이학박사 학위논문

Design, synthesis and evaluation of substituted N-methyl-N-(pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amines as JAK1-selective inhibitors for the treatment of rheumatoid arthritis

류마티스 관절염 치료를 위한 JAK1 선택적 억제제로서의 N-메틸-N-(피롤리딘-3-일)-7H-피롤로[2,3-d]피리미딘-4-아민의 설계, 합성 및 평가

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화학부 유기화학 전공

조 지 연

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Design, synthesis and evaluation of substituted *N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines as JAK1-selective inhibitors for the treatment of rheumatoid arthritis

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Based on (*R*)-*N*-methyl-*N*-(5-azaspiro[2.4]heptan-7-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine as a core scaffold, we identified (*R*)-3-(7-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-oxopropanenitrile [(*R*)-6c] as a JAK1 selective inhibitor. The structural design was based on the combination of tofacitinib's 7-deazapurine and 5-azaspiro[2.4]heptan-7-amine. Compound (*R*)-6c exhibited 8.5 nM IC$_{50}$ on JAK1 with a selectivity index of 48 over JAK2. To optimize (*R*)-6c as a lead compound, we performed cell-based functional assays, human whole blood tests, *in vitro* ADME, hERG, kinase profiling, and pharmacokinetic tests. Rat *in vivo* studies verified that (*R*)-6c exhibited desired efficacies on CIA and AIA models.

Key words: JAK inhibitor, rheumatoid arthritis, JAK1-selective, collagen-induced arthritis mouse model, adjuvant-induced arthritis rat model

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Design, synthesis and evaluation of substituted *N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines as JAK1-selective inhibitors for the treatment of rheumatoid arthritis

I.   Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that affects approximately 1~2% of the worldwide population.[1-2] Despite the high number afflicted by the disease, its pathogenesis and mechanism have still been elusive[3] and target-oriented fundamental therapy for this disease has not yet been made available. Considerable work has been conducted for therapeutic targets[4-5] and recently emerging molecular targets like cytokines,[6-7] G-protein coupled receptors,[8] and kinases[9-10] have surfaced. The drugs and developing candidates against these targets are categorized as disease-modifying antirheumatic drugs (DMARDs).[11] The most commonly used drugs for this disease include conventional synthetic DMARDs, such as methotrexate, sulfasalazine, leflunomide, etc.[12] However, they cannot be used for long-term treatment due to the low therapeutic response and severe side effects. To overcome such limitations, researchers have developed biological DMARDs[13] like etanercept, infliximab, and adalimumab. Although the biological DMARDs exhibit higher efficacies than synthetic ones, their applications also have several drawbacks due to the high cost, efficacy limitation on single administration,[14] limited accessibility due to intravenous (i.v.) administration,[15] etc.

To resolve the unmet medical needs in RA, many researchers have focused on developing new synthetic DMARDs equipped with high efficacy, low cost, and convenient administration regimen. As a result, Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signal pathways have been identified as new therapeutic targets. JAK kinases had first been isolated in 1989[16] and their roles were discovered in 1994.[17] In the immune system, the sequential processes of this signaling proceeds as follows: 1) cytokines interact with extracellular membrane receptors, 2) a receptor pair is dimerized, 3) the dimer is combined with JAKs dependent upon the cytokines and the receptors, 4) the combined JAKs and dimer are phosphorylated, 5) STATs are introduced into the phosphorylated dimer, 6) the STATs are

phosphorylated (pSTAT) and separated from the dimer, 7) the separated STATs are dimerized and translocated into a nucleus, and 8) transcription of inflammation factors is triggered through the binding.[18] The factors involved in the JAK-STAT signaling like the cytokines, receptors, STATs, and JAKs are related to many autoimmune diseases including rheumatoid arthritis, psoriasis, myelofibrosis, Crohn's disease, and ulcerative colitis. JAK3, out of four JAK isotypes, has received the most attention since it is mostly located in hematopoietic cells and affects the lymphoid cell function unlike others.[19] In 2003, researchers at Pfizer reported tofacitinib as a JAK3 inhibitor.[20] Its median inhibitory concentrations (IC_{50}) measured by ELISA were described as 1 nM for JAK3, 20 nM for JAK2, and 112 nM for JAK1. However, other Pfizer workers published different inhibitory activities by peptide mobility shift assay in 2010,[21] where the IC_{50}'s were 3.2, 4.1, 1.6, and 34.0 nM's for JAK1, JAK2, JAK3, and TYK2, respectively, rendering the tofacitinib a pan-JAK inhibitor. The fact that it suppresses all JAK-STAT signal pathways explains its excellent potencies in many preclinical[22] and clinical trials[23-25]. Finally, tofacitinib became the first US Food and Drug Administration (FDA) approved oral drug for the treatment of rheumatoid arthritis in 2012[26] with the trade name Xeljanz.

Using Pan-JAK inhibitors like tofacitinib for treatment is accompanied by some drawbacks since they inhibit all JAK isoenzymes. In particular, preclinical studies[27-29] and clinical trials[30-31] have revealed adverse effects derived from JAK2 inhibition like anemia, neutropenia, increased low and high density lipoprotein cholesterol levels, and elevated triglyceride levels. In the case of tofacitinib, similar adverse events have also been reported.[24-25,32] As a result, European Medicines Agency (EMA) refused the marketing authorization in Europe.[33] To avoid the undesirable events mentioned above, selective inhibitors of isoenzymes, except for JAK2, for treatment of rheumatoid arthritis have been brought to researchers' attention.[34] Nowadays, the search for JAK1-selective inhibition has been given considerable attention since it has been revealed that JAK1 inhibition plays a principal role on the

efficacies of tofacitinib.[35] So, many researchers have been focusing on developing JAK1-selective inhibitors. The representative JAK1-selective inhibitors are filgotinib (GLPG0634),[36,37-44] upadacitinib (ABT-494),[45-49] solcitinib (GSK2586184),[38,50-53] itacitinib (INCB039110),[54-57] PF-04965842.[58-59]

Among various JAK1-selective inhibitors,[60] the most advanced is filgotinib in phase III clinical trials by Galapagos found in 2009,[61-62] which is known to be highly selective for JAK1 over JAK2 by over 27.7 times. Its IC_{50}'s against IL-6/JAK1/pSTAT1 and GM-CSF/JAK2/pSTAT5 are 629 nM and 17453 nM, respectively.[36] From collagen-induced arthritis (CIA) mouse and rat models, its efficacy was shown to be similar to etanercept, a TNF-α blocker.[63] Through the phase IIa proof-of-concept study, the hypothesis was proven that rheumatoid arthritis can be ameliorated by treatment with JAK1-selective inhibitors.[64] Since 2016, Galapagos and Gilead have proceeded phase III clinical studies.[65-67] Despite its advantages, the reported preclinical results indicated that it induced testicular toxicity in rats and dogs. Thus, the US FDA approved a lower male maximum clinical dosage than for the female one.[68] Therefore, new JAK1-selective drugs overcoming the toxicological weakness need to be developed. Another promising candidate compound in this class is upadacitinib in phase III by AbbVie.[69-70] Although not much toxicological information on the preclinical and clinical trials of upadacitinib is available, its IC_{50}'s for JAK1 and JAK2 in cellular assay were reported to be 8 nM and 600 nM, respectively, indicating 74-fold selectivity.[71]

We have initiated our investigation on new JAK1-selective inhibitors based on the 5-azaspiro[2.4]heptan-7-amine core structure for subjugating the filgotinib limitation. New lead compounds were obtained, which met the criteria set by us for treating rheumatoid arthritis. In this paper, we describe the design, synthesis, and improved pharmaceutical efficacies of our inhibitors compared to filgotinib.

II.   Strategy
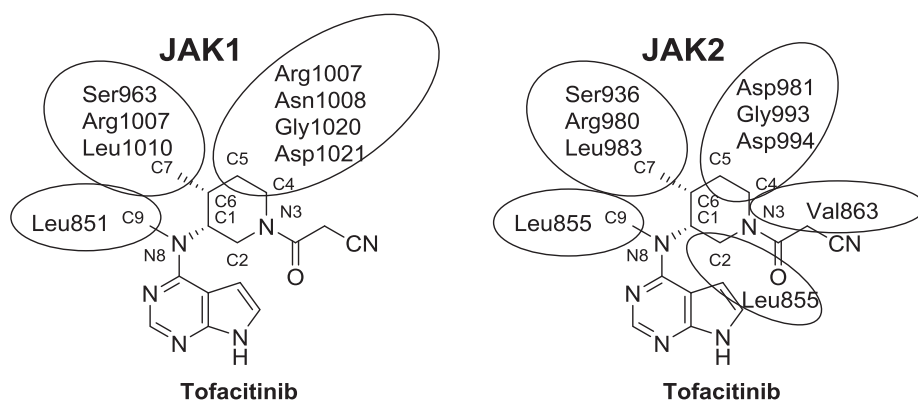
Figure 1 Interactions of tofacitinib with JAK1 or JAK2.

According to the tofacitinib's X-ray crystal structure reported by N. K. Williams et al.,[72] the interactions between the piperidine moiety of tofacitinib and each isozyme including JAK1 and JAK2 appear to be the basis for binding affinity differentiation. Especially, the carbon atoms C4, C5, and C7 of the piperidine ring may play an important role: notable interactions are those of C4 and C5 with Arg1007, Asn1008, Gly1020, and Asp1021 at JAK1 (Asp981, Gly993, and Asp994 at JAK2) and C7 with Ser963, Arg1007, and Leu1010 at JAK1 (Ser936, Arg980, and Leu983 at JAK2). However, the C2 and N3 atoms appear to be involved in binding JAK2, but not JAK1. Therefore, we hypothesized that changing the piperidine moiety of tofacitinib can alter the binding affinity with JAK2 more than the one with JAK1.
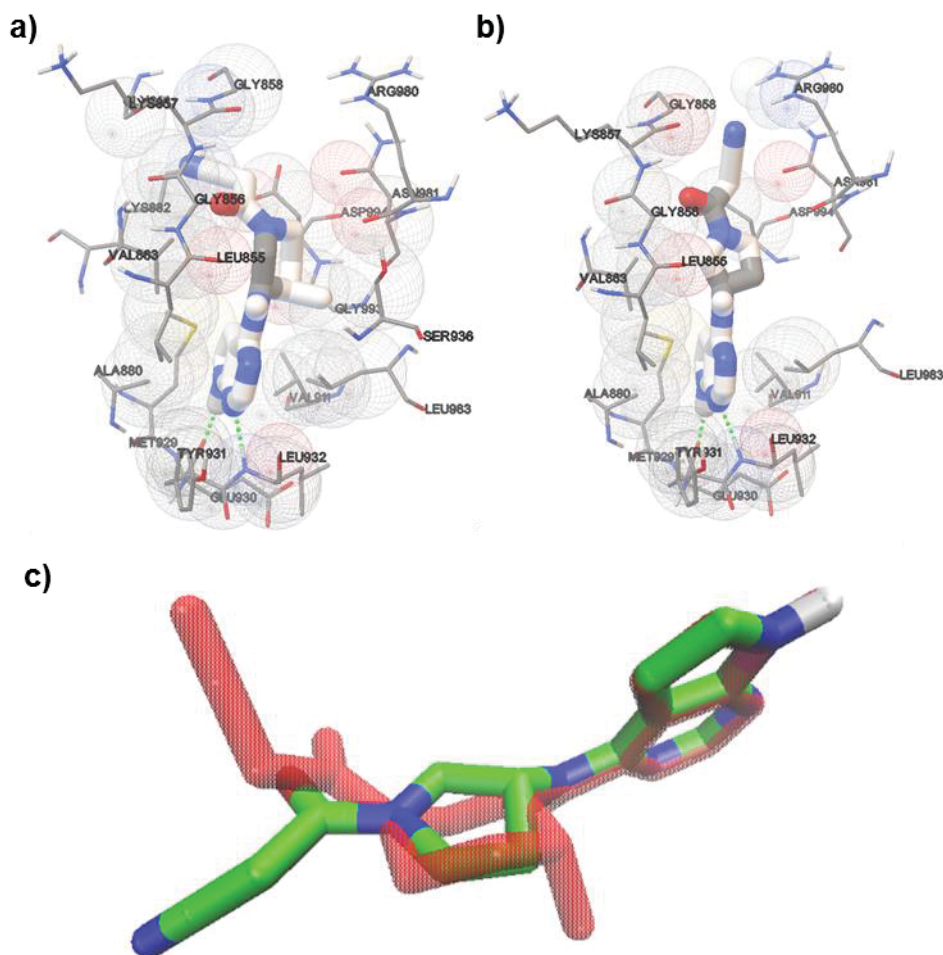
Figure 2 Docking simulation of a) tofacitinib and b) compound **12a** at JAK2 (PDB ID: 3FUP) and c) overray of the lowest conformations of tofactinib (red color) and compound **12a** at JAK2.

Based upon our hypothesis, we selected a pyrrolidine moiety in place of the piperidine of tofacitinib. A docking simulation using AutoDock 4.2 program[73] was performed to assess the effect of the pyrrolidine substitution at the piperidine site of the inhibitors. The estimated binding energies of tofacitinib and our representative compound **12a** at JAK1 (PDB ID: 3EYG) were -8.10 and -7.50 kcal/mol, respectively. And besides, estimated binding energies of -8.98 and -7.93 kcal/mol, respectively, for tofacitinib and compound

12a were obtained in the case of JAK2 binding (PDB ID: 3FUP, Figure 2). At JAK2, especially, the binding energy difference between tofacitinib and compound **12a** is influenced by their intermolecular energies composed of van der Waals, hydrogen bonding, electrostatic, and desolvation energies. Increasing intermolecular energy of compound **12a** seems to result from lacking the interactions with Ser963 and Leu983 at JAK2. From the above result, we expected that compound **12a** would exhibit lower binding affinity for JAK2 through the substitution into pyrrolidine moiety. In addition, since the methyl group of C9 at tofactinib appears to interact with Leu855 at JAK2, replacing the methyl group by another alkyl group may also influence the binding affinity at JAK2. According to the docking results, we designed inhibitors possessing several substituted pyrrolidine moieties equipped with various alkyl groups at the bridging amino group of compound **12a**.
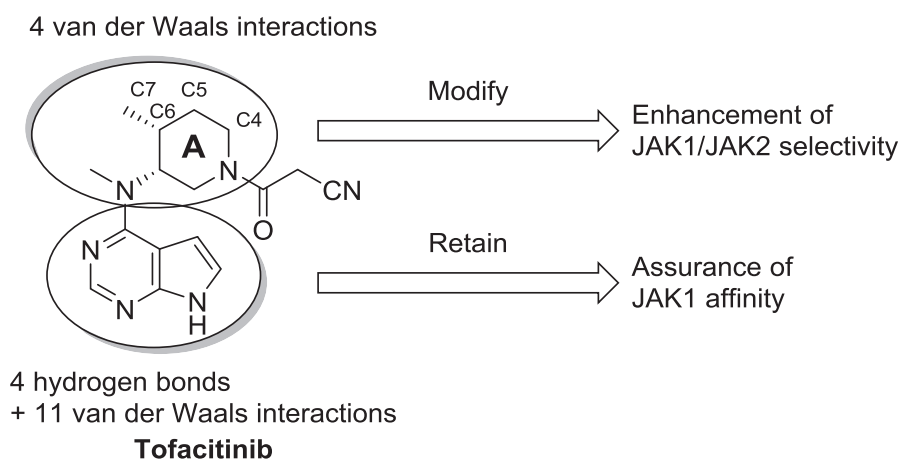
Figure 3 Design strategy by changing the piperidine moiety A.

III.   Synthesis

Methylation of primary amino groups
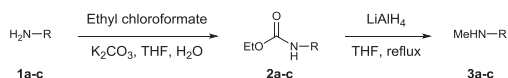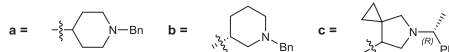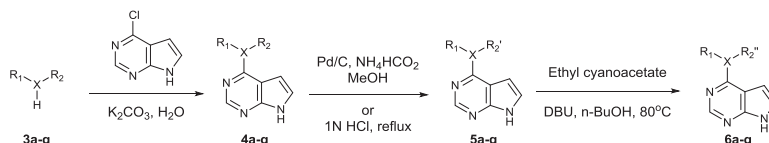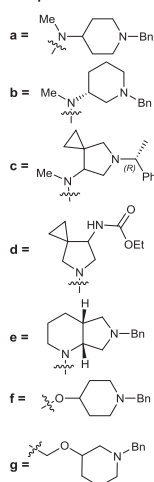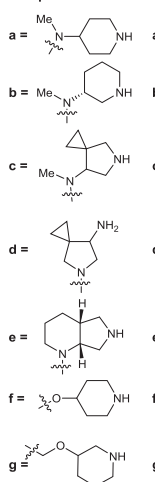
Synthesis of inhibitors
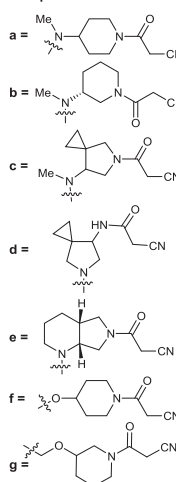
Scheme 1 Synthesis of inhibitors containing various heterocyclic core units replacing the aminopiperidine unit of tofacitinib and substituted *N*-methyl-*N*-(5-azaspiro[2.4]heptan-7-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines.

Various monocyclic and bicyclic nitrogen containing compounds **6a –
6g** were synthesized for the selection of the most optimal scaffold at the position
A in Figure 3 as shown in Scheme 1. Commercially available 4-amino-1-
benzylpiperidine and (*R*)-3-amino-1-benzylpiperidine were converted to the *N*-
ethyloxycarbonyl protected compounds, which were treated with lithium
aluminium hydride to result in the formation of methylamine derivatives **3a** and

3b. The key pyrrolidine component of compound **6c**, 5-((*R*)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-amine, was prepared according to the method reported by Y. Kimura and colleagues.[74] We obtained each diastereomer of 5-((*R*)-1-phenylethyl)-5-azaspiro-[2.4]heptan-7-amine with the carbon 7 as an epimeric center. Methylated compound **3c** was obtained through the above method from 5-((*R*)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-amine. Compound **3d** was synthesized through debenzylation of **2c**. A bis(hydrochloric acid) salt form of bicyclic amine **3e**, (*R*,*R*)-6-benzyl-octahydro-pyrrolo[3,4-*b*]pyridine dihydrochloride was purchased from Sigma-Aldrich, USA. Commercially available compounds such as 4-hydroxypiperidine and *rac*-3-hydroxymethylpiperidine were converted to the corresponding benzylated amines, **3f** and **3g**.

The obtained amines and alcohols, **3a** – **3g**, were used for the coupling with the 6-chloro-7-deazapurine in an aqueous solution, leading to compounds **4a** – **4g**. We then performed debenzylation of **4a-c** and **4e-f** using palladium on carbon and ammonium formate to remove benzyl and (*R*)-1-phenylethyl protection groups, obtaining **5a-c** and **5e-f**. In the case of **5d**, *N*-ethyloxycarbonyl group of **4d** was deprotected with 1 N aqueous hydrochloric acid under reflux condition. From compounds **5a** – **5g**, the corresponding amide couplings were carried out with ethyl cyanoacetate and 1,8-diazabicyclo[5.4.0]undec-7-ene at 80 °C, leading to compounds **6a** – **6g** according to the reaction pathway shown in Scheme 1.

Since the inhibitor (***R***)-**6c** synthesized from 7(*R*)-5-((*R*)-1-phenylethyl)-7-amino-5-azaspiro[2.4]heptane (***R***)-**1c** showed the most promising inhibition selectivity between JAK1 and JAK2, we focused our efforts on compound (***R***)-**6c**. Amine (***R***)-**5c** was transformed to the final compounds **6c** and **19** – **68** through various reactions at the pyrrolidine nitrogen.

Alkylation of primary amino groups

7a: R_1 = H, R_2 = H, R_3 = H
7b: R_1 = Me, R_2 = Me, R_3 = Me
7c: -R_1-R_2- = -CH$_2$CH$_2$-, R_3 = Me
7d: -R_1-R_2- = -CH$_2$CH$_2$CH$_2$-, R_3 = Me

8aa: R_1 = H, R_2 = H, R_3 = H, R_4 = tBuO
8ab: R_1 = H, R_2 = H, R_3 = H, R_4 = Me
8ac: R_1 = H, R_2 = H, R_3 = H, R_4 = cyclopropyl
8b: R_1 = Me, R_2 = Me, R_3 = Me, R_4 = tBuO
8c: -R_1-R_2- = -CH$_2$CH$_2$-, R_3 = Me, R_4 = tBuO

9aa: R_1 = H, R_2 = H, R_3 = H, R_5 = Me
9ab: R_1 = H, R_2 = H, R_3 = H, R_5 = Et
9ac: R_1 = H, R_2 = H, R_3 = H, R_5 = cyclopropyl-CH$_2$-
9b: R_1 = Me, R_2 = Me, R_3 = Me, R_5 = Me
9c: -R_1-R_2- = -CH$_2$CH$_2$-, R_3 = Me, R_5 = Me

Synthesis of inhibitors

9aa: R_1 = H, R_2 = H, R_3 = H, R_5 = Me
9ab: R_1 = H, R_2 = H, R_3 = H, R_5 = Et
9ac: R_1 = H, R_2 = H, R_3 = H, R_5 = cyclopropyl-CH$_2$-
7a: R_1 = H, R_2 = H, R_3 = H, R_5 = H
9b: R_1 = Me, R_2 = Me, R_3 = Me, R_5 = Me
9c: -R_1-R_2- = -CH$_2$CH$_2$CH$_2$-, R_3 = Me, R_5 = Me

10aa: R_1 = H, R_2 = H, R_3 = H, R_5 = Me
10ab: R_1 = H, R_2 = H, R_3 = H, R_5 = Et
10ac: R_1 = H, R_2 = H, R_3 = H, R_5 = cyclopropyl-CH$_2$-
10ad: R_1 = H, R_2 = H, R_3 = H, R_5 = H
10b: R_1 = Me, R_2 = Me, R_3 = Me, R_5 = Me
10c: -R_1-R_2- = -CH$_2$CH$_2$CH$_2$-, R_3 = Me, R_5 = Me

11aa: R_1 = H, R_2 = H, R_5 = Me
11ab: R_1 = H, R_2 = H, R_5 = Et
11ac: R_1 = H, R_2 = H, R_5 = cyclopropyl-CH$_2$-
11ad: R_1 = H, R_2 = H, R_5 = H
11b: R_1 = Me, R_2 = Me, R_5 = Me
11c: -R_1-R_2- = -CH$_2$CH$_2$CH$_2$-, R_5 = Me

12a - 18, 69 - 96

Scheme 2 Synthesis of substituted (*R*)-*N*-alkyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines.

For screening on the substituents at C4 atom of pyrrolidine moiety, four 3-aminopyrrolidine derivatives with varying R_1 and R_2 substituents at the 4-position were chosen for the studies, namely (*R*)-1-benzylpyrrolidin-3-amine (**7a**), (*R*)-4,4-dimethyl-1-((*R*)-1-phenylethyl)pyrrolidin-3-amine (**7b**), (*R*)-5-((*R*)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-amine (**7c**), and (*R*)-6-((*R*)-1-phenylethyl)-6-azaspiro[3.4]octan-8-amine (**7d**). Except for the commercially available (*R*)-3-amino-1-benzylpyrrolidine (**7a**), compounds **7b**, **7c** and **7d** were synthesized according to published methods.[74-75] Scheme 2 shows a synthetic sequence leading to the pyrrrolidines **11aa** − **11d**, from which a variety of derivatives (**12a** − **18** and **69** − **96**) were prepared as potential JAK1 inhibitors: 1) the primary amino group of **7a-d** was protected from the reaction with di-*tert*-butyl dicarbonate, acetic anhydride, or cyclopropanecarbonyl chloride, 2) the *N*-ethoxycarbonyl-, *N*-acetyl- or *N*-cyclopropanecarbonyl-protected compounds **8aa** − **8d** were treated with LiAlH$_4$ to yield alkylated amines **9aa** − **9d**, 3) the alkylamine **9aa** − **9d** and the unprotected amine **7a** were allowed to react with 6-chloro-7-deazapurine to produce compounds **10aa** − **10d**, 4) hydrogenolysis using palladium on carbon and ammonium formate removed the benzyl group of **10aa** − **10ad** or 1-phenylethyl moiety of **10b** −

10d. The desired inhibitors **12a** and **69 – 96** were obtained from **11aa – 11d** through amide coupling, sulfonylation, alkylation, carbonylation, etc.
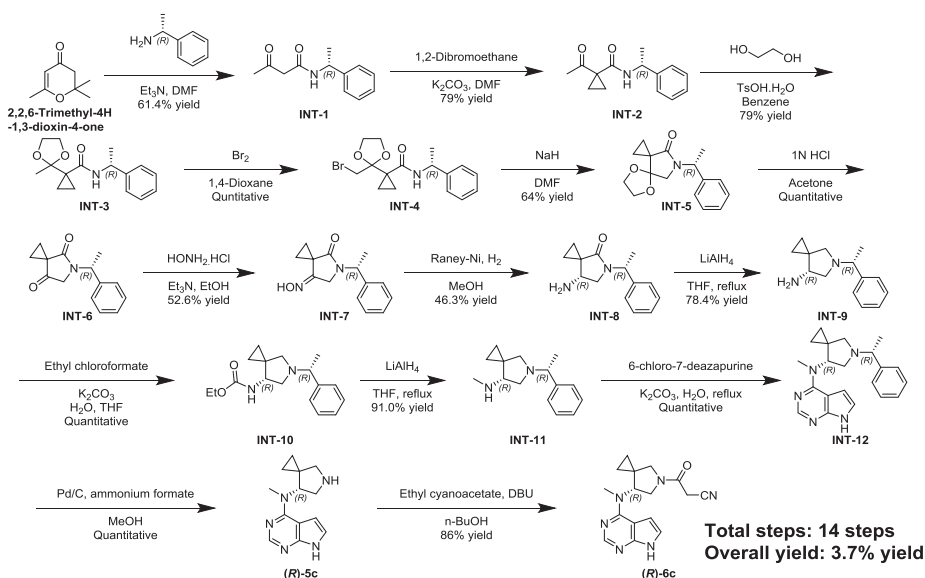
Scheme 3 Synthetic scheme of (*R*)-3-(7-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-oxopropanenitrile, **(*R*)-6c**.

Although we found that compound **(*R*)-6c** from (*R*)-*N*-methyl-*N*-(5-azaspiro[2.4]heptan-7-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine has a higher selectivity than compound **12a**, the reason that we tried to screen the derivatives from (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine is the synthetic cost of compound **(*R*)-6c**. By Y. Kimura's method[74] we synthesized intermediate INT-9 which was not commercially available so that the total synthetic steps of **(*R*)-6c** consist of 14 steps. Moreover, the overall synthetic yield of **(*R*)-6c** became 3.7%. Because of long synthetic steps and low overall yield, the synthetic cost of compound **(*R*)-6c** was calculated as 150 million Korean won per 1 kg. So we selected compound **11aa** as an alternative scaffold because of commercially available (*R*)-1-benzylpyrrolidin-3-amine (**7a**).

IV. Results and Discussions

Enzyme assay

Table 1 Screening of the hydrophobic moieties (moiety A).

Compound	A N O CN	Inhibition% at 1 μM	
		JAK1	JAK2
6a		49%	1%
6b		99%	83%
6c		95%	53%
6d		6%	10%
6e		54%	-7%
6f		35%	5%
6g		30%	10%

For the selection of a new scaffold, we first screened substituted piperidine and pyrrolidine scaffolds at the position A in Figure 3 (Table 1). Each compound was evaluated for inhibition against JAK1 and JAK2 at 1 μM concentration. In the case of **6a** and **6f**, the inhibition abilities against JAK1 and JAK2 do not appear to be influenced by the connecting atom (nitrogen vs oxygen) at the C(4) position of piperidine. However, the amino-substitution position at the piperidine ring appeared to be an important factor for determining affinities for not only JAK1 but also JAK2. Between **6a** and **6b**, the substitution at the C(3) position of piperidine (**6b**) was more favoured for both JAK1 and JAK2 inhibitions than the substitution at the C(4) position (**6a**) was. The substitution with methyloxy group (**6g**) was disfavoured for JAK1

affinity. In the case of **6e**, introducing (4a*R*,7a*R*)-octahydro-1*H*-pyrrolo[3,4-*b*]pyridine lowered the inhibition against JAK1. In introducing 5-azaspiro[2.4]heptan-7-amine moiety, the substitution position was important. While compound **6c** exhibited strong inhibition against JAK1, **6d** displayed very low inhibition against JAK1. Though both **6b** and **6c** showed strong inhibition against JAK1, **6b** showed high inhibition on JAK2 as well. Therefore, we selected **6c** as our scaffold for further SAR studies for finding inhibitors with high JAK1/JAK2 selectivity.

Table 2 Comparison of JAK1 IC_{50} values of 3-(7-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-oxopropanenitrile   racemate   and enantiomers.
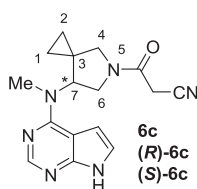
Compound	Configuration at 7-position	JAK1 IC_{50} (nM)
6c	racemate	29
(**R**)-**6c**	(*R*)	8.5
(**S**)-**6c**	(*S*)	7.9×10^2

Our further SAR study was based upon **5c** as a scaffold for derivatives on the pyrrolidine nitrogen. When a racemic mixture **6c** was tested against the JAK1 isozyme (Table 2), it showed an IC_{50} value of 29 nM, proving that it could be used as a good lead for new JAK1 inhibitors. Then we investigated both enantiomers of **6c**. Compound (**R**)-**6c** exhibited 8.5 nM against JAK1, whereas 7.9×10^2 nM IC_{50} was observed with the enantiomeric (**S**)-**6c**. As a result, for further SAR studies the (*R*)-configuration of 7-amino-5-azaspiro[2.4]heptane was chosen.

Table 3 The IC_{50} values of compound **(R)-6c** and **12a-c** against JAK1 and JAK2 and the selectivity indices of substituted (R)-N-methyl-N-(pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amines according to the substitution at the 4-position of the pyrrolidine ring.

Compound	A	IC_{50} (nM)		$SI^{a)}$
		JAK1	JAK2	
12a		19	1.3×10^2	6.8
12b		2.6×10^2	5.9×10^3	23
(R)-6c		8.5	4.1×10^2	48
12c		16	4.5×10^2	28
Tofacitinib		2.0	9.9	5.0

a) SI: Selectivity Index = JAK2 IC_{50} / JAK1 IC_{50}

The 7-deazapurine moiety of tofacitinib was considered to be critical in securing the ATP-binding site of JAK isozymes, therefore it was kept in our scaffold structure. First, to evaluate the effect of the substituents at the 4-position of the pyrrolidine ring, we prepared cyanoacetyl derivatives **(R)-6c**, and **12a-c** from the four parent pyrrolidine precursors, **(R)-5c**, **11aa**, **11b**, and **11c**. We then screened the inhibitory efficiencies of the derivatives substituted with dimethyl and spirocyclic moieties at the 4-position of the pyrrolidine core, which is believed to correspond to the 4-position of the piperidine of tofacitinib (Table 3). The unsubstituted inhibitor **12a** exhibited an IC_{50} value of 19 nM for JAK1 and its selectivity index was 6.8, which was higher than that of

tofacitinib. The dimethyl-substituted **12b** was 10-fold less potent against JAK1 than that of compound **12a**, however, the spirocyclic derivatives **(R)-6c** and **12c** had similar levels of IC_{50}'s to compound **12a**. This may indicate that the binding site around the 4-position of the pyrrolidine is rather small in volume. We identified the fact that the derivative **(R)-6c** having (R)-5-benzyl-5-azaspiro[2.4]heptan-7-amine moiety showed the best selectivity of JAK1 over JAK2.

Table 4 The IC_{50} values against JAK1 and JAK2 and the selectivity indices of substituted (R)-N-(pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amines with varying R_1 and R_2 groups.

Compound	R_1	R_2	IC_{50} (nM)		$SI^{a)}$
			JAK1	JAK2	
12a		Me	19	1.3×10^2	6.8
13		Et	62	1.3×10^3	21
14			1.6×10^2	1.7×10^3	11
15		H	5.1×10^2	1.9×10^3	3.7
16		Me	4.1	57	14
17		Et	19	1.1×10^2	5.8
18			17	1.4×10^3	82

a) SI: Selectivity Index = JAK2 IC_{50} / JAK1 IC_{50}

With the *N*-alkylated compounds in hand, we fixed the pyrrolidine nitrogen with either cyanoacetate or 3-cyanobenzenesulfonyl group as R_1 at 1-position and probed the inhibitory activities by changing the R_2 at 6-position from hydrogen to cyclopropylmethyl group. In both cyanoacetyl- and 3-cyanophenylsulfonyl-substituted pyrrolidine derivatives, increasing from

methyl to ethyl and to cyclopropylmethyl decreased the inhibitory activities against JAK1, although JAK2 inhibitions were not as much affected. In the case of compound **15**, where there is no alkyl substitution on the 3-amino group, quite low level of inhibition against JAK1 was observed. It turns out that the 3-cyanophenylsulfonyl substitution resulted in better inhibition on JAK1 than the cyanoacetyl one in all the cases examined, although mixed results were obtained in selectivity indices. After the results of Table 4, we chose methyl group as R_2 and (R)-N-methyl-N-(5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine as a scaffold for further SAR studies.

Table 5 The IC_{50} values against JAK1 and JAK2 with the selectivity indices of substituted (R)-N-methyl-N-(5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amines.

Compound	R	IC_{50} (nM)		SI[a]
		JAK1	JAK2	
19		1.4×10^3	3.0×10^4	21
20		2.3×10^2	1.5×10^4	65
21		1.2×10^2	9.8×10^4	8.2×10^2
(R)-6c		8.5	4.1×10^2	48
22		21	2.5×10^2	12
23		77	1.1×10^3	14

[a] SI, Selectivity Index = JAK2 IC_{50} / JAK1 IC_{50}

Table 6 The IC_{50} values against JAK1 and JAK2 with the selectivity indices of substituted (*R*)-*N*-methyl-*N*-(5-azaspiro[2.4]heptan-7-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines (continued).

Compound	R	IC$_{50}$ (nM)		SI[a]
		JAK1	JAK2	
24		1.7×10^2	2.8×10^3	16
25		1.7×10^2	1.2×10^3	7.1
26		5.2×10^2	1.6×10^4	31
27		6.7×10^2	1.3×10^4	19
28		1.8×10^2	2.5×10^3	14
29		53	9.3×10^2	18
30		1.5×10^2	6.3×10^3	42
31		1.6×10^2	7.8×10^3	49
32		1.4×10^2	6.5×10^3	46
33		1.0×10^2	7.4×10^3	74

[a] SI, Selectivity Index = JAK2 IC_{50} / JAK1 IC_{50}

25

Table 7 The IC$_{50}$ values against JAK1 and JAK2 with the selectivity indices of substituted (*R*)-*N*-methyl-*N*-(5-azaspiro[2.4]heptan-7-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines (continued).

Compound	R	IC$_{50}$ (nM)		SI[a]
		JAK1	JAK2	
34		1.9×10^2	4.5×10^3	24
35		5.0×10^2	9.1×10^3	18
36		4.2×10^2	1.1×10^4	26
37		20	1.6×10^2	8.0
38		34	1.2×10^3	35
39		75	2.8×10^3	37
40		69	4.4×10^3	64
41		12	4.2×10^2	35
42		23	1.0×10^3	43
43		1.2×10^2	4.7×10^3	39

[a] SI, Selectivity Index = JAK2 IC$_{50}$ / JAK1 IC$_{50}$

Table 8 The IC$_{50}$ values against JAK1 and JAK2 with the selectivity indices of substituted (*R*)-*N*-methyl-*N*-(5-azaspiro[2.4]heptan-7-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines (continued).

Compound	R	IC$_{50}$ (nM)		SI[a)]
		JAK1	JAK2	
44		40	1.3×10^3	33
45		54	2.8×10^3	52
46		74	1.3×10^3	18
47		85	1.3×10^3	15
48		1.5×10^3	9.1×10^3	6.1
49		4.7×10^2	1.1×10^4	23
50		7.2	1.8×10^2	25
51		20	1.3×10^2	6.5
52		6.9	36	5.2

[a)] SI, Selectivity Index = JAK2 IC$_{50}$ / JAK1 IC$_{50}$

Table 9 The IC$_{50}$ values against JAK1 and JAK2 with the selectivity indices of substituted (R)-N-methyl-N-(5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amines (continued).

Compound	R	IC$_{50}$ (nM)		SI[a]
		JAK1	JAK2	
53		9.0	51	5.7
54		13	97	7.5
55		2.8	8.4	3.0
56		9.4	50	5.3
57		50	4.8×10^2	9.6
58		5.8	59	10
59		9.8	1.1×10^3	1.1×10^2
60		26	4.7×10^2	18
61		1.4	5.5	3.9

[a] SI, Selectivity Index = JAK2 IC$_{50}$ / JAK1 IC$_{50}$

Table 10 The IC_{50} values against JAK1 and JAK2 with the selectivity indices of substituted (R)-N-methyl-N-(5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amines (continued).

Compound	R	IC_{50} (nM)		SI[a)]
		JAK1	JAK2	
62		6.3	30	4.8
63		12	66	5.5
64		9.3	1.8×10^2	19
65		16	1.4×10^3	88
66		17	1.5×10^2	8.8
67		15	1.5×10^2	10
68		31	2.6×10^2	8.4

a) SI, Selectivity Index = JAK2 IC_{50} / JAK1 IC_{50}

To find highly selective inhibitors for JAK1, we screened compounds possessing various substituent groups at the pyrrolidine nitrogen listed in Table 5 and comparing their IC_{50} values against JAK1 and JAK2. For this study, **(R)-5c** was used as a starting material. In the cases of N(5)-alkylated compounds **19**, **20**, and **21**, inhibitor **21** has an N-benzyl group with a higher affinity to JAK1 than those with short alkyl amine groups, but was disfavored against JAK2

(1.2x10^2 nM in JAK1 vs. 9.8x10^4 nM in JAK2). The inhibitor with the benzylamine group displayed its selectivity index of 8.2x10^2 for JAK1 over JAK2.

In the cases of inhibitors with amide groups at the pyrrolidine nitrogen, those with cyanoacetyl and azidoacetyl substitutions ((R)-6c and 22, respectively) were quite potent against JAK1 with the IC$_{50}$ values of 8.5 and 21 nM, respectively. Amine compounds possessing aliphatic side chains (ethyl-, n-butyl- and benzyl- compounds, 19 – 21, respectively) showed inferior inhibitory activities against JAK1 to that of (R)-6c with IC$_{50}$ values over 100 nM's. A slightly larger isovaleric amide 23 exhibited comparable activity for JAK1 inhibition. It is interesting to note that two similarly-sized amides, isobutyramide 24 and cyclopropanecarboxamide 25, exhibited similar JAK1 inhibitions. However, compound 24 showed a higher selectivity index against JAK2 than 25. This tells us that there may be a more sensitive structural interaction in JAK2 with the amide motif. Amides substituted with a polar group like compounds 26 – 28 did not show significant inhibition against JAK1. Aroyl amides 30 – 36 also showed IC$_{50}$ values in the 100-1000 nM ranges except for the small 2-furanoyl amide 29, which gave 53 nM IC$_{50}$ against JAK1. This indicates again that a large amide group at the pyrrolidine nitrogen is not tolerated well in the JAK1 binding site.

