



Discovery of a highly selective JAK2 inhibitor with a furopyrimidine scaffold

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ABSTRACT

Janus kinases (JAKs) mediate cytokine and growth factor signaling by phosphorylating STAT proteins, playing a critical role in immune regulation. Among JAK family, JAK2 drives hematopoietic and immune signaling. Its dysregulation promotes myeloproliferative neoplasms (MPN) by impairing hematopoietic stem cell function. Selective JAK2 inhibition offers a promising therapeutic strategy for effective and safe MPN treatment. Starting from a non-selective JAK inhibitor, we synthesized a series of furopyrimidine derivatives to discover potent, JAK2-selective inhibitors. Structural optimization led to potent JAK2 inhibitors with improved selectivity. Further refinements led to compound **14**, which exhibited approximately 10-fold selectivity for JAK2 over JAK1 and negligible inhibition of JAK3 and TYK2 in biochemical assays. These findings provide a framework for designing novel JAK2-selective inhibitors with improved potency and specificity for MPN therapy.

1. Introduction

The Janus kinase (JAK) family comprises of four non-receptor tyrosine kinases, JAK1, JAK2, JAK3, and TYK2. These kinases are associated with the intracellular domains of various cytokine receptors and are indispensable for transducing extracellular signals into cellular responses.¹ Despite their structural similarity, each JAK family enzyme partners with distinct receptor subtypes and regulates unique biological functions.^{1,2} Thus, selective inhibition of a single JAK could modulate a specific biological function while the activity of the other JAKs remains preserved.

Activation of the JAK family kinases occurs upon cytokine binding to their cognate receptors, leading to receptor dimerization and subsequent trans-phosphorylation of JAKs. Activated JAKs phosphorylate STAT proteins, which then dimerize and translocate into the nucleus to regulate the transcription of target genes [1]. This JAK–STAT pathway plays a pivotal role in orchestrating immune responses, inflammation, and cellular differentiation. Dysregulated JAK–STAT signaling has been strongly implicated in the pathogenesis of autoimmune and inflammatory diseases, including rheumatoid arthritis (RA), inflammatory bowel disease (IBD), psoriasis, alopecia areata (AA), and atopic dermatitis (AD) [2] [3]. Excessive activation of this pathway results in aberrant immune

cell proliferation and cytokine production, contributing to chronic inflammation and tissue damage. For this reason, several drugs targeting the JAK–STAT pathway are currently under development [2] [3].

Among the four members of the JAK family, JAK2 plays a particularly critical role in both hematologic and immune-mediated disorders. It mediates signaling through receptors for erythropoietin, thrombopoietin, and several interleukins, thereby regulating hematopoiesis and immune responses. Pathologically, a gain-of-function mutation such as JAK2 V617F is a well-established driver of myeloproliferative neoplasms (MPNs), including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) [4]. The V617F mutation has been detected in 50–60% of patients with ET and PMF [5]. In addition, aberrant activation of JAK2 has been observed in pediatric leukemia [6], where it promotes PD-L1 expression, leading to T-cell inhibition and enhanced leukemia cell survival.

Recently, several JAK inhibitors have been clinically approved for the treatment of diverse diseases, including myelofibrosis and leukemia, and numerous novel candidates are currently in preclinical or clinical development. The chemical structures of approved JAK inhibitors are illustrated in Fig. 1. Ruxolitinib, a JAK1/JAK2 dual inhibitor, was approved by the U.S. Food and Drug Administration (FDA) in 2011 for the treatment of myelofibrosis [7]. However, inhibition of JAK1 can

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suppress inflammatory cytokine production [8] and negatively affect T-cell function [9]. Therefore, achieving high selectivity for JAK2 over other JAK isoforms is crucial to minimize undesired immunosuppressive effects. Fedratinib with high selectivity for JAK2 and FLT3 was approved by US FDA in 2019 [10]. However, its inherent toxicity profile [10], [11], including thrombocytopenia, necessitates the discovery of novel JAK2 inhibitors. Exploring strategies to further enhance selectivity for JAK2 over other JAK family members remains a valuable approach in the discovery of next-generation JAK inhibitors. In this study, we describe the discovery of JAK2-selective compounds achieved by varying substituents on the furopyrimidine scaffold.

2. Results and discussion

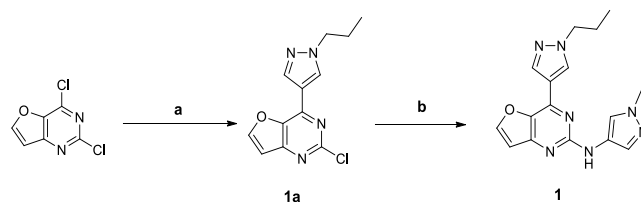
As shown in Scheme 1, compounds 1a was synthesized through Suzuki coupling reaction of 2,4-dichlorofuropyrimidines with 1-Propyl-1H-pyrazole-4-boronic acid. Compound 1 was synthesized by reacting intermediates 1a with 1-methyl-1H-pyrazole-4-amine via nucleophilic aromatic substitution (S_NAr) reaction.

In Scheme 2, 2a–5a were synthesized through Suzuki coupling reaction of 2,4-dichlorofuropyrimidines with 1H-pyrazole-4-boronic acid or pyrrole-3-boronic acid pinacol ester. Intermediates underwent alkylation with 3-bromopropionitrile, 2-bromomethyl methyl ether, or bromomethyl cyclopentane, yielding 2b and 5b from 3-bromopropionitrile, 3a and 6a from 2-bromomethyl methyl ether, and 4a and 7a from bromomethyl cyclopentane. The derivatives 2–7 were then synthesized by reacting intermediates (2b, 5b, 3a, 4a, 5a, 6a and 7a) with 1-methyl-1H-pyrazole-4-amine via nucleophilic aromatic substitution (S_NAr) reaction.

Further derivatives of furopyrimidines were synthesized as shown in Scheme 3. Derivatives 8–12 were obtained by either Suzuki coupling with various boronic acids or S_NAr reaction, followed by addition of aminopyrazole. Compound 12 was synthesized by deprotecting the BOC group from 12a, followed by acetylation and subsequent introduction of aminopyrazole.

Derivatives 13–17 were obtained as described in Scheme 3. Compound 13 was synthesized by Suzuki coupling using 1-(1-Boc-piperidino)pyrazole-4-boronic acid, followed by Buchwald-Hartwig coupling reaction and Boc deprotection. 14–17 were then synthesized from 13 via amide coupling, alkylation or acetylation (see Scheme 4).

To find JAK2 inhibitors, we commenced with screening in-house chemical library and identified compound 1, which exhibited strong JAK2 inhibition (99% inhibition) at 1 μ M, but poor selectivity (82% for JAK1, 72% for JAK3 and 94% for TYK2). To improve JAK2 selectivity, various substituents were introduced at the C-4 position of the furopyrimidine scaffold to explore the structure–activity relationships (SAR) (see Table 1). For a systematic analysis of the structural modifications, the scaffold of compound 1 was divided into two parts. The pyrazole ring was defined as part A, and the propyl chain was defined as part B. Structural modifications were then performed by introducing different ring structure at part A and diverse substituents at part B. First,



Scheme 1. Synthesis of compounds 1. Reagents and conditions: (a) 1-propyl-1H-pyrazole-4-boronic acid (1.2 equiv.), $Pd(PPh_3)_4$ (0.05 equiv.), K_2CO_3 (2 equiv.), dioxane:H₂O = 3:1, reflux, 6 h (b) 1-methyl-1H-pyrazole-4-amine (1.2 equiv.), 2N HCl, IPA, MW 160 $^{\circ}C$, 40 min.

compounds 2–5 were synthesized to evaluate the effect of substituents at part B while maintaining the pyrazole ring at part A. However, variation of the part B substituents did not lead to significant changes in either potency or selectivity. Next, to investigate the effect of the hetero-aromatic ring of the scaffold on biological activity, compounds 6 and 7 were synthesized by replacing the pyrazole moiety of part A with a pyrrole ring. Although a slight improvement in selectivity was observed, no significant overall difference in activity was detected. Based on these results, further optimization of part B was pursued while maintaining the pyrazole moiety in part A. During this process, compound 8 exhibiting pan-JAK inhibition was identified. This observation prompted us to consider introducing more rigid structural elements to improve binding stability. However, compounds 11 and 12, in which rigid moieties were incorporated at part A, did not exhibit favorable activity. In contrast, compound 9, which retained the aromatic scaffold at part A while introducing a morpholine substituent at part B, showed improved selectivity. Based on these SAR findings, further structural optimization was conducted by maintaining the pyrazole scaffold at part A while incorporating more rigid substituents at part B. Among the resulting compounds 13–17, 14 and 15, which possess terminal chain substituents, retained good potency while exhibiting the highest JAK2 selectivity. The IC_{50} values of compounds 14–16 were subsequently determined, and compound 14, which showed the most potent activity, was selected as the lead compound for further evaluation of selectivity across the JAK family (Table 2). As shown in Table 3, compound 14 exhibited the most potent JAK2 inhibitory activity, with approximately 10-fold selectivity over JAK1 and negligible inhibition of JAK3 and TYK2. In addition, the representative compound 14 displayed acceptable metabolic stability in mouse liver microsomes and was highly stable in human liver microsomes, indicating favorable drug-like properties and potential for further development (Table 4).

