* = 3.9 Hz), 131.9 (d, *J* = 5.0 Hz), 138.9, 139.5, 141.8, 142.9, 144.6, 149.0, 150.0, 152.8, 159.2 (s), 161.2 (d, *J* = 244.8 Hz), 165.4 (s). Anal. Calcd for $C_{25}H_{19}FN_6O$: C, 68.48; H, 4.37; N, 19.17. Found: C, 68.10; H, 4.25; N, 19.03.

***N*-{4-[2-(4-Fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}pyridin-3-carboxamide (10b).** This compound was prepared from **9b** as described in the synthesis of **10a** as a solid (36%). Mp: 221-222 °C (ethanol/tetrahydrofuran/H_2O). MS (ESI+): 425 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.29 (3H, d, *J* = 1.7 Hz), 6.95-7.01 (1H, m), 7.17 (1H, dd, *J* = 9.1, 4.4 Hz). 7.22 (1H, dd, *J* = 5.2, 1.7 Hz), 7.35-7.41 (1H, m), 7.44-7.49 (1H, m), 7.64 (1H, dd, *J* = 7.4, 1.9 Hz), 8.05 (1H, dd, *J* = 9.1, 1.7 Hz), 8.22-8.27 (1H, m), 8.30-8.34 (1H, m), 8.41 (1H, dd, *J* = 4.4, 1.7 Hz), 8.81 (1H, dd, *J* = 4.7, 1.7 Hz), 8.84-8.87 (1H, m), 8.89 (1H, s), 9.18 (1H, d, *J* = 1.9 Hz). Anal. Calcd for $C_{24}H_{17}FN_6O$: C, 67.92; H, 4.04; N, 19.80. Found: C, 67.59; H, 3.99; N, 19.59.

Methyl 2-methylpyridin-4-carboxylate (12a). Methyl 2-chloro-6-methylpyridin-4-carboxylate (15.0 g, 80.8 mmol), 10% palladium carbon powder (50% water containing product) (5.00 g), and triethylamine (22.5 mL, 162 mmol) were stirred in *N*, *N*-dimethylformamide (200 mL) under hydrogen atmosphere, at room temperature, for 15 h. The mixture was filtered, and the solvent was removed by evaporation under reduced pressure. The residue was purified by silica gel chromatography (ethyl acetate: methanol = 100: 0– 50: 1) to give the title compound (14.8 g, 61%) as an oil. ^{1}H NMR ($CDCl_3$) δ 2.64 (3H, s), 3.95 (3H, s), 7.64 (1H, dd, *J* = 5.2, 0.8 Hz), 7.72 (1H, s), 8.65 (1H, d, *J* = 4.7 Hz).

Methyl 2-methylpyridin-4-carboxylate 1-oxide (13a). To a solution of **12a** (9.00 g, 59.5 mmol) in acetic acid (120 mL) was added hydroxyperoxide urea (22.4 g, 238 mmol) at room temperature. The

mixture was stirred at 70 °C, under nitrogen atmosphere, for 15 h. After being cooled to room temperature, the mixture was poured onto ice (90 g). The mixture was neutralized with an 8N aqueous solution of sodium hydroxide at 0 °C. To the mixture were added ethyl acetate and sodium hydrogen carbonate. The organic layer was washed with a 5% aqueous solution of sodium hydrogen carbonate, dried over sodium sulfate, and evaporated to give **13a** as a solid (10 g); this compound was used in the next reaction. ^{1}H NMR ($CDCl_3$) δ 2.54 (3H, s), 3.94 (3H, s), 7.74 (1H, dd, J = 6.7, 2.4 Hz), 7.89 (1H, d, J = 2.4 Hz), 8.27 (1H, d, J = 6.8 Hz).

Methyl 6-methylpyridin-3-carboxylate 1-oxide (13b). This compound was prepared from methyl 6-methylpyridin-3-carboxylate (**12b**) as described in the synthesis of **13a** as a solid**,** and used in the next reaction. ^{1}H NMR (DMSO-d_6) δ 2.42 (3H, s), 3.88 (3H, s), 7.61-7.66 (1H, m), 7.68-7.74 (1H, m), 8.58 (1H, d, J = 1.1 Hz).