The transition from an amide to a thioamide lowered the inhibition activity against JAK1, but not so much against JAK2 that the inhibitor possessing 2-cyanoethanethioamide exhibited a lower selectivity index than that with 2-cyanoacetate ((R)-6c vs 37). Introducing a urethane (38) into the pyrrolidine nitrogen resulted in considerable potency (IC$_{50}$ = 34 nM) against JAK1 with a selectivity index of 35. Urea compounds (39 – 48), except for 43 and 48, exhibited two-digit nanomolar IC$_{50}$'s against JAK1. For ureas made up with aliphatic amines, 1-butylurea 39 and 1-cyclohexylurea 40 showed similar affinities for JAK1 with IC$_{50}$ values of 75 and 69 nM, respectively. However, compound 40 with a cyclic alkyl urea group was inferior in the JAK2 inhibition

with IC$_{50}$'s of 4.4x10^3 nM to the acyclic urea **39** with 2.8x10^3 IC$_{50}$. Comparing 1-cyclohexyl urea **40** and phenyl urea **41**, indicates that the inhibition abilities of the latter were higher in both JAK1 and JAK2 with IC$_{50}$'s of 12 nM and 4.2x10^2 nM, respectively. Halide-substituted phenyl ureas **42 – 47** did not show any improvements in inhibitory activities compared to the parent phenyl urea, **41**. When the phenyl group of the phenyl urea was substituted with an *ortho*-phenyl group (compound **48**), the inhibition of JAK1 and JAK2 decreased precipitously with IC$_{50}$ values of 1.5x10^3 and 9.1x10^3 nM, respectively, presumably due to increased steric hindrance.
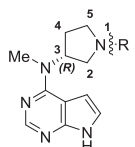
In the case of sulfonamides, most compounds displayed strong inhibition against the two enzymes. Some inhibitors showed single digit nanomolar range IC$_{50}$'s against JAK1. When amides and sulfonamides of similar sizes were compared, in all cases the inhibitors possessing a sulfonamide showed increased affinities for JAK1: the IC$_{50}$'s of **24** vs **51**, **30** vs **53**, **33** vs **58**, and **34** vs **59** were 1.7x10^2 vs 20, 1.5x10^2 vs 9.0, 1.0x10^2 vs 5.8, and 1.9x10^2 vs 9.8 nM, respectively. However, with the elevated affinities for JAK1, the sulfonamide inhibitors also increased their inhibition against JAK2, leading to lower selectivity indexes than those of amide inhibitors. The JAK1 affinity appeared to be quite sensitive towards the substituent on benzenesulfonamide (**54 – 62**): the *meta*-substitution gave the best inhibition whereas the *ortho*-substitution showed the lowest affinities for the JAK1 isozyme.
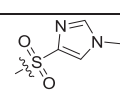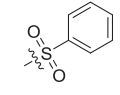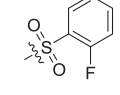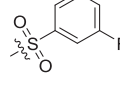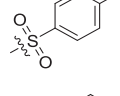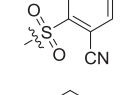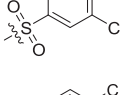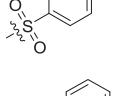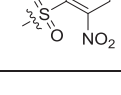
Table 11 The IC_{50} values against JAK1 and JAK2 and the selectivity indices of substituted (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines.

Compound	R	IC$_{50}$ (nM)		SI[a]
		JAK1	JAK2	
12a		19	1.3×10^2	6.8
69		53	1.9×10^3	36
70		7.4×10^2	2.7×10^4	36
71		10	1.7×10^2	17
72		70	3.9×10^3	56
73		1.1×10^2	4.3×10^3	39
74		22	5.5×10^2	25
75		70	4.7×10^3	67
76		1.4×10^2	4.5×10^3	32
77		79	2.4×10^3	30
78		1.7×10^2	4.8×10^3	28
79		34	4.6×10^2	14

[a] SI, Selectivity Index = JAK2 IC_{50} / JAK1 IC_{50}
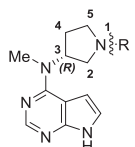
Table 12 The IC$_{50}$ values against JAK1 and JAK2 and the selectivity indices of substituted (R)-N-methyl-N-(pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amines (continued).
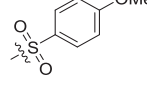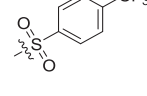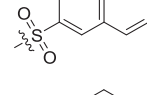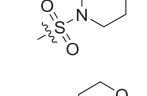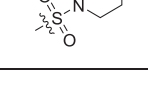
Compound	R	IC$_{50}$ (nM)		SI[a)]
		JAK1	JAK2	
80		6.5×10^2	1.9×10^4	29
81		28	4.3×10^2	15
82		25	1.3×10^2	5.2
83		14	53	3.8
84		11	1.1×10^2	10
85		52	3.7×10^2	7.1
16		4.1	57	14
86		11	1.2×10^3	1.1×10^2
87		1.1×10^2	1.1×10^3	10

a) SI, Selectivity Index = JAK2 IC$_{50}$ / JAK1 IC$_{50}$

Table 13 The IC_{50} values against JAK1 and JAK2 and the selectivity indices of substituted (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines (continued).

Compound	R	IC$_{50}$ (nM)		SI[a]
		JAK1	JAK2	
88		1.9	18	9.5
89		3.6	89	25
90		29	7.3×10^2	25
91		19	1.8×10^3	95
92		26	1.5×10^3	58
93		9.5	3.4×10^3	3.6×10^2
94		13	6.9×10^2	53
95		66	3.9×10^3	59
96		2.7×10^2	4.4×10^3	16

[a] SI, Selectivity Index = JAK2 IC$_{50}$ / JAK1 IC$_{50}$
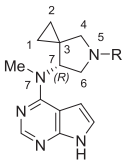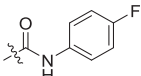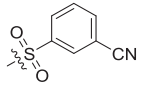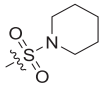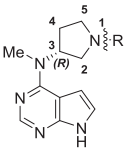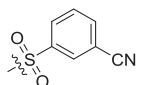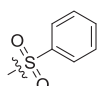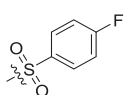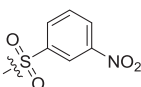
To find a new lead compound, we screened the inhibitory activities for JAK1 and JAK2 of compounds possessing a variety of substituents at the 1-nitrogen of pyrrolidine moiety (Table 6). First, a comparison between amide and alkylamine groups of similar size (**12a** vs **69**) was attempted and the amide group appeared to increase the affinity for JAK1 isozyme. This hypothesis also appears to apply to the urea functionality with compound **74** exhibiting 22 nM IC$_{50}$ value for JAK1. If the inhibitors contain an amide or urea side chain bulkier than the cyanomethyl group as in **12a**, their inhibitions for JAK1 isozyme were less effective (**70**, **72**, and **73**). However, in the case of **74**, its inhibitory activity was similar to that of compound **12a** although it has an *N*-phenyl side chain, which is larger than that of compound **12a**. With compounds **12a** and **71**, similar inhibitory activities were observed, which suggests that the planar or linear group at the side chain of amide offsets the ill effect the side chain length. The introduction of the sulfonamide on the 1-nitrogen of the pyrrolidine core improved the inhibitory activities for JAK1 (**70** vs **79**). Moreover, the arenesulfonamides (**16** and **81** – **94**) exhibited higher inhibitory activities than the sulfonamides having alkyl or heterocyclic groups (**75** – **80**). As for the substitutions at the benzene ring, inhibitors with substituents at *ortho*-position (**85** and **87**) showed lower inhibition than the *meta*- or *para*-counterparts, presumably due to steric interaction with JAK1 except for the fluorine substitution cases (**82** – **84**). In the case of the selectivity for JAK1 over JAK2, the inhibitors with substitution at *para*-position (**84**, **86**, and **89**) showed 2.5 to 7.9-fold improved JAK1 selectivity compared to those having *meta*-substitutions. Consequently, compounds **86** and **93** were the most selective for JAK1 over JAK2.

According to our enzyme assays, (***R***)**-6c** and **58** seemed to be more selective for JAK1 over JAK2 than filgotinib, of which the IC$_{50}$'s are 10, 28, 810, and 116 nM for JAK1, JAK2, JAK3, and TYK2, respectively.[63] Therefore, we selected two representative compounds, (***R***)**-6c** and **58,** for evaluation against JAK3 and TYK2. The IC$_{50}$ of (***R***)**-6c** on JAK3 and TYK2 were 1.1×10^3

and 2.5×10^2 nM and the selectivity indices over JAK1 were 130 and 30, respectively. For **58**, 1.1×10^2 and 25 nM IC_{50}'s were observed for JAK3 and TYK2, respectively, with its selectivity indices as 19 and 4.3 for JAK3 and TYK2 over JAK1.
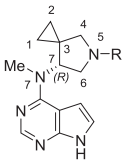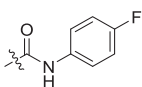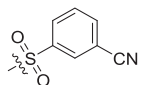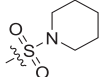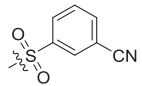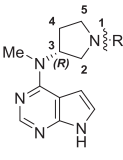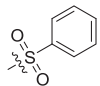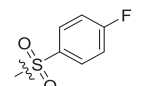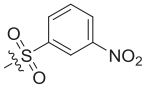
Table 14 The IC$_{50}$ values against cellular JAK1-JAK3 and JAK2 activity of substituted (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines.

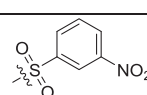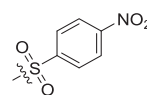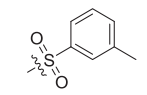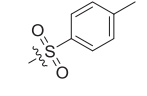| Compound			IC$_{50}$ (μM)		
			JAK1/3 (THP-1, IL-4-pSTAT6)		JAK2 (IL-3-Ba/F3 Proliferation)
Number	Scaffold	R	Facs Tube	96 well	
	Tofacitinib citrate		0.090	ND	~ 4.7
	Filgotinib		1.8	0.56	>10
(*R*)-6c		C(O)CH$_2$CN	0.84	0.21	>10
29		C(O)-furan	11	4.3	>10
42	(scaffold)	C(O)NH-C$_6$H$_4$-F	2.8	1.4	>10
50		SO$_2$Et	ND	0.73	ND
58		SO$_2$-C$_6$H$_4$-CN	1.1	0.24	>10
67		SO$_2$-piperidine	3.7	1.7	>10
12a		C(O)CH$_2$CN	0.92	0.30	>10
16		SO$_2$-C$_6$H$_4$-CN	0.61	0.20	>10
81	(scaffold)	SO$_2$-C$_6$H$_5$	1.1	0.98	>10
84		SO$_2$-C$_6$H$_4$-F	<0.010	0.40	>10
88		SO$_2$-C$_6$H$_4$-NO$_2$	0.13	0.040	>10

Encouraged by the enzyme assay results, we selected several compounds to see if they can inhibit JAK activity in a cell-based assay. We used the THP-1 cell to read the phosphorylation of STAT6, indicative of JAK activation.[63] When THP-1 cells are treated with IL-4 as a JAK-STAT pathway trigger, IL-4 receptors are dimerized. Consequently, one JAK1 and one JAK3 are recruited in the cytoplasmic domain. After the bound JAKs are phosphorylated and activated, STAT6 (pSTAT6) is phosphorylated. Thus, we performed FACS analysis to measure the pSTAT6 level in THP-1 cells upon IL-4 stimulation (Table 7). As expected, tofacitinib citrate, which strongly inhibits both JAK1 and JAK3 in the biochemical assay, showed potent inhibitory activity on the phosphorylation of STAT6 (IC_{50} = 0.09 μM). Compared to tofacitinib citrate, the JAK1-selective inhibitor filgotinib showed lower activity against JAK1-JAK3, which could be due to its relatively poor activity against JAK3 in the biochemical enzymatic assay (IC_{50} = 810 nM). Among the compounds tested in this study, (**R**)-**6c**, **58**, **12a**, **16**, **81**, **84**, and **88** inhibited the phosphorylation of STAT6 with the IC_{50} values 0.84, 1.1, 0.92, 0.61, 1.1, <0.010, and 0.13 μM's, respectively.

We evaluated the inhibitory activities of our representative compounds against JAK2 by performing the Ba/F3 cell proliferation assay. Ba/F3 cells proliferate upon the JAK2-STAT pathway activated by IL-3 stimulation.[63] Tofacitinib citrate exhibited an IC_{50} value of 4.7 μM, whereas our tested compounds except for **50** and filgotinib had IC_{50} values of >10 μM in this system. Taken together, these results suggest that our compounds possess higher selectivity for JAK1 or JAK3 than JAK2.

Table 15 Selectivity for individual JAK isozymes of substituted (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines in cellular assays.

Compound			GI$_{50}$ (µM)			
			TEL-JAKs-Ba/F3 (Proliferation)			
Number	Scaffold	R	JAK1	JAK2	JAK3	TYK2
Tofacitinib citrate			1.0	4.3	3.1	> 10
Filgotinib			7.0	>10	>10	> 10
(*R*)-6c			4.1	>10	>10	> 10
29			>10	>10	>10	> 10
42			>10	>10	>10	> 10
50			4.2	>10	>10	> 10
58			1.1	>10	>10	> 10
67			>10	>10	>10	> 10
12a			7.5	>10	>10	>10
16			1.0	>10	>10	>10
81			4.6	>10	>10	>10
84			4.2	>10	>10	>10
88			0.41	>10	>10	>10

We next examined 11 compounds, (*R*)-6c, 29, 42, 50, 58, 67, 12a, 16, 81, 84, and 88, in the selectivity for individual JAK isozymes by using Ba/F3

cell lines expressing constitutively active individual JAK isozymes (TEL-JAKs). We found that the tofacitinib citrate has growth inhibitory activity in cells expressing either JAK1, JAK2, or JAK3 with the median growth inhibitory concentrations (GI_{50}) of 1.0, 4.3, and 3.1 µM, respectively, but not in cells expressing TYK2 (Table 8). However, filgotinib and our compounds including (*R*)-6c, 50, 58, 12a, 16, 81, 84, and 88 showed growth inhibitory activity only in cells expressing JAK1 with GI_{50} values of 7.0, 4.1, 4.2, 1.1, 7.5, 1.0, 4.6, 4.2, and 0.41 µM, respectively. These results suggest that our tested compounds (*R*)-6c, 50, 58, 12a, 16, 81, 84, and 88 are more potent for JAK1 than filgotinib.

Human whole blood tests

Table 16 Selectivity for JAK1 over JAK2 of substituted (*R*)-*N*-alkyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines in human whole blood assays.
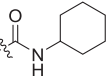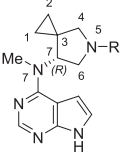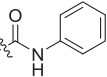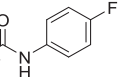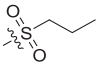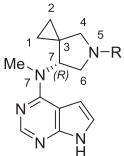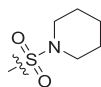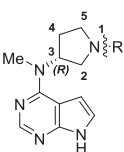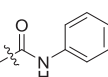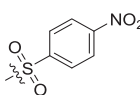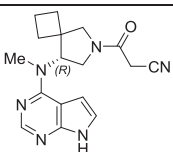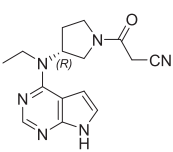
Compound			IC$_{50}$ (nM)		Selectivity index
Number	Scaffold	R	JAK1 (IL-6/pSTAT1)	JAK2 (GM-CSF/pSTAT5)	(JAK2/JAK1)
	Filgotinib		$3.2 \times 10^2 - 1.1 \times 10^3$	$7.8 \times 10^3 - 2.2 \times 10^4$	$9.1 - 25$
	Baricitinib		$8.0 - 40$	$17 - 1.4 \times 10^2$	$2.0 - 5.9$
(*R*)-6c		(CN acyl)	1.7×10^4	$>2.0 \times 10^4$	>1.2
40		(cyclohexyl amide)	1.5×10^4	$>2.0 \times 10^4$	>1.4
41		(phenyl amide)	6.3×10^3	$>2.0 \times 10^4$	>3.2
42		(4-fluorophenyl amide)	1.2×10^4	$>2.0 \times 10^4$	>1.6
50		(ethylsulfonyl)	4.4×10^2	2.4×10^3	5.5
52		(propylsulfonyl)	5.6×10^2	$>2.0 \times 10^4$	>36

Table 17 Selectivity for JAK1 over JAK2 of substituted (*R*)-*N*-alkyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines in human whole blood assays (continued).

Compound			IC$_{50}$ (nM)		Selectivity index
			JAK1	JAK2	
Number	Scaffold	R	(IL-6/pSTAT1)	(GM-CSF/pSTAT5)	(JAK2/JAK1)
67	*(structure)*	*(structure)*	4.8×10^3	$>2.0 \times 10^4$	>4.1
12a		*(structure)*	2.5×10^2	7.4×10^2	2.9
71		*(structure)*	2.4×10^2	1.5×10^4	60
74	*(structure)*	*(structure)*	3.6×10^3	$>2.0 \times 10^4$	>5.5
79		*(structure)*	4.3×10^2	$>2.0 \times 10^4$	>47
89		*(structure)*	3.0×10^2	1.5×10^4	51
12c	*(structure)*		4.3×10^2	6.0×10^3	14
13	*(structure)*		5.8×10^2	7.0×10^4	12

To identify the inhibition of the JAK-STAT signal pathway by our compounds in human blood environment, we performed human whole blood tests for 18 compounds. We selected two pathways: IL-6/JAK1/pSTAT1 and GM-CSF/JAK2/pSTAT5. For screening JAK1 inhibition in human blood, whole blood is treated with IL-6 and the pathway activated by IL-6 was

inhibited through JAK1 inhibition by inhibitors so that the inhibition percentage against the phosphorylation of STAT1 displays the inhibition portion of the pathway by JAK1 inhibitors. Similarly, the inhibition against the phosphorylation of STAT5 activated by GM-CSF shows the result from JAK2 inhibition of the pathway. As the result, we can obtain the selectivity indices for JAK1 over JAK2 in human whole blood environment.

We used filgotinib, JAK1-selective inhibitor, and baricitinib, JAK1/JAK2 inhibitor, as positive controls. The selectivity for JAK1 over JAK2 of filgotinib was distributed from 9.1 to 25.0. Of our test compounds, the tests of **18**, **44**, **93**, and **94** were not carried out because of low water solubility. Of other compounds, 6 compounds, **52**, **71**, **79**, **89**, **12c**, and **13** showing the selectivity indices ranged from 12 to 60 were more selective for JAK1 over JAK2 than filgotinib. However, our representative compound (**R**)-**6c** had the IC_{50} value of 1.6×10^4 nM for inhibition of IL-6/JAK1/pSTAT1 pathway so that we could not calculate the selectivity index. So we guessed that this system was not appropriate for identifying the selectivity of compound (**R**)-**6c**. In the case of compound **12a**, it showed the lower JAK1 IC_{50} value of 2.5×10^2 nM than filgotinib did, but it had a lower selectivity index of 2.9 than filgotinib.

In vitro ADME studies
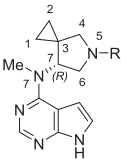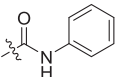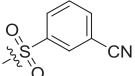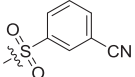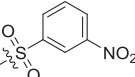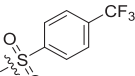
Table 18 Plasma stabilities.

			Human		Rat	
	Compound		(% remaining)		(% remaining)	
Number	Scaffold	R	30 min	120 min	30 min	120 min
(**R**)-**6c**			95.0	98.5	98.4	89.6
41			> 100	> 100	> 100	95.5
50			> 100	> 100	> 100	> 100
58			96.6	98.6	> 100	> 100
12a			96.3	99.8	> 100	> 100
16			94.8	96.9	> 100	> 100
88			90.6	93.0	> 100	> 100
93			> 100	> 100	98.9	99.8

Several *in vitro* ADME profiles for selected JAK1 inhibitors, (**R**)-**6c**, **41**, **50**, **58**, **12a**, **16**, **88**, and **93**, were investigated for plasma stability, protein binding, liver microsomal stability, Caco-2 permeability, and CYP inhibition. First, over 90% of all test compounds except for (**R**)-**6c** in rat plasma during 120 minutes remained in human and rat plasma for 120 minutes in plasma stability tests (Table 10). In the comparison between derivatives of (R)-N-alkyl-N-(pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine and (R)-N-methyl-N-(5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine ((**R**)-**6c** vs **12a**, and **58** vs **16**), whether the spiro moiety at pyrrolidine ring exist cannot
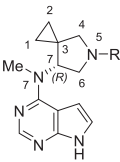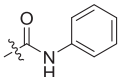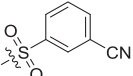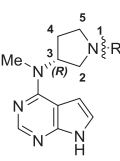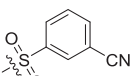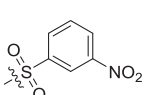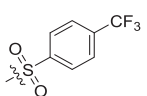
affect their plasma stability. Therefore, it can be concluded that most compounds are kept as their parent drug structures in plasma.

Table 19 Plasma protein binding abilities and Log P values.

Compound			% bound		LogP
Number	Scaffold	R	Human	Rat	(neutral X)
(**R**)-6c			29.8	18.7	1.69
41			92.5	88.1	3.07 (cLogP)
50			44.4	49.5	2.07
58			87.4	85.8	3.69
12a			14.7	17.7	0.61
16			83.1	82.7	2.73
88			90.4	91.9	3.03
93			98.7	95.9	3.58 (cLogP)
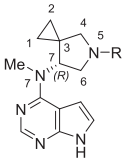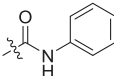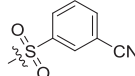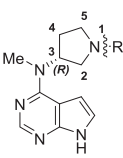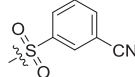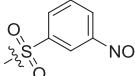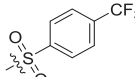
In human plasma protein binding tests (Table 11), the bound proportion of (**R**)-6c was 29.8%, which was similar to that of tofacitinib citrate and filgotinib, 39% and 31.8%, respectively.[33, 36] Compounds, **50**, **58**, **16**, **88**, and **93**, all of which have sulfonamide groups, showed higher protein binding of over 44.4%. The results correlate well with their lipophilicities: the LogP values of their neutral forms gradually increase in the order of amides **12a** and (**R**)-6c, aliphatic sulfonamide **50**, and aromatic sulfonamides **16** and **55**.

Table 20 Liver microsomal stabilities.

Compound			% Remaining after 30 min (%)			
Number	Scaffold	R	Human	Dog	Rat	Mouse
(**R**)-**6c**			94.6	99.9	84.8	84.9
41			67.3	62.2	41.6	18.5
50			79.7	56.1	22.9	33.9
58			6.0	5.5	2.4	3.2
12a			97.6	92.8	92.8	> 100
16			26.3	31.6	3.7	10.0
88			13.4	10.4	2.5	3.1
93			21.0	14.2	11.4	8.4
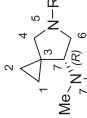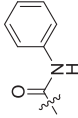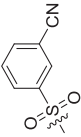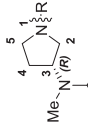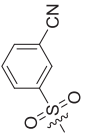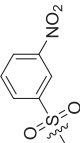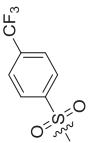
To probe the stability of the selected compounds in the liver first-pass, liver microsomal stabilities were examined (Table 12). The remaining compounds (**R**)-**6c** and **12a** (94.6 and 97.6%, respectively) after 30 minute incubation in human liver microsomes were similar to those of filgotinib (87% after 60 minute incubation).[36] While compound **50** possessing an ethanesulfonamide group was less stable than filgotinib, with 79.7% remaining after 30 minutes, the benzenesulfonamide-containing compounds, **58**, **16**, **88**, and **93** were heavily metabolized in human liver microsomes and only below 26.3% remained after 30 min incubation.

Table 21 Caco-2 permeabilities.

Compound			P_{app}, (x10^{-5} cm/sec)		Efflux ratio	Remark
Number	Scaffold	R	A to B	B to A		
(**R**)-**6c**			0.66	1.9	2.8	moderate
41			2.6	2.1	0.80	high
50			2.5	2.6	1.0	High
58			1.3	1.6	1.2	high
12a			0.38	0.77	2.0	moderate
16			1.7	2.3	1.4	high
88			1.4	1.9	1.3	high
93			0.19	0.42	2.3	moderate

Moderate permeabilities were observed for (**R**)-**6c**, **12a**, **93**, and filgotinib in Caco-2 permeability tests with the permeability coefficients 0.66, 0.38, 0.19 and 0.37x10^{-5} cm/sec, respectively (Table 13).[36] On the other hand, **41**, **50**, **58**, **16**, and **88** had high permeability coefficients ranged from 1.3x10^{-5} cm/sec to 2.6x10^{-5} cm/sec. As the results, the sulfonamide groups and the urea group at R group position, except for compound **93**, improved passive cell permeability from A to B. The efflux ratios of all test compounds were below 3.0 and they did not seem to be heavily affected by the efflux mechanism.

Table 22 The inhibition percentages against CYP$_{450}$ isoforms.

Number	Scaffold	R	CYP$_{450}$ isoforms (% of control activity @ 10 μM)								
			1A2	2A6	2B6	2C8	2C9	2C19	2D6	2E1	3A4
(*R*)-6c			92.5	92.0	91.5	81.0	84.4	66.0	91.6	50.3	89.7
41			70.1	85.8	92.5	63.6	60.7	92.1	95.8	48.7	73.5
50			99.9	96.7	>100	>100	90.7	93.8	>100	63.9	95.4
58			65.7	87.2	92.0	66.2	50.4	55.8	90.3	59.8	33.2
12a			89.3	99.1	>100	>100	>100	91.8	>100	57.7	98.2
16			69.2	85.7	>100	>100	62.3	76.2	98.9	66.0	55.3
88			71.6	85.1	90.1	>100	56.4	65.0	94.1	63.5	50.2
93			85.1	79.7	86.5	78.1	63.0	58.0	70.3	51.8	76.7

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To probe drug-drug interaction possibilities, we screened 8 test compounds against representative CYP_{450} isoforms at 10 µM concentrations for each compound (Table 14). In the cases of (**R**)-**6c** and **12a**, CYP 2C19 and 2E1 were influenced at the same concentrations. Compound **50** seemed to inhibit only CYP 2E1 isoform at the concentration. However, the treatments of the benzenesulfonamide-containing **41**, **58**, **16**, **88**, and **93** were likely to affect many isoforms including CYP 1A2, 2C8, 2C9, 2C19, 2E1, and 3A4, which may lead to possible interactions with many drugs. The CYP 2E1 isoform, which test compounds commonly inhibited, has substrates for some anaesthetics like halothane, enflurane, methoxyflurane, sevoflurane, etc.[76] Representative substrates of CYP 2C19 comprise of proton pump inhibitors including esomeprazole, lansoprazole, etc. In the case of the proton pump inhibitors, their prescription frequencies are decreasing in recent times, so that the combined prescription would not be a problem.

Due to high plasma protein binding, low liver microsomal stability, and high CYP_{450} inhibition rates, we excluded compounds with sulfonamide groups from further studies. Therefore, (**R**)-**6c** and **12a** were chosen for studies on hERG, kinase profiling, and *in vivo* efficacy tests.

Human ether-a-go-go related gene (hERG) potassium channel assays and kinase profiling

Next, we investigated the hERG binding of (**R**)-**6c** and **12a** for its cardiotoxicity prediction. The binding test was carried out with HEK293 cells according to the automated patch clamp method. Compound (**R**)-**6c** and **12a** showed with IC_{50} of 1.2×10^2 and 93 µM, respectively. Under the same conditions, filgotinib gave IC_{50} of 85 µM. In the case of tofacitinib citrate, IC_{50} was reported above 100 µM.[77] From these results, (**R**)-**6c** appears to be superior to filgotinib in the cardiotoxicity predicted by the hERG assay.

Kinase inhibitors targeting an ATP-binding site have a probability of

inhibiting other kinases in addition to the targeted one, which can result in some predictable side effects. Therefore, we screened (**R**)-**6c** and **12a** against 323 kinases at the 10 μM concentration. Under this condition, only four JAK family kinases including JAK1, JAK2, JAK3, and TYK2 showed over 90% inhibition by compound (**R**)-**6c**. The kinases with 80 – 90% inhibition were only ROCK-IIs derived from rats and humans. In the case of compound **12a**, the kinases inhibited to over 90% were only three kinases, JAK1, JAK2, and TYK2. And the kinases with 80 – 90% inhibition included 6 kinases: JAK3, ROCK-II (human), ROCK-II (rat), DCAMKL3, CLK1, and Flt4. On the other hand, it was reported that tofacitinib citrate inhibits 26 kinases above 90%,[20] so it is likely that (**R**)-**6c** and **12a** would have less side effects than tofacitinib citrate.
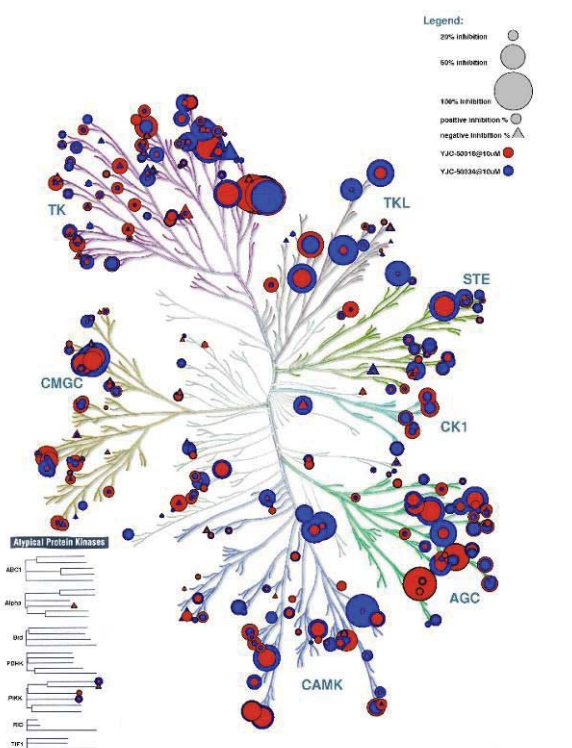
Figure 4 The kinome tree of (**R**)-**6c** and **12a** against 323 kinases at the 10 μM concentration drawn by the web accessible Kinome Render program.[78]

Pharmacokinetics

Table 23 Pharmacokinetic profiles of (*R*)-6c.

Species	Beagle dog		S. D. rat		ICR mouse	
Route	P.O.	I.V.	P.O.	I.V.	P.O.	I.V.
N	4M	4M	4M	4M	4M	4M
Dose (mg/kg)	5	3	10	5	10	5
C_{max} (ng/mL)	1.9×10^3		1.0×10^3		1.8×10^3	
T_{max} (h)	1.8		0.30		1.3	
$t_{1/2}$ (h)	3.3	3.0	2.1	1.2	1.7	0.9
$AUC_{0 \rightarrow inf}$ (ng·h/mL)	1.5×10^4	5.2×10^3	2.1×10^3	9.2×10^2	4.8×10^3	6.8×10^2
$AUC_{0 \rightarrow t}$ (ng·h/mL)	1.4×10^4	5.0×10^3	1.9×10^3	9.0×10^2	4.7×10^3	6.0×10^2
MRT (h)	7.3	4.7	3.1	1.1	2.9	1.3
F (%)	1.7×10^2		1.1×10^2		1.9×10^2	

Table 24 Pharmacokinetic profiles of **12a**.

Species	Beagle dog		S. D. rat		ICR mouse	
Route	P.O.	I.V.	P.O.	I.V.	P.O.	I.V.
N	4M	4M	4M	4M	4M	4M
Dose (mg/kg)	5	3	10	5	10	5
C_{max} (ng/mL)	1.9×10^3		1.9×10^3		1.0×10^3	
T_{max} (h)	1.1		0.30		0.30	
$t_{1/2}$ (h)	1.7	1.6	2.1	0.70	2.1	0.9
$AUC_{0 \rightarrow inf}$ (ng·h/mL)	6.5×10^3	2.2×10^3	4.3×10^3	9.5×10^2	2.1×10^3	6.8×10^2
$AUC_{0 \rightarrow t}$ (ng·h/mL)	6.4×10^3	2.1×10^3	4.1×10^3	9.3×10^2	1.9×10^3	5.1×10^2
MRT (h)	3.1	2.4	2.9	0.9	3.1	1.4
F (%)	1.8×10^2		2.2×10^2		1.9×10^2	
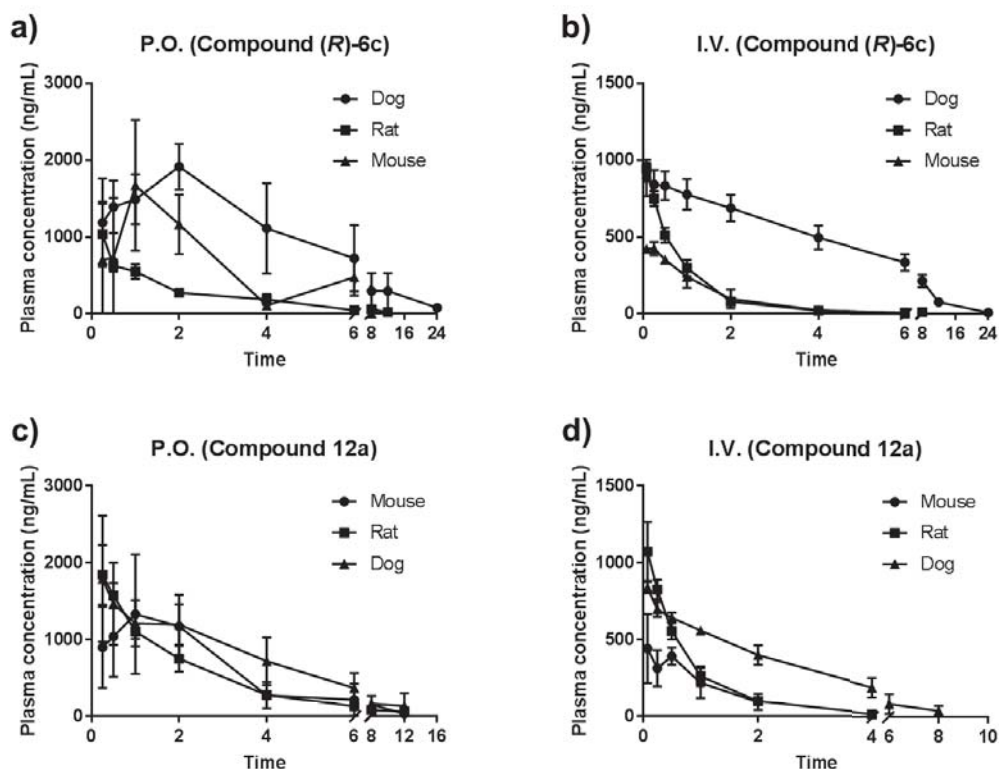
Figure 5 Plasma concentrations after oral administration and intravenous injection of (*R*)-6 and **12a** in Beagle dogs, Sprague-Dawley rats, and ICR mice.

To address oral bioavailability of our representative compounds, (*R*)-**6c** and **12a**, we then carried out the pharmacokinetic tests in dogs, rats, and mice. The vehicles for oral administration and intravenous injection were corn oil and the solution of 10% ethanol and 90% PEG400, respectively, because of low solubility of (*R*)-**6c** and **12a** in water. In the case of pharmacokinetics through intravenous injection, the drug exposure generally tended to be decreased so that the bioavailability at all species became over 100%, which is similar to the results reported by K. W. Ward *et al.*[79] and R. Weaver *et al.*[80]

In the case of oral administration at 10 mg/kg dosage in male Sprague Dawley rats, compound (*R*)-**6c** showed 2.1 hours of half-life ($t_{1/2}$), 4.3×10^3 ng·h/mL of area under curve from 0 to infinite ($AUC_{0 \to inf}$), 1.9×10^3 ng/mL of

maximum concetration (C_{max}), and 0.30 hour of the time to reach the maximum concentration (T_{max}). Compound **12a** had similar profiles to compound **(R)-6c** except for C_{max} and AUC which had about twice the values of compound **(R)-6c**. Though the profiles of $t_{1/2}$, C_{max}, and T_{max} were similar to the reported tofacitinib's ones ($t_{1/2} = 2.0$ h, $C_{max} = 2.4 \times 10^3$ ng/mL, $T_{max} = 0.31$ h), **(R)-6c** and **12a** surpassed tofacitinib with $AUC_{0 \to inf}$ value of 2.8×10^3 ng·h/mL on drug exposure.[77] In comparison with the reported profiles of filgotinib through oral treatment at 5 mg/kg dosage, filgotinib has a longer half-life ($t_{1/2} = 3.9$ h), but a lower drug exposure ($AUC_{0 \to t} = 1.7 \times 10^3$ ng·h/mL) than compound **12a**,[36] although direct comparison with filgotinib and **12a** is impossible because of their different oral administration dosages. Compound **(R)-6c** and **12a** showed a superior drug exposure to tofacitinib ($AUC_{0 \to inf} = 2.3 \times 10^3$ ng·h/mL)[77] in the PK study in male beagle dogs at 5 mg/kg dosage. However, PK profiles of **(R)-6c** and **12a** in dogs are inferior to the reported values of filgotinib, which features 5.2 hours of half-life ($t_{1/2}$) and 1.4×10^4 ng·h/mL of $AUC_{0 \to t}$.[36]

Table 25 Pharmacokinetic parameters of the free base and the salt forms of (***R***)-6c in Sprague-Dawley rats.

Sample	(***R***)-6c							
Salt form	Free base		Hydrochloride		Citrate		Tartrate	
Route	P.O.	I.V.	P.O.	I.V.	P.O.	I.V.	P.O.	I.V.
N (S.D. Rat)	4M	4M	4M	4M	4M	4M	4M	4M
Dose (mg/kg)	10	5	10	5	10	5	10	5
C_{max} (ng/mL)	1.0×10^3		1.3×10^3		1.2×10^3		1.0×10^3	
T_{max} (h)	0.30		0.40		0.50		0.40	
$t_{1/2}$ (h)	2.1	1.2	1.1	0.60	3.6	4.3	1.0	1.0
$AUC_{0 \to \infty}$ (ng·h/mL)	2.1×10^3	9.2×10^2	2.4×10^3	1.9×10^3	2.4×10^3	2.0×10^3	1.9×10^3	1.4×10^3
$AUC_{0 \to t}$ (ng·h/mL)	1.9×10^3	9.0×10^2	2.4×10^3	1.8×10^3	2.4×10^3	2.0×10^3	1.9×10^3	1.4×10^3
MRT (h)	3.1	1.1	1.6	0.7	2.3	1.2	1.5	0.9
F (%)	1.1×10^2		65		58		68	

Table 26 Pharmacokinetic parameters of the free base and the salt forms of **12a** in Sprague-Dawley rats.

Sample	12a							
Salt form	Free base		Hydrochloride		Citrate		Tartrate	
Route	P.O.	I.V.	P.O.	I.V.	P.O.	I.V.	P.O.	I.V.
N (S.D. Rat)	4M	4M	4M	4M	4M	4M	4M	4M
Dose (mg/kg)	10	5	10	5	10	5	10	5
C_{max} (ng/mL)	1.9×10^3		1.3×10^3		2.0×10^3		3.4×10^3	
T_{max} (h)	0.30		0.60		0.60		0.50	
$t_{1/2}$ (h)	2.1	0.70	2.9	4.1	3.2	7.7	4.2	4.2
$AUC_{0 \to \infty}$ (ng·h/mL)	4.3×10^3	9.5×10^2	2.8×10^3	2.1×10^3	3.8×10^3	4.6×10^3	4.8×10^3	6.7×10^3
$AUC_{0 \to t}$ (ng·h/mL)	4.1×10^3	9.3×10^2	2.7×10^3	2.0×10^3	3.8×10^3	4.6×10^3	4.7×10^3	6.7×10^3
MRT (h)	2.9	0.90	2.7	2.4	2.4	1.9	2.8	1.4
F (%)	2.2×10^2		67		41		35	

Figure 6 Plasma concentrations after oral administration and intravenous injection of the free base and the salt forms of (***R***)-**6c** and **12a** in Sprague-Dawley rats.

To improve exposures of (***R***)-**6c** and **12a** in the *in vivo* model, we made several different salts using hydrochloride, citric acid, and tartaric acid. For hydrochloride and citrate salts, their drug exposures were increased compared to the free base form. However, the tartrate salt of (***R***)-**6** and the hydrochloride and citrate salts of **12a** were less exposed than the free base in the oral administration. Moreover, the citrate form of (***R***)-**6c** and three salts of **12a** had the additive advantage that their half-lives were elongated to 2.9 – 4.2 hours. Hence, the citrate of (***R***)-**6c** and the tartrate of **12a** appear to be the preferred formulations in oral administration.