Next, a docking study was performed to understand potency and selectivity of compound 14.

JAK1 binding mode: compound 14 (selective for JAK2) and the non-selective inhibitor 8 were docked into the ATP-binding site of JAK1 (Fig. 2). The binding mode of compound 8 revealed hydrogen-bonding interactions with the hinge residue Leu959, consistent with a canonical hinge-binding pattern and supporting its favorable docking score

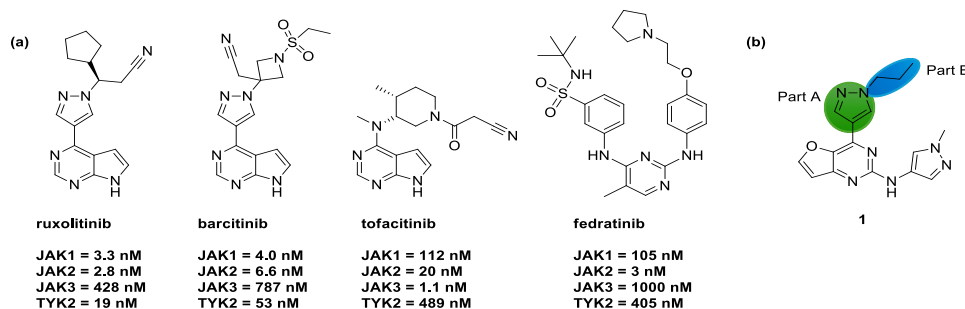
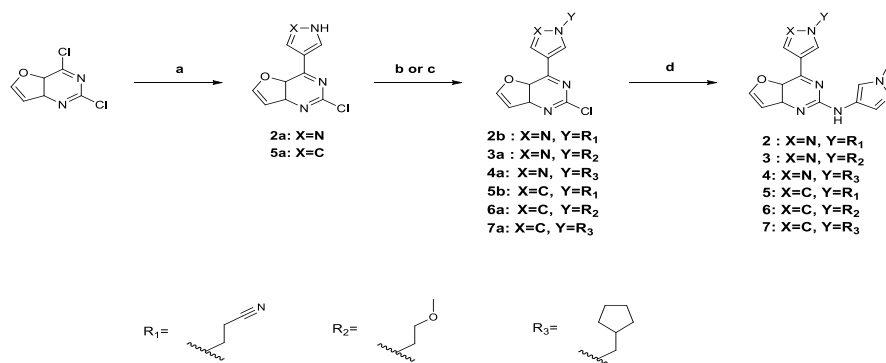
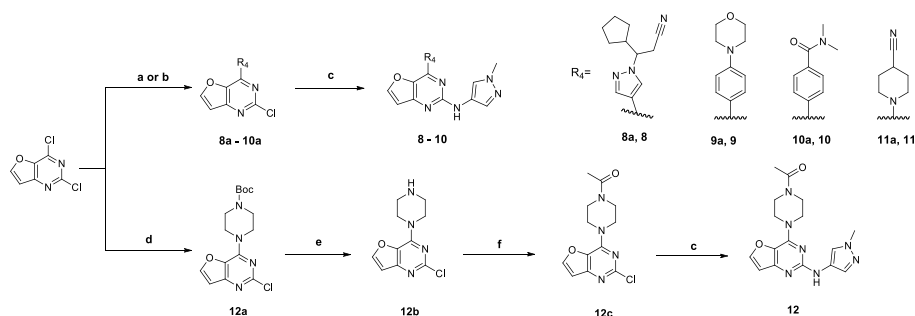


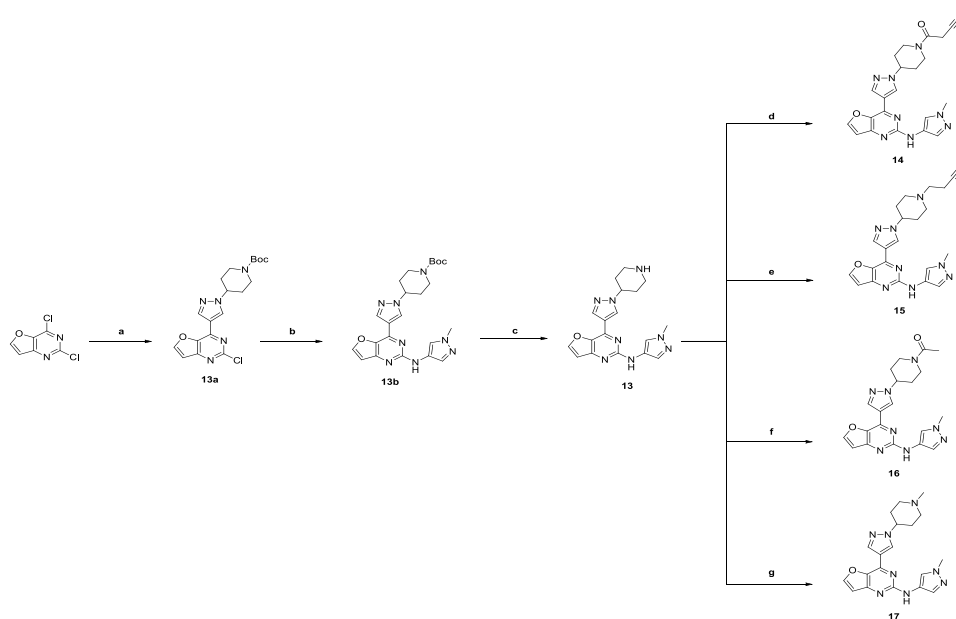
Fig. 1. (a) Structures of approved JAK2 inhibitors with their IC_{50} data [12–15]. (b) Strategy of derivatization of Compound 1. The aminopyrimidine motif was retained as a putative hinge-binding element, while part A and B modifications were explored to achieve target selectivity.



Scheme 2. Synthesis of compounds **2–7**. Reagents and conditions: (a) 1*H*-pyrazole-4-boronic acid or pyrrole-3-boronic acid pinacol ester (1.2 equiv.), Pd(PPh₃)₄ (0.05 equiv.), K₂CO₃ (2 equiv.), dioxane:H₂O = 3:1, reflux, 6 h (b) R₁ (1.2 equiv.), K₂CO₃ (5 equiv.), DMF, 50 °C, 16 h (c) R₂ or R₃ (1.2 equiv.), Cs₂CO₃ (2 equiv.), ACN, 80 °C, 16 h (d) 1-methyl-1*H*-pyrazole-4-amine (1.2 equiv.), 2*N* HCl, IPA, MW 160 °C, 40 min.



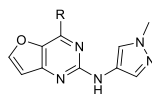
Scheme 3. Synthesis of compounds **8–12**. Reagents and conditions: (a) R₄B(OH)₂ or R₄B(OC₃H₇)₂ (1.2 equiv.), Pd(PPh₃)₄ (0.05 equiv.), K₂CO₃ (2 equiv.), dioxane: H₂O (3:1), reflux, 6 h (b) piperidine-4-carbonitrile (1.2 equiv.), Cs₂CO₃ (2 equiv.), ACN, 80 °C, 16 h (c) 1-methyl-1*H*-pyrazole-4-amine (1.2 equiv.), 2*N* HCl, IPA, MW 160 °C, 40 min (d) 1-Boc-piperazine (1.2 equiv.), Cs₂CO₃ (2 equiv.), ACN, 80 °C, 16 h (e) TFA, DCM, rt, 2 h (f) Acetyl chloride, TEA, DCM, rt, 2 h.



Scheme 4. Synthesis of compounds **13–17**. Reagents and conditions: (a) 1-(1-Boc-piperidino)pyrazole-4-boronic acid (1.2 equiv.), Pd(PPh₃)₄ (0.05 equiv.), K₂CO₃ (2 equiv.), dioxane:H₂O (3:1), reflux, 6 h (b) 1-methyl-1*H*-pyrazole-4-amine (1.2 equiv.), Pd₂(dba)₃ (0.1 equiv.), Cs₂CO₃ (2 equiv.), Xantphos (0.2 equiv.), anhydrous dioxane, reflux, 16 h (c) TFA, DCM, rt, 2 h (d) Cyanoacetic acid (1.2 equiv.), HATU (1.5 equiv.), DIPEA (3 equiv.), DCM, rt, 16 h (e) 3-bromopropionitrile (1.2 equiv.), Cs₂CO₃ (2 equiv.), ACN, 80 °C, 16 h (f) Acetyl chloride (1.2 equiv.), TEA (2 equiv.), DCM, rt, 16 h (g) formaldehyde (3 equiv.), acetic acid (1 equiv.), sodium borohydride (5 equiv.), MeOH, rt, 5 h

(−10.0 kcal/mol). In contrast, compound **14** adopted a distinct binding orientation, in which the carbonyl oxygen and the nitrogen atom of the

terminal nitrile group formed hydrogen bonds with the hydrophilic residues Lys970 and Asn971, located distal to the hinge region.

Table 1*In vitro* enzymatic kinase assays of compounds 1–17 at 1 μ M.

compound	R	% inhibition ^a				compound	R	% inhibition ^a			
		JAK1	JAK2	JAK3	TYK2			JAK1	JAK2	JAK3	TYK2
1		82	99	72	94	9		74	93	43	80
2		87	98	62	97	10		19	43	0	15
3		78	94	50	87	11		39	39	0	24
4		92	99	85	97	12		8	9	0	0
5		80	98	75	95	13		40	76	11	33
6		53	83	41	74	14		55	94	40	51
7		75	90	53	85	15		60	91	24	37
8		100	100	100	100	16		52	89	25	29
						17		21	67	4	9

^a All kinase assays were carried out at K_m of ATP by Reaction Biology Co, (USA).**Table 2** IC_{50} values of the selected derivatives for JAK2 *in vitro* kinase assays.