2-Methylpyridin-4-carboxylic acid 1-oxide (14a). A solution of **13a** (10.0 g) and an 8N aqueous solution of sodium hydroxide (30 mL) in tetrahydrofuran (60 mL), methanol (60 mL), and water (50 mL) was stirred at 70 °C for 15 h. The mixture was evaporated. To the residual solution was added a 1N aqueous solution of hydrogen chloride (240 mL) at room temperature. The resulting solid was collected on a filter, washed with water, and dried in vacuo to give **14a** as a solid (3.90 g, 43% from **10a**). ^{1}H NMR ($CDCl_3$) δ 2.38 (3H, s), 7.70 (1H, dd, J = 6.5, 2.5 Hz), 7.95 (1H, d, J = 2.4 Hz), 8.32 (1H, d, J = 6.8 Hz), 13.48 (1H, brs).

6-Methylpyridin-3-carboxylic acid 1-oxide (14b). This compound was prepared from **13b** as described in the synthesis of **14a** as a solid**,** and used in the next reaction. ^{1}H NMR (DMSO-d_6) δ 2.41 (3H, s), 7.56-7.64 (1H, m), 7.67-7.72 (1H, m), 8.54 (1H, d, J = 1.5 Hz), 13.75 (1H, brs).

***N*-{4-[2-(4-Fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}-2-methylpyridin-4-carboxamide 1-oxide (15a).** To a suspension of **7d** (3.00 g, 9.40 mmol), **14a** (2.16 g, 14.1 mmol), and *N*,*N*-diisopropylethylamine (5.51 mL, 31.9 mmol) in pyridine (50 mL) was added 1-

[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU; 6.07 g, 16.0 mmol) at 70 °C. The mixture was stirred at 70 °C for 3 h and then evaporated. The residue was washed with a saturated aqueous solution of sodium hydrogen carbonate, water, and ethyl acetate to give a solid. The solid was purified by silica gel chromatography (ethyl acetate: hexane = 1: 6– 4: 1) and recrystallized from hexane and ethyl acetate to give **15a** (2.50 g, 59%) as a white solid. The filtrate was recrystallized from acetic acid and ethanol to give **15a** (1.51 g, 35%) as a white solid. Mp: 245–246 °C (hexane-ethyl acetate). MS (ESI+): 455 (M+H). ^{1}H NMR ($CDCl_3$) δ 2.25 (3H, s), 2.40 (3H, s), 7.16 (1H, t, *J* = 9.0 Hz), 7.31-7.45 (3H, m), 7.66-7.72 (1H, m), 7.88 (1H, dd, *J* = 6.5, 2.5 Hz), 8.15 (1H, brs), 8.26 (1H, d, *J* = 9.4 Hz), 8.36 (1H, d, *J* = 6.8 Hz), 8.46-8.62 (3H, m), 11.13 (1H, brs). ^{13}C NMR ($CDCl_3$) δ 14.6 (d, *J* = 3.3 Hz), 18.0, 77.2, 114.7 (s), 115.3 (d, *J* = 23.1 Hz), 118.0, 121.4 (s), 123.3 (d, *J* = 237.1 Hz), 121.9, 125.4, 125.6 (s), 127.9 (d, *J* = 8.8 Hz), 129.2 (d, *J* = 3.9 Hz), 129.6 (s), 132.0 (d, *J* = 5.5 Hz), 139.6 (d, *J* = 8.8 Hz), 139.7, 143.2, 144.5, 148.2, 149.7, 151.5, 160.1, 162.1, 163.3 (s). Anal. Calcd for $C_{25}H_{19}FN_6O_2 \cdot 0.2H_2O$: C, 65.55; H, 4.27; N, 18.35. Found: C, 65.35; H, 4.22; N, 18.28.