In vivo efficacy studies on (*R*)-6c and 12a
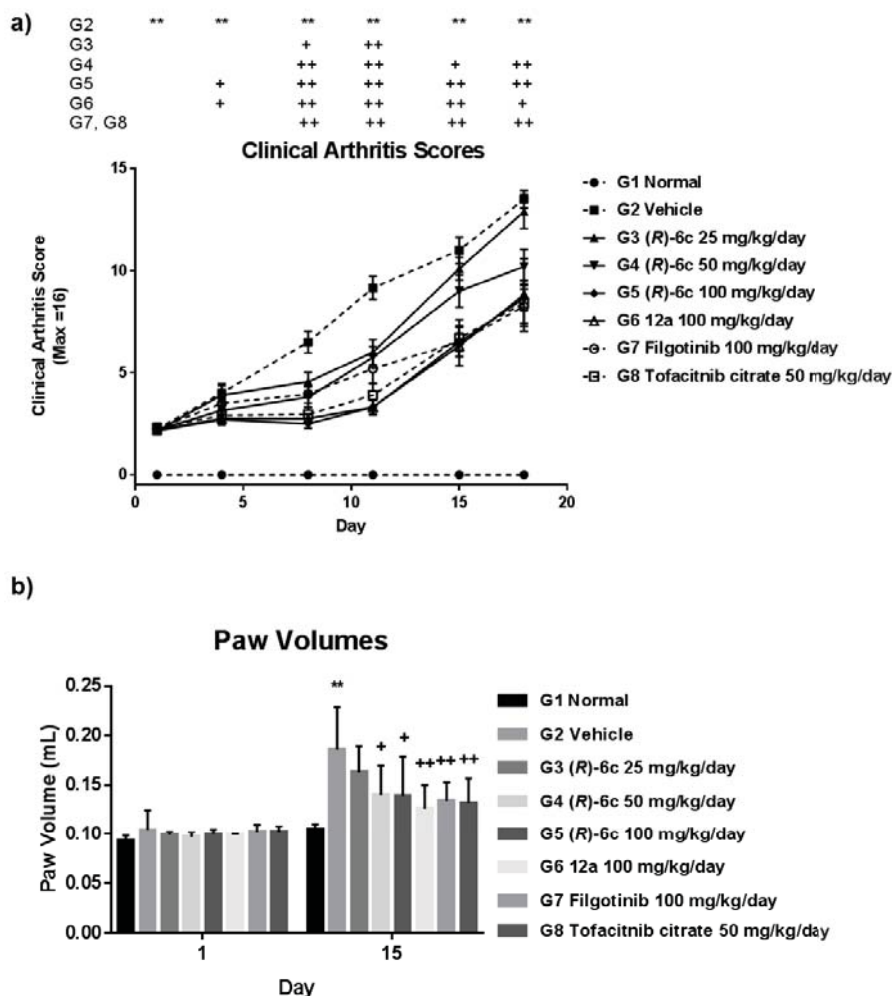
Figure 7 a) Clinical arthritis scores and b) paw volumes of (*R*)-6c and 12a treatment on collagen-induced arthritis in DBA/1J mice for 18 days. The significance symbols are ** = significantly different between G1 and G2 (P <0.01), + = significantly different from G2 (P <0.05), and ++ = significantly different from G2 (P <0.01).

Both collagen-induced arthritis (CIA) and adjuvant-induced arthritis (AIA) are well-established animal models for the testing and development of new RA therapeutics.[81-82] We used these to evaluate the efficacies for the treatment with (*R*)-6c and 12a in a free base form. In the mouse CIA study, the effect of (*R*)-6c and 12a treatments were evaluated by using the following

indices: clinical arthritis score, paw volume, serum cytokine concentration, bone surface/volume ratio, and histopathological data of ankles. Filgotinib (100 mg/kg/day)[63] and tofacitinib citrate (50 mg/kg/day)[22] were used as positive controls. Treatment with **(R)-6c** (25, 50 or 100 mg/kg/day) and **12a** (100 mg/kg/day) resulted in significant attenuation of arthritis in DBA1/J mice when compared to vehicle treatment (Figure 7). In the clinical arthritis scores, the treatments of **(R)-6c** and **12a** at 100 mg/kg/day dosage seemed to be faster relieve the symptom than filgotinib's one. However, the results of the treatments of **(R)-6c** and **12a** in clinical scores and paw volume at day 18 showed no significant difference between two test articles and two positive controls.
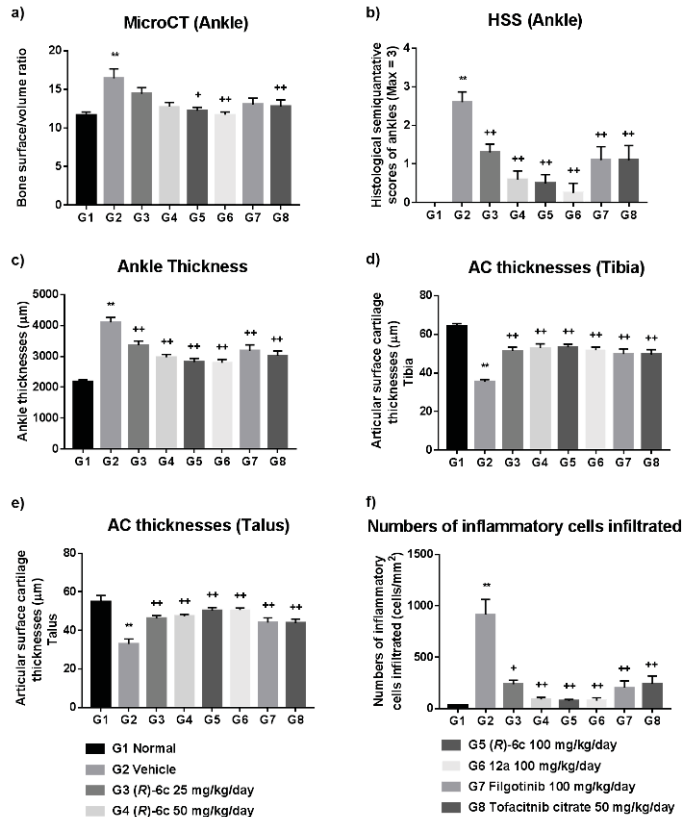
Figure 8 Effects of **(R)-6c** and **12a** treatment on collagen-induced arthritis in DBA/1J mice: a) the bone surface/volume ratios of right hind ankle joints measured by micro-CT, b) the histopathological semiquantitative scores of right hind ankle joints, c) the

right hind ankle joint thicknesses, d-e) the articular surface cartilage thicknesses (tibia and talus) in right hind ankle joints, and f) the numbers of inflammatory cells infiltrated in the right hind ankle joints. The significance symbols are ** = significantly different between G1 and G2 (P <0.01), + = significantly different from G2 (P <0.05), and ++ = significantly different from G2 (P <0.01).

We performed the micro-CT assay and the histopathological assays for further studies on the effects of compound (*R*)-6c and 12a. We measured the bone surface/volume ratios of right hind ankle joints measured by micro-CT, and identified the following histopathological factors: the histopathological semiquantitative scores of right hind ankle joints, the right hind ankle joint thicknesses, the articular surface cartilage thicknesses (tibia and talus) in right hind ankle joints, and the numbers of inflammatory cells infiltrated in the right hind ankle joints. As the resuls, all treatments of (*R*)-6c and 12a except for one at 25 mg/kg/day displayed higher alleviation efficacy than two positive controls. So, in the faces of micro-CT analysis and histopathological assay, (*R*)-6c, a JAK1-selective inhibitor, seemed to have better efficacy than tofacitinib citrate, a pan-JAK inhibitor, at same dosages.
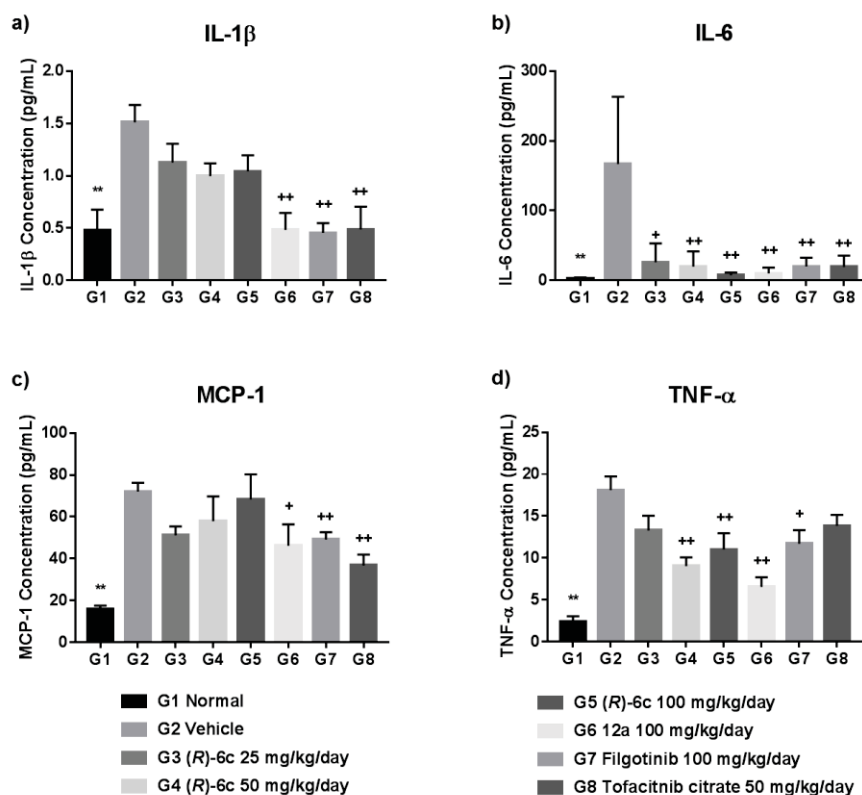
Figure 9 cytokine concentration changes in plasma by (***R***)-**6c** and **12a** treatment on collagen-induced arthritis in DBA/1J mice for 18 days: a) IL-1β, b) IL-6, c) MCP-1, and d) TNF-α. The significance symbols are ** = significantly different between G1 and G2 (P <0.01), + = significantly different from G2 (P <0.05), and ++ = significantly different from G2 (P <0.01).

We identified concentration changes of cytokines, including IL-1β, IL-6, MCP-1 and TNF-α, related to rheumatoid arthritis in plasma through the treatments of (***R***)-**6c** and **12a** for 18 days. As a result, compound (***R***)-**6c** and **12a** clearly influenced IL-6 and TNF-α levels in plasma. On these cytokines, treatments of (***R***)-**6c** and **12a** at all dosages more alleviated cytokine levels than positive controls' ones. The treatment of **12a** seemed to decrease the levels of

IL-1β and MCP-1 in plasma although its alleviation was inferior to one of tofacitinib citrate. However, compound (**R**)-**6c** could not significantly affect IL-6 and TNF-α levels in plasma. As the results, compound (**R**)-**6c** and **12a** may alleviate the clinical and histopathological symptons in mouse CIA model through lowering IL-6 level.
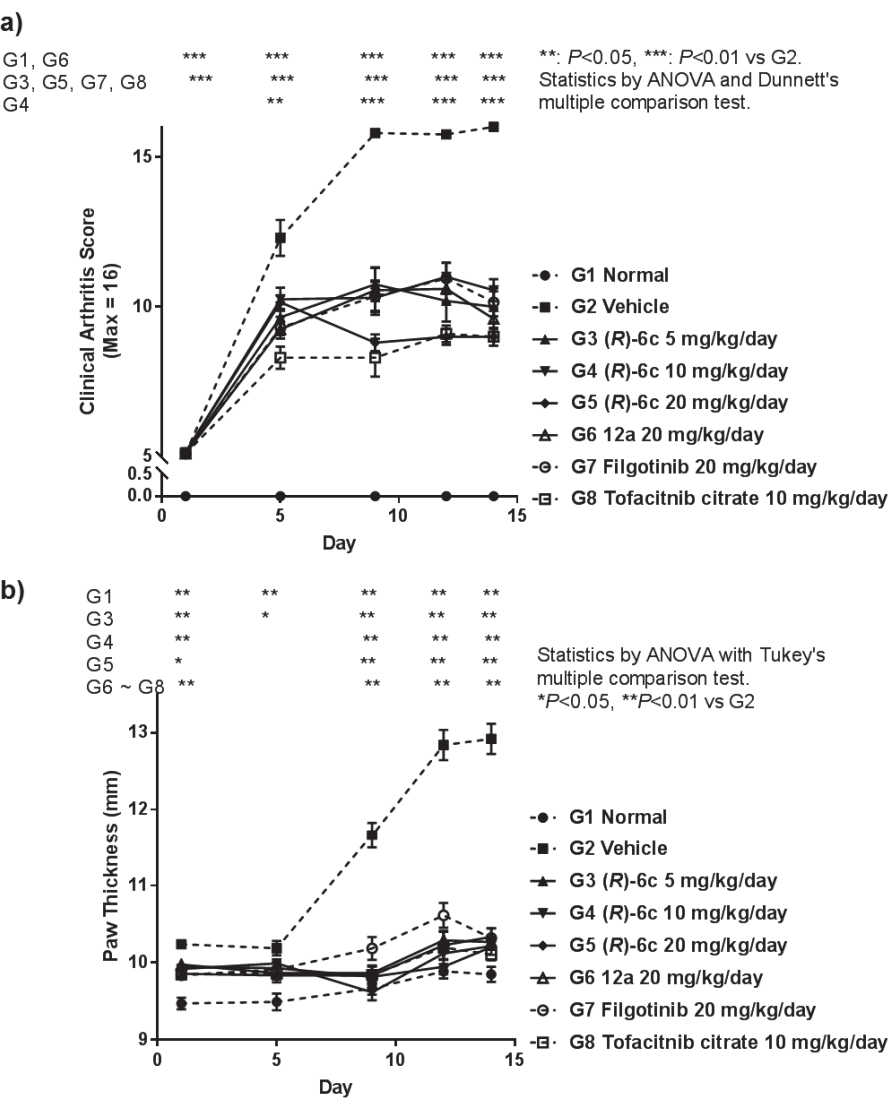
Figure 10 Effects of (**R**)-**6c** and **12a** treatment on adjuvant-induced arthritis in Lewis rats: a) the clinical arthritis scores and b) the volumes of right hind paws. The data were measured twice per week for 14 days.

In the rat AIA study, all treatments with test articles significantly suppressed the arthritis symptoms versus vehicle treatment for 14 days. The treatment with 20 mg/kg/day of (*R*)-6c demonstrated nearly equal efficacy as that of tofacitinib citrate (10 mg/kg/day). Their clinical arthritis scores reached the same 9.0 value at day 14 and paw thicknesses were similar ((*R*)-6c at 10.20 mm and tofacitinib citrate at 10.10 mm). However, filgotinib (20 mg/kg/day) and (*R*)-6c in lower concentrations (5 and 10 mg/kg) showed slightly inferior clinical arthritis scores (10.32, 10.21, and 10.33, respectively) to the former case. Treatment with compound **12a** (20 mg/kg/day) significantly attenuated arthritis symptoms to a similar extent as filgotinib (20 mg/kg/day) treatment and significantly reduced paw swelling to a similar extent as tofacitinib citrate (10 mg/kg/day) treatment.

V.   Conclusions

We have shown the efficacy of (**R**)-**6c** and **12a** through *in vitro* and *in vivo* tests. In the enzyme assays, the JAK1 IC_{50} value of (**R**)-**6c** was 8.5 nM and the selectivity indices of JAK2, JAK3, and TYK2 over JAK1 were 48.5, 128.5, and 29.6, respectively. And compound **12a** also showed a better JAK1-selectivity than tofacitinib citrate (IC_{50} value of 19 nM and selectivity index of 6.8). In the cell based functional assay, it inhibited the JAK1 isozyme more effectively than filgotinib, but less so than tofacitinib citrate. In the kinase profiling, the inhibitory activities on other kinases except for JAK series were less than those of tofacitinib citrate. From the above *in vitro* tests, we obtained highly JAK1-selective profiles for our inhibitor, which presumably would lead to lower toxicity than tofacitinib citrate.

In the *in vitro* ADME tests, its profiles were similar to those of tofacitinib citrate and filgotinib. The compound (**R**)-**6c** and **12a** showed good human plasma stability along with two positive controls to exhibit similar profiles on the bound percentages on human plasma protein and the stability against human liver microsomes. Thus, there was a moderate permeability coefficient from A to B in Caco-2 permeability tests like filgotinib, but less efflux ratio so that it seems to be more highly permeable to cells than filgotinib. In the CYP_{450} isozyme screening, the compound showed inhibition of 2C19 and 2E1 isoforms at 10 μM concentrations. In pharmacokinetic studies in rats through oral administration, the profiles of the free base were at acceptable levels.

In the *in vivo* studies, we observed that a double dose of (**R**)-**6c** and **12a**, JAK1-selective inhibitors, gave similar or superior efficacies to that of tofacitinib citrate, a pan-JAK inhibitor. Moreover, (**R**)-**6c** and **12a** relieved the arthritis symptoms more than an equivalent dose of filgotinib, the JAK1-selective inhibitor belonging to the same category, did. Taken together, our present study indicates that (**R**)-**6c** and **12a** have desirable physicochemical

properties and efficacy via selective inhibition of the JAK1 pathway. These findings suggest that **(R)-6c** and **12a** have therapeutic potential for the treatment of rheumatoid arthritis.

VI. Experimental Section

Synthesis

All reagents for the syntheses were obtained from commercially available sources and used without any further purification. A diastereomeric pair of the key starting material, 5-((R)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-amine, was purchased from custom-synthesis through Sundia Meditech, China. All final products were purified by flash column chromatography and Merck silica gel 60 (0.040 – 0.063 mm) was used for flash column chromatography. The structures of the compounds were identified through ^{1}H and ^{13}C NMR spectroscopy and high resolution mass spectrometry (MS). NMR spectra were taken from Agilent NMR system 400 MHz DD2MR400, Bruker Biospin AVANCE II 400, and Varian NMR System 500 MHz. Bruker Compact Ultra High Resolution ESI Q-TOF mass spectrometer was used for the MS data. The purities of synthesized compounds were analyzed through the use of 256 nm-wavelength absorption spectra on Agilent HPLC 1100 and 1260 infinity with 6120 Quadrupole LC/MS detector. Additionally, their optical rotation data were obtained by JASCO's P-1030 Polarimeter.

*Synthesis of ethyl ((R)-5-((R)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-yl)carbamate, **(R)-2c***

Potassium carbonate (25.9 g, 187 mmol) in 140 mL of deionized water was added to a (R)-5-((R)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-amine **(R)-1c** (20.1 g, 92.9 mmol) solution in 250 mL of tetrahydrofuran and stirred at room temperature for 10 minutes. Ethyl chloroformate (9.46 mL, 99.4 mmol) was then added and the mixture was stirred at room temperature overnight. After the reaction, the solution was evaporated and the residue was extracted with dichloromethane three times. The combined organic layers were dried over anhydrous sodium sulfate and the solid was filtered off. The filtered solution was evaporated. Removing the solvent in vacuo provided 26.7 g of

ethyl ((R)-5-((R)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-yl)carbamate (quantitatively yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.27 (m, 5H), 5.20 (d, J = 8.9 Hz, 1H), 4.04 (dd, J = 14.2, 7.1 Hz, 2H), 3.82 (ddd, J = 8.7, 5.8, 2.8 Hz, 1H), 3.21 (dd, J = 13.1, 6.5 Hz, 1H), 2.84 (dd, J = 9.6, 6.0 Hz, 1H), 2.78 (d, J = 8.9 Hz, 1H), 2.46 (dd, J = 9.6, 2.5 Hz, 1H), 2.30 (d, J = 8.8 Hz, 1H), 1.34 (d, J = 6.6 Hz, 3H), 1.20 (t, J = 7.1 Hz, 3H), 0.75 (m, 2H), 0.59 (m, 1H), 0.47 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 156.4, 144.8, 128.5, 127.2, 125.5, 77.5, 77.2, 76.9, 65.6, 61.0, 60.8, 60.7, 56.1, 26.4, 22.7, 14.7, 14.2, 8.9.

*Synthesis of (R)-N-methyl-5-((R)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-amine, (**R**)-3c*

An ethyl ((R)-5-((R)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-yl)carbamate **(R)-2c** (26.6 g, 92.2 mmol) solution in 345 mL of tetrahydrofuran was placed in a 1 L round bottom flask. After it was cooled at -40 ºC, lithium aluminum hydride (7.01 g, 185 mmol) was slowly added and stirred. The reaction mixture was refluxed for 4 hours then cooled down to -40 ºC. The reaction was quenched with 40 mL of deionized water, 40 mL of 15% sodium hydroxide solution, and 40 mL of deionized water. Then, celite 545 was added and the mixture was stirred for 30 minutes before being filtered through a celite 545 pad. The filtered solution was evaporated and extracted with dichloromethane three times. Combined organic layers were dried over anhydrous sodium sulfate and the solid was filtered off. And the filtered solution was evaporated. Removing the solvent in vacuo provided 19.4 g of (R)-N-methyl-5-((R)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-amine (91.0% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.27 (m, 5H), 3.22 (q, J = 6.6 Hz, 1H), 3.11 (dd, J = 9.4, 6.1 Hz, 1H), 2.86 (t, J = 5.5 Hz, 1H), 2.61 (d, J = 8.9 Hz, 1H), 2.39 (m, 1H), 2.30 (d, J = 4.9 Hz, 3H), 2.28 (d, J = 9.0 Hz, 1H), 1.37 (d, J = 6.6 Hz, 3H), 0.78 (m, 1H), 0.56 (dd, J = 8.6, 7.1 Hz, 2H), 0.35 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 145.4, 128.4, 127.3, 127.0, 66.2, 63.8, 61.9, 59.8, 34.4, 25.9, 23.0, 14.1, 7.3.

Synthesis of N-methyl-N-((R)-5-((R)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, (R)-4c

A (*R*)-*N*-methyl-5-((*R*)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-amine **(R)-3c** (18.3 g, 79.4 mmol) solution in 330 mL of deionized water was placed in a 500 mL round bottom flask. Consequently, 6-chloro-7-deazapurine (12.8 g, 83.3 mmol) and potassium carbonate (22.0 g, 159 mmol) were added and refluxed for 18 hours. After the reaction, it was cooled at room temperature and the aqueous mixture was extracted with 250 mL of dichloromethane three times. Combined organic layers were dried over anhydrous sodium sulfate and the solid was filtered off while the filtered solution was evaporated. Removing the solvent in vacuo provided 27.7 g of *N*-methyl-*N*-((*R*)-5-((*R*)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (quantitatively yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.24 (s, 1H), 8.20 (s, 1H), 7.36 (d, *J* = 7.1 Hz, 2H), 7.30 (t, *J* = 7.4 Hz, 2H), 7.22 (dd, *J* = 13.4, 6.2 Hz, 1H), 7.03 (t, *J* = 3.7 Hz, 1H), 6.53 (d, *J* = 3.4 Hz, 1H), 5.52 (s, 1H), 3.49 (s, 3H), 3.18 (dd, *J* = 13.0, 6.5 Hz, 1H), 2.87 (t, *J* = 9.5 Hz, 2H), 2.73 (dd, *J* = 10.5, 2.8 Hz, 1H), 2.45 (d, *J* = 8.8 Hz, 1H), 1.38 (d, *J* = 6.5 Hz, 3H), 0.94 (m, 1H), 0.63 (m, 2H), 0.47 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 158.0, 151.8, 150.6, 145.5, 128.6, 127.1, 127.1, 120.2, 102.7, 102.2, 66.2, 62.1, 59.7, 58.6, 33.8, 24.5, 23.2, 12.6, 11.6.

Synthesis of (R)-N-methyl-N-(5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, (R)-5c

A *N*-methyl-*N*-((*R*)-5-((*R*)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **(R)-4c** (27.7 g, 79.7 mmol) solution in 890 mL of methanol was placed in a 2 L round bottom flask. Then, 10w/w% palladium on charcoal (14.0 g, 5 wt%) and 10.1 g of ammonium formate (10.1 g, 160 mmol) were added and the reaction mixture was stirred at 60~70 °C overnight. After the reaction, it was filtered through a celite 545 pad before the

solution was evaporated. Removing the solvent in vacuo provided 22.2 g of (R)-N-methyl-N-(5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (quantitatively yield). ¹H NMR (400 MHz, CDCl₃) δ 12.13 (s, 1H), 8.25 (s, 1H), 7.08 (t, J = 6.0 Hz, 1H), 6.54 (d, J = 3.4 Hz, 1H), 5.35 (m, 1H), 4.52 (s, 1H), 3.64 (dd, J = 12.2, 8.2 Hz, 1H), 3.48 (t, J = 8.4 Hz, 1H), 3.41 (s, 3H), 3.27 (d, J = 10.9 Hz, 1H), 2.96 (m, 1H), 0.91 (d, J = 9.8 Hz, 1H), 0.71 (m, 2H), 0.62 (d, J = 10.1 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 157.9, 151.8, 150.5, 120.7, 103.1, 102.1, 62.4, 56.2, 51.3, 34.8, 25.1, 14.9, 9.4.

Syntheses of the 3-(7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-oxopropanenitrile enantiomers

In a 5 mL round bottom flask, (R)-N-methyl-N-(5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine **(R)-5c** (210 mg, 0.863 mmol) was placed and solved with 2.50 mL of *n*-butanol. Ethyl cyanoacetate (0.918 mL, 8.63 mmol) was added before 1,8-diazabicyclo[5.4.0]undec-7-ene (0.0654 mL, 0.437 mmol), then heated at 80 ºC for 24 hours. The reaction solution was evaporated and the residue was purified with flash column chromatography (methanol:dichloromethane=2:98). Finally, collected fragments were evaporated. Removing the solvent in vacuo provided 238 mg of (R)-3-(7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-oxopropanenitrile (88.8% yield).

In the cases of racemic mixture and (S)-enantiomer, the racemic mixture and the (S)-enantiomer of 5-(1-phenylethyl)-5-azaspiro[2.4]heptan-7-amine were purchased at Sundia Meditech, China, and the desired products were synthesized from them by the processes similar to the synthesis of (R)-form.

*(R)-3-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-oxopropanenitrile, **(R)-6c***

100% purity by HPLC. ^{1}H NMR (500 MHz, CDCl$_3$) δ 12.16 (s, 1H), 8.26 (d, J = 6.0 Hz, 1H), 7.13 (s, 1H), 6.56 (d, J = 8.6 Hz, 1H), 5.44 (dd, J = 41.1, 5.6 Hz, 1H), 4.26 – 4.00 (m, 1H), 3.98 – 3.70 (m, 2H), 3.58 – 3.32 (m, 6H), 1.16 – 0.94 (m, 1H), 0.82 (dd, J = 21.5, 10.3 Hz, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 159.9, 157.5, 151.9, 150.3, 120.9, 113.8, 103.1, 101.8, 77.4, 77.1, 76.8, 61.5, 54.8, 51.3, 33.5, 25.7, 22.6, 16.7, 8.1. HRMS (ESI) calcd for C$_{16}$H$_{19}$N$_6$O: 311.1620. Obsd 311.1616. [α]$_D$ +51.6° (c 1.49, CHCl$_3$).

3-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-oxopropanenitrile, 6c

Yield: 70.0 mg (79.2%). 96.8% purity by HPLC. ^{1}H NMR (500 MHz, CDCl$_3$) δ 12.09 (s, 1H), 8.26 (d, J = 6.1 Hz, 1H), 7.13 (s, 1H), 6.56 (d, J = 8.9 Hz, 1H), 5.56 – 5.27 (m, 1H), 4.25 – 4.02 (m, 1H), 4.00 – 3.70 (m, 2H), 3.60 – 3.29 (m, 6H), 1.15 – 0.94 (m, 1H), 0.82 (dd, J = 19.5, 9.6 Hz, 3H). ^{13}C NMR (125 MHz, CDCl$_3$) δ 159.5, 157.3, 151.7, 150.1, 120.5, 113.3, 102.8, 101.6, 61.4, 54.6, 52.6, 33.2, 25.4, 22.5, 16.5, 8.0. HRMS (ESI) calcd for C$_{16}$H$_{19}$N$_6$O: 311.1620. Obsd: 311.1616.

(S)-3-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-oxopropanenitrile, (S)-6c

Yield: 117 mg (80.1%). 97.4% purity by HPLC. ^{1}H NMR (500 MHz, CDCl$_3$) δ 12.18 (s, 1H), 8.26 (s, 1H), 7.12 (s, 1H), 6.55 (s, 1H), 5.38 (t, J = 37.8 Hz, 1H), 4.10 (d, J = 42.3 Hz, 1H), 3.82 (dd, J = 74.7, 15.0 Hz, 2H), 3.63 – 3.18 (m, 6H), 1.02 (d, J = 32.1 Hz, 1H), 0.78 (s, 3H). ^{13}C NMR (125 MHz, CDCl$_3$) δ 160.0, 157.5, 151.9, 150.2, 120.9, 113.9, 103.1, 101.8, 61.5, 54.8, 51.3, 33.4, 25.7, 22.6, 16.7, 8.1. HRMS (ESI) calcd for C$_{16}$H$_{19}$N$_6$O: 311.1620. Obsd: 311.1616. [α]$_D$ +35.6° (c 0.980, CHCl$_3$).

In the cases of **6a**, **6b** and **6d** – **6g**, the desired products were synthesized with 4-amino-1-benzylpiperidine, (R)-3-amino-1-

benzylpiperidine, ethyl (5-benzyl-5-azaspiro[2.4]heptan-7-yl)carbamate, (*R,R*)-6-benzyl-octahydro-pyrrolo[3,4-*b*]pyridine dihydrochloride, 4-hydroxypiperidine, and 3-hydroxymethylpiperidine, respectively, instead of (*R*)-5-((*R*)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-amine according to the aforementioned process (vide supra).

3-(4-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidin-1-yl)-3-oxopropanenitrile, **6a**

Yield: 6.2 mg (3.2%). 91.1% purity by HPLC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.65 (s, 1H), 8.38 (s, 1H), 7.43 (s, 1H), 6.90 (d, J = 1.6 Hz, 1H), 4.75 (s, 1H), 4.52 (d, J = 12.8 Hz, 1H), 4.08 (s, 2H), 3.81 (d, J = 13.6 Hz, 1H), 3.27 − 3.20 (m, 3H), 2.82 − 2.75 (m, 1H), 2.03 − 1.91 (m, 1H), 1.77 (s, 2H), 1.23 − 1.18 (m, 2H). LRMS (ESI) calcd for $C_{15}H_{19}N_6O$: 299.2. Obsd: 299.1.

(R)-3-(3-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidin-1-yl)-3-oxopropanenitrile, **6b**

Yield: 42.0 mg (36.2%). 97.2% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.41 (d, J = 84 Hz, 1H), 8.32 (s, 1H), 7.13 − 7.12 (m, 1H), 6.62 − 6.59 (m, 1H), 4.79 − 4.72 (m, 1H), 4.67 (d, J = 11.2 Hz, 1H), 3.91 − 3.55 (m, 3H), 3.38 (s, 2H), 3.27 (s, 1H), 3.12 (q, J = 13.2, 11.2 Hz, 1H), 2.65 − 2.58 (m, 1H), 2.11 − 2.08 (m, 1H), 2.02 − 1.69 (m, 3H). LRMS (ESI) calcd for $C_{15}H_{19}N_6O$: 299.2. Obsd: 299.1.

N-(5-(7H-Pyrrolo[2,3-d]pyrimidin-4-yl)-5-azaspiro[2.4]heptan-7-yl)-2-cyanoacetamide, **6d**

Yield: 30.0 mg (44.0%). 100% purity by HPLC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.62 (s, 1H), 8.68 (d, J = 7.2 Hz, 1H), 8.18 (s, 1H), 7.13 (s, 1H), 6.55 (s, 1H), 3.99 (s, 2H), 3.85 (d, J = 10.0 Hz, 1H), 3.64 (s, 2H), 3.62 − 3.55 (m, 1H), 3.16 − 3.10 (m, 1H), 0.84 − 0.70 (m, 4H). LRMS (ESI) calcd for $C_{15}H_{17}N_6O$: 297.1. Obsd: 297.1.

3-((4aR,7aR)-1-(7H-Pyrrolo[2,3-d]pyrimidin-4-yl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-3-oxopropanenitrile, **6e**

Yield: 50.0 mg (63.3%). 97.0% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.04 (s, 1H), 8.36 (d, J = 2.8 Hz, 1H), 7.15 – 7.08 (m, 1H), 6.58 – 6.54 (m, 1H), 5.65 – 5.51 (m, 1H), 4.65 – 4.57 (m, 1H), 4.01 – 3.49 (m, 4H), 3.46 (d, J = 3.6 Hz, 2H), 3.41 – 3.20 (m, 1H), 2.53 – 2.39 (m, 1H), 2.01 – 1.94 (m, 2H), 1.80 – 1.69 (m, 1H), 1.54 – 1.42 (m, 1H). LRMS (ESI) calcd for C$_{16}$H$_{19}$N$_6$O: 311.2. Obsd: 311.1.

3-(4-((7H-Pyrrolo[2,3-d]pyrimidin-4-yl)oxy)piperidin-1-yl)-3-oxopropanenitrile, **6f**

Yield: 35.4 mg (49.4%). 100% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.03 (s, 1H), 8.34 (s, 1H), 7.35 (t, J = 3.2 Hz, 1H), 6.47 (q, J = 3.6, 2.0 Hz, 1H), 5.58 – 5.43 (m, 1H), 4.08 (s, 2H), 3.90 – 3.86 (m, 1H), 3.63 – 3.59 (m, 1H), 3.47 – 3.32 (m, 2H), 2.09 – 2.00 (m, 2H), 1.82 – 1.77 (m, 1H), 1.69 – 1.61 (m, 1H). LRMS (ESI) calcd for C$_{14}$H$_{16}$N$_5$O$_2$: 286.1. Obsd: 286.1.

3-(3-(((7H-Pyrrolo[2,3-d]pyrimidin-4-yl)oxy)methyl)piperidin-1-yl)-3-oxopropanenitrile, **6g**

Yield: 54.9 mg (30.0%). 89.2% purity by HPLC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (s, 1H), 8.34 (s, 1H), 7.36 (s, 1H), 6.54 – 6.46 (m, 1H), 4.43 – 4.39 (m, 1H), 4.35 – 4.26 (m, 2H), 4.06 – 4.02 (m, 2H), 3.07 – 3.01 (m, 1H), 2.76 – 2.67 (m, 2H), 1.98 – 1.91 (m, 1H), 1.90 – 1.84 (m, 1H), 1.74 – 1.66 (m, 1H), 1.45 – 1.37 (m, 2H). LRMS (ESI) calcd for C$_{15}$H$_{18}$N$_5$O$_2$: 300.1. Obsd: 300.1.

Synthesis of (R)-N-(5-ethyl-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **19**

In a 5 mL round-bottom flask, (*R*)-*N*-methyl-*N*-(5-azaspiro[2.4]heptan-7-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **(*R*)-5c** (70.0 mg, 0.288 mmol) was

placed and solved with 1.00 mL of dichloromethane. The solution was treated with bromoethane (0.0320 mL, 0.432 mmol) and *N,N*-diisopropylethylamine (0.100 mL, 0.574 mmol) was added. The reaction solution was stirred at room temperature overnight then evaporated. The residue was purified by flash column chromatography (methanol:dichloromethane=2:98) and collected fragments were evaporated. Removing the solvent in vacuo provided 23.9 mg of (*R*)-*N*-(5-ethyl-5-azaspiro[2.4]heptan-7-yl)-*N*-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (30.7% yield). 100% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.99 (s, 1H), 8.24 (s, 1H), 7.06 (d, *J* = 3.2 Hz, 1H), 6.57 (s, 1H), 5.58 (s, 1H), 3.46 (d, *J* = 2.8 Hz, 3H), 3.08 (s, 1H), 2.94 (s, 1H), 2.80 (d, *J* = 5.9 Hz, 1H), 2.58 (d, *J* = 5.4 Hz, 2H), 1.19 – 1.12 (m, 3H), 0.90 (dd, *J* = 21.8, 5.3 Hz, 2H), 0.68 (s, 2H), 0.50 (d, *J* = 5.7 Hz, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.9, 151.8, 150.5, 120.0, 102.6, 102.0, 63.3, 59.9, 58.3, 50.4, 33.9, 24.1, 13.4, 13.2, 10.4. HRMS (ESI) calcd for C$_{15}$H$_{22}$N$_5$: 272.1875. Obsd: 272.1872. [α]$_D$ +43.2° (*c* 0.560, CHCl$_3$).

In the cases of **20** and **21**, the desired products were synthesized through substitution reactions with *n*-butyl bromide and benzyl bromide, respectively, instead of ethyl bromide according to the aforementioned process (vide supra).

(R)-N-(5-Butyl-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **20**

Yield: 35.0 mg (40.7%). 100% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.46 (s, 1H), 8.24 (s, 1H), 7.06 (d, *J* = 3.5 Hz, 1H), 6.58 (d, *J* = 3.4 Hz, 1H), 5.55 (s, 1H), 3.48 (s, 3H), 3.02 (s, 2H), 2.86 (s, 1H), 2.67 – 2.40 (m, 3H), 1.65 – 1.47 (m, 2H), 1.38 (dq, *J* = 14.4, 7.3 Hz, 2H), 0.94 (t, *J* = 7.3 Hz, 4H), 0.69 (s, 2H), 0.52 (t, *J* = 10.2 Hz, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.8, 151.9, 150.7, 119.9, 102.6, 102.2, 63.4, 60.0, 58.6, 56.2, 34.1, 30.3, 24.0, 20.6, 14.0, 13.1, 10.6. HRMS (ESI) calcd for C$_{17}$H$_{26}$N$_5$: 300.2188. Obsd: 300.2188. [α]$_D$ +55.6° (*c* 0.410, CHCl$_3$).

(R)-N-(5-Benzyl-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 21

Yield: 40.0 mg (38.8%). 100% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.52 (s, 1H), 8.21 (s, 1H), 7.44 – 7.21 (m, 5H), 7.03 (d, J = 3.5 Hz, 1H), 6.56 (d, J = 3.4 Hz, 1H), 5.57 (s, 1H), 3.64 (dd, J = 31.2, 12.9 Hz, 2H), 3.52 (s, 3H), 3.01 – 2.87 (m, 2H), 2.76 (d, J = 8.9 Hz, 1H), 2.51 (d, J = 8.9 Hz, 1H), 1.01 – 0.90 (m, 1H), 0.70 – 0.56 (m, 2H), 0.51 – 0.40 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.9, 151.8, 150.7, 138.9, 128.6, 128.3, 127.0, 119.7, 102.2, 63.3, 60.6, 59.1, 33.7, 24.4, 12.5, 11.2. HRMS (ESI) calcd for C$_{20}$H$_{24}$N$_5$: 334.2032. Obsd: 334.2025. [α]$_D$ +52.9° (*c* 3.07, CHCl$_3$).

Synthesis of (R)-3-methyl-1-(7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)butan-1-one, 23

In a 5 mL round-bottom flask, (*R*)-*N*-methyl-*N*-(5-azaspiro[2.4]heptan-7-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **(R)-5c** (60.0 mg, 0.247 mmol) was placed and solved with 1.00 mL of *N*,*N*-dimethylformamide. The solution was treated with isovaleryl chloride (46.6 mg, 0.386 mmol) and *N*,*N*-diisopropylethylamine (0.0860 mL, 0.494 mmol) was added. The reaction solution was stirred at room temperature overnight and then evaporated. The residue was purified by column chromatography (methanol:dichloromethane=2:98) and collected fragments were evaporated. Removing the solvent in vacuo provided 32.0 mg of (*R*)-3-methyl-1-(7-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)butan-1-one (39.1% yield). 100% purity by HPLC. ^{1}H NMR (500 MHz, CDCl$_3$) δ 12.06 (d, J = 16.4 Hz, 1H), 8.27 (d, J = 8.9 Hz, 1H), 7.11 (d, J = 9.6 Hz, 1H), 6.56 (d, J = 9.9 Hz, 1H), 5.37 (t, J = 38.6 Hz, 1H), 4.05 (ddd, J = 43.9, 27.1, 11.7 Hz, 2H), 3.82 (dd, J = 66.1, 11.9 Hz, 1H), 3.65 (dd, J = 178.8, 11.3 Hz, 1H), 3.45 – 3.33 (m, 3H), 2.20 (d, J = 13.8 Hz, 3H), 0.99 (s, 6H), 0.78 (t, J = 21.2 Hz, 4H). ^{13}C NMR (125 MHz, CDCl$_3$) δ 171.0, 157.5, 151.8, 150.2,

120.4, 102.8, 101.6, 61.6, 55.1, 52.5, 43.3, 33.1, 25.4, 24.8, 22.6, 16.6, 8.0. HRMS (ESI) calcd for $C_{18}H_{26}N_5O$: 328.2137. Obsd: 328.2126. $[\alpha]_D$ +45.2° (*c* 1.21, CHCl$_3$).