Compound	IC_{50} (nM)
	JAK2
14	54
15	208
16	138
Staurosporine	0.2

Table 3 IC_{50} values of compound 14 for JAK family kinases *in vitro* kinase assays.

Compound	IC_{50} (nM)			
	JAK1	JAK2	JAK3	TYK2
14	496	54	>1000	>1000
Staurosporine	0.6	0.2	0.1	0.3

Consequently, compound 14 exhibited a less favorable docking score (−7.6 kcal/mol), likely due to its failure to establish hinge hydrogen-bonding interactions, resulting in a suboptimal binding mode for

JAK1. These docking results suggest that hinge-region hydrogen bonding plays a critical role in JAK1 binding affinity, and the absence of such interactions in compound 14 may account for its weaker inhibitory activity against JAK1.

JAK2 binding mode: The docking simulation of compound 14 was

Table 4
Metabolic stability of compound 14.

Compound	Metabolic stability % remaining after 30 min	
	Human	Mouse
14	98.7	65.8
Verapamil	20.2	-

compared with that of the weak inhibitor **11** in the JAK2 active site (Fig. 3). Compound **14** formed favorable hydrogen-bonding interactions with the hinge residues Leu932. In addition, the presence of a bulky substituent in compound **14** allowed improved accommodation within the hydrophobic pocket of JAK2, where the terminal nitrile group contributed to additional stabilizing interactions, including a hydrogen bond with Gly861 and hydrophobic contacts with Phe860, resulting in an enhanced binding stability (-10.7 kcal/mol). In contrast, compound **11** lacked stabilizing hydrophobic interactions and exhibited a poorer fit within the hydrophobic region, despite retaining a potential hinge interaction, leading to a less favorable docking score (-8.9 kcal/mol).

JAK3 and TYK2 binding mode: Finally, docking simulations of compounds **8** and **14** were conducted at the ATP-binding sites of JAK3 and TYK2 to elucidate their differential binding modes (Fig. 4). In JAK3, compound **8** adopted a favorable binding pose in which the aminopyrimidine core positioned near the hinge region Leu905, enabling the formation of a hydrogen bond that may contribute to binding stability.

In contrast, compound **14** exhibited reduced hinge engagement in JAK3, as steric hindrance from the piperidine ring prevented effective hydrogen-bonding interactions, resulting in a less favorable predicted binding affinity. In TYK2, While the aminopyrimidine core of both compounds showed aligned similarly toward the hinge region, their terminal substituents adopted markedly different conformations. The nitrile and cyclopentyl groups of **8** were oriented toward the interior of the ATP-binding pocket. By contrast, steric repulsion introduced by the piperidine moiety in compound **14** appears to shift the terminal substituents toward an outward orientation, leading to suboptimal pocket occupancy and reduced binding stability. These docking studies suggest that the piperidine substituent of compound **14** selectively disrupts binding to JAK3 and TYK2 while maintaining favorable interactions in JAK2, consistent with the observed selectivity profile.

Taken together, these structural features suggest that the improved inhibitory potency of compound **14** toward JAK2 arise from its dual contribution of hinge-region hydrogen bonding and enhanced hydrophobic pocket fitting. Therefore, the molecular modeling results indicate that the introduction of the bulky substituent confers steric and spatial complementarity uniquely suited to the JAK2 binding pocket, leading to stronger and more selective inhibition of JAK2.

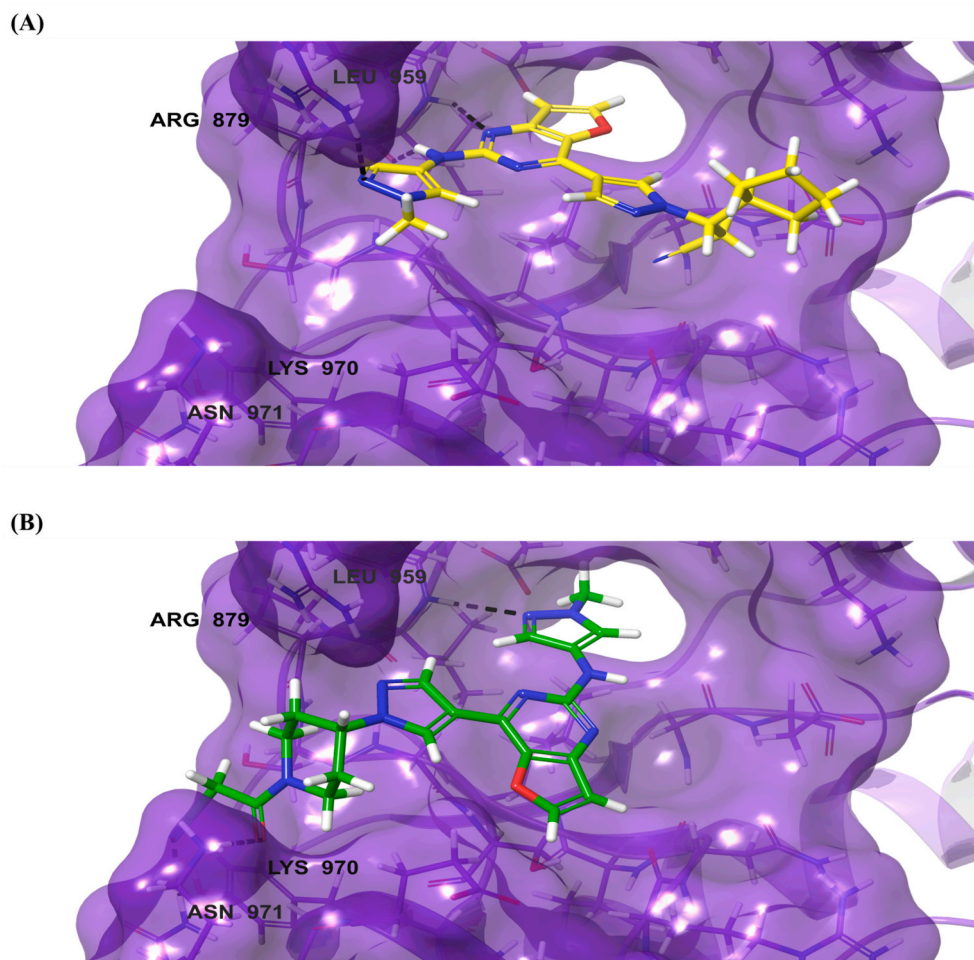


Fig. 2. Predicted docking orientation in JAK1. Comparison of the JAK1 (PDB: 6N7B) binding models of **8** (Yellow) and **14** (Green). (A) Binding model of **8** (-10.0 kcal/mol). (B) Binding model of **14** (-7.6 kcal/mol). Hydrogen bonds are displayed by black dashed lines. The aminopyrimidine moiety of compound **8** forms a bidentate hydrogen-bond with the hinge residue Leu959, consistent with a canonical hinge-binding pattern commonly observed in ATP-competitive kinase inhibitors. In contrast, compound **14** adopts a different binding orientation, in which the carbonyl oxygen and terminal nitrile nitrogen form hydrogen bonds with hydrophilic residues Lys970 and Asn971, located distal to the hinge region, thereby preventing hinge hydrogen-bonding interactions with JAK1.