***N*-{4-[2-(4-Fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}-6-methylpyridin-3-carboxamide 1-oxide (15b).** This compound was prepared from **14b** as described in the synthesis of **15a** as a solid (52%). Mp: 262–263 °C. MS (ESI+): 455 (M+H). ^{1}H NMR (DMSO-d_6) δ 2.25 (3H, s), 2.42 (3H, s), 7.16 (1H, t, *J* = 9.0 Hz), 7.31-7.45 (3H, m), 7.62 (1H, d, *J* = 7.9 Hz), 7.69 (1H, dd, *J* = 7.2, 1.5 Hz), 7.80 (1H, dd, *J* = 7.9, 1.5 Hz), 8.26 (1H, dd, *J* = 9.0, 1.5 Hz), 8.47 (1H, s), 8.52 (1H, d, *J* = 4.9 Hz), 8.58 (1H, dd, *J* = 4.3, 1.3 Hz), 8.78 (1H, s), 11.25 (1H, s). ^{13}C NMR ($CDCl_3$) δ 14.6 (d, *J* = 3.3 Hz), 18.1, 77.2, 114.9 (s), 115.2 (d, *J* = 23.1 Hz), 118.0, 121.4, 121.9, 123.7, 125.3, 125.6, 126.4 (s), 127.8 (d, *J* = 8.2 Hz), 129.1 (d, *J* = 3.8 Hz), 131.4 (s), 131.9 (d, *J* = 5.5 Hz), 138.9 (s), 139.5 (d, *J* = 14.8 Hz), 143.2, 144.5, 148.3, 151.5, 152.5, 161.6, 163.3 (s). Anal. Calcd for $C_{25}H_{19}FN_6O_2 \cdot 0.6H_2O$: C, 64.54; H, 4.38; N, 18.06. Found: C, 64.51; H, 4.17; N, 17.80.

***N*-{4-[2-(4-Fluoro-3-methylphenyl)imidazo[1,2-*b*]pyridazin-3-yl]pyridin-2-yl}-2-methylpyridin-4-carboxamide 1-oxide sulfate (16).** To a solution of **15a** (1.00 g, 2.20 mmol) in acetic acid (4 mL) were added sulfuric acid (0.120 mL, 2.24 mmol) and ethanol (16 mL) at 100 °C. The mixture was stirred at 100 °C for 2 h. After being cooled to room temperature, the resulting solid was collected on a filter, washed with ethanol, and dried in vacuo to give **16** (1.05 g, 87%) as a pale yellow solid. Mp: 276–277°C. ^{1}H NMR (DMSO-d_6) δ 2.25 (3H, s), 2.41 (3H, s), 7.17 (1H, t, *J* = 9.1 Hz), 7.36 (1H, dd, *J* = 5.2, 1.5 Hz), 7.38-7.47 (2H, m), 7.69 (1H, dd, *J* = 7.4, 1.5 Hz), 7.89 (1H, dd, *J* = 6.8, 2.4 Hz), 8.16 (1H, d, *J* = 2.4 Hz), 8.29 (1H, dd, *J* = 9.1, 1.5 Hz), 8.38 (1H, d, *J* = 6.8 Hz), 8.48 (1H, s), 8.53 (1H, d, *J* = 5.2 Hz), 8.62 (1H, dd, *J* = 4.3, 1.5 Hz), 11.22 (1H, brs). ^{13}C NMR (DMSO-d_6) δ 14.7 (d, *J* = 2.8 Hz), 17.7, 115.7, 115.8, 116.0, 120.2 (s), 121.7 (d, *J* = 34.7 Hz), 123.5 (s), 125.3 (d, *J* = 17.6 Hz), 125.8, 126.1 (s), 128.2 (d, *J* = 8.8 Hz), 129.0, 129.4 (s), 132.0 (d, *J* = 5.5 Hz), 139.3, 139.4, 142.5, 145.0, 148.0, 148.5, 152.4 (s), 161.4 (d, *J* = 245.4 Hz), 161.6, 163.7 (s). Anal. Calcd for $C_{25}H_{19}FN_6O_2 \cdot H_2SO_4$: C, 54.34; H, 3.83; N, 15.21. Found: C, 54.09; H, 3.82; N, 15.21.