In the cases of **24**, **25**, and **29** – **36**, the desired products were synthesized through substitution reactions with isobutyryl chloride, cyclopropane carbonyl chloride, 2-furoyl chloride, benzoyl chloride, nicotinoyl chloride hydrochloride, isonicotinoyl chloride hydrochloride, 3-cyanobenzoyl chloride, 4-cyanobenzoyl chloride, 2-(trifluoromethyl)benzoyl chloride, and 3-(trifluoromethyl)benzoyl chloride, respectively, instead of isovaleryl chloride according to the aforementioned process (vide supra).

*(R)-2-Methyl-1-(7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)propan-1-one, **24***

Yield: 38.0 mg (42.2%). 100% purity by HPLC. ^{1}H NMR (500 MHz, CDCl$_3$) δ 12.06 (d, *J* = 17.4 Hz, 1H), 8.27 (d, *J* = 9.1 Hz, 1H), 7.11 (d, *J* = 10.0 Hz, 1H), 6.56 (d, *J* = 10.8 Hz, 1H), 5.37 (dd, *J* = 42.3, 38.0 Hz, 1H), 4.23 – 3.74 (m, 3H), 3.43 (t, *J* = 21.8 Hz, 4H), 2.79 – 2.56 (m, 1H), 1.16 (dd, *J* = 9.8, 5.6 Hz, 6H), 1.10 – 0.94 (m, 1H), 0.79 (td, *J* = 25.2, 8.8 Hz, 3H). ^{13}C NMR (125 MHz, CDCl$_3$) δ 175.4, 157.2, 151.9, 150.3, 120.4, 102.9, 101.7, 61.6, 54.2, 50.3, 32.8, 32.0, 24.9, 18.7, 16.8, 8.0. HRMS (ESI) calcd for $C_{17}H_{24}N_5O$: 314.1981. Obsd: 314.1971. $[\alpha]_D$ +50.0° (*c* 1.12, CHCl$_3$).

*(R)-Cyclopropyl(7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)methanone, **25***

Yield: 41.0 mg (46.1%). 93.1% purity by HPLC. ^{1}H NMR (500 MHz, CDCl$_3$) δ 12.28 (d, *J* = 15.5 Hz, 1H), 8.28 (d, *J* = 10.3 Hz, 1H), 7.12 (d, *J* = 10.0 Hz, 1H), 6.56 (d, *J* = 14.6 Hz, 1H), 5.44 (d, *J* = 15.0 Hz, 1H), 4.40 – 4.24 (m, 1H), 4.11 – 3.92 (m, 2H), 3.74 (dd, *J* = 141.4, 10.9 Hz, 2H), 3.45 (t, *J* = 21.3 Hz, 3H), 1.63 (d, *J* = 40.6 Hz, 1H), 1.16 – 0.94 (m, 3H), 0.80 (s, 4H). ^{13}C NMR (125 MHz, CDCl$_3$) δ 172.1, 157.7, 152.0, 150.4, 120.6, 103.0, 101.8,

61.5, 54.5, 52.3, 33.2, 22.9, 16.8, 12.4, 12.3, 7.7. HRMS (ESI) calcd for C$_{17}$H$_{22}$N$_5$O: 312.1824. Obsd: 312.1823. [α]$_D$ +60.0° (*c* 1.31, CHCl$_3$).

(R)-Furan-2-yl(7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)methanone, **29**

Yield: 60.0 mg (54.0%). 99.2% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.49 (s, 1H), 8.28 (s, 1H), 7.52 (s, 1H), 7.15 (d, *J* = 2.5 Hz, 1H), 7.10 (d, *J* = 3.4 Hz, 1H), 6.58 (d, *J* = 2.6 Hz, 1H), 6.51 (s, 1H), 5.48 (s, 1H), 4.60 – 4.41 (m, 1H), 4.25 (d, *J* = 10.0 Hz, 2H), 4.18 – 4.02 (m, 1H), 3.72 (dd, *J* = 70.6, 11.6 Hz, 1H), 3.44 (s, 2H), 1.06 (s, 1H), 0.83 (dd, *J* = 22.6, 14.3 Hz, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.8, 157.6, 151.5, 150.2, 144.4, 144.3, 120.5, 116.6, 111.5, 102.9, 102.1, 61.8, 58.5, 51.8, 33.3, 25.5, 16.7, 8.0. HRMS (ESI) calcd for C$_{18}$H$_{20}$N$_5$O$_2$: 338.1617. Obsd: 338.1616. [α]$_D$ +56.0° (*c* 0.360, CHCl$_3$).

(R)-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)(phenyl)methanone, **30**

Yield: 77.7 mg (67.8%). 95.5% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.45 (s, 1H), 8.25 (d, *J* = 25.1 Hz, 1H), 7.54 (s, 2H), 7.39 (s, 2H), 7.28 (s, 1H), 7.06 (s, 1H), 6.53 (d, *J* = 21.5 Hz, 1H), 5.45 (d, *J* = 74.9 Hz, 1H), 4.40 – 3.97 (m, 2H), 3.89 – 3.54 (m, 2H), 3.42 (d, *J* = 20.4 Hz, 3H), 1.01 (s, 1H), 0.79 (dd, *J* = 26.4, 14.3 Hz, 2H), 0.63 (s, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 169.5, 157.5, 151.9, 150.1, 136.1, 130.1, 128.4, 127.0, 120.7, 102.9, 101.7, 61.4, 57.5, 50.6, 33.2, 22.9, 16.5, 8.5. HRMS (ESI) calcd for C$_{20}$H$_{22}$N$_5$O: 348.1824. Obsd: 348.1819. [α]$_D$ +22.5° (*c* 2.85, CHCl$_3$).

(R)-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)(pyridin-3-yl)methanone, **31**

Yield: 32.0 mg (32.0%). 100% purity by HPLC. ^{1}H NMR (500 MHz, CDCl$_3$) δ 11.87 (s, 1H), 8.83 (s, 1H), 8.68 (d, *J* = 12.5 Hz, 1H), 8.25 (d, *J* = 31.9 Hz, 1H), 7.90 (s, 1H), 7.47 – 7.31 (m, 1H), 7.10 (d, *J* = 12.4 Hz, 1H), 6.56

(d, J = 27.7 Hz, 1H), 5.40 (dd, J = 73.5, 68.8 Hz, 1H), 4.37 – 4.03 (m, 2H), 3.94 – 3.59 (m, 2H), 3.43 (t, J = 15.8 Hz, 3H), 1.05 (d, J = 8.0 Hz, 1H), 0.96 – 0.73 (m, 2H), 0.67 (s, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.9, 157.5, 152.0, 151.1, 150.4, 148.0, 135.0, 131.9, 123.4, 120.6, 103.0, 101.8, 61.5, 54.5, 50.7, 33.4, 22.6, 16.5, 8.4. HRMS (ESI) calcd for C$_{19}$H$_{21}$N$_6$O: 349.1777. Obsd: 349.1764. [α]$_D$ +15.1° (c 1.12, CHCl$_3$).

(R)-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)(pyridin-4-yl)methanone, **32**

Yield: 41.0 mg (28.7%). 94.1% purity by HPLC. ^{1}H NMR (500 MHz, CDCl$_3$) δ 12.14 (s, 1H), 8.72 (d, J = 15.1 Hz, 2H), 8.25 (d, J = 31.9 Hz, 1H), 7.41 (s, 2H), 7.11 (d, J = 12.7 Hz, 1H), 6.55 (d, J = 24.5 Hz, 1H), 5.46 (d, J = 111.2 Hz, 1H), 4.19 (dt, J = 26.7, 13.8 Hz, 2H), 3.64 (d, J = 12.1 Hz, 1H), 3.56 (dd, J = 220.1, 10.6 Hz, 1H), 3.44 (d, J = 14.5 Hz, 3H), 1.04 (d, J = 6.5 Hz, 1H), 0.95 – 0.72 (m, 2H), 0.67 (s, 1H). ^{13}C NMR (125 MHz, CDCl$_3$) δ 166.9, 157.5, 152.0, 150.4, 150.3, 143.5, 121.1, 120.7, 103.0, 101.7, 61.4, 57.2, 50.6, 33.4, 22.6, 16.6, 8.5. HRMS (ESI) calcd for C$_{19}$H$_{21}$N$_6$O: 349.1777. Obsd: 349.1772. [α]$_D$ +24.5° (c 1.16, CHCl$_3$).

(R)-3-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carbonyl)benzonitrile, **33**

Yield: 87.8 mg (71.4%). 97.7% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.28 (s, 1H), 8.27 (d, J = 23.4 Hz, 1H), 7.85 (s, 1H), 7.79 (d, J = 7.2 Hz, 1H), 7.76 – 7.64 (m, 1H), 7.62 – 7.46 (m, 1H), 7.11 (d, J = 5.6 Hz, 1H), 6.56 (d, J = 18.0 Hz, 1H), 5.46 (d, J = 82.3 Hz, 1H), 4.39 – 4.02 (m, 2H), 3.65 (t, J = 12.3 Hz, 1H), 3.58 (dd, J = 185.8, 10.4 Hz, 1H), 3.44 (d, J = 14.8 Hz, 3H), 1.05 (d, J = 10.3 Hz, 1H), 0.97 – 0.74 (m, 2H), 0.68 (s, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.9, 157.5, 152.0, 150.2, 137.3, 133.5, 131.4, 130.8, 129.5, 120.8, 117.9, 112.8, 103.1, 101.7, 61.5, 54.5, 50.8, 33.4, 22.6, 16.6, 8.4.

HRMS (ESI) calcd for $C_{21}H_{21}N_6O$: 373.1777. Obsd: 373.1772. $[\alpha]_D$ +10.9° (c 0.963, CHCl$_3$).

(R)-4-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carbonyl)benzonitrile, **34**

Yield: 78.0 mg (72.9%). 100% purity by HPLC. ^{1}H NMR (500 MHz, CDCl$_3$) δ 12.35 (s, 1H), 8.26 (d, J = 32.1 Hz, 1H), 7.78 – 7.67 (m, 2H), 7.64 (d, J = 6.7 Hz, 2H), 7.11 (d, J = 12.4 Hz, 1H), 6.55 (d, J = 25.3 Hz, 1H), 5.45 (d, J = 116.1 Hz, 1H), 4.19 (dt, J = 98.3, 17.9 Hz, 2H), 3.64 (d, J = 12.2 Hz, 1H), 3.55 (dd, J = 225.6, 10.4 Hz, 1H), 3.44 (d, J = 17.0 Hz, 3H), 1.03 (d, J = 6.0 Hz, 1H), 0.97 – 0.72 (m, 2H), 0.66 (s, 1H). ^{13}C NMR (125 MHz, CDCl$_3$) δ 167.4, 157.5, 152.0, 150.2, 140.3, 132.4, 127.8, 120.8, 118.0, 113.8, 103.0, 101.7, 61.5, 57.3, 50.7, 33.4, 22.6, 16.6, 8.5. HRMS (ESI) calcd for $C_{21}H_{21}N_6O$: 373.1777. Obsd: 373.1766. $[\alpha]_D$ +15.7° (c 2.54, CHCl$_3$).

(R)-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)(2-(trifluoromethyl)phenyl)methanone, **35**

Yield: 82.9 mg (69.7%). 98.8% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.19 (s, 1H), 8.30 (d, J = 32.9 Hz, 1H), 7.71 (dd, J = 13.4, 7.9 Hz, 1H), 7.66 – 7.56 (m, 1H), 7.52 (dt, J = 14.8, 7.6 Hz, 1H), 7.42 (d, J = 7.4 Hz, 1H), 7.09 (dd, J = 9.7, 3.5 Hz, 1H), 6.57 (dd, J = 18.0, 3.1 Hz, 1H), 5.69 – 5.31 (m, 1H), 4.07 (ddd, J = 19.1, 12.6, 7.8 Hz, 1H), 3.87 (dd, J = 163.0, 12.6 Hz, 1H), 4.18 – 3.35 (m, 1H), 3.45 (s, 3H), 3.31 (dd, J = 158.2, 10.8 Hz, 1H), 1.04 (dd, J = 11.7, 6.7 Hz, 1H), 0.94 – 0.68 (m, 2H), 0.68 – 0.56 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 167.0, 157.5, 151.8, 150.0, 135.4 (d, J = 26.1 Hz), 132.3, 129.3 (d, J = 4.1 Hz), 127.1 (d, J = 12.0 Hz), 126.9 – 126.6 (m), 123.6 (q, J = 273.8 Hz), 120.7, 103.2, 101.9, 61.2, 56.6, 50.2, 33.3, 24.8, 16.8, 8.3. HRMS (ESI) calcd for $C_{21}H_{21}F_3N_5O$: 416.1698. Obsd: 416.1695. $[\alpha]_D$ +25.1° (c 1.71, CHCl$_3$).

(R)-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-
azaspiro[2.4]heptan-5-yl)(3-(trifluoromethyl)phenyl)methanone, 36

Yield: 55.0 mg (46.2%). 98.4% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.67 (s, 1H), 8.27 (s, 1H), 7.82 (s, 1H), 7.71 (dd, J = 17.7, 7.8 Hz, 2H), 7.62 – 7.46 (m, 1H), 7.09 (s, 1H), 6.58 (d, J = 20.8 Hz, 1H), 5.48 (d, J = 77.8 Hz, 1H), 4.39 – 4.01 (m, 2H), 3.66 (t, J = 13.7 Hz, 1H), 3.59 (dd, J = 173.8, 10.5 Hz, 1H), 3.46 (d, J = 18.1 Hz, 3H), 1.05 (d, J = 10.5 Hz, 1H), 0.98 – 0.73 (m, 2H), 0.68 (s, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 167.8, 157.5, 151.4, 149.9, 136.8, 131.0 (q, J = 32.8 Hz), 130.4, 129.1, 126.9, 124.1, 123.6 (q, J = 273.0 Hz), 120.7, 103.4, 102.0, 61.5, 54.5, 50.7, 33.4, 22.8, 16.6, 8.4. HRMS (ESI) calcd for C$_{21}$H$_{21}$F$_3$N$_5$O: 416.1698. Obsd: 416.1692. [α]$_D$ +20.7º (c 0.730, CHCl$_3$).

Synthesis of (R)-2-azido-1-(7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)ethan-1-one, 22

In a 10 mL round-bottom flask, 2-azidoacetic acid (208 mg, 2.06 mmol) was placed and solved with 6.0 mL of *N,N*-dimethylformamide. *N,N'*-dicyclohexylcarbodiimide (423 mg, 2.05 mmol) and *N,N*-diisopropylethylamine (0.716 mL, 4.11 mmol) were added and the reaction mixture was stirred for 15 minutes. In a second 25 mL round-bottom flask, (*R*)-*N*-methyl-*N*-(5-azaspiro[2.4]heptan-7-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **(R)-5c** (300 mg, 1.23 mmol) was placed and the reaction mixture of 2-azidoacetic acid was transferred to this second flask. The reaction mixture was refluxed overnight and then cooled at room temperature before being filtered through a celite 545 pad and the solution evaporated. The residue was purified with column chromatography (methanol:dichloromethane=2:98) and collected fragments were evaporated. Removing the solvent in vacuo provided 41.0 mg of   (*R*)-2-azido-1-(7-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)ethan-1-one (11.9% yield). 100% purity by HPLC. ^{1}H

NMR (500 MHz, CDCl$_3$) δ 11.80 (s, 1H), 8.23 (d, J = 22.5 Hz, 1H), 7.10 (d, J = 17.0 Hz, 1H), 6.56 (s, 1H), 5.55 – 5.22 (m, 1H), 4.17 – 3.94 (m, 1H), 3.90 (d, J = 16.4 Hz, 2H), 3.81 – 3.47 (m, 2H), 3.38 (dd, J = 33.1, 19.3 Hz, 4H), 1.03 (d, J = 46.0 Hz, 1H), 0.91 – 0.58 (m, 3H). ^{13}C NMR (125 MHz, CDCl$_3$) δ 165.7, 157.5, 151.9, 150.4, 120.8, 103.1, 101.8, 61.7, 54.5, 51.6, 51.0, 33.4, 22.4, 16.8, 8.1. HRMS (ESI) calcd for C$_{15}$H$_{19}$N$_8$O: 327.1682. Obsd: 327.1673. [α]$_D$ +37.3º (c 1.49, CHCl$_3$).

In the cases of **26 – 28**, the desired products were synthesized through amide coupling reactions with *N*-acetylglycine, 3-(methylamino)-3-oxopropanoic acid, and 1-acetyl-4-piperidinecarboxylic acid, respectively, instead of 2-azidoacetic acid according to the aforementioned process (vide supra).

*(R)-N-(2-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-2-oxoethyl)acetamide, **26***

Yield: 62.1 mg (44.0%). 98.6% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.07 (s, 1H), 8.26 (s, 1H), 7.30 (s, 1H), 7.12 (s, 1H), 6.55 (s, 1H), 5.43 (d, J = 11.0 Hz, 1H), 4.09 (d, J = 15.0 Hz, 2H), 3.88 (ddd, J = 47.9, 37.2, 16.8 Hz, 2H), 3.45 (dd, J = 42.9, 13.8 Hz, 3H), 2.04 (s, 3H), 1.26 (s, 2H), 1.15 – 0.96 (m, 1H), 0.96 – 0.61 (m, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 170.5, 167.0, 157.5, 151.9, 150.2, 120.9, 103.1, 101.7, 61.3, 54.4, 51.2, 42.0, 33.3, 24.6, 22.8, 16.6, 8.1. HRMS (ESI) calcd for C$_{17}$H$_{23}$N$_6$O$_2$: 343.1882. Obsd: 343.1879. [α]$_D$ +42.2º (c 1.00, CHCl$_3$).

*(R)-N-Methyl-3-(7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-oxopropanamide, **27***

Yield: 39.1 mg (13.0%). 100% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.96 (d, J = 30.6 Hz, 1H), 8.24 (dd, J = 6.6, 1.8 Hz, 1H), 8.13 (s, 1H), 7.11 (s, 1H), 6.54 (s, 1H), 5.51 – 5.34 (m, 1H), 4.13 (ddd, J = 21.2, 12.6, 7.5 Hz, 1H), 4.00 – 3.80 (m, 2H), 3.49 (t, J = 11.6 Hz, 1H), 3.40 (d, J = 14.5

Hz, 3H), 3.35 (t, J = 19.8 Hz, 2H), 2.83 (dd, J = 4.5, 2.1 Hz, 3H), 1.11 – 0.94 (m, 1H), 0.90 – 0.68 (m, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 167.0, 166.7, 157.5, 152.0, 150.4, 120.8, 103.0, 101.7, 59.3, 55.6, 52.7, 41.3, 33.3, 26.1, 22.9, 16.3, 8.1. HRMS (ESI) calcd for C$_{17}$H$_{23}$N$_6$O$_2$: 343.1882. Obsd: 343.1872. [α]$_D$ +37.2° (c 1.30, CHCl$_3$).

(R)-1-(4-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carbonyl)piperidin-1-yl)ethan-1-one, 28

Yield: 104 mg (64.0%). 95.3% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.29 (s, 1H), 8.36 – 8.21 (m, 1H), 7.13 (dd, J = 9.8, 2.7 Hz, 1H), 6.62 – 6.51 (m, 1H), 5.54 – 5.31 (m, 1H), 4.62 (dd, J = 17.9, 8.6 Hz, 1H), 4.19 (dd, J = 9.8, 6.5 Hz, 1H), 4.11 – 3.76 (m, 3H), 3.54 – 3.46 (m, 1H), 3.46 – 3.33 (m, 3H), 3.19 – 3.01 (m, 1H), 2.75 – 2.51 (m, 2H), 2.17 – 2.02 (m, 3H), 1.97 – 1.63 (m, 4H), 1.15 – 0.95 (m, 1H), 0.92 – 0.71 (m, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.6, 168.8, 157.5, 152.0, 150.3, 120.8, 103.1, 101.7, 61.9, 58.9, 50.6, 45.7, 40.9, 40.2, 33.4, 24.9, 21.4, 16.0, 8.1. HRMS (ESI) calcd for C$_{21}$H$_{29}$N$_6$O$_2$: 397.2352. Obsd: 397.2343. [α]$_D$ +45.2° (c 1.63, CHCl$_3$).

Synthesis of (R)-3-(7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-thioxopropanenitrile, 37

In a 5 mL round-bottom flask, (*R*)-3-(7-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-oxopropanenitrile **(*R*)-6c** (44.7 mg, 0.144 mmol) was placed and solved with 1.40 mL of dichloromethane. The solution was treated with Lawesson's reagent (32.0 mg, 0.0791 mmol) and stirred for 3 days before being evaporated. The residue was purified with column chromatography (methanol:dichloromethane=2:98) and collected fragments were evaporated. Removing the solvent in vacuo provided 37.0 mg of (*R*)-3-(7-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-thioxopropanenitrile (78.7% yield). 100% purity

by HPLC. ^{1}H NMR (400 MHz, CDCl3) δ 12.07 (s, 1H), 8.27 (d, J = 3.4 Hz, 1H), 7.20 − 7.08 (m, 1H), 6.57 (dd, J = 5.7, 3.7 Hz, 1H), 5.45 (tt, J = 186.7, 93.9 Hz, 1H), 4.42 − 4.25 (m, 1H), 4.20 (dd, J = 131.3, 13.0 Hz, 1H), 4.01 (dd, J = 162.4, 14.4 Hz, 1H), 3.98 − 3.85 (m, 2H), 3.90 (dd, J = 211.1, 11.8 Hz, 1H), 3.44 (t, J = 19.7 Hz, 3H), 1.16 − 1.00 (m, 1H), 0.97 − 0.74 (m, 3H). ^{13}C NMR (100 MHz, CDCl3) δ 184.8, 157.4, 152.0, 150.2, 121.0, 114.1, 103.2, 101.8, 62.3, 59.1, 57.0, 34.2, 33.7, 25.2, 16.9, 8.1. HRMS (ESI) calcd for $C_{16}H_{19}N_6S$: 327.1392. Obsd: 327.1380. $[α]_D$ +48.8° (c 1.23, CHCl3).

Synthesis of isobutyl (R)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxylate, **38**

In a 5 mL round-bottom flask, (*R*)-*N*-methyl-*N*-(5-azaspiro[2.4]heptan-7-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **(R)-5c** (100 mg, 0.411 mmol) was placed and solved with 1.00 mL of *N,N*-dimethylformamide. The solution was treated with isobutyl chloroformate (84.2 mg, 0.616 mmol) and *N,N*-diisopropylethylamine (0.138 mL, 0.792 mmol) was added. The reaction solution was stirred at room temperature overnight and evaporated. The residue was purified by column chromatography (methanol:dichloromethane=2:98) and collected fragments were evaporated. Removing the solvent in vacuo provided 107.0 mg of isobutyl (*R*)-7-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxylate (76.4% yield). 100% purity by HPLC. ^{1}H NMR (500 MHz, CDCl3) δ 12.56 (s, 1H), 8.28 (s, 1H), 7.11 (s, 1H), 6.55 (s, 1H), 5.41 (s, 1H), 4.08 − 3.97 (m, 1H), 3.91 (d, J = 4.7 Hz, 2H), 3.85 − 3.66 (m, 2H), 3.42 (s, 3H), 3.39 − 3.29 (m, 1H), 2.02 − 1.87 (m, 1H), 1.01 (s, 1H), 0.94 (s, 6H), 0.75 (d, J = 9.4 Hz, 3H). ^{13}C NMR (125 MHz, CDCl3) δ 157.7, 154.9, 152.0, 150.3, 120.6, 102.9, 101.8, 71.4, 61.0, 54.6, 51.1, 33.0, 28.0, 23.6, 19.1, 16.5, 8.1. HRMS (ESI) calcd for $C_{18}H_{26}N_5O_2$: 344.2087. Obsd: 344.2077. $[α]_D$ +33.2° (c 4.45, CHCl3).

Synthesis of (R)-N-butyl-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, 39

In a 5 mL round-bottom flask, (*R*)-*N*-methyl-*N*-(5-azaspiro[2.4]heptan-7-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **(R)-5c** (49.2 mg, 0.202 mmol) was placed and solved with 2.00 mL of dichloromethane. *N,N*-diisopropylethylamine (0.0370 mL, 0.212 mmol) was added and the mixture was treated with 0.0241 mL of butyl isocyanate (0.0241 mL, 0.214 mmol). The reaction solution was stirred for 2 hours before being evaporated. The residue was purified by column chromatography (methanol:dichloromethane=2:98) and collected fragments were evaporated. Removing the solvent in vacuo provided 67.7 mg of (*R*)-*N*-butyl-7-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide (97.8% yield). 100% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.31 (s, 1H), 8.26 (s, 1H), 7.10 (s, 1H), 6.54 (s, 1H), 5.38 (d, *J* = 5.5 Hz, 1H), 4.46 (s, 1H), 3.98 (dd, *J* = 10.4, 7.6 Hz, 1H), 3.71 (dd, *J* = 31.5, 10.3 Hz, 2H), 3.41 (s, 3H), 3.33 (d, *J* = 9.8 Hz, 1H), 3.26 (d, *J* = 5.1 Hz, 2H), 1.50 (dt, *J* = 14.5, 7.2 Hz, 2H), 1.34 (td, *J* = 14.5, 7.2 Hz, 2H), 0.99 (d, *J* = 7.7 Hz, 1H), 0.92 (t, *J* = 7.2 Hz, 3H), 0.85 (s, 1H), 0.75 (s, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 156.6, 151.5, 150.0, 120.7, 102.9, 101.9, 61.0, 54.4, 51.1, 40.4, 33.1, 32.5, 24.1, 20.0, 16.7, 13.8, 8.1. HRMS (ESI) calcd for C$_{18}$H$_{27}$N$_6$O: 343.2246. Obsd: 343.2241. [α]$_D$ +43.2° (*c* 2.89, CHCl$_3$).

In the cases from **40** to **48**, the desired products were synthesized through substitution reactions with cyclohexyl isocyanate, phenyl isocyanate, isocyanic acid 4-fluorophenyl ester, isocyanic acid 2,4-dichlorophenyl ester, 3,4-dichlorophenyl isocyanate, 2,5-dichlorophenyl isocyanate, 2,3-dichlorophenyl isocyanate, 3-chloro-4-methylphenyl isocyanate, and 2-biphenyl isocyanate, respectively, instead of butyl isocyanate according to the aforementioned process (vide supra).

(R)-N-Cyclohexyl-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, 40

Yield: 59.1 mg (78.4%). 96.4% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.25 (s, 1H), 8.28 (s, 1H), 7.10 (d, J = 2.1 Hz, 1H), 6.56 (s, 1H), 5.39 (d, J = 5.8 Hz, 1H), 4.18 (d, J = 7.6 Hz, 1H), 3.97 (dd, J = 10.8, 7.5 Hz, 1H), 3.66 (t, J = 10.8 Hz, 2H), 3.53 (dd, J = 163.5, 9.8 Hz, 2H), 3.43 (s, 3H), 1.97 (d, J = 11.4 Hz, 2H), 1.70 (d, J = 9.8 Hz, 2H), 1.60 (d, J = 12.8 Hz, 1H), 1.36 (td, J = 14.2, 2.5 Hz, 2H), 1.22 − 1.04 (m, 3H), 1.03 − 0.93 (m, 1H), 0.76 (s, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 155.9, 151.5, 150.0, 120.6, 103.0, 101.9, 61.0, 54.4, 51.1, 49.2, 34.1, 33.1, 25.6, 25.0, 24.0, 16.7, 8.1. HRMS (ESI) calcd for C$_{20}$H$_{29}$N$_6$O: 369.2403. Obsd: 369.2398. [α]$_D$ +39.1° (c 2.39, CHCl$_3$).

(R)-7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-phenyl-5-azaspiro[2.4]heptane-5-carboxamide, **41**

Yield: 70.6 mg (94.8%). 98.5% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.63 (s, 1H), 8.29 (s, 1H), 7.44 (d, J = 7.2 Hz, 2H), 7.29 (s, 2H), 7.09 (s, 1H), 7.02 (t, J = 7.2 Hz, 1H), 6.52 (d, J = 45.2 Hz, 2H), 5.43 (s, 1H), 4.11 (s, 1H), 3.84 (dd, J = 21.3, 10.0 Hz, 2H), 3.68 − 3.25 (m, 1H), 3.46 (s, 3H), 1.04 (d, J = 8.6 Hz, 1H), 0.95 − 0.69 (m, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.5, 153.7, 150.9, 149.6, 138.8, 128.9, 123.1, 120.7, 119.7, 103.1, 102.2, 61.2, 54.6, 51.4, 33.3, 24.0, 16.8, 8.2. HRMS (ESI) calcd for C$_{20}$H$_{23}$N$_6$O: 363.1933. Obsd: 363.1928. [α]$_D$ +38.0° (c 0.707, CHCl$_3$).

(R)-N-(4-Fluorophenyl)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, **42**

Yield: 50.8 mg (46.6%). 99.2% purity by HPLC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (s, 1H), 8.41 − 8.28 (m, 1H), 8.18 (s, 1H), 7.52 (dd, J = 6.9, 5.2 Hz, 2H), 7.26 (s, 1H), 7.07 (t, J = 7.9 Hz, 2H), 6.72 (s, 1H), 5.20 (s, 1H), 4.03 (dd, J = 11.1, 7.4 Hz, 1H), 3.80 (t, J = 10.6 Hz, 2H), 3.49 (d, J = 68.5 Hz, 10H), 3.37 (s, 9H), 0.95 (d, J = 10.2 Hz, 1H), 0.84 (d, J = 13.6 Hz, 2H), 0.68 (d, J = 10.1 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.7 (d, J = 237.8 Hz), 154.2, 149.9, 148.6, 137.1 (d, J = 2.4 Hz), 122.2, 121.5 (d, J = 7.6 Hz),

115.2 (d, J = 22.0 Hz), 102.9, 102.5, 61.6, 54.5, 51.5, 33.6, 24.2, 16.5, 8.2. HRMS (ESI) calcd for $C_{20}H_{22}FN_6O$: 381.1839. Obsd: 381.1835. $[\alpha]_D$ +54.3° (c 0.223, MeOH).

*(R)-N-(2,4-Dichlorophenyl)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, **43***

Yield: 82.7 mg (67.2%). 97.3% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.01 (s, 1H), 8.28 (d, J = 8.9 Hz, 2H), 7.40 – 7.31 (m, 1H), 7.22 (dd, J = 8.9, 2.1 Hz, 1H), 7.13 (d, J = 2.6 Hz, 1H), 6.82 (s, 1H), 6.59 (s, 1H), 5.53 (d, J = 6.3 Hz, 1H), 4.16 (dd, J = 10.9, 7.5 Hz, 1H), 3.87 (dd, J = 23.7, 10.4 Hz, 2H), 3.48 (s, 3H), 3.42 (s, 1H), 1.08 (d, J = 10.3 Hz, 1H), 0.84 (s, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 152.6, 151.5, 150.0, 134.4, 128.3, 127.9, 127.4, 122.3, 121.2, 120.7, 103.0, 102.0, 60.7, 54.4, 51.1, 33.2, 24.1, 16.8, 8.2. HRMS (ESI) calcd for $C_{20}H_{21}Cl_2N_6O$: 431.1154. Obsd: 431.1146. $[\alpha]_D$ +47.8° (c 0.970, CHCl$_3$).

*(R)-N-(3,4-Dichlorophenyl)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, **44***

Yield: 34.7 mg (38.0%). 99.1% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.85 (d, J = 18.4 Hz, 1H), 8.24 (s, 1H), 7.64 (s, 1H), 7.26 (d, J = 4.1 Hz, 2H), 7.07 (d, J = 2.2 Hz, 1H), 6.72 (d, J = 12.8 Hz, 1H), 6.52 (d, J = 2.7 Hz, 1H), 5.37 (d, J = 4.8 Hz, 1H), 4.05 (dd, J = 10.6, 7.0 Hz, 1H), 3.79 (dd, J = 25.3, 10.5 Hz, 2H), 3.40 (s, 4H), 1.00 (s, 1H), 0.82 (d, J = 42.1 Hz, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 153.2, 151.8, 150.3, 138.5, 132.4, 130.2, 125.9, 121.2, 120.6, 118.9, 103.0, 101.9, 61.0, 54.6, 51.5, 33.2, 23.8, 16.8, 8.2. HRMS (ESI) calcd for $C_{20}H_{21}Cl_2N_6O$: 431.1154. Obsd: 431.1148. $[\alpha]_D$ +48.5° (c 0.850, CHCl$_3$).

*(R)-N-(2,5-Dichlorophenyl)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, **45***

Yield: 77.6 mg (86.0%). 98.4% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.32 (s, 1H), 8.36 (d, J = 53.7 Hz, 2H), 7.12 (s, 1H), 7.07 (dd, J = 124.5, 8.5 Hz, 2H), 6.88 (s, 1H), 6.57 (s, 1H), 5.53 (d, J = 5.8 Hz, 1H), 4.24 – 4.07 (m, 1H), 3.87 (dd, J = 21.7, 10.3 Hz, 2H), 3.48 (s, 1H), 3.46 (s, 3H), 1.07 (d, J = 8.7 Hz, 1H), 0.83 (s, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 152.4, 152.1, 150.3, 136.5, 133.5, 129.3, 122.9, 120.7, 120.2, 119.9, 103.0, 101.8, 60.6, 54.4, 51.0, 33.2, 24.1, 16.7, 8.1. HRMS (ESI) calcd for C$_{20}$H$_{21}$Cl$_2$N$_6$O: 431.1154. Obsd: 431.1148. [α]$_D$ +34.6° (c 2.36, DMSO).

(R)-N-(2,3-Dichlorophenyl)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, 46

Yield: 72.2 mg (78.2%). 98.8% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.36 (s, 1H), 8.45 – 8.16 (m, 2H), 7.23 – 7.06 (m, 3H), 6.97 (s, 1H), 6.58 (s, 1H), 5.54 (d, J = 5.8 Hz, 1H), 4.17 (dd, J = 10.8, 7.6 Hz, 1H), 3.88 (dd, J = 19.3, 10.4 Hz, 2H), 3.50 (s, 1H), 3.47 (s, 3H), 1.07 (d, J = 8.0 Hz, 1H), 0.86 (d, J = 18.6 Hz, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 152.6, 151.7, 150.0, 137.3, 132.3, 127.7, 123.7, 120.8, 120.4, 118.4, 103.1, 101.9, 60.7, 54.4, 51.1, 33.2, 24.2, 16.7, 8.2. HRMS (ESI) calcd for C$_{20}$H$_{21}$Cl$_2$N$_6$O: 431.1154. Obsd: 431.1148. [α]$_D$ +31.7° (c 3.17, CHCl$_3$).

(R)-N-(3-Chloro-4-methylphenyl)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, 47

Yield: 82.1 mg (93.8%). 98.3% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.26 (s, 1H), 8.24 (s, 1H), 7.49 (s, 1H), 7.20 (d, J = 6.6 Hz, 1H), 7.13 – 6.98 (m, 2H), 6.87 (s, 1H), 6.49 (s, 1H), 5.36 (s, 1H), 4.04 (s, 1H), 3.79 (d, J = 8.2 Hz, 2H), 3.42 (d, J = 32.5 Hz, 1H), 3.36 (s, 3H), 2.25 (s, 3H), 0.97 (d, J = 8.3 Hz, 1H), 0.72 (s, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.5, 153.8, 151.5, 150.0, 137.9, 134.1, 130.7, 130.3, 120.7, 120.5, 118.4, 103.0, 101.9, 61.0, 54.5, 51.3, 33.2, 23.9, 19.3, 16.6, 8.1. HRMS (ESI) calcd for C$_{21}$H$_{24}$ClN$_6$O: 411.1700. Obsd: 411.1694. [α]$_D$ +41.1° (c 3.38, CHCl$_3$).

(R)-N-([1,1'-Biphenyl]-2-yl)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, 48

Yield: 29.7 mg (32.5%). 97.9% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.27 (s, 1H), 8.24 (s, 1H), 8.20 (d, J = 8.3 Hz, 1H), 7.51 – 7.30 (m, 6H), 7.21 (d, J = 7.2 Hz, 1H), 7.10 (dd, J = 9.2, 5.5 Hz, 2H), 6.55 (d, J = 2.9 Hz, 1H), 6.38 (s, 1H), 5.34 (d, J = 6.4 Hz, 1H), 3.82 (dd, J = 10.9, 7.5 Hz, 1H), 3.64 (d, J = 10.0 Hz, 1H), 3.48 (d, J = 10.1 Hz, 1H), 3.38 (s, 3H), 3.20 (d, J = 9.9 Hz, 1H), 1.04 – 0.95 (m, 1H), 0.78 – 0.59 (m, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 157.4, 153.4, 149.6, 148.6, 138.5, 135.8, 131.5, 129.6, 129.2, 129.1, 128.5, 128.0, 123.0, 120.9, 120.6, 102.5, 61.1, 54.2, 50.9, 31.9, 22.7, 16.8, 8.1. HRMS (ESI) calcd for C$_{26}$H$_{27}$N$_6$O: 439.2246. Obsd: 439.2242. [α]$_D$ +29.2° (c 0.587, CHCl$_3$).

Synthesis of (R)-N-(3,5-bis(trifluoromethyl)phenyl)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carbothioamide, 49

In a 5 mL round-bottom flask, (*R*)-*N*-methyl-*N*-(5-azaspiro[2.4]heptan-7-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (***R***)-**5c** (49.8 mg, 0.205 mmol) was placed and solved with 2.00 mL of dichloromethane. *N,N*-diisopropylethylamine (0.0374 mL, 0.215 mmol) was added and the mixture was treated with 3,5-bis(trifluoromethyl)phenyl isothiocyanate (0.0400 mL, 0.219 mmol). The reaction solution was stirred for 2 hours before being evaporated. The residue was purified by column chromatography (methanol:dichloromethane=2:98) and collected fragments were evaporated. Removing the solvent in vacuo provided 109.8 mg of (*R*)-*N*-(3,5-bis(trifluoromethyl)phenyl)-7-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carbothioamide (quantitatively yield). 99.5% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.02 (s, 1H), 8.27 (s, 1H), 7.97 (s, 2H), 7.69 (s, 1H), 7.62 (s, 1H), 7.08 (s, 1H), 6.55 (s, 1H), 5.42 (s,

1H), 4.30 (s, 1H), 4.15 (s, 2H), 3.72 (s, 1H), 3.43 (s, 3H), 1.03 (s, 1H), 0.83 (s, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 177.8, 157.5, 151.4, 149.7, 140.7, 131.6 (q, J = 33.6 Hz), 124.8, 123.0 (q, J = 272.9 Hz), 120.9, 118.6, 103.4, 102.0, 61.0, 55.3, 33.4, 29.7, 23.3, 16.8, 8.0. HRMS (ESI) calcd for C$_{22}$H$_{21}$F$_6$N$_6$S: 515.1453. Obsd: 515.1446. [α]$_D$ +51.4° (c 3.37, CHCl$_3$).