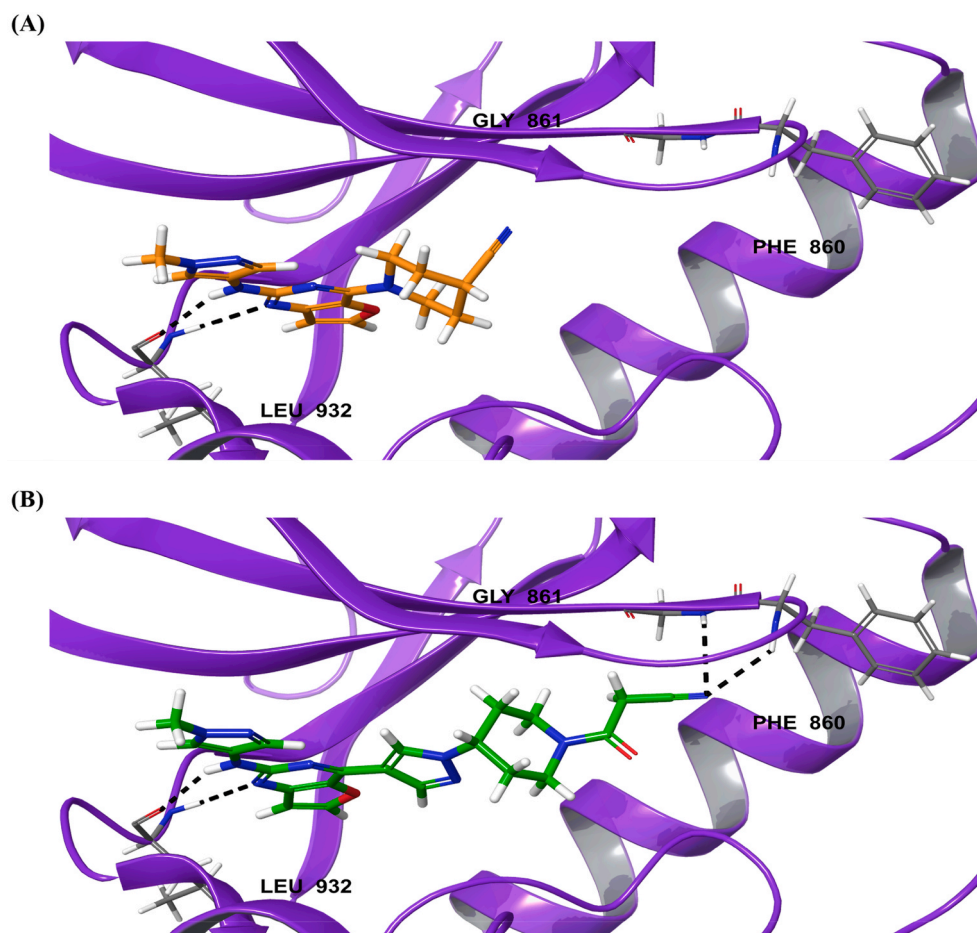


Fig. 3. Predicted docking orientation in JAK2. Comparison of the JAK2 (6VNE) binding models of **11** (Orange) and **14** (Green). (A) Binding model of **11** (−8.9 kcal/mol). (B) Binding model of **14** (−10.7 kcal/mol). Hydrogen bonds are displayed by black dashed lines. **14** exhibited strong hydrogen-bonding with the hinge residue Leu932 and additional hydrogen-bonding interactions via terminal nitrile group with Phe860 and Gly861, while a bulky substituent contributed to enhanced hydrophobic stabilization within the pocket. Conversely, **11** showed weaker hydrophobic contacts and poor accommodation in the hydrophobic region, despite retaining hinge region interactions.

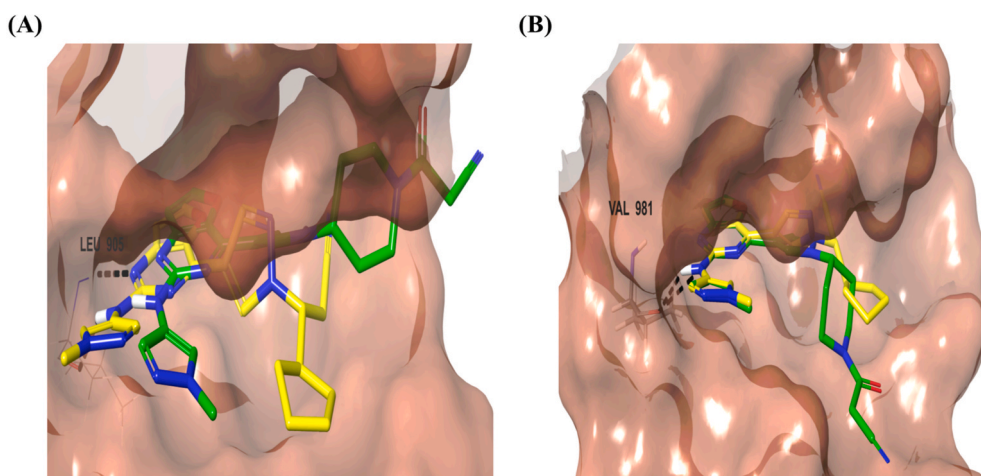


Fig. 4. Predicted docking orientation in JAK3 and TYK2. Comparison of the binding models of **8** (Yellow) and **14** (Green). (A) Comparison of the JAK3 (PDB:6GLB) binding models of **8** (−8.8 kcal/mol) and **14** (−7.4 kcal/mol). (B) Comparison of the TYK2 (PDB:7UYU) binding models of **8** (−8.7 kcal/mol) and **14** (−7.6 kcal/mol). Hydrogen bonds are displayed by black dashed lines. In (A), Compound **8** formed a hydrogen bond with the hinge residue Leu905, whereas steric hindrance from the piperidine ring in compound **14** prevented proper hinge interaction. In (B), Both aminopyrimidine cores formed a hydrogen bond with the hinge residue Val981, however, compound **8** oriented its terminal groups toward the pocket interior, whereas steric repulsion in piperidine moiety of compound **14** appears to introduce the substituents outward, leading to reduced binding stability.

3. Conclusion

Starting from non-selective JAK inhibitors, we discovered JAK2-selective compounds by optimizing the furopyrimidine scaffold through substituent modifications. Docking studies predicted that incorporating heteroatoms at the extended substituent's terminus enhances JAK2 binding, improving selectivity. Unlike fedratinib, the most selective JAK2 inhibitor in clinical use, our compounds feature a novel furopyrimidine scaffold. Compound **14**, with high selectivity for JAK2 over JAK1, JAK3 and TYK2 in kinase assays, provides structural insights for designing next-generation JAK2 inhibitors with enhanced specificity and safety.

4. Experimental

4.1. Chemistry

General information. All reagents and solvents were purchased from, Alfa Aesar, BLD Pharm, Combi-Blocks, Sigma Aldrich®, Tokyo Chemical Industry, and Samchun Chemicals. Microwave irradiations were conducted using Anton Paar Monowave 300. Reactions were monitored by UV light visualization and utilized TLC silica gel 60 F₂₅₄ 250 µm plates from Merck. Flash column chromatography was conducted by CombiFlash® from Teledyne Isco using ZEOprep 60 silica gel (Zeochem, 40–63 µm) loaded with pre-packed Welux™ Flash Column ultra-pure silica gel 40-63 µm 60 Å or RediSep® Silver from Inter-technologies Co., Ltd. ¹H and ¹³C NMR spectra were collected using a JEOL NMR spectrometer (¹H, 600 MHz; ¹³C, 150 MHz). In parts per million (ppm, δ), chemical shifts are reported using tetramethylsilane (TMS) as an internal standard. Coupling constants (*J*) is expressed in hertz (Hz). The splitting patterns are denoted using the following abbreviations: singlet (s); doublet (d); triplet (t); quartet (q); doublet of doublets (dd); triplet of doublets (td); multiplet (m). High-resolution mass spectroscopy (HRMS) was conducted in Chungnam National Univ. Center of Research Facilities, using a 1290 infinity II/TripleTOF 5600 plus (Sciex) coupled with an AB SCIEX TripleTOF® 5600 plus.

General Procedure A. To a solution of compounds fused pyrimidine (1.0 equiv.) and variety of boronic acids or boronic acid pinacol ester (1.2 equiv.) in the microwave sealed tube, and K₂CO₃ (2.0 equiv.) was dissolved in 3:1 mixture of 1,4-Dioxane and H₂O and degassed with N₂ for 5 min. Then, Pd(PPh₃)₄ (0.05 equiv.) was added to the tube. At 110 °C for 6 h, following reaction mixture was stirred. The reaction was monitored by TLC and then cooled down to room temperature. Next, the resulting mixture was filtered through Celite, and the concentrated filtrate was purified by was purified by MPLC.

General Procedure B. To a solution of substrate (1.0 equiv.) in the 20 mL vial, 3-bromopropionitrile (1.2 equiv.) and K₂CO₃ (5 equiv.) were dissolved in DMF. The vial was stirred at 50 °C for 16 h. The reaction was monitored by TLC and then cooled down to room temperature. The resulting mixture was added to water and extracted twice with ethyl acetate (EA). The collected EA layer was dried over sodium sulfate. After filtering off the drying agent, the solution was evaporated, and the product was purified by MPLC.

General Procedure C. To a solution of substrate (1.0 equiv.) in the 20 mL vial, alkyl bromide, piperidine-4-carbonitrile or 1-Boc-piperazine (1.2 equiv.) and Cs₂CO₃ (2 equiv.) were dissolved in ACN. The vial was stirred at 80 °C for 16 h. The reaction was monitored by TLC and then cooled down to room temperature. Next, the resulting mixture was filtered through Celite, and the concentrated filtrate was purified by was purified by MPLC.

General Procedure D. Substrate (1.0 equiv.), 1-methyl-1H-pyrazole-4-amine (1.2 equiv.), 2N HCl, and IPA were added to a microwave sealed tube and stirred at 160 °C for 40 min using microwave reactor. The reaction mixture was added to water and extracted twice with DCM. The collected DCM layer was dried over sodium sulfate. After filtering off the drying agent, the solution was evaporated, and the product was

isolated in high purity using MPLC.

General Procedure E. To a solution of substrate (1.0 equiv.) in the microwave sealed tube, 1-methyl-1H-pyrazole-4-amine (1.2 equiv.), Xantphos (0.2 equiv.) and Cs₂CO₃ (2.0 equiv.) were dissolved in anhydrous 1,4-Dioxane and degassed with N₂ for 5 min. Then, Pd₂(dba)₃ (0.1 equiv.) was added to the tube. The tube was purged with N₂ gas and stirred at 110 °C for 16 h. The reaction was monitored by TLC and then cooled down to room temperature. Next, the resulting mixture was filtered through Celite, and the concentrated filtrate was purified by was MPLC.