5.2. DMPK

Solubility. Small volumes of the compound solution dissolved in DMSO were added to the aqueous buffer solution (pH 6.8). After incubation, precipitates were separated by filtration. The solubility was determined by HPLC analysis of each filtrate.

Microsomal stability. Human and rat liver microsomes were purchased from Sekisui XenoTech, LLC. (Kansas City, KS, USA). The microsomes (0.2 mg protein/mL), and the compound (1 μM) were mixed in phosphate buffer (pH 7.4) under ice-cold conditions. The reactions were initiated by adding an NADPH generating system (a mixture of $MgCl_2$, glucose-6-phosphate, β-$NADP^+$, and glucose-6-phosphate dehydrogenase) to the mixtures before incubation. Incubations were conducted at 37°C for 20 min and terminated by adding acetonitrile. The zero-time incubations, which served as the controls, were terminated by adding ice-cold acetonitrile before adding each compound. After the samples were

mixed and centrifuged, the compound concentration in the supernatant fractions were measured by HPLC.

Inhibition of CYP3A4. The inhibitory activity of the compounds against CYP3A4 was evaluated by incubating 100 μM testosterone with CYP3A4-expressing microsomes (Corning Incorporated, Corning, NY, USA) in the presence of 10 μM of each compound. The concentration of 6β-hydroxytestosterone was measured by HPLC.

Pharmacokinetic profile in rats. Each compound was intravenously (0.2 mg/kg) and orally (3 mg/kg) administered to male Crl:CD (SD) rats (8 weeks old, Charles River Laboratories Japan, Inc., Yokohama, Japan). After the administration, blood samples were collected and centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatants were diluted with 0.01 M ammonium formate solution containing 0.2% formic acid and centrifuged again. The concentrations of the compounds in the supernatant were measured by LC/MS/MS (SIL-10AHc, Shimadzu, Kyoto, Japan; API3000, AB Sciex, Framingham, MA, USA).

5.3. Metabolite

Instrument. The LC/MS system consisted of an Agilent 1100 system (Agilent Technologies, Santa Clara, CA) and an LCQ-Deca ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) equipped with an electrospray ionization source.

Materials and methods

In vitro metabolism for structural analysis. In vitro metabolic reaction mixture (250 μL) contained 50 mM potassium phosphate buffer (pH 7.4), 10 μM substrate, and human liver microsomes (at a final protein concentration of 0.4 mg/mL; XenoTech, LLC., Lenexa, KS, USA). The reactions were initiated by the addition of NADPH-regenerating reagents (at final concentrations as 0.5 mM β-NADP+, 5 mM β-D-glucose 6-phosphate, 1.5 unit/mL glucose-6-phosphate dehydrogenase, and 5 mM magnesium

chloride) and conducted at 37 °C. At 0 and 30 min, the reactions were terminated by the addition of an equal volume of acetonitrile. After centrifugation at 21,600 g for 10 min, 10 μL of the supernatant was injected into an LC/MS system for metabolite profiling.

LC/MS analysis. Microsomal incubations were separated on an Inertsil ODS-3 column (5 μm, 2.1 x 150 mm; GL Sciences, Tokyo, Japan) using solvent A (10% acetonitrile in 10 mM aqueous ammonium acetate) and solvent B (90% acetonitrile in 10 mM aqueous ammonium acetate). At a flow rate of 0.2 mL/min, the initial elution gradient was 80% solvent A and 20% solvent B, with a linear gradient of 60% solvent B over 45 min, and a return to initial conditions. The column was allowed to equilibrate at 20% solvent B for 15 min before the next injection. The column temperature was 40 °C and UV detection was conducted at 300 nm. The mass spectrometer was run in positive ion mode; the source settings were as follows: spray voltage = 5 kV, sheath gas flow rate = 80, auxiliary gas flow rate = 20, capillary voltage = 11 V, capillary temperature = 350 °C.