Synthesis of (R)-N-(5-(ethylsulfonyl)-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 50

In a 5 mL round bottom flask, (*R*)-*N*-methyl-*N*-(5-azaspiro[2.4]heptan-7-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **(R)-5c** (70.0 mg, 0.288 mmol) was placed and solved with 0.700 mL of *N*,*N*-dimethylformamide. The solution was treated with ethanesulfonyl chloride (55.5 mg, 0.432 mmol) and *N*,*N*-diisopropylethylamine (0.208 mL, 1.19 mmol) was added. Then, the reaction solution was stirred at room temperature overnight before being evaporated. The residue was purified by flash column chromatography (methanol:dichloromethane=2:98) and collected fragments were evaporated. Removing the solvent in vacuo provided 80.0 mg of (*R*)-*N*-(5-(ethylsulfonyl)-5-azaspiro[2.4]heptan-7-yl)-*N*-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (82.5% yield). 100% purity by HPLC. ^{1}H NMR (500 MHz, CDCl$_3$) δ 12.42 (s, 1H), 8.26 (s, 1H), 7.12 (s, 1H), 6.56 (s, 1H), 5.55 (s, 1H), 3.92 (t, J = 9.1 Hz, 1H), 3.68 (d, J = 9.9 Hz, 2H), 3.48 (s, 3H), 3.33 (d, J = 9.8 Hz, 1H), 3.14 – 3.03 (m, 2H), 1.42 (t, J = 7.0 Hz, 3H), 1.03 (d, J = 9.9 Hz, 1H), 0.77 (d, J = 11.9 Hz, 2H), 0.73 (d, J = 10.9 Hz, 1H). ^{13}C NMR (125 MHz, CDCl$_3$) δ 157.7, 152.0, 150.2, 120.7, 103.0, 101.8, 60.5, 55.9, 52.4, 44.2, 33.5, 24.4, 15.4, 9.2, 7.9. HRMS (ESI) calcd for C$_{15}$H$_{22}$N$_5$O$_2$S: 336.1494. Obsd: 336.1485. [α]$_D$ +34.7° (c 3.25, CHCl$_3$).

In the cases from **51** to **68**, the desired products were synthesized through substitution reactions with 2-propanesulfonyl chloride, 1-propanesulfonyl chloride, benzenesulfonyl chloride, 2-fluorobenzene-1-

sulfonyl chloride, 3-fluorobenzene-1-sulfonyl chloride, 4-fluorobenzenesulfonyl chloride, 2-cyanobenzenesulfonyl chloride, 3-cyanobenzenesulfonyl chloride, 4-cyanobenzenesulfonyl chloride, 2-nitrobenzenesulfonyl chloride, 3-nitrobenzenesulfonyl chloride, 4-nitrobenzenesulfonyl chloride, 3-toluenesulfonyl chloride, 4-methoxybenzenesulfonyl chloride, 4-(trifluoromethyl)benzenesulfonyl chloride, 2-naphthalenesulfonyl chloride, piperidine-1-sulfonyl chloride, and morpholine-4-sulfonyl chloride, respectively, instead of ethylsulfonyl chloride according to the aforementioned process (vide supra).

*(R)-N-(5-(Isopropylsulfonyl)-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **51***

Yield: 54.0 mg (54.0%). 100% purity by HPLC. ^{1}H NMR (500 MHz, CDCl$_3$) δ 12.17 (s, 1H), 8.26 (s, 1H), 7.11 (s, 1H), 6.57 (s, 1H), 5.51 (s, 1H), 3.99 (t, J = 8.2 Hz, 1H), 3.71 (d, J = 8.7 Hz, 2H), 3.48 (s, 3H), 3.38 (d, J = 9.6 Hz, 1H), 3.28 (d, J = 4.9 Hz, 1H), 1.40 (s, 6H), 1.03 (d, J = 8.7 Hz, 1H), 0.85 – 0.69 (m, 3H). ^{13}C NMR (125 MHz, CDCl$_3$) δ 157.6, 151.9, 150.3, 120.5, 102.9, 101.8, 60.6, 56.4, 53.4, 52.9, 33.5, 24.4, 16.6, 15.5, 8.9. HRMS (ESI) calcd for C$_{16}$H$_{24}$N$_5$O$_2$S: 350.1651. Obsd: 350.1639. [α]$_D$ +36.0° (*c* 1.82, CHCl$_3$).

*(R)-N-Methyl-N-(5-(propylsulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **52***

Yield: 71.0 mg (71.0%). 99.4% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.17 (s, 1H), 8.29 (s, 1H), 7.12 (d, J = 3.3 Hz, 1H), 6.59 (s, 1H), 5.65 – 5.46 (m, 1H), 3.91 (dd, J = 10.9, 7.6 Hz, 1H), 3.77 – 3.61 (m, 2H), 3.49 (s, 3H), 3.32 (d, J = 9.8 Hz, 1H), 3.11 – 2.94 (m, 2H), 1.91 (dd, J = 15.4, 7.6 Hz, 2H), 1.09 (t, J = 7.4 Hz, 3H), 1.07 – 0.98 (m, 1H), 0.76 (dt, J = 11.3, 9.9 Hz, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.7, 151.8, 150.1, 120.7, 103.1, 101.9, 60.5, 55.8, 52.3, 51.3, 33.6, 24.4, 17.0, 15.4, 13.1, 9.2. HRMS (ESI) calcd for C$_{16}$H$_{24}$N$_5$O$_2$S: 350.1651. Obsd: 350.1650. [α]$_D$ +34.9° (*c* 1.97, CHCl$_3$).

(R)-N-Methyl-N-(5-(phenylsulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 53

Yield: 70.0 mg (63.6%). 99.4% purity by HPLC. ^{1}H NMR (500 MHz, CDCl$_3$) δ 12.22 (s, 1H), 8.11 (s, 1H), 7.75 (s, 2H), 7.56 (s, 1H), 7.48 (s, 2H), 7.00 (s, 1H), 6.42 (s, 1H), 5.34 (s, 1H), 3.51 (s, 2H), 3.43 (d, J = 8.4 Hz, 1H), 3.24 (s, 3H), 2.99 (d, J = 8.3 Hz, 1H), 0.79 (s, 1H), 0.64 (s, 1H), 0.52 (s, 2H). ^{13}C NMR (125 MHz, CDCl$_3$) δ 157.6, 152.0, 150.2, 135.1, 133.0, 129.1, 127.9, 120.6, 102.9, 101.8, 59.9, 56.0, 52.7, 33.3, 23.9, 14.5, 9.6. HRMS (ESI) calcd for C$_{19}$H$_{22}$N$_5$O$_2$S: 384.1494. Obsd: 384.1483. [α]$_D$ -3.5° (*c* 2.73, CHCl$_3$).

(R)-N-(5-((2-Fluorophenyl)sulfonyl)-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 54

Yield: 57.0 mg (49.6%). 97.3% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.84 (s, 1H), 8.21 (s, 1H), 7.91 (t, J = 7.3 Hz, 1H), 7.68 – 7.56 (m, 1H), 7.39 – 7.20 (m, 2H), 7.09 (d, J = 3.4 Hz, 1H), 6.54 (d, J = 3.0 Hz, 1H), 5.47 (d, J = 5.4 Hz, 1H), 3.82 (dd, J = 10.9, 7.8 Hz, 1H), 3.77 – 3.67 (m, 1H), 3.48 (dd, J = 160.2, 9.9 Hz, 2H), 3.41 (s, 3H), 0.98 – 0.89 (m, 1H), 0.76 (dt, J = 13.1, 6.5 Hz, 1H), 0.72 – 0.61 (m, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 159.1 (d, J = 255.8 Hz), 157.6, 151.7, 150.2, 135.2 (d, J = 8.4 Hz), 131.5, 125.0 (d, J = 14.8 Hz), 124.5 (d, J = 3.8 Hz), 120.6, 117.3 (d, J = 22.0 Hz), 102.9, 102.0, 60.3, 55.7, 52.3, 33.4, 24.2, 14.9, 9.3. HRMS (ESI) calcd for C$_{19}$H$_{21}$FN$_5$O$_2$S: 402.1400. Obsd: 402.1393. [α]$_D$ -7.2° (*c* 0.803, CHCl$_3$).

(R)-N-(5-((3-Fluorophenyl)sulfonyl)-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 55

Yield: 46.0 mg (39.9%). 99.6% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.90 (s, 1H), 8.20 (s, 1H), 7.67 – 7.62 (m, 1H), 7.56 (dt, J = 13.4, 6.6 Hz, 2H), 7.36 (ddd, J = 10.0, 5.3, 1.5 Hz, 1H), 7.10 (d, J = 3.5 Hz, 1H), 6.53 (d, J = 3.4 Hz, 1H), 5.44 (t, J = 5.0 Hz, 1H), 3.62 (d, J = 5.2 Hz, 2H), 3.37 (s, 3H), 3.33 (dd, J = 185.2, 9.6 Hz, 2H), 0.96 – 0.85 (m, 1H), 0.77 (dd, J = 10.2,

4.9 Hz, 1H), 0.69 – 0.54 (m, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 162.5 (d, J = 252.1 Hz), 157.6, 151.7, 150.1, 137.4 (d, J = 6.5 Hz), 130.9 (d, J = 7.7 Hz), 123.6 (d, J = 3.4 Hz), 120.6, 120.2 (d, J = 21.2 Hz), 115.1 (d, J = 24.1 Hz), 102.9, 101.9, 60.0, 56.0, 52.8, 33.4, 23.9, 14.6, 9.7. HRMS (ESI) calcd for C$_{19}$H$_{21}$FN$_5$O$_2$S: 402.1400. Obsd: 402.1391. [α]$_D$ -6.7° (c 0.880, CHCl$_3$).

(R)-N-(5-((4-Fluorophenyl)sulfonyl)-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 56

Yield: 59.0 mg (51.3%). 96.3% purity by HPLC. ^{1}H NMR (500 MHz, CDCl$_3$) δ 12.06 (s, 1H), 8.11 (d, J = 3.9 Hz, 1H), 7.78 (ddd, J = 8.8, 4.6, 2.0 Hz, 2H), 7.22 – 7.11 (m, 2H), 7.02 (d, J = 3.5 Hz, 1H), 6.44 (d, J = 3.4 Hz, 1H), 5.35 (s, 1H), 3.51 (d, J = 5.1 Hz, 2H), 3.44 (d, J = 9.6 Hz, 1H), 3.28 (s, 3H), 2.98 (d, J = 9.6 Hz, 1H), 0.87 – 0.77 (m, 1H), 0.71 – 0.61 (m, 1H), 0.60 – 0.48 (m, 2H). ^{13}C NMR (125 MHz, CDCl$_3$) δ 164.8 (d, J = 255.8 Hz), 157.1, 151.5, 149.8, 130.9, 130.1 (d, J = 9.0 Hz), 120.1, 115.9 (d, J = 21.4 Hz), 102.4, 101.4, 59.5, 55.6, 52.3, 33.0, 23.4, 14.1, 9.2. HRMS (ESI) calcd for C$_{19}$H$_{21}$FN$_5$O$_2$S: 402.1400. Obsd: 402.1388. [α]$_D$ -3.0° (c 2.17, CHCl$_3$).

(R)-2-((7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)sulfonyl)benzonitrile, 57

Yield: 56.0 mg (47.9%). 99.2% purity by HPLC. ^{1}H NMR (500 MHz, CDCl$_3$) δ 12.05 (s, 1H), 8.12 (s, 1H), 8.00 (d, J = 7.5 Hz, 1H), 7.83 (d, J = 7.3 Hz, 1H), 7.66 (dt, J = 22.3, 7.4 Hz, 2H), 7.02 (d, J = 3.1 Hz, 1H), 6.45 (d, J = 3.0 Hz, 1H), 5.41 (d, J = 4.8 Hz, 1H), 3.74 (dd, J = 10.9, 7.7 Hz, 1H), 3.68 (d, J = 9.8 Hz, 1H), 3.61 (dd, J = 11.0, 2.7 Hz, 1H), 3.32 (s, 3H), 3.29 (d, J = 9.8 Hz, 1H), 0.88 (t, J = 7.0 Hz, 1H), 0.72 (dd, J = 8.7, 5.5 Hz, 1H), 0.69 – 0.57 (m, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 151.9, 150.2, 140.0, 135.6, 133.0, 132.9, 130.3, 120.7, 116.4, 110.9, 103.0, 101.8, 60.3, 56.1, 52.6, 33.5, 24.1, 14.9, 9.3. HRMS (ESI) calcd for C$_{20}$H$_{21}$N$_6$O$_2$S: 409.1447. Obsd: 409.1435. [α]$_D$ +2.2° (c 1.91, CHCl$_3$).

(R)-3-((7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)sulfonyl)benzonitrile, 58

Yield: 62.0 mg (53.0%). 100% purity by HPLC. ^{1}H NMR (500 MHz, CDCl$_3$) δ 12.03 (s, 1H), 8.19 (s, 1H), 8.14 (s, 1H), 8.06 (d, J = 7.9 Hz, 1H), 7.91 (d, J = 7.7 Hz, 1H), 7.70 (t, J = 7.8 Hz, 1H), 7.11 (d, J = 2.8 Hz, 1H), 6.52 (d, J = 2.8 Hz, 1H), 5.40 (s, 1H), 3.65 (t, J = 5.1 Hz, 2H), 3.60 (d, J = 9.8 Hz, 1H), 3.36 (s, 3H), 3.10 (d, J = 9.7 Hz, 1H), 0.90 (dd, J = 9.8, 4.4 Hz, 1H), 0.83 – 0.74 (m, 1H), 0.73 – 0.58 (m, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.5, 151.9, 150.2, 137.5, 136.0, 131.6, 131.2, 130.2, 120.7, 117.1, 113.8, 103.0, 101.8, 60.1, 56.1, 52.8, 33.5, 23.8, 14.8, 9.6. HRMS (ESI) calcd for C$_{20}$H$_{21}$N$_6$O$_2$S: 409.1447. Obsd: 409.1435. [α]$_D$ -7.5° (*c* 1.82, CHCl$_3$).

(R)-4-((7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)sulfonyl)benzonitrile, 59

Yield: 62.0 mg (53.0%). 94.1% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.59 (s, 1H), 8.18 (s, 1H), 8.00 – 7.90 (m, 2H), 7.89 – 7.81 (m, 2H), 7.08 (dd, J = 3.5, 1.8 Hz, 1H), 6.52 (d, J = 2.4 Hz, 1H), 5.36 (dd, J = 5.7, 4.4 Hz, 1H), 3.65 (s, 1H), 3.64 (d, J = 1.9 Hz, 1H), 3.59 (d, J = 9.7 Hz, 1H), 3.34 (s, 3H), 3.10 (d, J = 9.7 Hz, 1H), 0.91 (ddd, J = 10.2, 6.2, 4.0 Hz, 1H), 0.82 – 0.75 (m, 1H), 0.73 – 0.59 (m, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.5, 151.9, 150.2, 134.0, 132.9, 128.3, 120.8, 117.2, 116.7, 103.0, 101.8, 60.2, 56.0, 52.9, 33.5, 23.8, 14.9, 9.5. HRMS (ESI) calcd for C$_{20}$H$_{21}$N$_6$O$_2$S: 409.1447. Obsd: 409.1433. [α]$_D$ -12.7° (*c* 1.66, CHCl$_3$).

(R)-N-Methyl-N-(5-((2-nitrophenyl)sulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 60

Yield: 68.8 mg (76.5%). 96.9% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.38 (s, 1H), 8.23 (s, 1H), 8.00 (d, J = 7.3 Hz, 1H), 7.69 (dd, J = 16.0, 7.9 Hz, 2H), 7.62 (d, J = 7.3 Hz, 1H), 7.10 (s, 1H), 6.53 (s, 1H), 5.50 (d, J = 6.2 Hz, 1H), 4.03 – 3.87 (m, 1H), 3.75 (d, J = 9.6 Hz, 2H), 3.39 (s, 3H),

3.38 – 3.27 (m, 1H), 0.96 (d, J = 5.5 Hz, 1H), 0.91 – 0.59 (m, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 152.0, 150.2, 148.4, 133.9, 131.6, 130.8, 124.1, 120.7, 103.0, 101.8, 60.4, 55.9, 52.6, 33.4, 24.2, 15.1, 9.2. HRMS (ESI) calcd for C$_{19}$H$_{21}$N$_6$O$_4$S: 429.1345. Obsd: 429.1340. [α]$_D$ +6.1^o (c 2.21, CHCl$_3$).

(R)-N-Methyl-N-(5-((3-nitrophenyl)sulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **61**

Yield: 61.6 mg (68.5%). 94.8% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.49 (s, 1H), 8.67 (d, J = 1.4 Hz, 1H), 8.49 (d, J = 8.2 Hz, 1H), 8.16 (d, J = 3.4 Hz, 2H), 7.78 (td, J = 8.1, 3.3 Hz, 1H), 7.06 (s, 1H), 6.52 (s, 1H), 5.47 – 5.32 (m, 1H), 3.79 – 3.57 (m, 3H), 3.37 (d, J = 3.2 Hz, 3H), 3.14 (d, J = 9.7 Hz, 1H), 0.90 (dd, J = 6.2, 3.2 Hz, 1H), 0.84 – 0.74 (m, 1H), 0.74 – 0.58 (m, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.5, 152.0, 150.6, 148.4, 138.1, 133.2, 130.5, 127.4, 122.7, 120.3, 102.8, 102.1, 60.1, 56.1, 52.9, 33.6, 23.8, 14.8, 9.6. HRMS (ESI) calcd for C$_{19}$H$_{21}$N$_6$O$_4$S: 429.1345. Obsd: 429.1339. [α]$_D$ -6.4^o (c 0.117, CHCl$_3$).

(R)-N-Methyl-N-(5-((4-nitrophenyl)sulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **62**

Yield: 52.1 mg (58.0%). 97.1% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.61 (s, 1H), 8.50 – 8.30 (m, 2H), 8.19 (d, J = 3.4 Hz, 1H), 8.02 (s, 2H), 7.09 (s, 1H), 6.50 (s, 1H), 5.36 (d, J = 3.5 Hz, 1H), 3.67 (s, 2H), 3.65 – 3.56 (m, 1H), 3.36 (d, J = 3.5 Hz, 3H), 3.19 – 3.05 (m, 1H), 0.83 – 0.75 (m, 1H), 0.67 (dd, J = 14.9, 9.8 Hz, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.5, 152.0, 150.3, 150.2, 141.6, 128.9, 124.3, 120.6, 103.0, 101.9, 60.2, 56.1, 52.9, 33.5, 23.8, 14.9, 9.5. HRMS (ESI) calcd for C$_{19}$H$_{21}$N$_6$O$_4$S: 429.1345. Obsd: 429.1338. [α]$_D$ -19.0^o (c 1.38, CHCl$_3$).

(R)-N-Methyl-N-(5-(m-tolylsulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **63**

Yield: 124 mg (81.6%). 98.0% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.57 (s, 1H), 8.22 (d, *J* = 4.7 Hz, 1H), 7.65 (s, 2H), 7.43 (s, 2H), 7.10 (s, 1H), 6.50 (s, 1H), 5.41 (s, 1H), 3.61 (s, 2H), 3.52 (dd, *J* = 9.3, 4.4 Hz, 1H), 3.33 (d, *J* = 4.3 Hz, 3H), 3.08 (dd, *J* = 9.3, 4.5 Hz, 1H), 2.42 (d, *J* = 4.3 Hz, 3H), 0.87 (d, *J* = 4.6 Hz, 1H), 0.75 (d, *J* = 9.6 Hz, 1H), 0.61 (d, *J* = 2.9 Hz, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 151.9, 150.1, 139.3, 134.9, 133.8, 129.0, 128.2, 125.0, 120.7, 102.9, 101.8, 60.0, 56.0, 52.8, 33.3, 23.9, 21.4, 14.4, 9.7. HRMS (ESI) calcd for C$_{20}$H$_{24}$N$_5$O$_2$S: 398.1651. Obsd: 398.1645. [α]$_D$ -5.6° (*c* 3.88, CHCl$_3$).

(R)-N-(5-((4-Methoxyphenyl)sulfonyl)-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **64**

Yield: 99.5 mg (83.8%). 96.3% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.94 (s, 1H), 8.19 (s, 1H), 7.78 (d, *J* = 8.8 Hz, 2H), 7.09 (d, *J* = 3.4 Hz, 1H), 7.02 (d, *J* = 8.8 Hz, 2H), 6.53 (d, *J* = 3.1 Hz, 1H), 5.43 (t, *J* = 4.7 Hz, 1H), 3.89 (s, 3H), 3.56 (d, *J* = 4.9 Hz, 2H), 3.36 (s, 3H), 3.27 (dd, *J* = 174.5, 9.5 Hz, 2H), 0.95 – 0.84 (m, 1H), 0.78 – 0.68 (m, 1H), 0.62 (t, *J* = 7.5 Hz, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 163.2, 157.6, 151.7, 150.2, 130.1, 126.6, 120.5, 114.2, 102.9, 102.0, 59.9, 56.0, 55.6, 52.7, 33.4, 23.9, 14.3, 9.9. HRMS (ESI) calcd for C$_{20}$H$_{24}$N$_5$O$_3$S: 414.1600. Obsd: 414.1591. [α]$_D$ -14.2° (*c* 0.983, CHCl$_3$).

(R)-N-Methyl-N-(5-((4-(trifluoromethyl)phenyl)sulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **65**

Yield: 70.5 mg (54.2%). 97.5% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.77 (s, 1H), 8.23 (s, 1H), 7.90 (dd, *J* = 54.7, 8.2 Hz, 4H), 7.10 (d, *J* = 3.1 Hz, 1H), 6.54 (s, 1H), 5.41 (dd, *J* = 6.0, 3.1 Hz, 1H), 3.65 (dd, *J* = 15.1, 4.8 Hz, 2H), 3.37 (s, 3H), 3.34 (dd, *J* = 191.6, 9.6 Hz, 2H), 0.99 – 0.87 (m, 1H), 0.82 – 0.72 (m, 1H), 0.71 – 0.57 (m, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.5, 151.5, 149.9, 139.1, 134.7 (q, *J* = 33.1 Hz), 128.3, 126.3 (q, *J* = 3.6 Hz), 123.2

(q, J = 272.9 Hz), 120.7, 103.1, 102.0, 60.2, 56.0, 52.8, 33.5, 23.9, 14.7, 9.7. HRMS (ESI) calcd for $C_{20}H_{21}F_3N_5O_2S$: 452.1368. Obsd: 452.1361. $[\alpha]_D$ -6.8° (*c* 0.970, CHCl$_3$).

(R)-N-Methyl-N-(5-(naphthalen-2-ylsulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **66**

Yield: 124 mg (99.0%). 98.5% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.58 (s, 1H), 8.42 (s, 1H), 8.19 (d, J = 1.7 Hz, 1H), 7.96 (d, J = 8.1 Hz, 2H), 7.93 – 7.77 (m, 2H), 7.62 (dt, J = 16.4, 7.3 Hz, 2H), 7.06 (s, 1H), 6.43 (s, 1H), 5.39 (s, 1H), 3.67 (d, J = 3.8 Hz, 2H), 3.36 (dd, J = 171.8, 9.6 Hz, 2H), 3.30 (s, 3H), 0.81 (d, J = 7.0 Hz, 1H), 0.71 (d, J = 4.6 Hz, 1H), 0.57 (t, J = 6.5 Hz, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.5, 151.9, 150.1, 134.9, 132.3, 132.1, 129.3, 129.2, 128.9, 127.9, 127.6, 123.1, 120.7, 102.9, 101.8, 60.0, 56.1, 52.9, 33.4, 23.9, 14.5, 9.7. HRMS (ESI) calcd for $C_{23}H_{24}N_5O_2S$: 434.1651. Obsd: 434.1644. $[\alpha]_D$ -17.1° (*c* 4.58, CHCl$_3$).

(R)-N-Methyl-N-(5-(piperidin-1-ylsulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **67**

Yield: 73.0 mg (65.2%). 95.4% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.36 (s, 1H), 8.25 (s, 1H), 7.09 (s, 1H), 6.58 (s, 1H), 5.56 (d, J = 5.8 Hz, 1H), 3.92 – 3.76 (m, 1H), 3.54 (d, J = 9.5 Hz, 2H), 3.48 (s, 4H), 3.28 (d, J = 4.5 Hz, 4H), 1.61 (dd, J = 26.6, 3.9 Hz, 6H), 1.03 (d, J = 9.7 Hz, 1H), 0.76 (d, J = 9.9 Hz, 2H), 0.69 (d, J = 9.9 Hz, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.7, 152.0, 150.6, 120.3, 102.8, 102.0, 60.2, 56.6, 52.9, 47.2, 33.5, 25.5, 24.0, 23.8, 15.1, 9.5. HRMS (ESI) calcd for $C_{18}H_{27}N_6O_2S$: 391.1916. Obsd: 391.1913. $[\alpha]_D$ +32.7° (*c* 0.297, CHCl$_3$).

(R)-N-Methyl-N-(5-(morpholinosulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **68**

Yield: 44.0 mg (38.9%). 94.6% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.33 (s, 1H), 8.27 (d, J = 2.8 Hz, 1H), 7.13 (s, 1H), 6.57 (s, 1H), 5.63

– 5.51 (m, 1H), 3.90 (ddd, J = 10.6, 7.6, 2.7 Hz, 1H), 3.75 (dd, J = 5.8, 3.1 Hz, 4H), 3.65 – 3.55 (m, 2H), 3.48 (d, J = 2.8 Hz, 3H), 3.37 – 3.22 (m, 5H), 1.04 (d, J = 9.6 Hz, 1H), 0.85 – 0.75 (m, 2H), 0.75 – 0.65 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.7, 152.0, 150.3, 120.7, 102.9, 101.8, 66.4, 60.2, 56.8, 53.1, 46.4, 33.5, 24.0, 15.3, 9.3. HRMS (ESI) calcd for $C_{17}H_{25}N_6O_3S$: 393.1709. Obsd: 393.1704. [α]$_D$ +32.9° (c 1.57, CHCl$_3$).

Synthesis of (R)-4,4-dimethyl-1-((R)-1-phenylethyl)pyrrolidin-3-amine, 7b

Benzyl 3-oxobutanoate

Ethyl acetoacetate → **Benzyl 3-oxobutanoate** (BnOH, Ph$_3$P, Toluene, reflux)
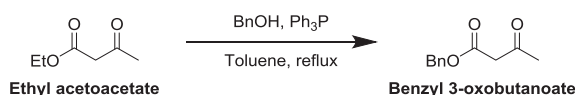
Benzyl alcohol (4.78 mL, 46.0 mmol) was added to ethyl acetoacetate (6.00 g, 46.1 mmol) solution in 60.0 mL of toluene. The solution was treated with triphenylphosphine (1.21 g, 4.61 mmol) and then refluxed for 12 hours. The mxiture was concentrated under reduced pressure. The residue was purified with flash column chromatography (ethyl acetate:n-hexane = 1:20). Removing the solvent in vacuo provided 6.69 g of benzyl 3-oxobutanoate (75.1% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.39 – 7.23 (m, 5H), 5.17 (s, 2H), 3.50 (s, 2H), 2.24 (s, 3H).

Benzyl 2,2-dimethyl-3-oxobutanoate

Benzyl 3-oxobutanoate → **Benzyl 2,2-dimethyl-3-oxobutanoate** (CH$_3$I, NaH, THF, r.t.)
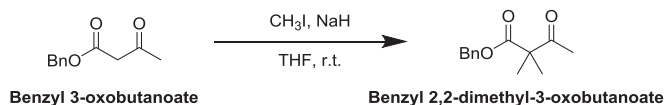
Sodium hydride, 60wt% (3.48 g, 87.0 mmol) was slowly added to benzyl 3-oxobutanoate (6.69 g, 34.8 mmol) solution in 67.0 mL of tetrahydrofuran at 0 °C. The mixture was stirred at room temperature for 1 hour. Iodomethane (6.48 mL, 104 mmol) was slowly added at 0 °C. The reaction mixture was stirred at room temperature for 12 hours. The mixture was

concentrated under reduced pressure. To the residue were added 100 mL of brine and 50 mL of saturated ammonium chloride solution. The aqueous mixture was extracted with 100 mL of ethyl acetate three times. The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified with flash column chromatography (ethyl acetate:*n*-hexane = 1:8). Removing the solvent in vacuo provided 6.69 g of benzyl 2,2-dimethyl-3-oxobutanoate (90.1% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.39 – 7.30 (m, 5H), 5.17 (s, 2H), 2.08 (s, 3H), 1.38 (s, 6H).

Benzyl 2-methyl-2-(2-methyl-1,3-dioxolan-2-yl)propanoate

Benzyl 2,2-dimethyl-3-oxobutanoate → **Benzyl 2-methyl-2-(2-methyl-1,3-dioxolan-2-yl)propanoate**
(Ethylene glycol, TsOH·H$_2$O, Benzene, reflux)
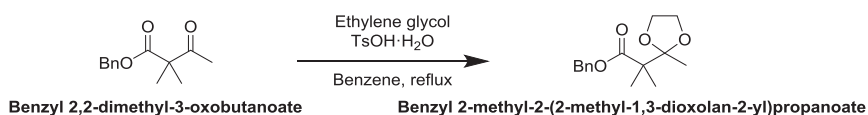
Ethylene glycol (4.90 mL, 87.6 mmol) and *p*-toluenesulfonic acid monohydrate (0.410 g, 2.16 mmol) were added to benzyl 2,2-dimethyl-3-oxobutanoate (9.69 g, 44.0 mmol) solution in 195 mL of benzene. The reaction flask was equipped with a Dean-Stark trap. The reaction solution was refluxed stirred for 24 hours. The solution was concentrated under reduced pressure. To the residue were added 200 mL of brine and 100 mL of saturated sodium bicarbonate solution. The aqueous mixture was extracted with 100 mL of ethyl acetate three times. The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified with flash column chromatography (ethyl acetate:*n*-hexane = 1:10). Removing the solvent in vacuo provided 9.47 g of benzyl 2-methyl-2-(2-methyl-1,3-dioxolan-2-yl)propanoate (81.6% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.39 – 7.26 (m, 5H), 5.15 (s, 2H), 3.99 – 3.93 (m, 2H), 3.89 – 3.84 (m, 2H), 1.33 (s, 3H), 1.29 (s, 6H).

2-Methyl-2-(2-methyl-1,3-dioxolan-2-yl)propanoic acid

Benzyl 2-methyl-2-(2-methyl -1,3-dioxolan-2-yl)propanoate → **2-Methyl-2-(2-methyl-1,3-dioxolan -2-yl)propanoic acid**
(Pd/C, H$_2$ (g), MeOH, r.t.)

95
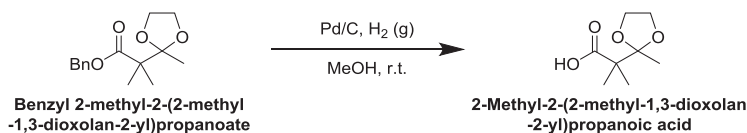
Palladium on charcoal (9.47 g, 10wt/wt%) was added to benzyl 2-methyl-2-(2-methyl-1,3-dioxolan-2-yl)propanoate (9.47 g, 35.8 mmol) solution in 95.0 mL of methanol. The reaction flask was equipped with a hydrogen gas balloon. The reaction mixture was vigorously stirred for 24 hours. The mixture was filtered through a celite 545 pad. Removing the solvent in vacuo provided 5.94 g of 2-methyl-2-(2-methyl-1,3-dioxolan-2-yl)propanoic acid (95.2% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.09 – 4.01 (m, 4H), 1.37 (s, 3H), 1.29 (s, 6H).

(R)-2-Methyl-2-(2-methyl-1,3-dioxolan-2-yl)-N-(1-phenylethyl)propanamide

1) Ethyl chloroformate
Et$_3$N, DCM, -20°C

2) (R)-(+)-1-phenylethylamine, r.t.

2-Methyl-2-(2-methyl-1,3-dioxolan-2-yl)propanoic acid

(R)-2-Methyl-2-(2-methyl-1,3-dioxolan-2-yl)-N-(1-phenylethyl)propanamide
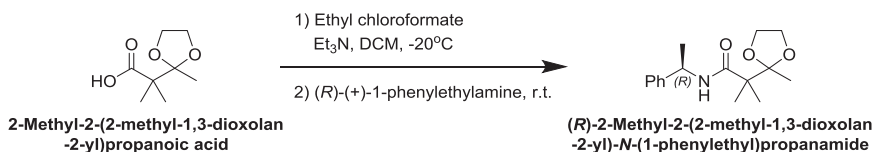
Triethylamine (9.51 mL, 68.2 mmol) was added to 2-methyl-2-(2-methyl-1,3-dioxolan-2-yl)propanoic acid (5.94 g, 21.4 mmol) solution in 53.0 mL of dichloromethane at -20 °C. Ethyl chloroformate (3.59 mL, 37.7 mmol) was slowly added at -20 °C. The reaction solution was stirred at -20 °C for 40 minutes. To the reaction solution was dropwise added (R)-(+)-phenylethylamine (4.78 mL, 37.6 mmol) at -20 °C. After the addition, the reaction solution was stirred at room temperature for 12 hours. To the reaction solution was poured 50.0 mL of deionized water and then the organic layer was separated. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified with flash column chromatography (ethyl acetate:n-hexane = 1:8). Removing the solvent in vacuo provided 1.89 g of (R)-2-methyl-2-(2-methyl-1,3-dioxolan-2-yl)-N-(1-phenylethyl)propanamide (20.0% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.35 – 7.21 (m, 5H), 7.11 (d, J = 6.8 Hz, 1H), 5.14 – 5.04 (m, 1H), 4.04 – 3.97 (m, 2H), 3.96 – 3.90 (m, 2H), 1.47 (d, J = 6.8 Hz, 3H), 1.24 (s, 3H), 1.23 (s, 3H), 1.20 (s, 3H).

<u>(*R*)-2-(2-(Bromomethyl)-1,3-dioxolan-2-yl)-2-methyl-*N*-(1-phenylethyl)propanamide</u>

(*R*)-2-Methyl-2-(2-methyl-1,3-dioxolan-2-yl)-*N*-(1-phenylethyl)propanamide

(*R*)-2-(2-(Bromomethyl)-1,3-dioxolan-2-yl)-2-methyl-*N*-(1-phenylethyl)propanamide
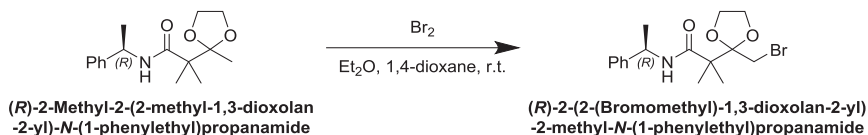
A solution of bromine (0.590 mL, 11.5 mmol) in 30.0 mL of 1,4-dioxane was slowly added to (*R*)-2-methyl-2-(2-methyl-1,3-dioxolan-2-yl)-*N*-(1-phenylethyl)propanamide (1.89 g, 6.81 mmol) in 18.0 mL of diethyl ether and 8.00 mL of 1,4-dioxane at 0 °C. The reaction solution was stirred at room temperature for 12 hours. The solution was concentrated under reduced pressure. The residue was extracted with 18.0 mL of ethyl acetate, 18.0 mL of brine, and 18.0 mL of saturated sodium thiosulfate solution. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified with flash column chromatography (ethyl acetate:*n*-hexane = 1:10). Removing the solvent in vacuo provided 2.29 g of (*R*)-2-(2-(bromomethyl)-1,3-dioxolan-2-yl)-2-methyl-*N*-(1-phenylethyl)propanamide (94.2% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.36 – 7.22 (m, 5H), 7.02 (d, *J* = 7.6 Hz, 1H), 5.11 – 5.01 (m, 1H), 4.43 – 4.36 (m, 2H), 4.14 – 4.05 (m, 2H), 3.62 (d, *J* = 11.2 Hz, 1H), 3.51 (d, *J* = 10.0 Hz, 1H), 1.54 (d, *J* = 7.6 Hz, 3H), 1.25 (s, 6H).

<u>(*R*)-9,9-Dimethyl-7-(1-phenylethyl)-1,4-dioxa-7-azaspiro[4.4]nonan-8-one</u>

(*R*)-2-(2-(Bromomethyl)-1,3-dioxolan-2-yl)-2-methyl-*N*-(1-phenylethyl)propanamide

(*R*)-9,9-Dimethyl-7-(1-phenylethyl)-1,4-dioxa-7-azaspiro[4.4]nonan-8-one
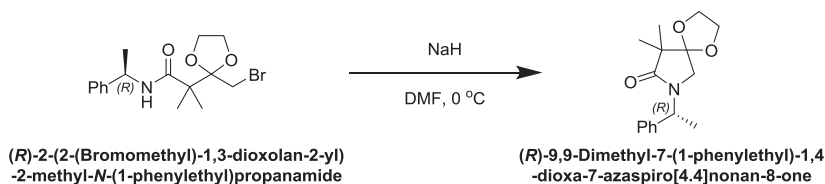
An (*R*)-2-methyl-2-(2-methyl-1,3-dioxolan-2-yl)-*N*-(1-phenylethyl)propanamide (2.29 g, 6.43 mmol) was solved in 22.0 mL of *N,N*-dimethylformamide. The solution was treated with sodium hydride, 60wt% (440 mg, 11.0 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 3

hours. To the reaction mixture was poured 500 mL of brine and the mixture was extracted with 200 mL of ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified with flash column chromatography (ethyl acetate:dichloromethane = 1:100). Removing the solvent in vacuo provided 1.07 g of (R)-9,9-dimethyl-7-(1-phenylethyl)-1,4-dioxa-7-azaspiro[4.4]nonan-8-one (60.5% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.38 – 7.23 (m, 5H), 5.58 (q, J = 14.0 Hz, 1H), 4.03 – 3.83 (m, 4H), 3.20 (d, J = 10.0 Hz, 1H), 2.95 (s, 1H), 1.51 (d, J = 7.2 Hz, 3H), 1.15 (s, 3H), 1.10 (s, 3H).

<u>(R)-3,3-Dimethyl-1-(1-phenylethyl)pyrrolidine-2,4-dione</u>

(R)-9,9-Dimethyl-7-(1-phenylethyl)-1,4
-dioxa-7-azaspiro[4.4]nonan-8-one

(R)-3,3-Dimethyl-1-(1-phenylethyl)pyrrolidine-2,4-dione

To a solution of (R)-9,9-dimethyl-7-(1-phenylethyl)-1,4-azaspiro[4.4]nonan-8-one (1.07 g, 3.89 mmol) in 11.0 mL of acetone was added 4.67 mL of 1N hydrochloric acid solution at room temperature. The reaction solution was heated at 60 ºC for 12 hours. The solution was concentrated under reduced pressure. The residue was extracted with 50.0 mL of brine and 50.0 mL of ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and filtered. Removing the solvent in vacuo provided 840 mg of (R)-3,3-dimethyl-1-(1-phenylethyl)pyrrolidine-2,4-dione (93.2% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.40 – 7.26 (m, 5H), 5.81 (q, J = 7.2 Hz, 1H), 3.77 (d, J = 17.6 Hz, 1H), 3.41 (d, J = 17.6 Hz, 1H), 1.59 (d, J = 7.2 Hz, 3H), 1.26 (s, 3H), 1.20 (s, 3H).

<u>(R)-4-(Hydroxyimino)-3,3-dimethyl-1-(1-phenylethyl)pyrrolidin-2-one</u>

(R)-3,3-Dimethyl-1-(1-phenylethyl)-
pyrrolidine-2,4-dione

(R)-4-(Hydroxyimino)-3,3-dimethyl
-1-(1-phenylethyl)pyrrolidin-2-one

Hydroxylamine hydrochloride (395 mg, 5.68 mmol), and triethylamine (0.782 mL, 5.61 mmol) were added to (R)-3,3-dimethyl-1-(1-phenylethyl)pyrrolidine-2,4-dione (840 mg, 3.63 mmol) solution in 9.00 mL of ethanol. The reaction solution was stirred at room temperature for 5 hours. The reaction solution was concentrated under reduced pressure. The residue was purified with flash column chromatography (ethyl acetate:n-hexane = 1:3). Removing the solvent in vacuo provided 730 mg of (R)-4-(hydroxyimino)-3,3-dimethyl-1-(1-phenylethyl)pyrrolidin-2-one (94.1% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.37 – 7.26 (m, 5H), 7.26 (s, 1H), 5.65 (q, J = 7.2 Hz, 1H), 4.10 (d, J = 16.8 Hz, 1H), 3.71 (d, J = 16.4 Hz, 1H), 1.55 (s, 3H), 1.34 (s, 3H), 1.30 (s, 3H).