22-Chloro-4-(1-propyl-1H-pyrazol-4-yl)furo [3,2-d] pyrimidine (1a). Compound **1a** was synthesized via Suzuki coupling, following **General Procedure A**. Yield: 73%. ¹H NMR (600 MHz, CDCl₃) δ 8.37 (s, 1H), 8.33 (s, 1H), 8.03 (d, *J* = 2.1 Hz, 1H), 6.92 (d, *J* = 1.7 Hz, 1H), 4.20 (t, *J* = 7.2 Hz, 2H), 1.98 (td, *J* = 14.5, 7.6 Hz, 2H), 0.96 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 155.3, 155.1, 152.4, 144.4, 141.2, 140.0, 131.1, 115.9, 107.5, 54.3, 23.3, 10.9.

N-(1-Methyl-1H-pyrazol-4-yl)-4-(1-propyl-1H-pyrazol-4-yl)furo [3,2-d] pyrimidin-2-amine (1). Compound **1** was prepared by **General Procedure D**. Yield: 15%. ¹H NMR (600 MHz, CDCl₃) δ 8.34 (s, 1H), 8.25 (s, 1H), 7.92 (s, 1H), 7.85 (d, *J* = 2.1 Hz, 1H), 7.54 (s, 1H), 6.87 (s, 1H), 6.77 (d, *J* = 2.1 Hz, 1H), 4.19 (t, *J* = 6.9 Hz, 2H), 3.92 (s, 3H), 1.98 (td, *J* = 14.5, 7.6 Hz, 2H), 0.97 (t, *J* = 7.6 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 157.2, 154.4, 150.9, 143.0, 139.7, 138.4, 130.7, 130.6, 123.7, 121.0, 117.3, 107.2, 54.3, 39.3, 23.6, 11.1; HRMS (ESI): *m/z* calcd for C₁₆H₁₈N₇O [M+H]⁺ 324.1568, found 324.1578.

2-Chloro-4-(1H-pyrazol-4-yl)furo [3,2-d] pyrimidine (2a). Compound **2a** was prepared by General Procedure A. Yield: 69%. ¹H NMR (600 MHz, CDCl₃) δ 8.55 (s, 2H), 8.05 (d, *J* = 1.4 Hz, 1H), 6.98 (d, *J* = 1.4 Hz, 1H); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 156.1, 155.6, 155.1, 154.5, 144.8(5), 144.8(4), 141.4, 115.4, 107.8.

3-(4-(2-Chlorofuro [3,2-d] pyrimidin-4-yl)-1H-pyrazol-1-yl)propanenitrile (2b). Compound **2b** was prepared by **General Procedure B**. Yield: 93%. ¹H NMR (600 MHz, CDCl₃) δ 8.48 (d, *J* = 5.5 Hz, 2H), 8.04 (d, *J* = 2.1 Hz, 1H), 6.97 (d, *J* = 2.1 Hz, 1H), 4.52 (t, *J* = 6.5 Hz, 2H), 3.06 (t, *J* = 6.5 Hz, 2H); ¹³C NMR (150 MHz, CDCl₃) δ 155.8, 155.3, 152.7, 143.8, 141.5, 141.4, 132.1, 117.1, 116.5, 107.8, 48.1, 19.2.

3-(4-(2-(1-Methyl-1H-pyrazol-4-yl)amino)furo [3,2-d] pyrimidin-4-yl)-1H-pyrazol-1-yl)propanenitrile (2). Compound **2** was prepared by **General Procedure D**. Yield: 23%. ¹H NMR (600 MHz, CDCl₃) δ 8.39 (s, 1H), 8.38 (s, 1H), 7.92 (s, 1H), 7.85 (d, *J* = 2.1 Hz, 1H), 7.54 (s, 1H), 6.97 (s, 1H), 6.77 (d, *J* = 2.1 Hz, 1H), 4.51 (t, *J* = 6.9 Hz, 2H), 3.92 (s, 3H), 3.04 (t, *J* = 6.5 Hz, 2H); ¹³C NMR (150 MHz, CDCl₃) δ 157.3, 154.7, 151.2, 142.3, 141.0, 138.4, 131.4, 130.8, 123.7, 121.1, 118.3, 116.7, 107.3, 48.0, 39.3, 19.3; HRMS (ESI): *m/z* calcd for C₁₆H₁₅N₈O [M+H]⁺ 335.1364, found 335.1373.

2-Chloro-4-(1-(2-methoxyethyl)-1H-pyrazol-4-yl)furo [3,2-d] pyrimidine (3a). Compound **3a** was prepared by **General procedure C**. Yield: 74%. ¹H NMR (600 MHz, CDCl₃) δ 8.44 (s, 1H), 8.41 (s, 1H), 8.03 (d, *J* = 2.1 Hz, 1H), 6.94 (d, *J* = 2.8 Hz, 1H), 4.40 (t, *J* = 5.2 Hz, 2H), 3.81 (t, *J* = 5.2 Hz, 2H), 3.36 (s, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 155.5, 155.2, 152.4, 144.5, 141.4, 140.4, 132.4, 116.3, 107.6, 70.6, 59.1, 52.8.

4-(1-(2-Methoxyethyl)-1H-pyrazol-4-yl)-N-(1-methyl-1H-pyrazol-4-yl)furo [3,2-d] pyrimidin-2-amine (3). Compound **3** followed **General Procedure D**. Yield: 35%. ¹H NMR (600 MHz, CDCl₃) δ 8.35 (s, 2H), 7.94 (s, 1H), 7.84 (d, *J* = 2.1 Hz, 1H), 7.53 (s, 1H), 6.96 (s, 1H), 6.77 (d, *J* = 2.8 Hz, 1H), 4.40 (t, *J* = 5.2 Hz, 2H), 3.92 (s, 3H), 3.82 (t, *J* = 5.2 Hz, 2H), 3.36 (s, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 157.2, 154.4, 150.9, 142.9, 139.9, 138.4, 131.8, 130.6, 123.7, 120.9, 117.5, 107.2, 70.9, 59.1, 52.7, 39.3; HRMS (ESI): *m/z* calcd for C₁₆H₁₈N₇O₂ [M+H]⁺ 340.1517, found 340.1522.

2-Chloro-4-(1-(cyclopentylmethyl)-1H-pyrazol-4-yl)furo [3,2-d] pyrimidine (4a). Compound **4a** was by following according to **General Procedure C**. Yield: 40%. ¹H NMR (600 MHz, CDCl₃) δ 8.39 (s, 1H), 8.35 (s, 1H), 8.03 (d, *J* = 2.8 Hz, 1H), 6.95 (d, *J* = 2.8 Hz, 1H), 4.15 (d, *J*

= 7.6 Hz, 2H), 2.50-2.56 (m, 1H), 1.74-1.78 (m, 2H), 1.65-1.69 (m, 2H), 1.59-1.62 (m, 2H), 1.25-1.34 (m, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ 155.4, 155.2, 152.4, 144.5, 141.3, 140.1, 131.2, 116.0, 107.6, 57.6, 40.5, 30.2, 24.4.

4-(1-(Cyclopentylmethyl)-1H-pyrazol-4-yl)-N-(1-methyl-1H-pyrazol-4-yl)furo [3,2-d] pyrimidin-2-amine (4). Compound 4 followed General Procedure D. Yield: 17%. ^1H NMR (600 MHz, CDCl_3) δ 8.32 (s, 1H), 8.25 (s, 1H), 7.92 (s, 1H), 7.85 (d, J = 2.8 Hz, 1H), 7.54 (s, 1H), 6.86 (s, 1H), 6.77 (d, J = 2.1 Hz, 1H), 4.14 (d, J = 7.6 Hz, 2H), 3.92 (s, 3H), 2.50-2.55 (m, 1H), 1.74-1.79 (m, 2H), 1.66-1.71 (m, 2H), 1.58-1.63 (m, 2H), 1.28-1.34 (m, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ 157.2, 154.4, 150.9, 143.0, 139.6, 138.4, 130.5, 124.6, 123.7, 121.0, 117.3, 107.2, 57.5, 40.6, 39.3, 30.3, 25.0; HRMS (ESI): m/z calcd for $\text{C}_{19}\text{H}_{22}\text{N}_7\text{O}$ $[\text{M}+\text{H}]^+$ 364.1881, found 364.1892.

2-Chloro-4-(1H-pyrrol-3-yl)furo [3,2-d] pyrimidine (5a). Compound 5a was produced as in General Procedure A. Yield: 33%. ^1H NMR (600 MHz, CDCl_3) δ 7.98 (d, J = 2.1 Hz, 1H), 7.92 (d, J = 1.4 Hz, 1H), 7.16 (d, J = 1.4 Hz, 1H), 6.94 (t, J = 2.4 Hz, 1H), 6.91 (d, J = 2.1 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3) δ 155.3, 155.2, 152.0, 141.8, 134.3, 128.1, 123.0, 119.9, 109.3, 107.6.

3-(3-(2-Chlorofuro [3,2-d] pyrimidin-4-yl)-1H-pyrrol-1-yl)propanenitrile (5b). Compound 5b was prepared according to General Procedure B. Yield: 79%. ^1H NMR (600 MHz, CDCl_3) δ 7.97 (d, J = 2.1 Hz, 1H), 7.80 (t, J = 2.1 Hz, 1H), 7.10 (q, J = 1.4 Hz, 1H), 6.90 (d, J = 2.1 Hz, 1H), 6.85 (t, J = 2.4 Hz, 1H), 4.30 (t, J = 6.5 Hz, 2H), 2.89 (t, J = 6.9 Hz, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ 155.2, 155.1, 152.0, 146.4, 141.5, 124.8, 122.5, 119.1, 116.6, 110.5, 107.5, 45.6, 20.6.