The LC/UV/MS analytical data for **10a** and the main metabolite, MS3, are summarized in Table 4. The compound **10a,** eluting at 33.4 min, produced the protonated molecule $[M+H]^+$ at *m/z* 439 in full scan mass spectrum. The product ion spectra of **10a** exhibited fragment ions at *m/z* 411 as a base peak and at *m/z* 303, *m/z* 321, and *m/z* 421. The main metabolite, MS3, eluted at the retention time of 23.0 min, produced the protonated molecule $[M+H]^+$ at *m/z* 455, which was a gain of 16u over that of **10a**. The product ion spectra of *m/z* 455 contained *m/z* 438 as a base peak and *m/z* 303, *m/z* 321, and *m/z* 409. In particular, the product ion, at *m/z* 438, was considered to result from the cleavage of the hydroxyl radical. The other fragment ions were similar to **10a**, indicating that the amide side chain was oxidized. The main metabolite, MS3, was the predicted *N*-oxide analog (Figure 5).

5.4. Biology

p38 MAP kinase enzyme assay. The FLAG-tagged human p38-α protein was expressed with a baculovirus system and activated by constitutive active human MKK3. Then, the recombinant p38-α

protein was purified using the anti-FLAG antibody affinity agarose gel (Sigma). Kinase reactions were evaluated with LanthaScreen assay system (Life Technologies, USA); 2.5 μL of test compounds, diluted with DMSO (final concentration 1% DMSO), were added to the reaction mixture (25 mM HEPES [pH7.5], 10 mM Mg acetate, 1 mM DTT, 0.01% Tween-20, and 0.01% BSA) containing 125 pg human p38-α protein and 8 nM GFP-ATF2 (19-96) (Life Technologies) in 384-well plates (Nunc, USA). After a 5 min incubation at room temperature, the reaction was started by adding 5 μL of 580 μM ATP. After a 20 min incubation, the reaction was terminated by adding 5 μL of 80 mM EDTA. Then, 5 μL of the Tb-anti-phospho ATF2 (pThr71) antibody (Life Technologies) was added and incubated for 60 min at room temperature. The time-resolved fluorescence resonance energy transfer (TR-FRET) signal was measured using EnVision Multilabel Plate Reader (PerkinElmer).

TNF-α production assay. THP-1 cells were suspended in RPMI 1640 medium (Life Technologies) containing 1% fetal bovine serum (Morgate, Australia). Then, 40 μL of the cell suspension ($0.625X10^6$ cells/mL) was added to 384-well plates (Corning, USA) and mixed with 5 μL of test compounds diluted with 10% DMSO. After 60 min incubation at 37 °C and 5% CO_2, the cells were stimulated with 5 μL of 100 μg/mL LPS (Wako). After incubating for 4 h at 37°C and 5% CO_2, the concentration of TNF-α in the medium was measured with the TNF-α HTRF kit (CisBio, USA). The TR-FRET signal was measured with EnVision Multilabel Plate Reader (PerkinElmer).

Rat collagen model of induced arthritis. Rat CIA was induced in female, 6-week-old Lewis rats as follows. Bovine type II collagen (Collagen research center, Tokyo, Japan) was dissolved in 0.05 M acetic acid at a concentration of 3 mg/mL and emulsified in an equal volume of Freund's Incomplete Adjuvant (FIA, Difco, Detroit, USA). Then, 0.5 mL of the emulsion was administered intradermally into five sites on the backs of the rats. After 7 days (Day 0), 0.2 mL of the emulsion was re-injected into the base of the tail. On day 0 and 4, the volumes of both hind paws were measured using a Plethysmometer (UGO BASIL, Varese, Italy). The CIA rats were divided based on body weight and increase in paw volume (n=8 per group) on day 4: one group received corn oil, while the other five

groups received compounds suspended in corn oil at a dosage of 0.1-10 mg/kg. The increase in paw volume was expressed as the value of paw volume measured on day 4, minus the value of the paw volume measured on day 0, in each CIA rat. The compounds were administered to rats twice a day from day 4 to day 14. The same paw was subsequently measured on day 14. Differences between the means of the control and drug-treated groups were analyzed using the Dunnett test.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. A. K., M. T., T. T., O. U., and S. M. contributed design and synthesis of compounds; M. G., T. M., Y. H., S. U., T. K., T. H., and M. S. conducted in vitro and in vivo study; T. T., S. I., T. T., K. O., G. S., K. B., and B. S. contributed x-ray crystallography.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

MAP, mitogen-activated protein; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; RA, rheumatoid arthritis; IBD, inflammatory bowel disease; COPD, chronic obstructive pulmonary disease; LPS, lipopolysaccharide; DMPK, drug metabolism and pharmacokinetics; CIA, collagen-induced arthritis; SAR, structure-activity relationships.