(R)-4-Amino-3,3-dimethyl-1-((R)-1-phenylethyl)pyrrolidin-2-one

(R)-4-(Hydroxyimino)-3,3-dimethyl
-1-(1-phenylethyl)pyrrolidin-2-one

(R)-4-Amino-3,3-dimethyl-1-((R)-1
-phenylethyl)pyrrolidin-2-one
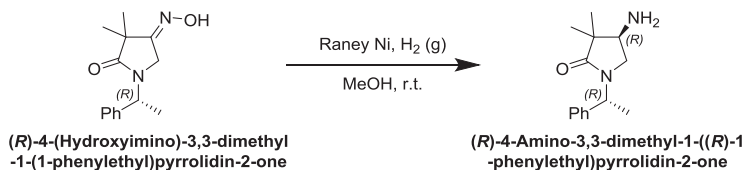
Raney®-nickel slurry (1.56 mL, Raney® 2400) was added to (R)-4-(hydroxyimino)-3,3-dimethyl-1-(1-phenylethyl)pyrrolidin-2-one (730 mg, 2.96 mmol) solution in 36.5 mL of methanol. The reaction flask was equipped with a hydrogen gas balloon. The reaction mixture was vigorously stirred for 12 hours. The mixture was filtered through a celite 545 pad. The filtered solution was concentrated under reduced pressure. The residue was purified with flash column chromatography (methanol:dichloromethane = 2:98). Removing the solvent in vacuo provided 264 mg of (R)-4-amino-3,3-dimethyl-1-((R)-1-phenylethyl)pyrrolidin-2-one (38.4% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.31 – 7.26 (m, 5H), 5.50 (q, J = 6.8 Hz, 1H), 3.09 – 3.03 (m, 2H),

2.83 – 2.76 (m, 1H), 1.50 (d, *J* = 6.8, 3H), 1.12 (s, 3H), 1.03 (s, 3H). Also was obtained 300 mg of (*S*)-4-amino-3,3-dimethyl-1-((*R*)-1-phenylethyl)pyrrolidin-2-one (38.4% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.31 – 7.26 (m, 5H), 5.50 (q, *J* = 6.8 Hz, 1H), 3.10 – 3.03 (m, 2H), 2.83 – 2.76 (m, 1H), 1.50 (d, *J* = 6.8 Hz, 3H), 1.12 (s, 3H), 1.03 (s, 3H).

(*R*)-4,4-Dimethyl-1-((*R*)-1-phenylethyl)-pyrrolidin-3-amine, **7b**

(*R*)-4-Amino-3,3-dimethyl-1-((*R*)-1 -phenylethyl)pyrrolidin-2-one

(*R*)-4,4-Dimethyl-1-((*R*)-1-phenylethyl) -pyrrolidin-3-amine, 1b
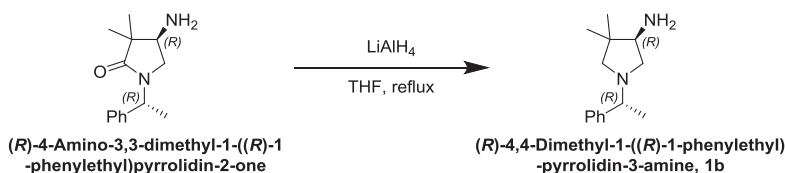
Lithium aluminum hydride (189 mg, 4.98 mmol) was slowly added to (*R*)-4-amino-3,3-dimethyl-1-((*R*)-1-phenylethyl)pyrrolidin-2-one (264 mg, 1.14 mmol) solution in 13.0 mL of tetrahydrofuran at 0 °C. The reaction solution was refluxed for 12 hours and then cooled down to 0 °C. The reaction was quenched with 1.15 mL of deionized water, 1.15 mL of 15% sodium hydroxide solution, and 3.45 mL of deionized water. Then, celite 545 was added and the mixture was stirred for 30 minutes before being filtered through a celite 545 pad. The filtered solution was concentrated under reduced pressure and the residue was extracted with 10.0 mL of brine and 10.0 mL of ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and filtered. Removing the solvent in vacuo provided 240 mg of (*R*)-4,4-dimethyl-1-((*R*)-1-phenylethyl)-pyrrolidin-3-amine, **1b** (96.8% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.38 – 7.16 (m, 5H), 3.26 (q, *J* = 6.8 Hz, 1H), 3.08 (dd, *J* = 9.2, 7.2 Hz, 1H), 2.98 (t, *J* = 7.2 Hz, 1H), 2.36 (s, 2H), 2.20 (dd, *J* = 9.6, 6.8 Hz, 1H), 1.30 (d, *J* = 6.4 Hz, 3H), 1.03 (s, 3H), 0.92 (s, 3H).

*Synthesis of tert-butyl (R)-(1-benzylpyrrolidin-3-yl)carbamate, **8aa***

Sodium bicarbonate (5.92 g, 70.5 mmol) in 118 mL of deionized water was added to (3*R*)-(+)-benzylaminopyrrolidine **7a** (5.00 g, 28.4 mmol) solution in 118 mL of acetonitirile and the mixture was stirred at room temperature for

10 minutes. Di-*tert*-butyl dicarbamate (6.22 g, 28.5 mmol) was then added and the mixture was stirred at room temperature overnight. After the reaction, the solution was concentrated under reduced pressure and the residue was extracted with dichloromethane three times. Combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified with flash column chromatography (methanol:dichloromethane = 2:98). Removing the solvent in vacuo provided 4.24 g of *tert*-butyl (*R*)-(1-benzylpyrrolidin-3-yl)carbamate (65.2% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.36 – 7.26 (m, 5H), 4.86 (bs, 1H), 4.18 (bs, 1H), 3.61 (s, 2H), 2.79 (bs, 1H), 2.65 – 2.61 (m, 1H), 2.54 (d, *J* = 8.0 Hz, 1H), 2.34 – 2.25 (m, 2H), 1.61 – 1.51 (m, 1H), 1.46 (s, 9H). [α]$_D$ +2.5° (*c* 0.620, CHCl$_3$).

In the cases of **8b** and **8c**, the desired products were synthesized from (*R*)-4,4-dimethyl-1-((*R*)-1-phenylethyl)pyrrolidin-3-amine (**7b**) and (*R*)-6-((*R*)-1-phenylethyl)-6-azaspiro[3.4]octan-8-amine (**7c**), respectively, instead of (3*R*)-(+)-benzylaminopyrrolidine **7a** according to the aforementioned process (vide supra).

tert-Butyl ((*R*)-4,4-dimethyl-1-((*R*)-1-phenylethyl)pyrrolidin-3-yl)carbamate, **8b**

Yield: 335 mg (95.7%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.29 – 7.20 (m, 5H), 4.61 (d, *J* = 10.4 Hz, 1H), 3.79 – 3.74 (m, 1H), 3.23 (m, 1H), 2.89 (q, *J* = 9.6, 7.2 Hz, 1H), 2.51 (d, *J* = 9.2 Hz, 1H), 2.31 – 2.19 (m, 2H), 1.43 (s, 9H), 1.30 (d, *J* = 6.4 Hz, 3H), 1.10 (s, 3H), 0.98 (s, 3H). [α]$_D$ +7.4° (*c* 0.153, CHCl$_3$).

tert-Butyl ((*R*)-6-((*R*)-1-phenylethyl)-6-azaspiro[3.4]octan-8-yl)carbamate, **8c**

Yield: 563 mg (quantitative yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.32 – 7.22 (m, 5H), 4.71 (d, *J* = 8.8 Hz, 1H), 3.95 – 3.90 (m, 1H), 3.21 (q, *J* = 6.4 Hz, 1H), 2.77 – 2.70 (m, 2H), 2.51 (d, *J* = 9.6 Hz, 1H), 2.21 (dd, *J* = 10.0, 3.6

Hz, 1H), 2.07 – 2.02 (m, 2H), 1.89 – 1.73 (m, 4H), 1.45 (s, 9H), 1.31 (d, $J = 6.4$ Hz, 3H). $[\alpha]_D$ +8.4° (c 0.387, CHCl$_3$).

In the cases of **8ab** and **8ac**, the desired products were synthesized through substitution reactions with acetic anhydride and cyclopropanecarbonyl chloride instead of di-*tert*-butyl dicarbamate according to the aforementioned process (vide supra).

(R)-N-(1-Benzylpyrrolidin-3-yl)acetamide, **8ab**

Yield: 2.12 g (85.0%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.34 – 7.24 (m, 5H), 5.93 (s, 1H), 4.46 – 4.42 (m, 1H), 3.60 (s, 2H), 2.90 – 2.86 (m, 1H), 2.62 – 2.51 (m, 2H), 2.30 – 2.22 (m, 2H), 1.93 (s, 3H), 1.64 – 1.60 (m, 1H). $[\alpha]_D$ +19.7° (c 0.410, CHCl$_3$).

(R)-N-(1-Benzylpyrrolidin-3-yl)cyclopropanecarboxamide, **8ac**

Yield: 3.02 g (quantitative yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.39 (d, $J = 5.2$ Hz, 1H), 7.62 – 7.48 (m, 5H), 4.96 (s, 1H), 4.28 – 4.21 (bs, 2H), 3.81 (s, 1H), 3.52 (d, $J = 11.2$ Hz, 1H), 3.05 – 2.88 (m, 2H), 2.54 – 2.47 (m, 1H), 2.30 – 2.23 (m, 1H), 0.94 – 0.91 (m, 2H), 0.89 – 0.84 (m, 1H), 0.78 – 0.75 (m, 2H). $[\alpha]_D$ +16.3° (c 0.397, CHCl$_3$).

Synthesis of (R)-1-benzyl-N-methylpyrrolidin-3-amine, **9aa**

A *tert*-butyl (R)-(1-benzylpyrrolidin-3-yl)carbamate **8aa** (3.20 g, 11.6 mmol) solution in 58.0 mL of tetrahydrofuran was placed in a 100 mL round bottom flask. After it was cooled at -40 °C, lithium aluminum hydride (2.64 g, 69.6 mmol) was slowly added to the stirred mixture. The reaction mixture was refluxed for 4 hours and then cooled down to -40 °C. The reaction was quenched with 2.70 mL of deionized water, 2.70 mL of 15% sodium hydroxide solution, and 8.10 mL of deionized water. Then, celite 545 was added and the mixture

was stirred for 30 minutes before being filtered through a celite 545 pad. The filtered solution was concentrated under reduced pressure and the residue was extracted with dichloromethane three times. Combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified with flash column chromatography (methanol:dichloromethane :ammonium hydroxide = 5:90:5). Removing the solvent in vacuo provided 2.17 g of (*R*)-1-benzyl-*N*-methylpyrrolidin-3-amine (98.6% yield). ^{1}H NMR (400 MHz, CDCl₃) δ 7.34 – 7.24 (m, 5H), 3.62 (s, 2H), 3.25 – 3.19 (m, 1H), 2.74 (dd, *J* = 9.4, 6.8 Hz, 1H), 2.64 (dt, *J* = 8.6, 6.0 Hz, 1H), 2.52 (dt, *J* = 8.4, 6.0 Hz, 1H), 2.41 – 2.37 (m, 1H), 2.38 (s, 3H), 2.19 – 2.09 (m, 1H), 2.02 (bs, 1H), 1.63 – 1.56 (m, 1H).

In the cases from **9ab** to **9c**, the desired products were synthesized from **8ab** – **8c**, respectively, instead of (*R*)-(1-benzylpyrrolidin-3-yl)carbamate **8aa** according to the aforementioned process (vide supra).

(R)-1-Benzyl-N-ethylpyrrolidin-3-amine, **9ab**

Yield: 1.61 g (94.0%). ^{1}H NMR (400 MHz, CDCl₃) δ 7.31 – 7.27 (m, 5H), 3.64 (s, 2H), 3.51 (d, *J* = 12.8 Hz, 1H), 2.67 – 2.58 (m, 2H), 2.53 – 2.50 (m, 1H), 2.29 – 2.25 (m, 2H), 2.06 – 1.95 (m, 2H), 1.69 – 1.60 (m, 2H), 1.13 – 1.04 (m, 3H).

(R)-1-Benzyl-N-(cyclopropylmethyl)pyrrolidin-3-amine, **9ac**

Yield: 1.68 g (64.0%). ^{1}H NMR (400 MHz, CDCl₃) δ 7.34 – 7.29 (m, 5H), 3.62 (d, *J* = 7.2 Hz, 2H), 3.66 – 3.33 (m, 1H), 2.80 – 2.76 (m, 1H), 2.65 – 2.55 (m, 2H), 2.44 – 2.38 (m, 2H), 2.36 – 2.32 (m, 2H), 1.61 – 1.55 (m, 2H), 0.97 – 0.93 (m, 1H), 0.51 – 0.47 (m, 2H), 0.12 – 0.09 (m, 2H).

(R)-N,4,4-Trimethyl-1-((R)-1-phenylethyl)pyrrolidin-3-amine, **9b**

Yield: 238 mg (97.9%). ^{1}H NMR (400 MHz, CDCl₃) δ 7.38 – 7.07 (m, 5H), 3.26 (q, *J* = 13.2, 6.4 Hz, 1H), 3.08 (q, *J* = 9.2, 7.2 Hz, 1H), 2.73 (t, *J* =

7.2 Hz, 1H), 2.37 (s, 3H), 2.36 (d, *J* = 4.4 Hz, 1H), 2.31 – 2.26 (m, 1H), 2.24 – 2.17 (m, 1H), 1.30 (d, *J* = 6.4 Hz, 3H), 1.06 (s, 3H), 0.96 (s, 3H).

(R)-N-Methyl-6-((R)-1-phenylethyl)-6-azaspiro[3.4]octan-8-amine, 9c

Yield: 308 mg (75.0%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.33 – 7.23 (m, 5H), 3.24 (q, *J* = 6.4 Hz, 1H), 3.03 (dd, *J* = 9.6, 6.4 Hz, 1H), 2.81 (t, *J* = 6.4 Hz, 1H), 2.66 (d, *J* = 9.2 Hz, 1H), 2.55 (d, *J* = 9.2 Hz, 1H), 2.41 (s, 3H), 2.21 (s, 1H), 2.17 (dd, *J* = 9.6, 5.6 Hz, 1H), 1.99 – 1.95 (m, 1H), 1.90 – 1.63 (m, 4H), 1.34 (d, *J* = 6.4 Hz, 4H).

Synthesis of (R)-N-(1-benzylpyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 10aa

A solution of (*R*)-1-benzyl-*N*-methylpyrrolidin-3-amine **9aa** (420 mg, 2.21 mmol) in 11.0 mL of deionized water was placed in a 50 mL round bottom flask. Consequently, 6-chloro-7-deazapurine (372 mg, 2.42 mmol) and potassium carbonate (609 mg, 4.41 mmol) were added and the mixture was refluxed for 18 hours. After the reaction, it was cooled at room temperature and the aqueous mixture was extracted with 20 mL of dichloromethane three times. Combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified with flash column chromatography (methanol:dichloromethane = 2:98). Removing the solvent in vacuo provided 507 mg of (*R*)-*N*-(1-benzylpyrrolidin-3-yl)-*N*-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (74.8% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.40 (s, 1H), 8.29 (s, 1H), 7.51 – 7.20 (m, 5H), 7.03 (s, 1H), 6.59 (d, *J* = 2.2 Hz, 1H), 5.66 (s, 1H), 3.65 (dd, *J* = 62.5, 12.9 Hz, 2H), 3.42 (s, 3H), 2.98 (dd, *J* = 13.5, 7.8 Hz, 1H), 2.83 (dd, *J* = 10.3, 3.4 Hz, 1H), 2.69 – 2.53 (m, 1H), 2.44 – 2.21 (m, 2H), 1.96 – 1.83 (m, 1H).

In the cases of **10ab**, **10ac**, **10ad**, **10b**, and **10c**, the desired products were synthesized from **9ab**, **9ac**, **7a**, **9b**, and **9c**, respectively, instead of (*R*)-1-

benzyl-*N*-methylpyrrolidin-3-amine **9aa** according to the aforementioned process (vide supra).

*(R)-N-(1-Benzylpyrrolidin-3-yl)-N-ethyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **10ab***

Yield: 296 mg (10.0%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.84 (s, 1H), 8.31 (s, 1H), 7.39 – 7.33 (m, 5H), 7.06 (d, *J* = 3.6 Hz, 1H), 6.51 (d, *J* = 3.6 Hz, 1H), 5.57 (bs, 1H), 3.93 – 3.85 (m, 2H), 3.78 – 3.75 (m, 1H), 3.65 – 3.58 (m, 1H), 2.98 (bs, 1H), 2.84 (bs, 1H), 2.70 (bs, 1H), 2.49 – 2.33 (m, 2H), 2.01 – 1.93 (m, 1H), 1.36 (t, *J* = 7.2 Hz, 3H).

*(R)-N-(1-Benzylpyrrolidin-3-yl)-N-(cyclopropylmethyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **10ac***

Yield: 313 mg (12.4%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 9.59 – 9.54 (bs, 1H), 8.30 (s, 1H), 7.36 – 7.30 (bs, 5H), 7.03 (bs, 1H), 6.69 (bs, 1H), 5.54 (bs, 1H), 3.78 – 3.68 (m, 3H), 3.63 (bs, 1H), 3.00 (bs, 1H), 2.62 (bs, 1H), 2.39 (bs, 2H), 2.01 (bs, 1H), 1.64 (bs, 1H), 0.62 – 0.54 (m, 1H), 0.44 – 0.41 (m, 1H), 0.39 – 0.36 (m, 1H).

*(R)-N-(1-Benzylpyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **10ad***

Yield: 292 mg (58.5%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.20 (s, 1H), 8.36 (s, 1H), 7.37 – 7.26 (m, 5H), 7.05 (d, *J* = 3.6 Hz, 1H), 6.40 (d, *J* = 3.2 Hz, 1H), 5.80 (d, *J* = 8.0 Hz, 1H), 4.89 (s, 1H), 3.71 (s, 2H), 3.02 – 3.00 (m, 1H), 2.88 (d, *J* = 8.4 Hz, 1H), 2.81 – 2.76 (m, 1H), 2.51 – 2.39 (m, 2H), 1.92 – 1.83 (m, 1H).

*N-((R)-4,4-Dimethyl-1-((R)-1-phenylethyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **10b***

Yield: 106 mg (30.7%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 9.03 (s, 1H), 8.21 (s, 1H), 7.38 – 7.19 (m, 5H), 6.96 (q, J = 3.6, 2.0 Hz, 1H), 6.61 (q, J = 3.2, 1.6 Hz, 1H), 5.14 (d, J = 8.4 Hz, 1H), 3.48 (s, 3H), 3.18 (q, J = 13.2, 6.4 Hz, 1H), 2.98 (d, J = 9.2 Hz, 1H), 2.68 (d, J = 12.0 Hz, 1H), 2.48 (q, J = 11.2, 8.4 Hz, 1H), 2.10 (d, J = 8.8 Hz, 1H), 1.39 (d, J = 6.4 Hz, 3H), 1.35 (s, 3H), 0.98 (s, 3H).

*N-Methyl-N-((R)-6-((R)-1-phenylethyl)-6-azaspiro[3.4]octan-8-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **10c***

Yield: 272 mg (60.0%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 9.06 (s, 1H), 8.25 (s, 1H), 7.37 – 7.22 (m, 5H), 6.98 (dd, J = 3.6, 2.4 Hz, 1H), 6.61 (dd, J = 3.6, 2.0 Hz, 1H), 5.48 (s, 1H), 3.29 (s, 3H), 3.18 (d, J = 6.8 Hz, 2H), 2.65 – 2.46 (m, 4H), 1.98 – 1.91 (m, 2H), 1.87 – 1.80 (m, 2H), 1.73 – 1.69 (m, 1H), 1.40 (d, J = 6.4 Hz, 3H).

*Synthesis of (R)-N-methyl-N-(pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **11aa***

A (R)-N-(1-benzylpyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine **10aa** (638 mg, 2.08 mmol) solution in 20.8 mL of methanol was placed in a 100 mL round bottom flask. Then, 10w/w% palladium on charcoal (638 mg, 5 wt%) and 10.1 g of ammonium formate (262 mg, 4.15 mmol) were added and the reaction mixture was stirred at 60 – 70 ºC overnight. After the reaction, it was filtered through a celite 545 pad before the solution was concentrated under reduced pressure. The residue was purified with flash column chromatography (methanol:dichloromethane:ammonium hydroxide = 10:88:2). Removing the solvent in vacuo provided 325 mg of (R)-N-methyl-N-(pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (72.0% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.16 (bs, 1H), 8.33 (s, 1H), 7.09 (d, J = 3.5 Hz, 1H), 6.58 (d, J = 3.4 Hz, 1H), 5.62 – 5.42 (m, 1H), 3.42 – 3.32 (m, 3H), 3.29 (dd, J = 11.5, 8.4 Hz, 1H), 3.24 – 3.12 (m, 1H), 3.10 – 3.01 (m, 1H), 2.98

(dd, J = 11.5, 6.2 Hz, 1H), 2.66 (bs, 1H), 2.26 – 2.10 (m, 1H), 1.91 (td, J = 14.9, 7.6 Hz, 1H).

In the cases from **11ab** to **11c**, the desired products were synthesized from **10ab** – **10c**, respectively, instead of (*R*)-*N*-(1-benzylpyrrolidin-3-yl)-*N*-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **10aa** according to the aforementioned process (vide supra).

(R)-N-Ethyl-N-(pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **11ab**

Yield: 189 mg (88.8%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.30 – 10.01 (bs, 1H), 8.31 (s, 1H), 7.09 (d, J = 3.6 Hz, 1H), 6.51 (d, J = 3.6 Hz, 1H), 5.10 – 5.04 (m, 1H), 3.81 (q, J = 7.2 Hz, 2H), 3.51 (s, 1H), 3.35 – 3.29 (m, 2H), 3.14 – 3.04 (m, 2H), 2.26 – 2.20 (m, 1H), 2.10 – 2.01 (m, 1H), 1.40 (t, J = 7.2 Hz, 3H).

(R)-N-(Cyclopropylmethyl)-N-(pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **11ac**

Yield: 162 mg (70.7%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.40 – 10.10 (bs, 1H), 8.32 (d, J = 4.4 Hz, 1H), 7.10 (d, J = 3.6 Hz, 1H), 6.67 (d, J = 3.6 Hz, 1H), 4.95 – 4.91 (m, 1H), 3.76 – 3.60 (m, 2H), 3.39 – 3.34 (m, 1H), 3.26 – 3.24 (m, 2H), 3.04 – 2.97 (m, 1H), 2.24 – 2.12 (m, 2H), 1.25 – 1.13 (m, 2H), 0.69 – 0.62 (m, 2H), 0.45 – 0.39 (m, 2H).

(R)-N-(Pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **11ad**

Yield: 191 mg (94.8%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.47 (s, 1H), 8.08 (s, 1H), 7.31 (d, J = 6.8 Hz, 1H), 7.05 (d, J = 3.2 Hz, 1H), 5.73 (d, J = 3.6 Hz, 1H), 4.63 – 4.50 (m, 1H), 3.12 – 3.07 (m, 1H), 3.02 – 2.95 (m, 1H), 2.87 – 2.81 (m, 1H), 2.75 (dd, J = 11.2, 3.2 Hz, 1H), 2.10 – 2.01 (m, 1H), 1.75 – 1.67 (m, 1H).

(R)-N-(4,4-Dimethylpyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 11b

Yield: 58.2 mg (79.1%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 9.57 (d, J = 24.8 Hz, 1H), 8.24 (d, J = 14.8 Hz, 1H), 7.01 – 6.98 (m, 1H), 6.67 – 6.61 (m, 1H), 5.34 – 5.23 (m, 1H), 3.56 – 3.40 (m, 3H), 3.19 – 3.16 (m, 1H), 2.97 – 2.55 (m, 2H), 2.32 (s, 1H), 1.33 (d, J = 25.2 Hz, 3H), 0.94 (d, J = 12.0 Hz, 3H).

(R)-N-Methyl-N-(6-azaspiro[3.4]octan-8-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 11c

Yield: 163 mg (84.5%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.62 (s, 1H), 8.29 (s, 1H), 7.07 (d, J = 3.6 Hz, 1H), 6.62 (d, J = 4.0 Hz, 1H), 5.40 (s, 1H), 3.43 (dd, J = 12.4, 8.4 Hz, 1H), 3.30 (s, 3H), 3.26 – 3.19 (m, 2H), 3.09 (dd, J = 12.4, 5.2 Hz, 1H), 2.37 – 2.32 (m, 2H), 2.03 (d, J = 7.2 Hz, 1H), 1.95 – 1.88 (m, 2H), 1.86 – 1.81 (m, 2H).

Syntheses of (R)-3-(3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile, 12a

To an (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **11aa** (103 mg, 0.474 mmol) solution in 4.70 mL of *n*-butanol in a 10 mL round bottom flask, ethyl cyanoacetate (0.505 mL, 4.75 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.0355 mL, 0.237 mmol) were added and the mixture was heated at 80 °C for 24 hours. The reaction solution was concentrated under reduced pressure and the residue was purified with flash column chromatography (methanol:dichloromethane = 2:98). Removing the solvent in vacuo provided 101 mg of (*R*)-3-(3-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile (74.8% yield). 98.7% purity by HPLC.

^{1}H NMR (400 MHz, CDCl$_3$) δ 10.98 (s, 1H), 8.33 (s, 1H), 7.13 (d, J = 3.6 Hz, 1H), 6.60 (d, J = 3.6 Hz, 1H), 5.76 (m, 1H), 3.90 (dt, J = 14.9, 8.0 Hz, 1H), 3.70 (ddd, J = 26.4, 16.1, 8.3 Hz, 1H), 3.51 (m, 4H), 3.35 (d, J = 14.8 Hz,

3H), 2.27 (m, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 160.3, 157.8, 152.3, 150.9, 120.9, 113.8, 103.6, 102.1, 55.0, 48.0, 45.2, 32.5, 26.9, 26.0. HRMS (ESI) calcd for C$_{14}$H$_{17}$N$_6$O: 285.1464. Obsd: 285.1452. [α]$_D$ +42.6° (*c* 1.00, CHCl$_3$).

In the cases of **12b**, **12c**, **13**, and **14**, the desired products were synthesized from **11b**, **11c**, **11ab**, and **11ac**, respectively, instead of (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **11aa** according to the aforementioned process (vide supra).

(R)-3-(3,3-Dimethyl-4-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile, **12b**

Yield: 41.4 mg (57.1%). 97.7% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.19 (s, 1H), 8.31 (s, 1H), 7.14 (s, 1H), 6.61 (s, 1H), 5.69 (dd, *J* = 39.2, 6.0 Hz, 1H), 4.03 (m, 1H), 3.82 (dd, *J* = 34.5, 12.6 Hz, 1H), 3.54 (m, 3H), 3.41 (m, 1H), 3.34 (d, *J* = 10.4 Hz, 3H), 1.25 (d, *J* = 2.8 Hz, 3H), 1.04 (m, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 160.4, 158.0, 152.3, 150.3, 120.8, 113.9, 103.1, 102.2, 62.2, 59.9, 49.1, 44.6, 33.9, 28.1, 26.0, 21.6. HRMS (ESI) calcd for C$_{16}$H$_{21}$N$_6$O: 313.1777. Obsd: 313.1772. [α]$_D$ -8.93° (*c* 0.864, CHCl$_3$).

(R)-3-(8-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-6-azaspiro[3.4]octan-6-yl)-3-oxopropanenitrile, **12c**

Yield: 23.7 mg (19.0%). 95.0% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.84 (s, 1H), 8.36 (s, 1H), 7.15 (s, 1H), 6.62 (s, 1H), 5.96 (dd, *J* = 19.0, 6.3 Hz, 1H), 3.94 (ddd, *J* = 39.2, 19.0, 12.0 Hz, 2H), 3.73 (m, 2H), 3.50 (d, *J* = 5.8 Hz, 2H), 3.28 (d, *J* = 4.9 Hz, 3H), 2.23 (m, 1H), 1.99 (m, 5H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 160.0, 158.2, 152.4, 150.6, 120.8, 113.7, 103.1, 102.3, 62.0, 58.9, 49.9, 47.7, 35.7, 33.6, 26.4, 26.0, 16.3. HRMS (ESI) calcd for C$_{17}$H$_{21}$N$_6$O: 325.1777. Obsd: 325.1770. [α]$_D$ +7.04° (*c* 0.557, CHCl$_3$).

(R)-3-(3-(Ethyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile, 13

Yield: 50.9 mg (55.0%). 96.1% purity by HPLC. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.66 (s, 1H), 8.11 (d, *J* = 2.5 Hz, 1H), 7.16 (s, 1H), 6.48 (m, 1H), 5.35 (ddd, *J* = 54.1, 16.5, 8.3 Hz, 1H), 3.95 (dd, *J* = 19.0, 8.5 Hz, 1H), 3.87 (dd, *J* = 18.9, 3.9 Hz, 1H), 3.70 (m, 1H), 3.62 (dd, *J* = 9.2, 6.9 Hz, 3H), 3.45 (dd, *J* = 17.5, 8.8 Hz, 1H), 3.24 (m, 1H), 2.16 (m, 1H), 2.07 (m, 1H), 1.19 (dd, *J* = 11.2, 6.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 161.3, 156.1, 151.8, 150.5, 121.5, 116.0, 101.7, 101.1, 54.4, 47.4, 44.2, 28.5, 26.8, 25.5, 15.7. HRMS (ESI) calcd for $C_{15}H_{19}N_6O$: 299.1620. Obsd: 299.1617. [α]$_D$ +78.5° (*c* 1.09, DMSO).

(R)-3-(3-((Cyclopropylmethyl)(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile, 14

Yield: 38.4 mg (54.0%). 95.3% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.03 (d, *J* = 14.0 Hz, 1H), 8.35 (d, *J* = 5.1 Hz, 1H), 7.17 (d, *J* = 5.0 Hz, 1H), 6.67 (m, 1H), 5.30 (ddt, *J* = 25.0, 16.8, 8.3 Hz, 1H), 3.93 (m, 2H), 3.63 (m, 3H), 3.49 (m, 3H), 2.33 (ddt, *J* = 17.5, 12.0, 9.6 Hz, 2H), 1.18 (m, 1H), 0.69 (t, *J* = 8.9 Hz, 2H), 0.36 (m, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 160.3, 157.1, 152.3, 150.4, 121.4, 114.0, 103.2, 101.8, 56.6, 50.4, 48.9, 45.1, 29.6, 26.0, 11.9, 5.1, 4.9. HRMS (ESI) calcd for $C_{17}H_{21}N_6O$: 325.1777. Obsd: 325.1775. [α]$_D$ +24.5° (*c* 1.08, CHCl$_3$).

Synthesis of (R)-3-((3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, 16

To an (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **11aa** (70.0 mg, 0.322 mmol) solution in 1.50 mL of dichloromethane in a 5 mL round bottom flask, 3-cyanobenzenesulfonyl chloride (68.6 mg, 0.340 mmol) and *N*,*N*-diisopropylethylamine (0.0590 mL, 0.339 mmol) were

added. Then, the reaction solution was stirred at room temperature overnight before being concentrated under reduced pressure. The residue was purified by flash column chromatography (methanol:dichloromethane=2:98). Removing the solvent in vacuo provided 88.6 mg of (*R*)-3-((3-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile (72.4% yield). 97.0% purity by HPLC.

^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.68 (s, 1H), 8.32 (d, *J* = 7.8 Hz, 1H), 8.23 (d, *J* = 7.7 Hz, 1H), 8.17 (d, *J* = 8.0 Hz, 1H), 8.06 (t, *J* = 4.6 Hz, 1H), 7.88 (td, *J* = 7.8, 2.2 Hz, 1H), 7.14 (d, *J* = 2.1 Hz, 1H), 6.48 (s, 1H), 5.27 (m, 1H), 3.51 (dd, *J* = 7.6, 4.4 Hz, 1H), 3.44 (m, 1H), 3.36 (s, 3H), 3.23 (m, 1H), 3.17 (s, 1H), 2.03 (dd, *J* = 15.0, 7.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 156.8, 151.7, 150.4, 137.1, 136.8, 131.9, 131.0, 130.9, 121.2, 117.6, 112.9, 102.5, 101.3, 54.0, 48.7, 46.8, 31.7, 27.5. HRMS (ESI) calcd for $C_{18}H_{19}N_6O_2S$: 383.1290. Obsd: 383.1285. [α]$_D$ -45.7° (*c* 0.530, CHCl$_3$).

In the cases of **15**, **17**, and **18**, the desired products were synthesized from **11ad**, **11ab**, and **11ac**, respectively, instead of (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **11aa** according to the aforementioned process (vide supra).

(R)-3-((3-((7H-Pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, 15

Yield: 42.0 mg (38.2%). 97.6% purity by HPLC. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.49 (s, 1H), 8.14 (s, 1H), 8.06 (s, 1H), 7.98 (d, *J* = 7.7 Hz, 1H), 7.92 (d, *J* = 7.5 Hz, 1H), 7.58 (t, *J* = 7.7 Hz, 1H), 7.13 (d, *J* = 4.1 Hz, 1H), 7.03 (s, 1H), 6.36 (s, 1H), 4.38 (d, *J* = 4.3 Hz, 1H), 3.49 (m, 2H), 3.31 (m, 1H), 3.25 (m, 1H), 2.06 (dd, *J* = 12.3, 6.2 Hz, 1H), 1.88 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d6) δ 155.2, 151.1, 150.2, 137.2, 136.4, 131.6, 130.7, 130.4, 121.0, 117.5, 112.7, 102.6, 98.7, 53.3, 50.0, 46.7, 30.3. HRMS (ESI) calcd for $C_{17}H_{17}N_6O_2S$: 369.1134. Obsd: 369.1128. [α]$_D$ -27.4° (*c* 1.09, DMSO).

*(R)-3-((3-(Ethyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, **17***

Yield: 84.3 mg (73.3%). 95.1% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.64 (s, 1H), 8.17 (d, J = 14.8 Hz, 2H), 8.11 (d, J = 7.9 Hz, 1H), 7.92 (d, J = 7.6 Hz, 1H), 7.72 (t, J = 7.8 Hz, 1H), 7.13 (d, J = 3.0 Hz, 1H), 6.42 (d, J = 3.0 Hz, 1H), 5.23 (m, 1H), 3.71 (m, 4H), 3.33 (m, 1H), 3.26 (m, 1H), 2.20 (m, 2H), 1.34 (t, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 156.5, 152.1, 150.4, 138.6, 136.1, 131.7, 131.2, 130.4, 121.2, 117.4, 114.0, 102.7, 101.5, 55.6, 49.5, 47.0, 40.6, 28.9, 16.0. HRMS (ESI) calcd for C$_{19}$H$_{21}$N$_6$O$_2$S: 397.1447. Obsd: 397.1442. [α]$_D$ -63.5° (c 0.568, CHCl$_3$).

*(R)-3-((3-((Cyclopropylmethyl)(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, **18***

Yield: 62.2 mg (61.0%). 95.0% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.91 (s, 1H), 8.19 (s, 1H), 8.11 (d, J = 7.7 Hz, 1H), 8.04 (s, 1H), 7.93 (d, J = 7.4 Hz, 1H), 7.73 (t, J = 7.3 Hz, 1H), 7.15 (s, 1H), 6.60 (s, 1H), 5.05 (m, 1H), 3.66 (m, 2H), 3.60 (d, J = 4.9 Hz, 2H), 3.48 (m, 1H), 3.31 (dd, J = 15.4, 7.6 Hz, 1H), 2.26 (d, J = 7.2 Hz, 2H), 1.12 (s, 1H), 0.65 (d, J = 2.7 Hz, 2H), 0.33 (d, J = 2.1 Hz, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 156.6, 152.0, 149.9, 138.3, 136.0, 131.8, 131.3, 130.3, 121.2, 117.4, 113.9, 103.2, 101.8, 56.9, 51.3, 49.7, 47.4, 29.2, 11.7, 4.8. HRMS (ESI) calcd for C$_{21}$H$_{23}$N$_6$O$_2$S: 423.1603. Obsd: 423.1598. [α]$_D$ -31.5° (c 1.49, CHCl$_3$).

*Synthesis of (R)-3-(3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)propanenitrile, **69***

To an (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **11aa** (60.0 mg, 0.276 mmol) solution in 1.00 mL of dichloromethane in a 5 mL round-bottom flask, 3-bromopropionitrile (0.0240 mL, 0.289 mmol)

and *N,N*-diisopropylethylamine (0.0720 mL, 0.413 mmol) were added. The reaction mixture was stirred at room temperature overnight and then concentrated under reduced pressure. The residue was purified by column chromatography (methanol:dichloromethane=2:98). Removing the solvent in vacuo provided 55.3 mg of (*R*)-3-(3-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)pyrrolidin-1-yl)propanenitrile (74.7% yield). 100% purity by HPLC.

^{1}H NMR (400 MHz, CDCl$_3$) δ 12.32 (s, 1H), 8.32 (s, 1H), 7.10 (d, *J* = 3.4 Hz, 1H), 6.58 (d, *J* = 3.3 Hz, 1H), 5.73 (s, 1H), 3.42 (s, 3H), 3.06 (t, *J* = 7.1 Hz, 1H), 2.94 (dd, *J* = 9.9, 3.1 Hz, 1H), 2.83 (m, 1H), 2.72 (m, 1H), 2.64 (t, *J* = 9.2 Hz, 1H), 2.56 (t, *J* = 6.8 Hz, 2H), 2.34 (m, 2H), 1.93 (dt, *J* = 13.0, 10.0 Hz, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 151.8, 150.6, 120.4, 118.8, 103.1, 102.1, 57.2, 54.4, 53.7, 50.8, 32.4, 29.3, 17.7. HRMS (ESI) calcd for C$_{14}$H$_{19}$N$_6$: 271.1671. Obsd: 271.1665. [α]$_D$ +35.3º (*c* 1.07, CHCl$_3$).

In the cases of compound **70**, the desired products were synthesized through substitution reactions with *n*-butyl bromide instead of 3-bromopropionitrile according to the aforementioned process (vide supra).

(R)-N-(1-Butylpyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **70**

Yield: 90.0 mg (83.3%). 100% purity by HPLC. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.69 (s, 1H), 8.11 (s, 1H), 7.11 (d, *J* = 3.1 Hz, 1H), 6.58 (d, *J* = 3.2 Hz, 1H), 5.53 (dt, *J* = 15.1, 7.7 Hz, 1H), 3.25 (s, 4H), 3.08 (m, 2H), 2.81 (m, 3H), 2.16 (m, 1H), 1.95 (m, 1H), 1.50 (dt, *J* = 15.2, 7.4 Hz, 2H), 1.25 (dq, *J* = 14.5, 7.3 Hz, 2H), 0.82 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 156.7, 151.6, 150.4, 121.1, 102.5, 101.5, 54.5, 54.3, 53.7, 52.9, 32.7, 28.2, 27.1, 19.7, 13.6. HRMS (ESI) calcd for C$_{15}$H$_{24}$N$_5$: 274.2032. Obsd: 274.2027. [α]$_D$ +10.6º (*c* 3.42, DMSO).

*Synthesis of (R)-2-azido-1-(3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)ethan-1-one, **71***

To a 2-azidoacetic acid (247 mg, 2.44 mmol) solution in 8.0 mL of *N,N*-dimethylformamide in a 25 mL round-bottom flask, *N,N'*-dicyclohexylcarbodiimide (503 mg, 2.44 mmol) and *N,N*-diisopropylethylamine (0.850 mL, 4.88 mmol) were added and the reaction mixture was stirred for 15 minutes. In a second 25 mL round-bottom flask, (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **11aa** (265 mg, 1.22 mmol) was placed and the reaction mixture of 2-azidoacetic acid was transferred to this second flask. The reaction mixture was refluxed overnight and then cooled at room temperature. The mixture was filtered through a celite 545 pad and the solution was concentrated under reduced pressure. The residue was purified with column chromatography (methanol:dichloromethane=2:98). Removing the solvent in vacuo provided 41.0 mg of (*R*)-2-azido-1-(3-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)pyrrolidin-1-yl)ethan-1-one (5.27% yield). 96.2% purity by HPLC.