3-(3-(2-((1-Methyl-1H-pyrazol-4-yl)amino)furo [3,2-d] pyrimidin-4-yl)-1H-pyrrol-1-yl)propanenitrile (5). Compound 5 was prepared by General Procedure D. Yield: 17%. ^1H NMR (600 MHz, CDCl_3) δ 7.94 (s, 1H), 7.82 (d, J = 2.1 Hz, 1H), 7.75 (d, J = 2.1 Hz, 1H), 7.54 (s, 1H), 7.07 (q, J = 1.6 Hz, 1H), 6.84 (t, J = 2.4 Hz, 1H), 6.75 (d, J = 2.1 Hz, 1H), 4.30 (t, J = 6.9 Hz, 2H), 3.92 (s, 3H), 2.87 (t, J = 6.9 Hz, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ 157.3, 154.3, 150.7, 145.0, 138.9, 130.8, 124.0, 122.1, 121.0, 120.7, 116.9, 110.3, 107.2, 45.8, 39.4, 20.9; HRMS (ESI): m/z calcd for $\text{C}_{17}\text{H}_{16}\text{N}_7\text{O}$ $[\text{M}+\text{H}]^+$ 334.1411, found 334.1419.

2-Chloro-4-(1-(2-methoxyethyl)-1H-pyrrol-3-yl)furo [3,2-d] pyrimidine (6a). Compound 6a was synthesized by using General Procedure C. Yield: 78%. ^1H NMR (600 MHz, CDCl_3) δ 7.96 (d, J = 2.1 Hz, 1H), 7.81 (t, J = 2.1 Hz, 1H), 7.07 (q, J = 1.4 Hz, 1H), 6.88 (d, J = 2.1 Hz, 1H), 6.82 (t, J = 2.8 Hz, 1H), 4.13 (t, J = 5.5 Hz, 2H), 3.71 (t, J = 5.5 Hz, 2H), 3.36 (s, 3H); ^{13}C NMR (150 MHz, CDCl_3) δ 155.1, 154.8, 151.7, 147.1, 141.6, 125.9, 123.3, 117.9, 109.5, 107.4, 72.0, 59.1, 50.2.

4-(1-(2-Methoxyethyl)-1H-pyrrol-3-yl)-N-(1-methyl-1H-pyrazol-4-yl)furo [3,2-d] pyrimidin-2-amine (6). Compound 6 followed General Procedure D. Yield: 56%. ^1H NMR (600 MHz, CDCl_3) δ 7.95 (s, 1H), 7.80 (d, J = 2.8 Hz, 1H), 7.73 (t, J = 1.7 Hz, 1H), 7.50 (s, 1H), 7.10 (s, 1H), 7.02 (q, J = 1.4 Hz, 1H), 6.81 (t, J = 2.4 Hz, 1H), 6.73 (d, J = 2.1 Hz, 1H), 4.13 (t, J = 5.2 Hz, 2H), 3.90 (s, 3H), 3.70 (t, J = 5.5 Hz, 2H), 3.36 (s, 3H); ^{13}C NMR (150 MHz, CDCl_3) δ 157.2, 153.8, 150.3, 145.5, 138.7, 130.5, 124.9, 124.1, 122.7, 120.8, 119.2, 108.9, 107.0, 72.2, 59.1, 50.1, 39.2; HRMS (ESI): m/z calcd for $\text{C}_{17}\text{H}_{19}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$ 339.1564, found 339.1563.

2-Chloro-4-(1-(cyclopentylmethyl)-1H-pyrrol-3-yl)furo [3,2-d] pyrimidine (7a). Compound 7a was synthesized by General Procedure C. Yield: 59%. ^1H NMR (600 MHz, CDCl_3) δ 7.96 (d, J = 1.7 Hz, 1H), 7.76 (t, J = 1.7 Hz, 1H), 7.05 (q, J = 1.4 Hz, 1H), 6.88 (d, J = 2.8 Hz, 1H), 6.76 (t, J = 2.8 Hz, 1H), 3.88 (d, J = 7.6 Hz, 2H), 2.32-2.40 (m, 1H), 1.56-1.78 (m, 6H), 1.23-1.29 (m, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ 155.2, 154.8, 151.6, 147.2, 141.6, 125.6, 123.0, 117.6, 109.3, 107.4, 55.3, 41.6, 30.3, 24.9.

4-(1-(Cyclopentylmethyl)-1H-pyrrol-3-yl)-N-(1-methyl-1H-pyrazol-4-yl)furo [3,2-d] pyrimidin-2-amine (7). Compound 7 followed General Procedure D. Yield: 34%. ^1H NMR (600 MHz, CDCl_3) δ 7.95 (s, 1H), 7.80 (d, J = 2.1 Hz, 1H), 7.69 (t, J = 1.7 Hz, 1H), 7.52 (s, 1H), 7.00

(t, J = 2.4 Hz, 1H), 6.88 (s, 1H), 6.76 (t, J = 2.4 Hz, 1H), 6.73 (d, J = 2.8 Hz, 1H), 3.91 (s, 3H), 3.88 (d, J = 7.6 Hz, 2H), 2.32-2.40 (m, 1H), 1.57-1.77 (m, 6H), 1.26-1.29 (m, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ 157.1, 153.7, 150.2, 145.7, 138.7, 130.6, 124.6, 124.1, 122.4, 120.8, 118.8, 108.6, 107.0, 55.19, 41.7, 39.2, 30.4, 24.9; HRMS (ESI): m/z calcd for $\text{C}_{20}\text{H}_{23}\text{N}_6\text{O}$ $[\text{M}+\text{H}]^+$ 363.1928, found 363.1926.

3-(4-(2-Chlorofuro [3,2-d] pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile (8a). Compound 8a was synthesized via Suzuki coupling, following General Procedure A. Yield: 99%. ^1H NMR (600 MHz, CDCl_3) δ 8.46 (s, 2H), 8.04 (d, J = 1.7 Hz, 1H), 6.96 (d, J = 2.1 Hz, 1H), 4.24-4.28 (m, 1H), 2.95-3.17 (m, 2H), 2.56-2.61 (m, 1H), 1.97 (tt, J = 11.9, 3.9 Hz, 1H), 1.51-1.76 (m, 4H), 1.17-1.33 (m, 3H); ^{13}C NMR (150 MHz, CDCl_3) δ 155.7, 155.3, 152.6, 144.1, 141.4, 141.1, 131.4, 116.5, 116.3, 107.7, 64.6, 44.3, 30.1, 30.0, 25.4, 24.7, 23.5.

3-Cyclopentyl-3-(4-(2-((1-methyl-1H-pyrazol-4-yl)amino)furo [3,2-d] pyrimidin-4-yl)-1H-pyrazol-1-yl)propanenitrile (8). Compound 8 followed General Procedure D. Yield: 22%. ^1H NMR (600 MHz, CDCl_3) δ 8.40 (s, 1H), 8.38 (s, 1H), 7.94 (s, 1H), 7.86 (d, J = 2.1 Hz, 1H), 7.53 (s, 1H), 7.09 (s, 1H), 6.78 (d, J = 2.1 Hz, 1H), 4.26 (td, J = 9.5, 3.4 Hz, 1H), 3.92 (s, 3H), 2.94-3.16 (m, 2H), 2.59 (q, J = 8.3 Hz, 1H), 1.95-1.98 (m, 1H), 1.52-1.75 (m, 5H), 1.26-1.31 (m, 1H), 1.21 (td, J = 8.4, 3.9 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3) δ 157.3, 154.7, 151.2, 142.6, 140.7, 138.5, 130.9, 130.8, 123.8, 121.1, 117.7, 116.9, 107.3, 64.5, 44.5, 39.4, 30.3, 30.1, 25.6, 25.0, 24.9, 23.7; HRMS (ESI): m/z calcd for $\text{C}_{21}\text{H}_{23}\text{N}_8\text{O}$ $[\text{M}+\text{H}]^+$ 403.1990, found 403.1991.

2-Chloro-4-(4-morpholinophenyl)furo [3,2-d] pyrimidine (9a). Compound 9a was synthesized via Suzuki coupling, following General Procedure A. Yield: 14%. ^1H NMR (600 MHz, CDCl_3) δ 8.44 (dd, J = 6.9, 2.1 Hz, 2H), 8.02 (d, J = 2.1 Hz, 1H), 6.98-7.00 (m, 2H), 6.93 (d, J = 2.1 Hz, 1H), 3.89 (t, J = 5.2 Hz, 4H), 3.34 (t, J = 5.2 Hz, 4H); ^{13}C NMR (150 MHz, CDCl_3) δ 156.1, 155.1, 153.5, 152.2, 148.9, 142.5, 130.9, 122.7, 114.0, 107.4, 66.5, 47.5.