REFERENCES

1. Paul, A.; Wilson, S.; Belham, C. M.; Robinson, C. J. M.; Scott, P. H.; Gould, G. W.; Plevin, R. Stress-activated Protein Kinases: Activation, Regulation and Function. *Cell. Signal.* **1997**, *9*, 403-410.

2. Lee, J. C.; Laydon, J. T.; McDonnell, P. C.; Gallagher, T. F.; Kimer, S.; Green, D.; McNulty, D.; Blumenthal, M. J.; Heys, J. R.; Landvatter, S. W.; Stickler, J. E.; McLaughlin, M. M.; Siemens, I. R.; Fisher, S. M.; Livi, G. P.; White, J. R.; Adams, J. L.; Young, P. R. A Protein Kinase Involved in the Regulation of Inflammatory Cytokine Biosynthesis. *Nature* **1994**, *372*, 739-745.

3. Suzuki, M.; Tetsuka, T.; Yoshida, S.; Watanabe, N.; Kobayashi, M.; Matsui, N.; Okamoto, T. The Role of p38 Mitogen-activated Protein Kinase in IL-6 and IL-8 Production from TNF-α or IL-1β-stimulated rheumatoid synovial fibroblasts. *FEBS Lett.* **2000**, *465*, 23-27.

4. Chen, Z.; Gibson, T. B.; Robinson, F.; Silvestro, L.; Rearson, G.; Xu, B.; Wright, A.; Vanderbilt, C.; Cobb, M. H. MAP Kinases. *Chem. Rev.* **2001**, *101*, 2449-2476.

5. Raingeaud, J.; Gupta, S.; Rogers, J. S.; Dickens, M.; Han, J.; Ulevitch, R. J.; Davis, R. J. Pro-inflammatory Cytokines and Environmental Stress Cause p38 Mitogen-activated Protein Kinase Activation by Dual Phosphorylation on Tyrosine and Threonine. *J. Biol. Chem.* **1995**, *270*, 7420-7426.

6. Görtz, B.; Hayer, S.; Tuerck, B.; Zwerina, J.; Smolen, J. S.; Schett, G. Tumour Necrosis Factor Activates the Mitogen-activated Protein Kinases p38α and ERK in the Synovial Membrane In Vivo. *Arthritis Res. Ther.* **2005**, *7*, R1140-R1147.

7. Schett, G.; Tohidast-Akrad, M.; Smalen, J. S.; Schmid, B. J.;Steiner, C. W.; Bitzan, P.; Zenz, P.; Redlich, K.; Xu, Q.; Steiner G. Activation, Differential Localization, and Regulation of the Stress-activated Protein Kinases, Extracellular Signal-regulated Kinase, c-Jun N-terminal Kinase, and p38 Mitogen-activated Protein Kinase, in Synovial Tissue and Cells in Rheumatoid Arthritis. *Arthritis Rheum.* **2000**, *43*, 2501-2512.

8. Kumar, S.; Boehm, J.; Lee, J. C. p38 MAP Kinases: key Signaling Molecules as Therapeutic Targets for Inflammatory Diseases. *Nature Rev. Drug Discov.* **2003**, *2*, 717-726.

9. Fitzgerald, M. F.; Fox, J. C. Emerging Trends in the Therapy of COPD: Novel Anti-inflammatory Agents in Clinical Development. *Drug Discov. Today* **2007**, *12*, 479-486.

10. Babu, K. S.; Morjaria, J. B. Emerging Therapeutic Strategies in COPD. *Drug Discov. Today* **2015**, *20*, 371-379.

11. Goldstein, D. M.; Kuglstatter, A.; Lou, Y.; Soth, M. J. Selective p38α Inhibitors Clinically Evaluated for the Treatment of Chronic Inflammatory Disorders. *J. Med. Chem.*, **2010**, *53*, 2345-2353.