^{1}H NMR (400 MHz, CDCl$_3$) δ 11.97 (d, *J* = 32.0 Hz, 1H), 8.34 (d, *J* = 1.9 Hz, 1H), 7.15 (dd, *J* = 6.5, 3.6 Hz, 1H), 6.59 (s, 1H), 5.75 (m, 1H), 3.92 (m, 3H), 3.79 (dd, *J* = 19.2, 10.6 Hz, 1H), 3.57 (tt, *J* = 11.9, 8.2 Hz, 2H), 3.34 (m, 3H), 2.21 (m, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.2, 157.8, 152.3, 150.7, 121.1, 103.6, 101.8, 55.0, 51.3, 46.7, 44.6, 32.3, 26.7. HRMS (ESI) calcd for C$_{13}$H$_{17}$N$_8$O: 301.1525. Obsd: 301.1522. [α]$_D$ +33.2° (*c* 0.753, CHCl$_3$).

*Synthesis of (R)-3-methyl-1-(3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)butan-1-one, **72***

To an (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **11aa** (70.0 mg, 0.322 mmol) solution in 1.00 mL of dichloromethane in a 5 mL round-bottom flask, isovaleryl chloride (38.8 mg, 0.322 mmol) and *N,N*-diisopropylethylamine (0.0590 mL, 0.339 mmol) were added. The reaction

mixture was stirred at room temperature overnight and then concentrated under reduced pressure. The residue was purified by column chromatography (methanol:dichloromethane=2:98). Removing the solvent in vacuo provided 66.7 mg of (*R*)-3-methyl-1-(3-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)pyrrolidin-1-yl)butan-1-one (68.7% yield). 98.7% purity by HPLC.

^{1}H NMR (400 MHz, CDCl$_3$) δ 11.67 (s, 1H), 8.38 (s, 1H), 7.14 (s, 1H), 6.60 (s, 1H), 5.72 (m, 1H), 3.81 (m, 2H), 3.49 (m, 2H), 3.34 (d, *J* = 11.5 Hz, 3H), 2.18 (m, 4H), 1.50 (d, *J* = 35.8 Hz, 1H), 0.94 (d, *J* = 44.9 Hz, 6H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 171.6, 157.7, 151.9, 150.2, 121.1, 103.5, 101.7, 54.9, 47.8, 45.4, 43.8, 32.1, 29.7, 25.5, 22.8. HRMS (ESI) calcd for C$_{16}$H$_{24}$N$_5$O: 302.1981. Obsd: 302.1977. [α]$_D$ +29.6º (*c* 1.47, CHCl$_3$).

*Synthesis of isobutyl (R)-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidine-1-carboxylate, **73***

To an (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **11aa** (70.0 mg, 0.322 mmol) solution in 1.00 mL of dichloromethane in a 5 mL round-bottom flask, isobutyl chloroformate (44.0 mg, 0.322 mmol) and *N,N*-diisopropylethylamine (0.0560 mL, 0.321 mmol) were added. The reaction solution was stirred at room temperature overnight and then concentrated under reduced pressure. The residue was purified by column chromatography (methanol:dichloromethane=2:98). Removing the solvent in vacuo provided 41.0 mg of isobutyl (*R*)-3-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)pyrrolidine-1-carboxylate (40.2% yield). 97.7% purity by HPLC.

^{1}H NMR (400 MHz, CDCl$_3$) δ 11.83 (s, 1H), 8.33 (s, 1H), 7.11 (d, *J* = 2.8 Hz, 1H), 6.58 (d, *J* = 3.3 Hz, 1H), 5.72 (d, *J* = 6.3 Hz, 1H), 3.90 (d, *J* = 6.5 Hz, 2H), 3.76 (d, *J* = 9.2 Hz, 1H), 3.69 (m, 1H), 3.45 (m, 2H), 3.33 (s, 3H), 2.15 (dd, *J* = 21.9, 12.3 Hz, 2H), 1.95 (d, *J* = 6.1 Hz, 1H), 0.94 (d, *J* = 6.2 Hz, 6H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.9, 155.5, 152.0, 150.6, 120.8, 103.4,

102.0, 71.5, 54.7, 46.8, 44.8, 32.0, 28.2, 19.2, 9.5. HRMS (ESI) calcd for C$_{16}$H$_{24}$N$_5$O$_2$: 318.1930. Obsd: 318.1924. [α]$_D$ +23.7^o (*c* 0.550, CHCl$_3$).

*Synthesis of (R)-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-phenylpyrrolidine-1-carboxamide, **74***

To an (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **11aa** (70.0 mg, 0.322 mmol) solution in 1.00 mL of dichloromethane in a 5 mL round-bottom flask, *N*,*N*-diisopropylethylamine (0.0590 mL, 0.339 mmol) was added and the mixture was treated with phenyl isocyanate (0.0350 mL, 0.322 mmol). The reaction solution was stirred for 2 hours before being concentrated under reduced pressure. The residue was purified by column chromatography (methanol:dichloromethane = 2:98). Removing the solvent in vacuo provided 81.5 mg of (*R*)-3-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)-*N*-phenylpyrrolidine-1-carboxamide (75.4% yield). 99.8% purity by HPLC.

^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.72 (s, 1H), 8.22 (s, 1H), 8.18 (s, 1H), 7.54 (d, *J* = 8.1 Hz, 2H), 7.23 (t, *J* = 7.8 Hz, 2H), 7.18 (d, *J* = 2.3 Hz, 1H), 6.92 (t, *J* = 7.2 Hz, 1H), 6.64 (d, *J* = 3.1 Hz, 1H), 5.56 (m, 1H), 3.75 (m, 1H), 3.67 (m, 1H), 3.45 (m, 2H), 3.25 (s, 3H), 2.16 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 157.1, 154.1, 151.8, 150.6, 140.5, 128.3, 121.7, 121.1, 119.5, 102.6, 101.6, 54.2, 46.8, 44.4, 31.6, 27.6. HRMS (ESI) calcd for C$_{18}$H$_{21}$N$_6$O: 337.1777. Obsd: 337.1772. [α]$_D$ +43.6^o (*c* 2.44, DMSO).

*Synthesis of (R)-N-methyl-N-(1-(methylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **75***

To an (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **11aa** (70.0 mg, 0.322 mmol) solution in 1.00 mL of dichloromethane in a 5 mL round bottom flask, methanesulfonyl chloride (36.9 mg, 0.322 mmol) and *N*,*N*-diisopropylethylamine (0.0590 mL, 0.339 mmol) were added. Then,

the reaction solution was stirred at room temperature overnight before being concentrated under reduced pressure. The residue was purified by flash column chromatography (methanol:dichloromethane=2:98). Removing the solvent in vacuo provided 40.0 mg of (*R*)-*N*-methyl-*N*-(1-(methylsulfonyl)pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (42.1% yield). 96.9% purity by HPLC.

^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.70 (s, 1H), 8.15 (d, *J* = 1.3 Hz, 1H), 7.17 (d, *J* = 2.9 Hz, 1H), 6.62 (d, *J* = 2.7 Hz, 1H), 5.58 (m, 1H), 3.52 (t, *J* = 9.0 Hz, 1H), 3.46 (m, 1H), 3.31 (dd, *J* = 17.4, 8.7 Hz, 1H), 3.23 (dd, *J* = 10.0, 4.4 Hz, 4H), 2.98 (d, *J* = 1.3 Hz, 3H), 2.14 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 157.0, 151.8, 150.5, 121.1, 102.6, 101.5, 54.2, 48.4, 46.3, 33.4, 31.8, 27.9. HRMS (ESI) calcd for $C_{12}H_{18}N_5O_2S$: 296.1181. Obsd: 296.1175. [α]$_D$ +23.0º (*c* 1.20, DMSO).

In the cases from **76** to **96**, the desired products were synthesized through substitution reactions with trifluoromethanesulfonyl chloride, ethanesulfonyl chloride, 2-propanesulfonyl chloride, 1-propanesulfonyl chloride, 1-methyl-1*H*-imidazole-4-sulfonyl chloride, benzenesulfonyl chloride, 2-fluorobenzene-1-sulfonyl chloride, 3-fluorobenzene-1-sulfonyl chloride, 4-fluorobenzenesulfonyl chloride, 2-cyanobenzenesulfonyl chloride, 4-cyanobenzenesulfonyl chloride, 2-nitrobenzenesulfonyl chloride, 3-nitrobenzenesulfonyl chloride, 4-nitrobenzenesulfonyl chloride, 3-toluenesulfonyl chloride, 4-toluenesulfonyl chloride, 4-methoxybenzenesulfonyl chloride, 4-(trifluoromethyl)benzenesulfonyl chloride, 2-naphthalenesulfonyl chloride, piperidine-1-sulfonyl chloride, and morpholine-4-sulfonyl chloride, respectively, instead of methanesulfonyl chloride according to the aforementioned process (vide supra).

(R)-N-Methyl-N-(1-((trifluoromethyl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **76**

Yield: 72.0 mg (64.3%). 97.4% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.77 (s, 1H), 8.37 (d, J = 8.8 Hz, 1H), 7.18 (m, 1H), 6.59 (m, 1H), 5.84 (m, 1H), 3.89 (m, 2H), 3.62 (m, 1H), 3.53 (m, 1H), 3.36 (d, J = 8.7 Hz, 3H), 2.29 (dd, J = 17.0, 8.6 Hz, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 152.2, 150.3, 121.3, 120.4 (q, J = 323.8 Hz), 103.7, 101.6, 54.7, 48.9, 47.7, 32.4, 28.5. HRMS (ESI) calcd for C$_{12}$H$_{15}$F$_3$N$_5$O$_2$S: 350.0899. Obsd: 350.0893. [α]$_D$ +19.4° (c 2.79, CHCl$_3$).

(R)-N-(1-(Ethylsulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 77

Yield: 53.4 mg (53.6%). 98.2% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.30 (s, 1H), 8.34 (s, 1H), 7.13 (s, 1H), 6.61 (s, 1H), 5.80 (m, 1H), 3.70 (dd, J = 19.4, 10.1 Hz, 2H), 3.43 (d, J = 10.3 Hz, 2H), 3.38 (s, 3H), 3.08 (q, J = 7.3 Hz, 2H), 2.28 (s, 1H), 2.19 (m, 1H), 1.43 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 151.6, 149.9, 121.1, 103.8, 102.0, 54.9, 48.6, 46.6, 44.6, 29.8, 28.8, 8.1. HRMS (ESI) calcd for C$_{13}$H$_{20}$N$_5$O$_2$S: 310.1338. Obsd: 310.1335. [α]$_D$ +13.1° (c 1.24, CHCl$_3$).

(R)-N-(1-(Isopropylsulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 78

Yield: 36.0 mg (34.6%). 99.1% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.23 (s, 1H), 8.35 (s, 1H), 7.14 (d, J = 3.2 Hz, 1H), 6.59 (d, J = 2.6 Hz, 1H), 5.78 (m, 1H), 3.75 (m, 2H), 3.46 (m, 2H), 3.36 (s, 3H), 3.28 (dt, J = 13.6, 6.8 Hz, 1H), 2.26 (m, 1H), 2.16 (m, 1H), 1.41 (d, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.8, 152.2, 150.6, 120.9, 103.5, 101.9, 55.0, 53.6, 49.0, 47.2, 32.3, 28.9, 16.8. HRMS (ESI) calcd for C$_{14}$H$_{22}$N$_5$O$_2$S: 324.1494. Obsd: 324.1487. [α]$_D$ +18.2° (c 0.950, CHCl$_3$).

(R)-N-Methyl-N-(1-(propylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 79

Yield: 49.0 mg (54.9%). 97.1% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.36 (s, 1H), 8.34 (s, 1H), 7.15 (s, 1H), 6.58 (s, 1H), 5.78 (m, 1H), 3.67 (m, 2H), 3.41 (d, J = 9.3 Hz, 2H), 3.36 (s, 3H), 3.01 (m, 2H), 2.27 (s, 1H), 2.19 (m, 1H), 1.90 (dd, J = 14.5, 7.2 Hz, 2H), 1.09 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.7, 152.1, 150.5, 121.0, 103.5, 101.8, 54.8, 51.5, 48.5, 46.5, 32.3, 28.7, 17.1, 13.3. HRMS (ESI) calcd for C$_{14}$H$_{22}$N$_5$O$_2$S: 324.1494. Obsd: 324.1488. [α]$_D$ +11.3º (*c* 1.52, CHCl$_3$).

(R)-N-Methyl-N-(1-((1-methyl-1H-imidazol-4-yl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 80

Yield: 17.0 mg (22.4%). 98.4% purity by HPLC. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.70 (s, 1H), 8.10 (s, 1H), 7.89 (s, 2H), 7.16 (s, 1H), 6.52 (s, 1H), 5.31 (dd, J = 15.6, 7.8 Hz, 1H), 3.74 (s, 3H), 3.50 (m, 2H), 3.27 (m, 2H), 3.12 (s, 3H), 1.99 (dd, J = 15.6, 8.0 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 156.8, 151.6, 150.3, 140.2, 135.8, 126.1, 121.2, 102.4, 101.4, 54.2, 48.7, 46.9, 33.6, 31.4, 27.7. HRMS (ESI) calcd for C$_{15}$H$_{20}$N$_7$O$_2$S: 362.1399. Obsd: 362.1393. [α]$_D$ +12.5º (*c* 0.477, DMSO).

(R)-N-Methyl-N-(1-(phenylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 81

Yield: 101 mg (84.2%). 99.3% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.18 (s, 1H), 8.26 (s, 1H), 7.87 (dd, J = 7.0, 1.4 Hz, 2H), 7.65 (m, 1H), 7.58 (m, 2H), 7.10 (d, J = 3.0 Hz, 1H), 6.50 (d, J = 2.9 Hz, 1H), 5.60 (m, 1H), 3.64 (dd, J = 12.1, 5.7 Hz, 1H), 3.45 (dd, J = 13.6, 5.3 Hz, 1H), 3.35 (m, 1H), 3.27 (d, J = 2.1 Hz, 3H), 3.12 (dt, J = 16.6, 4.7 Hz, 1H), 2.17 (ddd, J = 12.3, 10.0, 7.7 Hz, 1H), 2.05 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 152.1, 150.5, 135.8, 133.1, 129.3, 127.9, 120.9, 103.4, 101.8, 54.4, 49.3, 47.2,

32.2, 28.5. HRMS (ESI) calcd for $C_{17}H_{20}N_5O_2S$: 358.1338. Obsd: 358.1333. $[\alpha]_D$ -29.2° (*c* 1.21, CHCl₃).

(R)-N-(1-((2-Fluorophenyl)sulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 82

Yield: 96.8 mg (80.6%). 99.4% purity by HPLC. ¹H NMR (400 MHz, CDCl₃) δ 12.58 (s, 1H), 8.29 (s, 1H), 7.91 (t, *J* = 6.8 Hz, 1H), 7.60 (d, *J* = 5.0 Hz, 1H), 7.27 (m, 2H), 7.11 (s, 1H), 6.51 (s, 1H), 5.68 (m, 1H), 3.73 (d, *J* = 8.2 Hz, 1H), 3.63 (t, *J* = 9.1 Hz, 1H), 3.40 (m, 1H), 3.34 (d, *J* = 8.5 Hz, 1H), 3.28 (s, 3H), 2.20 (s, 1H), 2.10 (dd, *J* = 19.9, 9.1 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 160.3, 157.7, 157.5, 152.0, 150.3, 135.2 (d, *J* = 8.4 Hz), 131.4, 125.4 (d, *J* = 14.9 Hz), 124.6 (d, *J* = 3.6 Hz), 121.0, 117.4 (d, *J* = 22.0 Hz), 102.5 (d, *J* = 172.3 Hz), 54.5, 48.4, 46.6, 32.1, 28.5. HRMS (ESI) calcd for $C_{17}H_{19}FN_5O_2S$: 376.1243. Obsd: 376.1236. $[\alpha]_D$ -18.5° (*c* 3.38, CHCl₃).

(R)-N-(1-((3-Fluorophenyl)sulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 83

Yield: 101 mg (84.0%). 100% purity by HPLC. ¹H NMR (400 MHz, CDCl₃) δ 12.64 (s, 1H), 8.27 (s, 1H), 7.65 (d, *J* = 7.5 Hz, 1H), 7.55 (dd, *J* = 14.7, 6.6 Hz, 2H), 7.33 (m, 1H), 7.11 (d, *J* = 2.8 Hz, 1H), 6.49 (d, *J* = 2.9 Hz, 1H), 5.59 (dd, *J* = 14.9, 7.4 Hz, 1H), 3.63 (t, *J* = 7.3 Hz, 1H), 3.47 (t, *J* = 9.3 Hz, 1H), 3.33 (dd, *J* = 10.2, 6.6 Hz, 1H), 3.26 (s, 3H), 3.13 (dd, *J* = 16.7, 9.1 Hz, 1H), 2.15 (m, 1H), 2.06 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 163.8, 161.3, 157.4, 152.0, 150.2, 137.9 (d, *J* = 6.5 Hz), 131.1 (d, *J* = 7.7 Hz), 123.5 (d, *J* = 3.2 Hz), 121.0 (s), 120.2 (d, *J* = 21.2 Hz), 115.0 (d, *J* = 24.1 Hz), 102.5 (d, *J* = 172.4 Hz), 54.3, 49.1, 47.0, 32.1, 28.3. HRMS (ESI) calcd for $C_{17}H_{19}FN_5O_2S$: 376.1243. Obsd: 376.1239. $[\alpha]_D$ -39.8° (*c* 3.38, CHCl₃).

(R)-N-(1-((4-Fluorophenyl)sulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 84

Yield: 93.5 mg (77.8%). 98.0% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.08 (s, 1H), 8.24 (s, 1H), 7.89 (dd, *J* = 8.4, 5.1 Hz, 2H), 7.26 (dd, *J* = 9.2, 7.5 Hz, 2H), 7.09 (d, *J* = 2.7 Hz, 1H), 6.52 (d, *J* = 2.9 Hz, 1H), 5.61 (m, 1H), 3.65 (t, *J* = 7.5 Hz, 1H), 3.38 (dt, *J* = 10.1, 6.9 Hz, 2H), 3.31 (d, *J* = 8.7 Hz, 3H), 3.11 (m, 1H), 2.18 (d, *J* = 4.7 Hz, 1H), 2.08 (dd, *J* = 14.9, 6.4 Hz, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.8, 164.2, 157.6, 152.1, 150.5, 132.1, 130.6 (d, *J* = 9.6 Hz), 120.9, 116.6 (d, *J* = 22.5 Hz), 90.4, 54.4, 49.2, 47.2, 32.3, 28.5. HRMS (ESI) calcd for C$_{17}$H$_{19}$FN$_5$O$_2$S: 376.1243. Obsd: 376.1237. [α]$_D$ -35.9° (*c* 0.670, CHCl$_3$).

(R)-2-((3-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, 85

Yield: 68.8 mg (56.0%). 98.3% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.29 (s, 1H), 8.28 (d, *J* = 3.7 Hz, 1H), 8.11 (dd, *J* = 7.4, 2.8 Hz, 1H), 7.91 (m, 1H), 7.74 (m, 2H), 7.12 (s, 1H), 6.55 (s, 1H), 5.73 (d, *J* = 6.8 Hz, 1H), 3.80 (t, *J* = 8.8 Hz, 1H), 3.67 (td, *J* = 10.1, 3.5 Hz, 1H), 3.43 (m, 2H), 3.32 (d, *J* = 3.5 Hz, 3H), 2.22 (ddd, *J* = 18.3, 11.1, 4.5 Hz, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 152.1, 150.4, 140.7, 135.7, 133.2, 133.0, 130.4, 121.0, 116.5, 110.8, 103.5, 101.8, 54.6, 48.7, 47.2, 32.3, 28.5. HRMS (ESI) calcd for C$_{18}$H$_{19}$N$_6$O$_2$S: 383.1290. Obsd: 383.1287. [α]$_D$ -12.6° (*c* 2.34, CHCl$_3$).

(R)-4-((3-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, 86

Yield: 80.5 mg (65.8%). 100% purity by HPLC. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.66 (s, 1H), 8.09 (m, 2H), 8.03 (d, *J* = 3.0 Hz, 1H), 7.98 (dd, *J* = 4.7, 3.8 Hz, 2H), 7.09 (d, *J* = 1.3 Hz, 1H), 6.43 (s, 1H), 5.24 (m, 1H), 3.44 (m, 2H), 3.17 (ddd, *J* = 18.1, 10.9, 5.2 Hz, 2H), 3.06 (d, *J* = 2.8 Hz, 3H), 1.96

(m, 2H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 156.8, 151.7, 150.4, 139.8, 133.6, 128.2, 121.1, 117.7, 115.6, 102.6, 101.4, 54.1, 48.7, 46.8, 31.7, 27.6. HRMS (ESI) calcd for $C_{18}H_{19}N_6O_2S$: 383.1290. Obsd: 383.1284. [α]$_D$ -10.7° (*c* 2.72, DMSO).

(R)-N-Methyl-N-(1-((2-nitrophenyl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **87**

Yield: 101 mg (78.1%). 97.5% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.92 (s, 1H), 8.30 (s, 1H), 8.05 (dd, *J* = 7.3, 1.7 Hz, 1H), 7.72 (m, 2H), 7.64 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.12 (d, *J* = 3.2 Hz, 1H), 6.56 (d, *J* = 3.3 Hz, 1H), 5.75 (dd, *J* = 15.9, 7.8 Hz, 1H), 3.76 (m, 2H), 3.46 (ddd, *J* = 17.0, 9.8, 7.2 Hz, 2H), 3.32 (s, 3H), 2.26 (m, 1H), 2.18 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.7, 152.2, 150.6, 148.5, 133.9, 131.8, 131.6, 131.1, 124.2, 120.9, 103.5, 101.9, 54.7, 48.6, 47.0, 32.3, 28.7. HRMS (ESI) calcd for $C_{17}H_{19}N_6O_4S$: 403.1188. Obsd: 403.1182. [α]$_D$ +12.6° (*c* 2.17, CHCl$_3$).

(R)-N-Methyl-N-(1-((3-nitrophenyl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **88**

Yield: 104 mg (81.0%). 99.6% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.63 (s, 1H), 8.70 (s, 1H), 8.50 (d, *J* = 7.9 Hz, 1H), 8.22 (s, 1H), 8.19 (d, *J* = 8.1 Hz, 1H), 7.80 (t, *J* = 7.9 Hz, 1H), 7.08 (s, 1H), 6.54 (s, 1H), 5.60 (m, 1H), 3.73 (t, *J* = 7.5 Hz, 1H), 3.50 (dd, *J* = 11.6, 7.1 Hz, 1H), 3.40 (m, 1H), 3.31 (s, 3H), 3.18 (dd, *J* = 16.8, 9.2 Hz, 1H), 2.15 (m, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 152.2, 150.5, 148.6, 138.7, 133.2, 130.7, 127.5, 122.8, 121.0, 103.5, 101.9, 54.4, 49.1, 47.2, 32.5, 28.4. HRMS (ESI) calcd for $C_{17}H_{19}N_6O_4S$: 403.1188. Obsd: 403.1184. [α]$_D$ -41.1° (*c* 1.24, CHCl$_3$).

(R)-N-Methyl-N-(1-((4-nitrophenyl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **89**

Yield: 95.3 mg (74.0%). 99.1% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.99 (s, 1H), 8.42 (d, J = 8.6 Hz, 2H), 8.22 (s, 1H), 8.05 (d, J = 8.6 Hz, 2H), 7.09 (d, J = 2.1 Hz, 1H), 6.52 (d, J = 2.6 Hz, 1H), 5.59 (dt, J = 15.6, 7.9 Hz, 1H), 3.70 (m, 1H), 3.51 (m, 1H), 3.38 (dd, J = 10.2, 7.0 Hz, 1H), 3.30 (s, 3H), 3.18 (dd, J = 16.9, 9.2 Hz, 1H), 2.15 (m, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 152.2, 150.9, 150.5, 142.3, 129.0, 124.6, 120.7, 103.5, 102.1, 54.5, 49.1, 47.2, 32.5, 28.4. HRMS (ESI) calcd for C$_{17}$H$_{19}$N$_6$O$_4$S: 403.1188. Obsd: 403.1185. [α]$_D$ -63.5° (c 0.568, CHCl$_3$).

(R)-N-Methyl-N-(1-(m-tolylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 90

Yield: 101 mg (84.9%). 95.0% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.57 (s, 1H), 8.27 (s, 1H), 7.66 (d, J = 9.2 Hz, 2H), 7.44 (m, 2H), 7.10 (d, J = 3.0 Hz, 1H), 6.48 (d, J = 2.7 Hz, 1H), 5.58 (dt, J = 14.9, 7.4 Hz, 1H), 3.62 (t, J = 7.5 Hz, 1H), 3.44 (m, 1H), 3.33 (dd, J = 10.2, 6.4 Hz, 1H), 3.25 (s, 3H), 3.10 (dd, J = 16.9, 9.1 Hz, 1H), 2.44 (s, 3H), 2.14 (dd, J = 9.7, 5.3 Hz, 1H), 2.03 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.4, 152.0, 150.2, 139.4, 135.5, 133.8, 129.0, 128.1, 124.9, 120.9, 103.3, 101.6, 54.3, 49.2, 47.1, 32.0, 28.4, 21.4. HRMS (ESI) calcd for C$_{18}$H$_{22}$N$_5$O$_2$S: 372.1494. Obsd: 372.1495. [α]$_D$ -39.6° (c 3.34, CHCl$_3$).

(R)-N-Methyl-N-(1-tosylpyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 91

Yield: 87.4 mg (73.5%). 99.4% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.57 (s, 1H), 8.26 (s, 1H), 7.73 (d, J = 6.8 Hz, 2H), 7.35 (d, J = 7.1 Hz, 2H), 7.10 (s, 1H), 6.48 (s, 1H), 5.58 (m, 1H), 3.61 (d, J = 7.6 Hz, 1H), 3.41 (t, J = 9.0 Hz, 1H), 3.31 (dd, J = 8.7, 6.2 Hz, 1H), 3.25 (s, 3H), 3.08 (dd, J = 16.3, 7.7 Hz, 1H), 2.44 (s, 3H), 2.13 (s, 1H), 2.04 (dd, J = 18.2, 9.9 Hz, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.4, 151.9, 150.2, 143.8, 132.5, 129.8, 127.8,

120.8, 103.2, 101.6, 54.2, 49.2, 47.0, 32.0, 28.3, 21.5. HRMS (ESI) calcd for $C_{18}H_{22}N_5O_2S$: 372.1494. Obsd: 372.1490. $[\alpha]_D$ -41.5° (*c* 3.24, CHCl$_3$).

(R)-N-(1-((4-Methoxyphenyl)sulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **92**

Yield: 107 mg (86.2%). 100% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.56 (s, 1H), 8.27 (s, 1H), 7.79 (d, *J* = 6.6 Hz, 2H), 7.10 (s, 1H), 7.02 (d, *J* = 6.6 Hz, 2H), 6.48 (s, 1H), 5.58 (s, 1H), 3.87 (s, 3H), 3.59 (t, *J* = 8.5 Hz, 1H), 3.40 (t, *J* = 9.0 Hz, 1H), 3.31 (d, *J* = 6.3 Hz, 1H), 3.25 (s, 3H), 3.08 (m, 1H), 2.14 (s, 1H), 2.03 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 163.1, 157.4, 151.9, 150.2, 129.9, 127.1, 120.8, 114.3, 103.2, 101.6, 55.6, 54.2, 49.2, 47.0, 32.0, 28.3. HRMS (ESI) calcd for $C_{18}H_{22}N_5O_3S$: 388.1443. Obsd: 388.1441. $[\alpha]_D$ -37.5° (*c* 4.03, CHCl$_3$).

(R)-N-Methyl-N-(1-((4-(trifluoromethyl)phenyl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **93**

Yield: 99.2 mg (72.9%). 99.8% purity by HPLC. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.71 (s, 1H), 8.05 (m, 5H), 7.12 (s, 1H), 6.48 (s, 1H), 5.28 (dd, *J* = 14.4, 7.2 Hz, 1H), 3.51 (s, 1H), 3.43 (dd, *J* = 18.2, 8.2 Hz, 3H), 3.22 (m, 2H), 3.12 (m, 3H), 2.04 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 157.2, 152.1, 150.8, 140.0, 133.2 (q, *J* = 32.4 Hz), 128.9, 127.0 (d, *J* = 3.1 Hz), 123.9 (q, *J* = 272.7 Hz), 121.5, 102.9, 101.7, 54.5, 49.1, 47.2, 32.1, 28.0. HRMS (ESI) calcd for $C_{18}H_{19}F_3N_5O_2S$: 426.1212. Obsd: 426.1204. $[\alpha]_D$ -29.6° (*c* 3.29, CHCl$_3$).

(R)-N-Methyl-N-(1-(naphthalen-2-ylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **94**

Yield: 112 mg (84.7%). 96.8% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.57 (s, 1H), 8.43 (s, 1H), 8.21 (s, 1H), 7.97 (m, 2H), 7.91 (d, *J* =

7.9 Hz, 1H), 7.85 (d, J = 8.6 Hz, 1H), 7.61 (td, J = 15.0, 7.0 Hz, 2H), 7.02 (d, J = 2.5 Hz, 1H), 6.39 (d, J = 2.6 Hz, 1H), 5.52 (m, 1H), 3.68 (m, 1H), 3.50 (t, J = 9.4 Hz, 1H), 3.37 (dd, J = 9.9, 6.7 Hz, 1H), 3.20 (s, 3H), 3.16 (m, 1H), 2.10 (ddd, J = 18.9, 8.3, 6.1 Hz, 1H), 2.00 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.4, 151.9, 150.2, 134.9, 132.8, 132.2, 129.4, 129.2, 129.1, 128.9, 127.9, 127.6, 123.0, 120.8, 103.2, 101.6, 54.3, 49.2, 47.1, 32.0, 28.3. HRMS (ESI) calcd for C$_{21}$H$_{22}$N$_5$O$_2$S: 408.1494. Obsd: 408.1495. [α]$_D$ -40.3° (c 3.97, CHCl$_3$).

(R)-N-Methyl-N-(1-(piperidin-1-ylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 95

Yield: 91.0 mg (77.8%). 98.7% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.65 (s, 1H), 8.36 (s, 1H), 7.15 (s, 1H), 6.58 (s, 1H), 5.78 (m, 1H), 3.60 (m, 2H), 3.35 (s, 3H), 3.34 (m, 2H), 3.26 (d, J = 4.7 Hz, 4H), 2.28 (dd, J = 9.6, 7.4 Hz, 1H), 2.13 (m, 1H), 1.64 (d, J = 4.2 Hz, 4H), 1.56 (d, J = 3.4 Hz, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 152.0, 150.4, 120.9, 103.3, 101.7, 77.5, 77.2, 76.9, 54.6, 49.4, 47.3, 47.1, 32.0, 28.5, 25.5, 23.8. HRMS (ESI) calcd for C$_{16}$H$_{25}$N$_6$O$_2$S: 365.1760. Obsd: 365.1755. [α]$_D$ +13.7° (c 3.06, CHCl$_3$).

(R)-N-Methyl-N-(1-(morpholinosulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 96

Yield: 47.0 mg (40.2%). 96.8% purity by HPLC. ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.37 (s, 1H), 8.35 (s, 1H), 7.15 (s, 1H), 6.58 (s, 1H), 5.79 (m, 1H), 3.76 (m, 4H), 3.65 (m, 2H), 3.38 (m, 2H), 3.36 (s, 3H), 3.28 (m, 4H), 2.27 (dt, J = 10.4, 8.5 Hz, 1H), 2.16 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 157.7, 152.2, 150.5, 121.0, 103.5, 101.8, 66.5, 54.7, 49.4, 47.6, 46.5, 32.2, 28.6. HRMS (ESI) calcd for C$_{15}$H$_{23}$N$_6$O$_3$S: 367.1552. Obsd: 367.1547. [α]$_D$ +12.9° (c 1.53, CHCl$_3$).

In vitro enzyme assays and kinase profiling

All enzyme inhibition assay including kinase profiling results were obtained by commercially available kinase binding activity assay, KinaseProfiler[TM] services (Eurofins Scientific, UK).[83] All kinase binding activity assays were performed at K_m values for ATP. The 50% inhibitory concentration (IC_{50}) of each compound was determined with GraphPad Prism software. The kinome tree of the inhibition percentages of 323 kinases at the 10 μM concentration for (***R***)-**6c** and **12a** was drawn by R. Najmanovich's Kinome Render web accessible tool.[78]

Cell-based functional assays

Phosphorylation of STAT6

THP-1 cells were purchased from ATCC (ATCC TIB-202) and then grown in RPMI-1640 medium (Hyclone) containing 10% fetal bovine serum (FBS) (Hyclone) and 1% penicillin/streptomycin (Hyclone). Cells were pretreated with the indicated compound at 8 concentrations over a 3-fold serial dilution series (0 – 10 μM), 30 μM and 50 μM at 37 °C for 1 h. Cells were then stimulated with IL-4 (10 ng/mL) (Peprotech) at 37 °C for 60 min and fixed in Cytofix/Cytoperm (BD Biosciences) buffer. Thereafter, they were permeabilization in Phosflow perm buffer III (BD Biosciences) on ice for 30 min. After blocking with Fc blocking reagent (Miltenyi Biotec), cells were stained with PE-conjugated mouse anti-human pSTAT6 antibody (BD Phosflow) on ice for 30 min. pSTAT6 was detected by flow cytometry (Beckman, Gallios) after washing three times. All experiments were repeated at least twice to confirm the reproducibility. The 50% inhibitory concentration (IC_{50}) of each compound was determined with GraphPad Prism software.

Proliferation of Ba/F3 cells

Ba/F3 cells are dependent on IL-3 for proliferation. Thus, Ba/F3 cells

were grown and maintained in RPMI containing 10% FBS, mouse IL-3 (Peprotech), and 1% penicillin/streptomycin. Ba/F3 cell lines expressing TEL-JAK1, TEL-JAK2, TEL-JAK3, and TEL-TYK2 are independent of IL-3 for proliferation. These cell lines were grown and maintained in RPMI-1640 medium containing 10% FBS and antibiotics without IL-3. For the cell proliferation assay, each cell line ($1x10^4$/well) was grown in a 96-well plate overnight and then treated with the indicated compound at 10 concentrations over a 3-fold serial dilution series (0 – 10 μM). The cell proliferation was analyzed using the Cell Counting Kit-8 (CCK8) (Dojindo Laboratories) according to the manufacturer's instructions. All experiments were performed twice and the mean 50% inhibitory concentrations (IC_{50}) of each compound were determined with GraphPad Prism software.

Human whole blood tests

The 10 mM stock solutions of test articles in dimethyl sulfoxide were prepared. The solutions of test articles at desired concentrations were obtained through the dilution of stock solutions with 4% dimethyl sulfoxide solution. In a 1.75 mL Eppendorf tube, 90 μL of human whole blood was placed and it was treated with 5 μL of sample solution at the desired concentration. The human whole blood tube was incubated at 37 ℃ for 45 minutes. For the activation of JAK1 or JAK2 signals, 5 μL of IL-6 or GM-CSF, respectively, was added to the human whole blood tube. It was incubated at 37 ℃ for 15 minutes. To the blood tube was added 900 μL of lysis/fix buffer solution which was warmed at 37 ℃ and then it was placed at 37 ℃ for 20 minutes. The blood tube was centrifuged with a 500 xg force at 4 ℃ for 8 minutes. After removing the supernatant, 1 mL of wash buffer (500 mL of Dulbecco's phosphate-buffered saline + 0.5 g of bovine serum albumin + 0.5 g of sodium azide) was added to the tube. Then it was centrifuged with a 500 xg force at 4 ℃ for 8 minutes. The above washing process was carried out once more. After removing the

supernatant, 400 μL of BD phosflow perm buffer cooled at ice bath was added and it was vortexed. The it was placed at ice bath for 30 minutes. The tube was centrifuged with a 500 xg force at 4 °C for 8 minutes. Antibody solutions were prepared: 5.5 mL of BD pharmingen staining buffer + 110 μL of Anti-Human CD4 + 55 μL of pSTAT1 antibody for IL-6/JAK1/pSTAT1 signaling and 5.5 mL of BD pharmingen staining buffer + 110 μL of Anti-Human CD33 + 55 μL of pSTAT5 antibody for GM-CSF/JAK2/pSTAT5 signaling. After removing the supernatant in the blood tube, 250 μL of antibody solution was added and then the tube was placed at 4 °C for overnight. It was analyzed with fluorescence-activated cell sorting (FACS) method.

In vitro ADME assays

All *in vitro* ADME, including plasma stability, plasma protein binding, liver microsomal stability, and Caco-2 permeability assays, and hERG assays were performed by commercially available services at the New Drug Development Center, Daegu-Gyeongbuk Medical Innovation Foundation, South Korea and Drug Discovery Platform Technology Group, Korea Research Institute of Chemical Technology, South Korea.

Plasma stability assay

Human or rat plasma was treated with test articles at 10 μM concentration. Procaine and diltiazem were used for positive controls. The plasma tubes were incubated at 37 °C for 0, 30, and 120 minutes. Acetonitrile including internal standard, chlorpropamide, was added to the tube, which was vortexed and centrifuged with a power of 14,000 rpm at 4 °C. After the centrifugation, the supernatant was analysed by LC-MS/MS, Nexera XR system (Shimadzu, Japan) with TSQ vantage triple quadruple (Thermo, USA). The column was Kinetex XB-C18 column (2.1x100 mm, 2.6 μm particle size;

Phenomenex, USA) and the obtained data were analysed in Xcalibur program (version 1.6.1).

Plasma protein binding test

Rapid equilibrium dialysis (RED) method was used for plasma protein binding test. Positive controls were dexamethasone and warfarin. Human or rat plasma was treated with test articles at 10 μM concentration. The same volumes of the treated plasma and phosphate-buffered saline (PBS, pH 7.4) were placed in RED chamber. The chamber was incubated at 37 °C for 4 hours. The same volumes of the incubated plasma and buffer were sampled and the same volumes of buffer and blank plasma were added, respectively. Acetonitrile including internal standard, chlorpropamide, was added to each sample tube, which was vortexed and centrifuged with a power of 14,000 rpm at 4 °C. After the centrifugation, the supernatant was analysed by LC-MS/MS, Nexera XR system (Shimadzu, Japan) with TSQ vantage triple quadruple (Thermo, USA). The column was Kinetex XB-C18 column (2.1x100 mm, 2.6 μm particle size; Phenomenex, USA) and the obtained data were analysed in Xcalibur program (version 1.6.1).

Liver microsomal stability test

The liver microsome of human, dog, rat, or mouse (0.5 mg/mL), 0.1 M phosphate buffer, and a test article at 1 μM concentration were placed in a tube. Positive control was verapamil. The tube was incubated at 37 °C for 5 minutes. NADPH regeneration system solution was added to the tube, which were incubated at 37 °C for 30 minutes. Acetonitrile including internal standard, chlorpropamide, was added to the tube, which was vortexed and centrifuged with a power of 14,000 rpm at 4 °C. After the centrifugation, the supernatant was analysed by LC-MS/MS, Nexera XR system (Shimadzu, Japan) with TSQ vantage triple quadruple (Thermo, USA). The column was Kinetex XB-C18

column (2.1x100 mm, 2.6 μm particle size; Phenomenex, USA) and the obtained data were analysed in Xcalibur program (version 1.6.1).

Caco-2 permeability assay

In a 12-well transwell, $1x10^6$ cells of Caco-2 cells (ATCC® HTB-37™) were seeded and they were grown for 3 weeks. Test article was diluted to 25 μM concentration with transport buffer (10 mM glucose, 4 mM sodium bicarbonate, and 1 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid in Hank's balanced salt solution, pH 7.4). Positive controls were caffeine, ofloxacin, and atenolol. Of apical and basolateral chamber, test article was added to one chamber and then transport buffer was added to the other. During 60-minute incubation at 37 ºC, samples from each chamber were taken every 15 minutes. The samples were diluted to 5 μM concentration with acetonitrile including internal standard, chlorpropamide. the samples were analysed by LC-MS/MS, Nexera XR system (Shimadzu, Japan) with TSQ vantage triple quadruple (Thermo, USA). The column was Kinetex XB-C18 column (2.1x100 mm, 2.6 μm particle size; Phenomenex, USA) and the obtained data were analysed in Xcalibur program (version 1.6.1).