N-(1-Methyl-1H-pyrazol-4-yl)-4-(4-morpholinophenyl)furo [3,2-d] pyrimidin-2-amine (9). Compound 9 followed General Procedure D. Yield: 28%. ^1H NMR (600 MHz, CDCl_3) δ 8.40 (dd, J = 6.9, 2.1 Hz, 2H), 7.94 (s, 1H), 7.87 (d, J = 2.1 Hz, 1H), 7.56 (s, 1H), 7.03 (dd, J = 6.9, 2.1 Hz, 2H), 6.90 (s, 1H), 6.78 (d, J = 2.1 Hz, 1H), 3.92 (s, 3H), 3.90 (t, J = 4.8 Hz, 4H), 3.32 (t, J = 4.8 Hz, 4H); ^{13}C NMR (150 MHz, CDCl_3) δ 157.0, 155.1, 153.0, 150.9, 147.7, 139.6, 130.8, 130.3, 124.8, 123.8, 121.0, 114.4, 107.0, 66.7, 48.0, 39.3; HRMS (ESI): m/z calcd for $\text{C}_{20}\text{H}_{21}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$ 377.1721, found 377.1728.

4-(2-Chlorofuro [3,2-d] pyrimidin-4-yl)-N,N-dimethylbenzamide (10a). Compound 10a was synthesized via Suzuki coupling following General Procedure A. Yield: 99%. ^1H NMR (600 MHz, CDCl_3) δ 8.54 (d, J = 9.0 Hz, 2H), 8.14 (d, J = 2.1 Hz, 1H), 7.62 (d, J = 8.3 Hz, 2H), 7.01 (d, J = 2.1 Hz, 1H), 3.16 (s, 3H), 3.02 (s, 3H); ^{13}C NMR (150 MHz, CDCl_3) δ 170.7, 157.4, 155.3, 153.5, 148.0, 143.0, 139.7, 133.6, 129.4, 127.7, 107.7, 39.5, 35.4.

N,N-Dimethyl-4-(2-((1-methyl-1H-pyrazol-4-yl)amino)furo [3,2-d] pyrimidin-4-yl)benzamide (10). Compound 10 was prepared by General Procedure D. Yield: 21%. ^1H NMR (600 MHz, CDCl_3) δ 8.48 (dd, J = 8.3, 2.1 Hz, 2H), 7.93 (s, 1H), 7.92 (d, J = 2.1 Hz, 1H), 7.60 (dd, J = 6.5, 1.7 Hz, 2H), 7.57 (s, 1H), 6.99 (s, 1H), 6.82 (d, J = 2.1 Hz, 1H), 3.93 (s, 3H), 3.16 (s, 3H), 3.03 (s, 3H); ^{13}C NMR (150 MHz, CDCl_3) δ 170.9, 157.2, 156.1, 151.7, 146.7, 139.8, 138.6, 135.3, 130.8, 128.9, 127.4, 123.5, 121.1, 107.1, 39.5, 39.3, 35.3; HRMS (ESI): m/z calcd for $\text{C}_{19}\text{H}_{19}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$ 363.1564, found 363.1566.

1-(2-Chlorofuro [3,2-d] pyrimidin-4-yl)piperidine-4-carbonitrile (11a). Compound 11a was obtained by following General procedure C. Yield: 96%. ^1H NMR (600 MHz, CDCl_3) δ 7.77 (d, J = 2.1 Hz, 1H), 6.79 (d, J = 2.1 Hz, 1H), 4.23 (qd, J = 7.2, 3.6 Hz, 2H), 4.01 (qd, J = 7.3, 3.6 Hz, 2H), 3.00-3.04 (m, 1H), 2.07 (tt, J = 10.7, 3.4 Hz, 2H), 1.97-2.02 (m, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ 154.2, 153.7, 148.9, 148.2, 133.1, 120.6, 107.6, 43.2, 28.4, 26.3.

1-(2-((1-Methyl-1H-pyrazol-4-yl)amino)furo [3,2-d] pyrimidin-4-yl)piperidine-4-carbonitrile (11). Compound 11 followed General

Procedure D. Yield: 55%. ^1H NMR (600 MHz, CDCl_3) δ 7.76 (s, 1H), 7.62 (d, J = 1.7 Hz, 1H), 7.47 (s, 1H), 6.80 (s, 1H), 6.65 (d, J = 2.1 Hz, 1H), 4.20 (qd, J = 7.1, 3.6 Hz, 2H), 3.89-3.93 (m, 2H), 3.88 (s, 3H), 2.95-2.99 (m, 1H), 2.04 (qd, J = 7.0, 3.3 Hz, 2H), 1.96 (tt, J = 10.7, 3.8 Hz, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ 156.6, 153.3, 148.4, 148.0, 131.1, 130.5, 123.9, 121.1, 107.3, 43.3, 39.3, 28.6, 26.7; HRMS (ESI): m/z calcd for $\text{C}_{16}\text{H}_{18}\text{N}_7\text{O}$ $[\text{M}+\text{H}]^+$ 324.1568, found 324.1584.

tert-butyl 4-(2-Chlorofuro [3,2-*d*] pyrimidin-4-yl)piperazine-1-carboxylate (12a). Compound 12a was prepared by following General Procedure C. Yield: 98%. ^1H NMR (600 MHz, CDCl_3) δ 7.71 (d, J = 2.1 Hz, 1H), 6.72 (d, J = 2.1 Hz, 1H), 3.95 (s, 4H), 3.52 (t, J = 5.2 Hz, 4H), 1.44 (s, 9H); ^{13}C NMR (150 MHz, CDCl_3) δ 154.6, 154.4, 153.7, 149.0, 148.6, 133.3, 107.7, 80.5, 45.1, 43.0, 28.4.

1-(4-(2-Chlorofuro [3,2-*d*] pyrimidin-4-yl)piperazin-1-yl)ethenone (12c). Compound 12c was obtained via acetylation reaction by using compound 12b. Yield: 82%. ^1H NMR (600 MHz, CDCl_3) δ 7.78 (d, J = 2.1 Hz, 1H), 6.81 (d, J = 2.1 Hz, 1H), 4.04-4.07 (m, 4H), 3.78 (t, J = 5.2 Hz, 2H), 3.63 (t, J = 5.2 Hz, 2H), 2.18 (s, 3H); ^{13}C NMR (150 MHz, CDCl_3) δ 169.3, 154.4, 154.0, 149.2, 148.6, 133.3, 107.9, 46.1, 41.3, 21.5.

1-(4-(2-((1-Methyl-1H-pyrazol-4-yl)amino)furo [3,2-*d*] pyrimidin-4-yl)piperazin-1-yl)ethenone (12). Compound 12 was prepared by following General Procedure D. Yield: 50%. ^1H NMR (600 MHz, CDCl_3) δ 7.76 (s, 1H), 7.62 (d, J = 2.1 Hz, 1H), 7.47 (s, 1H), 6.80 (s, 1H), 6.65 (d, J = 2.1 Hz, 1H), 3.97 (td, J = 10.0, 5.0 Hz, 4H), 3.87 (s, 3H), 3.75 (t, J = 5.2 Hz, 2H), 3.59 (dd, J = 6.2, 4.1 Hz, 2H), 2.15 (s, 3H); ^{13}C NMR (150 MHz, CDCl_3) δ 169.3, 156.6, 153.4, 148.6, 148.2, 131.1, 130.5, 123.9, 121.1, 107.3, 46.2, 45.1, 45.0, 41.4, 39.4, 21.6; HRMS (ESI): m/z calcd for $\text{C}_{16}\text{H}_{20}\text{N}_7\text{O}_2$ $[\text{M}+\text{H}]^+$ 342.1673, found 342.1687.

tert-butyl 4-(4-(2-Chlorofuro [3,2-*d*] pyrimidin-4-yl)-1H-pyrazol-1-yl)piperidine-1-carboxylate (13a). Compound 13a was synthesized via Suzuki coupling reaction by following General Procedure A. Yield: 78%. ^1H NMR (600 MHz, CDCl_3) δ 8.39 (s, 2H), 8.04 (d, J = 2.1 Hz, 1H), 6.94 (d, J = 2.1 Hz, 1H), 4.30-4.42 (m, 3H), 2.94 (s, 2H), 2.23 (d, J = 11.0 Hz, 2H), 2.00 (qd, J = 12.3, 4.2 Hz, 2H), 1.49 (s, 9H); ^{13}C NMR (150 MHz, CDCl_3) δ 155.6, 155.3, 154.6, 152.6, 144.5, 141.4, 140.1, 129.0, 116.2, 107.8, 80.1, 60.5, 60.1, 32.3, 28.5, 21.1, 14.3.

tert-butyl 4-(4-(2-((1-Methyl-1H-pyrazol-4-yl)amino)furo [3,2-*d*] pyrimidin-4-yl)-1H-pyrazol-1-yl)piperidine-1-carboxylate (13b). Compound 13b was synthesized by General Procedure E via Buchwald-Hartwig coupling. Yield: 41%. ^1H NMR (600 MHz, CDCl_3) δ 8.35 (s, 1H), 8.29 (s, 1H), 7.92 (s, 1H), 7.85 (d, J = 2.1 Hz, 1H), 7.51 (s, 1H), 7.35 (s, 1H), 6.77 (d, J = 2.1 Hz, 1H), 4.25-4.39 (m, 3H), 3.91 (s, 3H), 2.92 (d, J = 6.2 Hz, 2H), 2.18-2.21 (m, 2H), 1.99 (t, J = 10.0 Hz, 2H), 1.49 (s, 9H); ^{13}C NMR (150 MHz, CDCl_3) δ 157.2, 154.5, 154.4, 150.9, 142.8, 139.5, 138.3, 130.6, 128.3, 123.7, 120.8, 117.3, 107.1, 79.9, 59.7, 42.9, 42.5, 39.2, 32.2, 32.1, 28.3.