12. Xing, L.; Devadas, B.; Devraj, R. V.; Selness, S. R.; Shieh, H.; Walker, J. K.; Mao, M.; Messing, D.; Samas, B.; Yang, J. Z.; Anderson, G. D.; Webb, E. G.; Monahan, J. B. Discovery and characterization of atropisomer PH-797804, a p38 MAP kinase inhibitor, as a clinical drug candidate. *ChemMedChem*, **2012**, *7*, 273-280.

13. Selness, S. R.; Devraj, R. V.; Devadas, B.; Walker, J. K.; Boehm, T. L.; Durley, R. C.; Shieh, H.; Xing, L.; Rucker, P. V.; Jerome, K. D.; Benson, A. G.; Marrufo, L. D.; Madsen, H. M.; Hitchcock, J.; Owen, T. J.; Christie, L.; Promo, M. A.; Hickory, B. S.; Alvira, E.; Naing, W.; Blevis-Bal, R.; Messing, D.; Yang, J. Z.; Mao, M. K.; Yalamanchili, G.; Embse, R. V.; Hirsch, J. Discovery of PH-797804, a highly selective and potent inhibitor of p38 MAP kinase. *Bioorg. Med. Chem. Lett.*, **2011**, *21*, 4066-4071.

14. Walker, J. K.; Selness, S. R.; Devraj, R. V.; Hepperle, M. E.; Naing, W.; Shieh, H.; Kurambail, R.; Yang, S.; Flynn, D. L.; Benson, A. G.; Messing, D. M.; Dice, T.; Kim, T.; Lindmark, R. J.; Monahan, J. B.; Portanova, J. Identification of SD-0006, a potent diaryl pyrazole inhibitor of p38 MAP kinase. *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 2634-2638.

15. Hasumi, K.; Sato, S.; Saito, T.; Kato, J.; Shirota, K.; Sato, J.; Suzuki, H.; Ohta, S. Design and synthesis of 5-[(2-chloro-6-fluorophenyl)acetylamino]-3-(4-fluorophenyl)-4-(4-pyrimidinyl)isoxazole (AKP-001), a novel inhibitor of p38 MAP kinase with reduced side effects based on the antedrug concept. *Bioorg. Med. Chem.*, **2014**, *22*, 4162-4176.

16. Asano, T.; Yamazaki, H.; Kasahara, C.; Kubota, H.; Kontani, T.; Harayama, Y.; Ohno, K.; Mizuhara, H.; Yokomoto, M.; Misumi, K.; Kinoshita, T.; Ohta, M.; Takeuchi, M. Identification, synthesis, and biological evaluation of 6-[(6*R*)-2-(4-fluorophenyl)-6-(hydroxymethyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (AS1940477), a potent p38 MAP kinase inhibitor. *J. Med. Chem.* **2012**, *55*, 7772-7785.

17. Heo, J.; Shin, H.; Lee, J.; Kim, T.; Inn, K. S.; Kim, N. J. Synthesis and biological evaluation of N-cyclopropylbenzamide-benzophenone hybrids as novel and selective p38 mitogen activated protein kinase (MAPK) inhibitors. *Bioorg. Med. Chem. Lett.*, **2015**, *25*, 3694-3698.

18. Choi, M. S.; Heo, J.; Yi, C. M.; Ban, J.; Lee, N. J.; Lee, N. R.; Kim, S. W.; Kim, N. J.; Inn, K. S. A novel p38 mitogen activated protein kinase (MAPK) specific inhibitor suppresses respiratory syncytial

virus and influenza A virus replication by inhibiting virus-induced p38 MAPK activation. *Biochem. Biophys. Res. Commun.*, **2016**, *477*, 311-316.

19. Lee, M. R.; Dominguez, C. MAP Kinase p38Inhibitors: Clinical Results and an Intimate Look at Their Interactions with p38αProtein. *Curr. Med. Chem.*, **2005**, *12*, 2979-2994.

20. Cuenda, A.; Rouse, J.; Doza, Y. N.; Meier, R.; Cohen, P.; Gallagher, T. F.; Young, P. R.; Lee, J. C. SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. *FEBS Lett.*, **1995**, *364*, 229-233.

21. Mavunkel, B. J.; Chakravarty, S.; Perumattam, J.; Dugar, S.; Lu, Q.; Liang, X. Preparation of 5-[4-Benzylpiperidinyl(piperazinyl)]-indolecarboxamides as Inhibitors of p38 Kinase. *WO2000071535*, **2000**.

22. Nikas, S. N.; Drosos, A. A. SCIO-469 Scios Inc. *Curr. Opin. Invest. Drugs*, **2004**, *5*, 1205–1212.

23. Haddad, J. J. VX-745. Vertex Pharmaceuticals. *Curr. Opin. Invest. Drugs*, **2001**, *2*, 1070–1076.

24. Natarajan, S. R.; Doherty, J. B. *Curr. Top. Med. Chem.*, **2005**, *5*, 987.

25. Regan, J.; Breitfelder, S.; Cirillo, P.; Gilmore, T.; Graham, A. G.; Hickey, E.; Klaus, B.; Madwed, J.; Moriak, M.; Moss, N.; Pargellis, C.; Pav, S.; Proto, A.; Swinamer, A.; Tong, L.; Torcellini, C. Pyrazole urea-based inhibitors of p38 MAP kinase: from lead compound to clinical candidate. *J. Med. Chem.*, **2002**, *45*, 2994-3008.

26. Miwatashi, S.; Arikawa, Y.; Kotani, E.; Miyamoto, M.; Naruo, K.; Kimura, H.; Tanaka, T.; Asahi, S.; Ohkawa, S. Novel Inhibitor o p38 MAP Kinase as an Anti-TNF-α drug: Discovery of *N*-[4-[2-ethyl-4-(3-methylphenyl)-1,3-thiazol-5-yl]-2-pyridyl]benzamide (TAK-715) as a Potent and Orally Active Anti-rheumatoid Arthritis Agent. *J. Med. Chem.* **2005**, *48*, 5966-5979.

27. Miwatashi, S.; Arikawa, Y.; Naruo, K.; Igaki, K.; Watanabe, Y.; Kimura, H.; Kawamoto, T.; Ohkawa, S. Synthesis and Biological Activities of 4-Phenyl-5-pyridyl-1,3-thiazole Derivatives as p38 MAP Kinase Inhibitors. *Chem. Pharm. Bull.* **2005**, *53*, 410-418.

28. Azevedo, R.; Zeeland, M. V.; Raaijmakers, H.; Kazemier, B.; Vlieg, J. D.; Oubrie, A. X-ray structure of p38a bound to TAK-715: comparison with three classic inhibitors. *Acta Crystallogr.D. Biol. Crystallogr.* **2012**, *68*, 1041-1050.

29. Verhoest, P. R.; Chapin, D. S.; Corman, M.; Fonseca, K.; Harms, J. F.; Hou, X.; Marr, E. S.; Menniti, F. S.; Nelson, F.; O'Connor, R.; Pandit, J.; Proulx-LaFrance, C.; Schmidt, A. W.; Schmidt, C. J.; Suiciak, J. A.; Liras, S. Discovery of a Novel Class of Phosphodiesterase 10A Inhibitors and Identification of Clinical Candidate 2-[4-(1-methyl-4-pyridin-4-yl-1*H*-pyrazol-3-yl)-phenoxymethyl]-quinoline (PF-2545920) for the Treatment of Schizophrenia. *J. Med. Chem.* **2009**, *52*, 5188-5196.

30. Zlokarnik, G.; Grootenhuis, P. D. J.; Watson, J. B. High Throughput P450 Inhibition Screens in Early Drug Discovery. *Drug Discov. Today* **2005**, *10*, 1443-1450.

Graphical Abstract

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Structure-based Design, Synthesis, and Biological Evaluation of Imidazo[1,2-*b*]pyridazine-based p38 MAP Kinase Inhibitors

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