N-(1-Methyl-1H-pyrazol-4-yl)-4-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)furo [3,2-*d*] pyrimidin-2-amine (13). Compound 13 was obtained by Boc deprotection of compound 13b. Yield: 76%. ^1H NMR (600 MHz, CDCl_3) δ 8.35 (s, 1H), 8.31 (s, 1H), 7.93 (s, 1H), 7.84 (d, J = 2.1 Hz, 1H), 7.52 (s, 1H), 7.25 (s, 1H), 6.77 (d, J = 1.7 Hz, 1H), 4.32 (tt, J = 11.7, 4.1 Hz, 1H), 3.91 (s, 3H), 3.26-3.28 (m, 2H), 2.79 (td, J = 12.4, 2.1 Hz, 2H), 2.21-2.23 (m, 2H), 1.95 (qd, J = 12.3, 4.0 Hz, 2H), 1.76 (s, 1H); ^{13}C NMR (150 MHz, CDCl_3) δ 157.2, 154.4, 150.9, 143.0, 139.4, 138.3, 130.6, 128.1, 123.7, 120.9, 117.2, 107.2, 60.2, 45.6, 39.2, 33.8; HRMS (ESI): m/z calcd for $\text{C}_{18}\text{H}_{21}\text{N}_8\text{O}$ $[\text{M}+\text{H}]^+$ 365.1833, found 365.1834.

3-(4-(4-(2-((1-Methyl-1H-pyrazol-4-yl)amino)furo [3,2-*d*] pyrimidin-4-yl)-1H-pyrazol-1-yl)piperidin-1-yl)-3-oxopropanenitrile (14). Compound 14 was prepared by amide coupling reaction by using cyanoacetic acid. Yield: 90%. ^1H NMR (600 MHz, CDCl_3) δ 8.35 (s, 1H), 8.31 (s, 1H), 7.90 (s, 1H), 7.86 (d, J = 2.1 Hz, 1H), 7.55 (s, 1H), 7.04 (s, 1H), 6.78 (d, J = 2.1 Hz, 1H), 4.72-4.63 (m, 1H), 4.54-4.47 (m, 1H), 3.95-3.89 (m, 4H), 3.58 (d, J = 6.9 Hz, 2H), 3.44-3.37 (m, 1H), 3.00-2.93 (m, 1H), 2.33 (dt, J = 40.6, 5.9 Hz, 2H), 2.20-2.06 (m, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ 160.0, 157.2, 154.5, 151.1, 142.7, 139.8,

138.3, 130.7, 128.5, 123.7, 120.9, 117.6, 113.7, 107.1, 58.7, 45.2, 41.4, 39.2, 32.3, 31.5, 25.0; HRMS (ESI): m/z calcd for $\text{C}_{21}\text{H}_{22}\text{N}_9\text{O}_2$ $[\text{M}+\text{H}]^+$ 432.1891, found 432.1887.

3-(4-(4-(2-((1-Methyl-1H-pyrazol-4-yl)amino)furo [3,2-*d*] pyrimidin-4-yl)-1H-pyrazol-1-yl)piperidin-1-yl)propanenitrile (15). Compound 15 was prepared by using compound 13 through General Procedure B. Yield: 12%. ^1H NMR (600 MHz, CDCl_3) δ 8.37-8.30 (m, 2H), 7.92-7.85 (m, 2H), 7.59 (d, J = 34.4 Hz, 1H), 6.97 (s, 1H), 6.77 (d, J = 2.8 Hz, 1H), 4.29-4.23 (m, 1H), 3.94 (d, J = 14.5 Hz, 3H), 3.08 (d, J = 11.7 Hz, 1H), 2.78 (t, J = 7.2 Hz, 1H), 2.57 (t, J = 6.9 Hz, 1H), 2.34 (t, J = 11.0 Hz, 1H), 2.26 (d, J = 11.0 Hz, 1H), 2.18-2.12 (m, 2H), 2.09-2.00 (m, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ 157.3, 154.5, 151.2, 143.2, 139.6, 139.4, 130.9, 128.4, 123.9, 121.2, 118.8, 117.5, 107.3, 59.7, 53.3, 52.2, 39.4, 32.5, 31.1, 29.9, 16.5; HRMS (ESI): m/z calcd for $\text{C}_{21}\text{H}_{23}\text{N}_9\text{O}$ $[\text{M}+\text{H}]^+$ 418.2099, found 418.2111.

1-(4-(4-(2-((1-Methyl-1H-pyrazol-4-yl)amino)furo [3,2-*d*] pyrimidin-4-yl)-1H-pyrazol-1-yl)piperidin-1-yl)ethenone (16). Compound 16 was prepared by using acetylation reaction of compound 13. Yield: 33%. ^1H NMR (600 MHz, CDCl_3) δ 8.35 (s, 1H), 8.30 (s, 1H), 7.91 (s, 1H), 7.85 (d, J = 2.1 Hz, 1H), 7.55 (s, 1H), 6.95 (s, 1H), 6.78 (d, J = 1.7 Hz, 1H), 4.81-4.78 (m, 1H), 4.46 (tt, J = 11.4, 4.1 Hz, 1H), 4.00 (d, J = 13.8 Hz, 1H), 3.92 (s, 3H), 3.31-3.26 (m, 1H), 2.82-2.78 (m, 1H), 2.32-2.24 (m, 2H), 2.16 (s, 3H), 2.07-2.01 (m, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ 169.0, 157.2, 154.5, 151.0, 142.8, 139.7, 138.4, 130.8, 128.4, 123.6, 121.0, 117.5, 107.2, 59.4, 45.2, 40.4, 39.3, 32.9, 31.8, 21.5; HRMS (ESI): m/z calcd for $\text{C}_{20}\text{H}_{22}\text{N}_8\text{O}_2$ $[\text{M}+\text{H}]^+$ 407.1939, found 407.1950.

N-(1-Methyl-1H-pyrazol-4-yl)-4-(1-(1-methylpiperidin-4-yl)-1H-pyrazol-4-yl)furo [3,2-*d*] pyrimidin-2-amine (17). Compound 17 was prepared by using methylation reaction of compound 13. Yield: 94%. ^1H NMR (600 MHz, CDCl_3) δ 8.34 (s, 1H), 8.31 (s, 1H), 7.93 (s, 1H), 7.84 (d, J = 2.1 Hz, 1H), 7.51 (s, 1H), 7.09 (s, 1H), 6.77 (d, J = 2.1 Hz, 1H), 4.25-4.20 (m, 1H), 3.91 (s, 3H), 3.01 (d, J = 11.0 Hz, 2H), 2.35 (s, 3H), 2.24 (d, J = 11.7 Hz, 2H), 2.20-2.11 (m, 4H); ^{13}C NMR (150 MHz, CDCl_3) δ 157.2, 154.4, 150.9, 143.0, 139.4, 138.3, 130.6, 128.0, 123.7, 120.9, 117.3, 107.2, 59.4, 54.5, 46.0, 39.2, 32.5; HRMS (ESI): m/z calcd for $\text{C}_{19}\text{H}_{22}\text{N}_8\text{O}$ $[\text{M}+\text{H}]^+$ 379.1990, found 379.1998.

4.2. In vitro kinase assay

Kinase profiles (IC_{50} or % inhibition) of final compounds were tested using the kinase HotSpot Profiling service of Reaction Biology Corporation (USA). All kinase assays were performed in the presence of Km concentration of ATP.

4.3. Molecular docking

The 2D structures of molecules were converted to 3D structures using Maestro LigPrep. The structures of JAK family kinases were obtained from the Protein Data Bank (6N7B for JAK1 and 6VNE for JAK2). The Grid files were obtained following the standard procedure recommended by Schrodinger. The 3D structures were docked flexibly using Glide in XP mode, and other docking parameters were set to default values. Five predicted poses were obtained during the docking process, and the Grid-based docking score was calculated for each pose. The image files were generated by PyMOL.

4.4. Metabolic stability assay

Human and mouse liver microsomes (0.5 mg ml⁻¹, Corning, cat No. #452117, #452701) were pre-incubated with compounds (1 μM) in 0.1 M phosphate buffer (pH 7) at 37 °C for 5 min. The reaction was initiated by adding the NADPH Regeneration System solution (Promega, #V9510) and incubating for 30 min at 37 °C. To stop the reaction, an acetonitrile solution containing chlorpropamide as an internal standard was added, followed by centrifugation at 15000 rpm and 4 °C for 15

min. The supernatant was injected into the LC-MS/MS system (Shimadzu Nexera XR) for analysis. Experiments were conducted duplicate with variation within 15%. The results were quantified by comparing the values obtained after 30 min to those obtained at 0 min, expressed as % remaining. A positive control group using Verapamil (1 μ M) in human microsomes showed internal reference values within 15% ($\pm$ 1), confirming the experimental accuracy.

CRedit authorship contribution statement

Mingi Kim: Validation, Methodology, Investigation. **Yong Jin Ahn:** Validation, Data curation. **Taek Jun Nam:** Visualization, Validation, Investigation. **Chanhee Han:** Validation. **Kyung Hoon Min:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejmcr.2026.100341>.

Data availability

No data was used for the research described in the article.

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