CYP inhibition test

A cocktail of human liver microsomes (0.25 mg/mL), 0.1 M phosphate buffer, each substrate for CYP_{450} isozymes, and test article at 0 or 10 μM concentration was incubated at 37 ºC for 5 minutes. The cocktails are as follows: Cocktail A, phenacentin 50 μM + coumarin 2.5 μM + *S*-mephenytoin 100 μM + dextromethorphan 5 μM + midazolam 2.5 μM; Cocktail B, bupropion 50 μM + aminodaquine 2.5 μM + tolbutamide 100 μM + chlorzoxazone 50 μM. Then NADPH generation system solution was added and it was incubated at 37 ºC for 15 minutes again. After the incubation, the reaction was quenched with acetonitrile including chlorphopamide as an internal standard. It was centrifuged with a power of 14,000 rpm at 4 ºC. After

the centrifugation, the supernatant was analysed by LC-MS/MS, Nexera XR system (Shimadzu, Japan) with TSQ vantage triple quadruple (Thermo, USA). The column was Kinetex XB-C18 column (2.1x100 mm, 2.6 μm particle size; Phenomenex, USA) and the obtained data were analysed in Xcalibur program (version 1.6.1).

Human ether-a-go-go related gene (hERG) potassium channel assay

hERG assay was performed with automated planar patch clamp method in PatchXpress® 7000A (Molecular Devices, LLC., USA). HERG – HEK293 cells ($2 - 4x10^6$ cells) were placed in a 384 well-plate. Amphotericin B solution was added for perforated patch clamp and then it was placed for 10 minutes. To measure hERG normal current, The HEK293 cell membrane was held at -80 mV, and the current of potassium channel was measured while voltage was changed as follows: -40 mV for 100 milliseconds (ms), +40 mV for 500 ms, and -50 mV for 2 seconds. After measuring normal current with the mentioned method, the HEK293 cells were treated with test article solution at desired concentration. After 5 minutes, the current of potassium channel treated with test article was measured with the aforementioned method.

Pharmacokinetic study

Beagle dogs (10 – 12 kg), Sprague Dawley rats (7 – 8 weeks old) and ICR mice (7 – 8 weeks old) were kept in an environmentally controlled breeding room (25 ± 2 °C, 60 ± 5% humidity, 12h dark/light cycle) with free access to food and water. All groups consisted of four males fed freely for intravenous tests, but had fasted for 16 hours beforehand per oral tests. The dosages for intravenous and per oral tests in dogs, rats, and mice were 5 and 3 mg/kg, 5 and 10 mg/kg, and 5 and 10 mg/kg, respectively. The free base form of (**R**)-**6c** or **12a** was clearly solved under the vehicle condition of 10% ethanol

and 90% PEG400 so the dose volume of 1 mL/kg is for intravenous administration. For oral administration, it became the suspension in corn oil, which has the 5 mL/kg dose volume. The salt forms include hydrochloride, citrate, and tartrate, which were clearly solved in 100% saline so that their dose volumes became 250 and 2500 µL/kg for IV and PO, respectively. After their administrations, the blood samplings were performed at 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours for IV and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours for PO. 20 µL of the sampled plasma was diluted with 180 µL of acetonitrile at an internal standard. It was then vortexed and centrifuged under 15000 rpm at 4 °C. After the centrifugation, the supernatant was analyzed by LC-MS/MS, Nexera XR system (Shimadzu, Japan) with TSQ vantage triple quadruple (Thermo, USA). The column was Kinetex XB-C18 column (2.1x100 mm, 2.6 µm particle size; Phenomenex, USA) and pharmacokinetic parameters were obtained by the non-compartmental analysis model in Phoenix WinNonlin 6.4 version (Pharsight, USA). The animal care and procedure of this study were approved by the Animal Research Care Committee of New Drug Development Center, Daegu-Gyeongbuk Medical Innovation Foundation.

Mouse collagen-induced arthritis

Male DBA1/J mice (6 weeks old) were purchased from Japan SLC, Inc and all mice were housed in specific pathogen-free (SPF) conditions with free access to food and water. After 7 days of acclimation, mice were immunized with 0.1 mL of 1:1 mixture of type II collagen emulsion (2 mg/mL) and complete Freund's adjuvant by subcutaneous injection at 1.5 cm distal from the tail base. After 21 days, immunized mice were boosted by another injection with 0.1 mL of type II collagen emulsion and incomplete Freund's adjuvant. The emulsions were prepared according to manufacturer's instruction.[84] When all mice indicated signs of arthritis, treatment with test articles and assessment of arthritis were initiated (day 1). The immunized and boosted mice were

randomized into 6 treatment groups (n = 10 each) and same-aged naïve mice were assigned to a normal group (n = 5). All test articles or vehicle were orally administered once daily and the clinical arthritis scores were assessed twice weekly for 18 days. Corn oil was used as a vehicle and all test articles were suspended in vehicle. The test article doses were 25, 50, and 100 mg/kg/day for (*R*)-6c, 100 mg/kg/day for **12a**, 100 mg/kg/day for filgotinib, and 50 mg/kg/day for tofacitinib citrate. Paw volumes were measured by LE7500 plethysmometer (Panlab, Spain) on days 1 and 15. The severity of each paw was evaluated and scored according to criteria where 0 = normal; 0.5 = redness of the toe, but not swollen; 1 = one toe inflamed and swollen; 2 = more than one toe, but entire paw, inflamed and swollen, or mild swelling of entire paw; 3 = entire paw inflamed and swollen; and 4 = very inflamed and swollen paw or ankylosed paw.[85] The clinical arthritis score was represented by the total scores of each paw. On day 19, all individuals were sacrificed and autopsies were performed. Serum cytokines including IL-1β, IL-6, MCP-1, and TNF-α were measured by ELISA kits (ProcartaPlex Mix and Match customized, Mouse 5 plex, BMS). For the histopathological studies, the right hind paws of each mouse were fixed by 10% formalin solution and the hematoxylin-eosin staining was performed on the ankle and third digit of the paw. The histopathological score was semiquantitatively measured according to criteria where 0 = normal; 1 = infiltration of inflammatory cells; 2 = synovial hyperplasia and pannus formation; and 3 = bone erosion and destruction.[86] The obtained images were analyzed by iSolution EL ver 9.1 (IMT i-solution Inc., Canada) and the micro-CT analyses of all individuals were performed by viviCT 80 micro-CT (SCANCO Medical, Switzerland) to measure bone surface/volume ratio. Student's *t*-test or one-way analysis of variance test was performed to determine statistically significant differences. The data for clinical arthritis scores were statistically analyzed by the Kruskal-Wallis test or Mann-Whitney test where a significant difference was defined as $P < 0.05$. The experimental protocol of

the mouse study was approved by the Animal Research Care Committee of Gyeonggi Biocenter (Approval No. 2015-11-0019).

Rat adjuvant-induced arthritis

AIA was induced in SPF Lewis LEW/SsNSlc rats (Japan SLC Inc., Japan). After 2 weeks of acclimation, 10 week old rats were immunized by the subcutaneous injection of 0.1 mL of complete Freund's adjuvant containing 10 mg/mL of heat-killed mycobacterium (Chondrex, Inc., USA) at a 2.0 cm distal from the rat tail base. After 12 days of immunization (day 1), the rats were randomized into 6 treatment groups (n = 10 each) and received test articles or vehicles alone once daily for 14 days. Same-aged naïve mice were assigned to a normal group (n = 5). Corn oil was used as a vehicle and test article doses were 5, 10, and 20 mg/kg/day for (**R**)-**6c**, 20 mg/kg/day for **12a**, 20 mg/kg/day for filgotinib, and 10 mg/kg/day for tofacitinib citrate. The clinical arthritis score and paw thicknesses were evaluated twice weekly for 14 days. The criteria for the clinical arthritis score are 0 = normal; 1 = mild edema or erythema; 2 = moderate edema; 3 = severe edema; and 4 = ankylosis. The paw thicknesses were measured by electric caliper CD-15CPX (Mitutoyo Corp., Japan). Kruskal-Wallis test or one-way analysis of variance test was performed to determine statistically significant differences, which were defined as $P < 0.05$. The experimental protocol of the rat study was approved by the Animal Research Care Committee of Qu-BEST BIO, Co., Ltd. (Approval No. QBSIACUC-A17001).

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NMR spectra

Ethyl ((R)-5-((R)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-yl)carbamate, **(R)-2c**

¹H NMR (400 MHz, CDCl₃) δ 7.27 (m, 5H), 5.20 (d, J = 8.9 Hz, 1H), 4.04 (dd, J = 14.2, 7.1 Hz, 2H), 3.82 (ddd, J = 8.7, 5.8, 2.8 Hz, 1H), 3.21 (dd, J = 13.1, 6.5 Hz, 1H), 2.84 (dd, J = 9.6, 6.0 Hz, 1H), 2.78 (d, J = 8.9 Hz, 1H), 2.46 (dd, J = 9.6, 2.5 Hz, 1H), 2.30 (d, J = 8.8 Hz, 1H), 1.34 (d, J = 6.6 Hz, 3H), 1.20 (t, J = 7.1 Hz, 3H), 0.75 (m, 2H), 0.59 (m, 1H), 0.47 (m, 1H).

¹³C NMR (100 MHz, CDCl₃) δ 156.4, 144.8, 128.5, 127.2, 125.5, 77.5, 77.2, 76.9, 65.6, 61.0, 60.8, 60.7, 56.1, 26.4, 22.7, 14.7, 14.2, 8.9.

(R)-N-Methyl-5-((R)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-amine, **(R)-3c**

PROTON_01
LSM-20-64

¹H NMR (400 MHz, CDCl₃) δ 7.27 (m, 5H), 3.22 (q, *J* = 6.6 Hz, 1H), 3.11 (dd, *J* = 9.4, 6.1 Hz, 1H), 2.86 (t, *J* = 5.5 Hz, 1H), 2.61 (d, *J* = 8.9 Hz, 1H), 2.39 (m, 1H), 2.30 (d, *J* = 4.9 Hz, 3H), 2.28 (d, *J* = 9.0 Hz, 1H), 1.37 (d, *J* = 6.6 Hz, 3H), 0.78 (m, 1H), 0.56 (dd, *J* = 8.6, 7.1 Hz, 2H), 0.35 (m, 1H).

CARBON_01
Compound R-2
LSM-20-64

¹³C NMR (100 MHz, CDCl₃) δ 145.4, 128.4, 127.3, 127.0, 66.2, 63.8, 61.9, 59.8, 34.4, 25.9, 23.0, 14.1, 7.3.

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N-Methyl-N-((R)-5-((R)-1-phenylethyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, (R)-4c

PROTON_01
Compound R-3
LSM-12-59

¹H NMR (400 MHz, CDCl₃) δ 12.24 (s, 1H), 8.20 (s, 1H), 7.36 (d, J = 7.1 Hz, 2H), 7.30 (t, J = 7.4 Hz, 2H), 7.22 (dd, J = 13.4, 6.2 Hz, 1H), 7.03 (t, J = 3.7 Hz, 1H), 6.53 (d, J = 3.4 Hz, 1H), 5.52 (s, 1H), 3.49 (s, 3H), 3.18 (dd, J = 13.0, 6.5 Hz, 1H), 2.87 (t, J = 9.5 Hz, 1H), 2.73 (dd, J = 10.5, 2.8 Hz, 1H), 2.45 (d, J = 8.8 Hz, 1H), 1.38 (d, J = 6.5 Hz, 3H), 0.94 (m, 1H), 0.63 (m, 2H), 0.47 (m, 1H).

CARBON_01
Compound R-3
LSM-12-59

¹³C NMR (100 MHz, CDCl₃) δ 158.0, 151.8, 150.6, 145.5, 128.6, 127.1, 127.1, 120.2, 102.7, 102.2, 66.2, 62.1, 59.7, 58.6, 33.8, 24.5, 23.2, 12.6, 11.6.

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(R)-N-Methyl-N-(5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine,
(R)-5c

PROTON_01
Compound R-4
LSM-12-60

¹H NMR (400 MHz, CDCl₃) δ 12.13 (s, 1H), 8.25 (s, 1H), 7.08 (t, *J* = 6.0 Hz, 1H), 6.54 (d, *J* = 3.4 Hz, 1H), 5.35 (m, 1H), 4.52 (s, 1H), 3.64 (dd, *J* = 12.2, 8.2 Hz, 1H), 3.48 (t, *J* = 8.4 Hz, 1H), 3.41 (s, 3H), 3.27 (d, *J* = 10.9 Hz, 1H), 2.96 (m, 1H), 0.91 (d, *J* = 9.8 Hz, 1H), 0.71 (m, 2H), 0.62 (d, *J* = 10.1 Hz, 1H).

CARBON_01
Compound R-4
LSM-12-60

¹³C NMR (100 MHz, CDCl₃) δ 157.9, 151.8, 150.5, 120.7, 103.1, 102.1, 62.4, 56.2, 51.3, 34.8, 25.1, 14.9, 9.4.

(R)-3-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-oxopropanenitrile, **(R)-6c**

PROTON_01
Compound R-6
LSM-97-31

^{1}H NMR (500 MHz, CDCl$_3$) δ 12.16 (s, 1H), 8.26 (d, *J* = 6.0 Hz, 1H), 7.13 (s, 1H), 6.56 (d, *J* = 8.6 Hz, 1H), 5.44 (dd, *J* = 41.1, 5.6 Hz, 1H), 4.26 − 4.00 (m, 1H), 3.98 − 3.70 (m, 2H), 3.58 − 3.32 (m, 6H), 1.16 − 0.94 (m, 1H), 0.82 (dd, *J* = 21.5, 10.3 Hz, 3H).

CARBON_01
Compound R-6
LSM-97-31

^{13}C NMR (100 MHz, CDCl$_3$) δ 159.9, 157.5, 151.9, 150.3, 120.9, 113.8, 103.1, 101.8, 77.4, 77.1, 76.8, 61.5, 54.8, 51.3, 33.5, 25.7, 22.6, 16.7, 8.1.

*3-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-oxopropanenitrile, **6c***

^{1}H NMR (500 MHz, CDCl$_3$) δ 12.09 (s, 1H), 8.26 (d, J = 6.1 Hz, 1H), 7.13 (s, 1H), 6.56 (d, J = 8.9 Hz, 1H), 5.56 − 5.27 (m, 1H), 4.25 − 4.02 (m, 1H), 4.00 − 3.70 (m, 2H), 3.60 − 3.29 (m, 6H), 1.15 − 0.94 (m, 1H), 0.82 (dd, J = 19.5, 9.6 Hz, 3H).

^{13}C NMR (125 MHz, CDCl$_3$) δ 159.5, 157.3, 151.7, 150.1, 120.5, 113.3, 102.8, 101.6, 61.4, 54.6, 52.6, 33.2, 25.4, 22.5, 16.5, 8.0.

(S)-3-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-oxopropanenitrile, **(S)-6c**

PROTON_01
Compound S-5
CYC-63-80C

1H NMR (500 MHz, CDCl₃) δ 12.18 (s, 1H), 8.26 (s, 1H), 7.12 (s, 1H), 6.55 (s, 1H), 5.38 (t, *J* = 37.8 Hz, 1H), 4.10 (d, *J* = 42.3 Hz, 1H), 3.82 (dd, *J* = 74.7, 15.0 Hz, 2H), 3.63 – 3.18 (m, 6H), 1.02 (d, *J* = 32.1 Hz, 1H), 0.78 (s, 3H).

CARBON_01
Compound S-5
CYC-63-80C

13C NMR (125 MHz, CDCl₃) δ 160.0, 157.5, 151.9, 150.2, 120.9, 113.9, 103.1, 101.8, 61.5, 54.8, 51.3, 33.4, 25.7, 22.6, 16.7, 8.1.

*(R)-3-(3-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile, **12a***

PROTON_01
Compound 7
LSM-07-42

¹H NMR (400 MHz, CDCl₃) δ 10.98 (s, 1H), 8.33 (s, 1H), 7.13 (d, *J* = 3.6 Hz, 1H), 6.60 (d, *J* = 3.6 Hz, 1H), 5.76 (m, 1H), 3.90 (dt, *J* = 14.9, 8.0 Hz, 1H), 3.70 (ddd, *J* = 26.4, 16.1, 8.3 Hz, 1H), 3.51 (m, 4H), 3.35 (d, *J* = 14.8 Hz, 3H), 2.27 (m, 2H).

CARBON_01
Compound 7
LSM-07-42

¹³C NMR (100 MHz, CDCl₃) δ 160.3, 157.8, 152.3, 150.9, 120.9, 113.8, 103.6, 102.1, 55.0, 48.0, 45.2, 32.5, 26.9, 26.0.

*(R)-3-(3,3-Dimethyl-4-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile, **12b***

PROTON_01
Compound 8
LSM-117-84

¹H NMR (400 MHz, CDCl₃) δ 12.19 (s, 1H), 8.31 (s, 1H), 7.14 (s, 1H), 6.61 (s, 1H), 5.69 (dd, J = 39.2, 6.0 Hz, 1H), 4.03 (m, 1H), 3.82 (dd, J = 34.5, 12.6 Hz, 1H), 3.54 (m, 3H), 3.41 (m, 1H), 3.34 (d, J = 10.4 Hz, 3H), 1.25 (d, J = 2.8 Hz, 3H), 1.04 (m, 3H).

CARBON_01
Compound 8
LSM-117-84

¹³C NMR (100 MHz, CDCl₃) δ 160.4, 158.0, 152.3, 150.3, 120.8, 113.9, 103.1, 102.2, 62.2, 59.9, 49.1, 44.6, 33.9, 28.1, 26.0, 21.6.

(R)-3-(8-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-6-azaspiro[3.4]octan-6-yl)-3-oxopropanenitrile, **12c**

PROTON_01
Compound 10
JMS-122-18

¹H NMR (400 MHz, CDCl₃) δ 11.84 (s, 1H), 8.36 (s, 1H), 7.15 (s, 1H), 6.62 (s, 1H), 5.96 (dd, *J* = 19.0, 6.3 Hz, 1H), 3.94 (ddd, *J* = 39.2, 19.0, 12.0 Hz, 2H), 3.73 (m, 2H), 3.50 (d, *J* = 5.8 Hz, 2H), 3.28 (d, *J* = 4.9 Hz, 3H), 2.23 (m, 1H), 1.99 (m, 5H).

CARBON_01
Compound 10
JMS-122-18

¹³C NMR (100 MHz, CDCl₃) δ 160.0, 158.2, 152.4, 150.6, 120.8, 113.7, 103.1, 102.3, 62.0, 58.9, 49.9, 47.7, 35.7, 33.6, 26.4, 26.0, 16.3.

(R)-3-(3-(Ethyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile, 13

PROTON_01
Compound 11
JMS-103-95

¹H NMR (400 MHz, DMSO-d6) δ 11.66 (s, 1H), 8.11 (d, *J* = 2.5 Hz, 1H), 7.16 (s, 1H), 6.48 (m, 1H), 5.35 (ddd, *J* = 54.1, 16.5, 8.3 Hz, 1H), 3.95 (dd, *J* = 19.0, 8.5 Hz, 1H), 3.87 (dd, *J* = 18.9, 3.9 Hz, 1H), 3.70 (m, 1H), 3.62 (dd, *J* = 9.2, 6.9 Hz, 3H), 3.45 (dd, *J* = 17.5, 8.8 Hz, 1H), 3.24 (m, 1H), 2.16 (m, 1H), 2.07 (m, 1H), 1.19 (dd, *J* = 11.2, 6.1 Hz, 3H).

CARBON_01
Compound 11
JMS-103-95

¹³C NMR (100 MHz, DMSO-d6) δ 161.3, 156.1, 151.8, 150.5, 121.5, 116.0, 101.7, 101.1, 54.4, 47.4, 44.2, 28.5, 26.8, 25.5, 15.7.

(R)-3-(3-((Cyclopropylmethyl)(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile, **14**

¹H NMR (400 MHz, CDCl₃) δ 12.03 (d, *J* = 14.0 Hz, 1H), 8.35 (d, *J* = 5.1 Hz, 1H), 7.17 (d, *J* = 5.0 Hz, 1H), 6.67 (m, 1H), 5.30 (ddt, *J* = 25.0, 16.8, 8.3 Hz, 1H), 3.93 (m, 2H), 3.63 (m, 3H), 3.49 (m, 3H), 2.33 (ddt, *J* = 17.5, 12.0, 9.6 Hz, 2H), 1.18 (m, 1H), 0.69 (t, *J* = 8.9 Hz, 2H), 0.36 (m, 2H).

¹³C NMR (100 MHz, CDCl₃) δ 160.3, 157.1, 152.3, 150.4, 121.4, 114.0, 103.2, 101.8, 56.6, 50.4, 48.9, 45.1, 29.6, 26.0, 11.9, 5.1, 4.9.

(R)-3-((3-((7H-Pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, **15**

PROTON_01
Compound 13
LSM-108-59-2

^{1}H NMR (400 MHz, DMSO-d6) δ 11.49 (s, 1H), 8.14 (s, 1H), 8.06 (s, 1H), 7.98 (d, *J* = 7.7 Hz, 1H), 7.92 (d, *J* = 7.5 Hz, 1H), 7.58 (t, *J* = 7.7 Hz, 1H), 7.13 (d, *J* = 4.1 Hz, 1H), 7.03 (s, 1H), 6.36 (s, 1H), 4.38 (d, *J* = 4.3 Hz, 1H), 3.49 (m, 2H), 3.31 (m, 1H), 3.25 (m, 1H), 2.06 (dd, *J* = 12.3, 6.2 Hz, 1H), 1.88 (m, 1H).

CARBON_01
Compound 13
LSM-108-59-2

^{13}C NMR (100 MHz, DMSO-d6) δ 155.2, 151.1, 150.2, 137.2, 136.4, 131.6, 130.7, 130.4, 121.0, 117.5, 112.7, 102.6, 98.7, 53.3, 50.0, 46.7, 30.3.

*(R)-3-((3-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, **16***

PROTON_01
Compound 14
JMS-103-28

^{1}H NMR (400 MHz, DMSO-d6) δ 11.68 (s, 1H), 8.32 (d, *J* = 7.8 Hz, 1H), 8.23 (d, *J* = 7.7 Hz, 1H), 8.17 (d, *J* = 8.0 Hz, 1H), 8.06 (t, *J* = 4.6 Hz, 1H), 7.88 (td, *J* = 7.8, 2.2 Hz, 1H), 7.14 (d, *J* = 2.1 Hz, 1H), 6.48 (s, 1H), 5.27 (m, 1H), 3.51 (dd, *J* = 7.6, 4.4 Hz, 1H), 3.44 (m, 1H), 3.36 (s, 3H), 3.23 (m, 1H), 3.17 (s, 1H), 2.03 (dd, *J* = 15.0, 7.6 Hz, 2H).

CARBON_01
Compound 14
JMS-103-28

^{13}C NMR (100 MHz, DMSO-d6) δ 156.8, 151.7, 150.4, 137.1, 136.8, 131.9, 131.0, 130.9, 121.2, 117.6, 112.9, 102.5, 101.3, 54.0, 48.7, 46.8, 31.7, 27.5.

(R)-3-((3-(Ethyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, **17**

PROTON_01
Compound 15
JMS-HW14-32

¹H NMR (400 MHz, CDCl₃) δ 11.64 (s, 1H), 8.17 (d, *J* = 14.8 Hz, 2H), 8.11 (d, *J* = 7.9 Hz, 1H), 7.92 (d, *J* = 7.6 Hz, 1H), 7.72 (t, *J* = 7.8 Hz, 1H), 7.13 (d, *J* = 3.0 Hz, 1H), 6.42 (d, *J* = 3.0 Hz, 1H), 5.23 (m, 1H), 3.71 (m, 4H), 3.33 (m, 1H), 3.26 (m, 1H), 2.20 (m, 2H), 1.34 (t, *J* = 6.9 Hz, 3H).

CARBON_01
Compound 15
JMS-HW14-32

¹³C NMR (100 MHz, CDCl₃) δ 156.5, 152.1, 150.4, 138.6, 136.1, 131.7, 131.2, 130.4, 121.2, 117.4, 114.0, 102.7, 101.5, 55.6, 49.5, 47.0, 40.6, 28.9, 16.0.

(R)-3-((3-((Cyclopropylmethyl)(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, 18

PROTON_01
Compound 16
JMS-103-96

^{1}H NMR (400 MHz, CDCl$_3$) δ 11.91 (s, 1H), 8.19 (s, 1H), 8.11 (d, J = 7.7 Hz, 1H), 8.04 (s, 1H), 7.93 (d, J = 7.4 Hz, 1H), 7.73 (t, J = 7.3 Hz, 1H), 7.15 (s, 1H), 6.60 (s, 1H), 5.05 (m, 1H), 3.66 (m, 2H), 3.60 (d, J = 4.9 Hz, 2H), 3.48 (m, 1H), 3.31 (dd, J = 15.4, 7.6 Hz, 1H), 2.26 (d, J = 7.2 Hz, 2H), 1.12 (s, 1H), 0.65 (d, J = 2.7 Hz, 2H), 0.33 (d, J = 2.1 Hz, 2H).

CARBON_01
Compound 16
JMS-103-96

^{13}C NMR (100 MHz, CDCl$_3$) δ 156.6, 152.0, 149.9, 138.3, 136.0, 131.8, 131.3, 130.3, 121.2, 117.4, 113.9, 103.2, 101.8, 56.9, 51.3, 49.7, 47.4, 29.2, 11.7, 4.8.

(R)-N-(5-Ethyl-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 19

PROTON_01
Compound 6
LSM-104-1-2

^{1}H NMR (400 MHz, CDCl$_3$) δ 11.99 (s, 1H), 8.24 (s, 1H), 7.06 (d, *J* = 3.2 Hz, 1H), 6.57 (s, 1H), 5.58 (s, 1H), 3.46 (d, *J* = 2.8 Hz, 3H), 3.08 (s, 1H), 2.94 (s, 1H), 2.80 (d, *J* = 5.9 Hz, 1H), 2.58 (d, *J* = 5.4 Hz, 2H), 1.19 – 1.12 (m, 3H), 0.90 (dd, *J* = 21.8, 5.3 Hz, 2H), 0.68 (s, 2H), 0.50 (d, *J* = 5.7 Hz, 1H).

CARBON_01
Compound 6
LSM-104-1-2

^{13}C NMR (100 MHz, CDCl$_3$) δ 157.9, 151.8, 150.5, 120.0, 102.6, 102.0, 63.3, 59.9, 58.3, 50.4, 33.9, 24.1, 13.4, 13.2, 10.4.

PROTON_01
Compound 7
LSM-97-100

¹H NMR (400 MHz, CDCl₃) δ 11.46 (s, 1H), 8.24 (s, 1H), 7.06 (d, J = 3.5 Hz, 1H), 6.58 (d, J = 3.4 Hz, 1H), 5.55 (s, 1H), 3.48 (s, 3H), 3.02 (s, 2H), 2.86 (s, 1H), 2.67 – 2.40 (m, 3H), 1.65 – 1.47 (m, 2H), 1.38 (dq, J = 14.4, 7.3 Hz, 2H), 0.94 (t, J = 7.3 Hz, 4H), 0.69 (s, 2H), 0.52 (t, J = 10.2 Hz, 1H).

CARBON_01
Compound 7
LSM-97-100

¹³C NMR (100 MHz, CDCl₃) δ 157.8, 151.9, 150.7, 119.9, 102.6, 102.2, 63.4, 60.0, 58.6, 56.2, 34.1, 30.3, 24.0, 20.6, 14.0, 13.1, 10.6.

(R)-N-(5-Benzyl-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **21**

¹H NMR (400 MHz, CDCl₃) δ 11.52 (s, 1H), 8.21 (s, 1H), 7.44 – 7.21 (m, 5H), 7.03 (d, *J* = 3.5 Hz, 1H), 6.56 (d, *J* = 3.4 Hz, 1H), 5.57 (s, 1H), 3.64 (dd, *J* = 31.2, 12.9 Hz, 2H), 3.52 (s, 3H), 3.01 – 2.87 (m, 2H), 2.76 (d, *J* = 8.9 Hz, 1H), 2.51 (d, *J* = 8.9 Hz, 1H), 1.01 – 0.90 (m, 1H), 0.70 – 0.56 (m, 2H), 0.51 – 0.40 (m, 1H).

¹³C NMR (100 MHz, CDCl₃) δ 157.9, 151.8, 150.7, 138.9, 128.6, 128.3, 127.0, 119.7, 102.2, 63.3, 60.6, 59.1, 33.7, 24.4, 12.5, 11.2.

(R)-2-Azido-1-(7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)ethan-1-one, **22**

PROTON_01
Compound 9
LSM-97-71

^{1}H NMR (500 MHz, CDCl$_3$) δ 11.80 (s, 1H), 8.23 (d, *J* = 22.5 Hz, 1H), 7.10 (d, *J* = 17.0 Hz, 1H), 6.56 (s, 1H), 5.55 − 5.22 (m, 1H), 4.17 − 3.94 (m, 1H), 3.90 (d, *J* = 16.4 Hz, 2H), 3.81 − 3.47 (m, 2H), 3.38 (dd, *J* = 33.1, 19.3 Hz, 4H), 1.03 (d, *J* = 46.0 Hz, 1H), 0.91 − 0.58 (m, 3H).

CARBON_01
Compound 9
LSM-97-71

^{13}C NMR (125 MHz, CDCl$_3$) δ 165.7, 157.5, 151.9, 150.4, 120.8, 103.1, 101.8, 61.7, 54.5, 51.6, 51.0, 33.4, 22.4, 16.8, 8.1.

(R)-3-Methyl-1-(7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)butan-1-one, **23**

PROTON_01
Compound 10
LSM-97-75

¹H NMR (500 MHz, CDCl₃) δ 12.06 (d, *J* = 16.4 Hz, 1H), 8.27 (d, *J* = 8.9 Hz, 1H), 7.11 (d, *J* = 9.6 Hz, 1H), 6.56 (d, *J* = 9.9 Hz, 1H), 5.37 (t, *J* = 38.6 Hz, 1H), 4.05 (ddd, *J* = 43.9, 27.1, 11.7 Hz, 2H), 3.82 (dd, *J* = 66.1, 11.9 Hz, 1H), 3.65 (dd, *J* = 178.8, 11.3 Hz, 1H), 3.45 − 3.33 (m, 3H), 2.20 (d, *J* = 13.8 Hz, 3H), 0.99 (s, 6H), 0.78 (t, *J* = 21.2 Hz, 4H).

CARBON_01
Compound 10
LSM-97-75

¹³C NMR (125 MHz, CDCl₃) δ 171.0, 157.5, 151.8, 150.2, 120.4, 102.8, 101.6, 61.6, 55.1, 52.5, 43.3, 33.1, 25.4, 24.8, 22.6, 16.6, 8.0.

*(R)-2-Methyl-1-(7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)propan-1-one, **24***

¹H NMR (500 MHz, CDCl₃) δ 12.06 (d, *J* = 17.4 Hz, 1H), 8.27 (d, *J* = 9.1 Hz, 1H), 7.11 (d, *J* = 10.0 Hz, 1H), 6.56 (d, *J* = 10.8 Hz, 1H), 5.37 (dd, *J* = 42.3, 38.0 Hz, 1H), 4.23 – 3.74 (m, 3H), 3.43 (t, *J* = 21.8 Hz, 4H), 2.79 – 2.56 (m, 1H), 1.16 (dd, *J* = 9.8, 5.6 Hz, 6H), 1.10 – 0.94 (m, 1H), 0.79 (td, *J* = 25.2, 8.8 Hz, 3H).

¹³C NMR (125 MHz, CDCl₃) δ 175.4, 157.2, 151.9, 150.3, 120.4, 102.9, 101.7, 61.6, 54.2, 50.3, 32.8, 32.0, 24.9, 18.7, 16.8, 8.0.

(R)-Cyclopropyl(7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)methanone, **25**

PROTON_01
Compound 12
LSM-97-95

¹H NMR (500 MHz, CDCl₃) δ 12.28 (d, J = 15.5 Hz, 1H), 8.28 (d, J = 10.3 Hz, 1H), 7.12 (d, J = 10.0 Hz, 1H), 6.56 (d, J = 14.6 Hz, 1H), 5.44 (d, J = 15.0 Hz, 1H), 4.40 – 4.24 (m, 1H), 4.11 – 3.92 (m, 2H), 3.74 (dd, J = 141.4, 10.9 Hz, 2H), 3.45 (t, J = 21.3 Hz, 3H), 1.63 (d, J = 40.6 Hz, 1H), 1.16 – 0.94 (m, 3H), 0.80 (s, 4H).

CARBON_01
Compound 12
LSM-97-95

¹³C NMR (125 MHz, CDCl₃) δ 172.1, 157.7, 152.0, 150.4, 120.6, 103.0, 101.8, 61.5, 54.5, 52.3, 33.2, 22.9, 16.8, 12.4, 12.3, 7.7.

167

(R)-N-(2-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-2-oxoethyl)acetamide, **26**

PROTON_01
Compound 13
JMS-103-14B

^{1}H NMR (400 MHz, CDCl$_3$) δ 12.07 (s, 1H), 8.26 (s, 1H), 7.30 (s, 1H), 7.12 (s, 1H), 6.55 (s, 1H), 5.43 (d, J = 11.0 Hz, 1H), 4.09 (d, J = 15.0 Hz, 2H), 3.88 (ddd, J = 47.9, 37.2, 16.8 Hz, 2H), 3.45 (dd, J = 42.9, 13.8 Hz, 3H), 2.04 (s, 3H), 1.26 (s, 2H), 1.15 – 0.96 (m, 1H), 0.96 – 0.61 (m, 3H).

CARBON_01
Compound 13
JMS-103-14B

^{13}C NMR (100 MHz, CDCl$_3$) δ 170.5, 167.0, 157.5, 151.9, 150.2, 120.9, 103.1, 101.7, 61.3, 54.4, 51.2, 42.0, 33.3, 24.6, 22.8, 16.6, 8.1.

(R)-N-Methyl-3-(7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-oxopropanamide, **27**

PROTON_01
Compound 14
CYC-96-55

¹H NMR (400 MHz, CDCl₃) δ 11.96 (d, *J* = 30.6 Hz, 1H), 8.24 (dd, *J* = 6.6, 1.8 Hz, 1H), 8.13 (s, 1H), 7.11 (s, 1H), 6.54 (s, 1H), 5.51 − 5.34 (m, 1H), 4.13 (ddd, *J* = 21.2, 12.6, 7.5 Hz, 1H), 4.00 − 3.80 (m, 2H), 3.49 (t, *J* = 11.6 Hz, 1H), 3.40 (d, *J* = 14.5 Hz, 3H), 3.35 (t, *J* = 19.8 Hz, 2H), 2.83 (dd, *J* = 4.5, 2.1 Hz, 3H), 1.11 − 0.94 (m, 1H), 0.90 − 0.68 (m, 3H).

CARBON_01
Compound 14
CYC-96-55

¹³C NMR (100 MHz, CDCl₃) δ 167.0, 166.7, 157.5, 152.0, 150.4, 120.8, 103.0, 101.7, 59.3, 55.6, 52.7, 41.3, 33.3, 26.1, 22.9, 16.3, 8.1.

(R)-1-(4-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carbonyl)piperidin-1-yl)ethan-1-one, **28**

¹H NMR (400 MHz, CDCl₃) δ 12.29 (s, 1H), 8.36 − 8.21 (m, 1H), 7.13 (dd, *J* = 9.8, 2.7 Hz, 1H), 6.62 − 6.51 (m, 1H), 5.54 − 5.31 (m, 1H), 4.62 (dd, *J* = 17.9, 8.6 Hz, 1H), 4.19 (dd, *J* = 9.8, 6.5 Hz, 1H), 4.11 − 3.76 (m, 3H), 3.54 − 3.46 (m, 1H), 3.46 − 3.33 (m, 3H), 3.19 − 3.01 (m, 1H), 2.75 − 2.51 (m, 2H), 2.17 − 2.02 (m, 3H), 1.97 − 1.63 (m, 4H), 1.15 − 0.95 (m, 1H), 0.92 − 0.71 (m, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 172.6, 168.8, 157.5, 152.0, 150.3, 120.8, 103.1, 101.7, 61.9, 58.9, 50.6, 45.7, 40.9, 40.2, 33.4, 24.9, 21.4, 16.0, 8.1.

(R)-Furan-2-yl(7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)methanone, 29

PROTON_01
Compound 16
JMS-103-10

¹H NMR (400 MHz, CDCl₃) δ 11.49 (s, 1H), 8.28 (s, 1H), 7.52 (s, 1H), 7.15 (d, J = 2.5 Hz, 1H), 7.10 (d, J = 3.4 Hz, 1H), 6.58 (d, J = 2.6 Hz, 1H), 6.51 (s, 1H), 5.48 (s, 1H), 4.60 – 4.41 (m, 1H), 4.25 (d, J = 10.0 Hz, 2H), 4.18 – 4.02 (m, 1H), 3.72 (dd, J = 70.6, 11.6 Hz, 1H), 3.44 (s, 2H), 1.06 (s, 1H), 0.83 (dd, J = 22.6, 14.3 Hz, 3H).

CARBON_01
Compound 16
JMS-103-10

¹³C NMR (100 MHz, CDCl₃) δ 157.8, 157.6, 151.5, 150.2, 144.4, 144.3, 120.5, 116.6, 111.5, 102.9, 102.1, 61.8, 58.5, 51.8, 33.3, 25.5, 16.7, 8.0.

171

(R)-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)(phenyl)methanone, **30**

PROTON_01
Compound 17
JMS-103-39

¹H NMR (400 MHz, CDCl₃) δ 12.45 (s, 1H), 8.25 (d, J = 25.1 Hz, 1H), 7.54 (s, 2H), 7.39 (s, 2H), 7.28 (s, 1H), 7.06 (s, 1H), 6.53 (d, J = 21.5 Hz, 1H), 5.45 (d, J = 74.9 Hz, 1H), 4.40 – 3.97 (m, 2H), 3.89 – 3.54 (m, 2H), 3.42 (d, J = 20.4 Hz, 3H), 1.01 (s, 1H), 0.79 (dd, J = 26.4, 14.3 Hz, 2H), 0.63 (s, 1H).

CARBON_01
Compound 17
JMS-103-39

¹³C NMR (100 MHz, CDCl₃) δ 169.5, 157.5, 151.9, 150.1, 136.1, 130.1, 128.4, 127.0, 120.7, 102.9, 101.7, 61.4, 57.5, 50.6, 33.2, 22.9, 16.5, 8.5.

(R)-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)(pyridin-3-yl)methanone, 31

PROTON_01
Compound 18
LSM-97-90

^{1}H NMR (500 MHz, CDCl$_3$) δ 11.87 (s, 1H), 8.83 (s, 1H), 8.68 (d, J = 12.5 Hz, 1H), 8.25 (d, J = 31.9 Hz, 1H), 7.90 (s, 1H), 7.47 – 7.31 (m, 1H), 7.10 (d, J = 12.4 Hz, 1H), 6.56 (d, J = 27.7 Hz, 1H), 5.40 (dd, J = 73.5, 68.8 Hz, 1H), 4.37 – 4.03 (m, 2H), 3.94 – 3.59 (m, 2H), 3.43 (t, J = 15.8 Hz, 3H), 1.05 (d, J = 8.0 Hz, 1H), 0.96 – 0.73 (m, 2H), 0.67 (s, 1H).

CARBON_01
Compound 18
LSM-97-90

^{13}C NMR (100 MHz, CDCl$_3$) δ 166.9, 157.5, 152.0, 151.1, 150.4, 148.0, 135.0, 131.9, 123.4, 120.6, 103.0, 101.8, 61.5, 54.5, 50.7, 33.4, 22.6, 16.5, 8.4.

173

(R)-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)(pyridin-4-yl)methanone, 32

PROTON_01
Compound 19
LSM-97-91

^{1}H NMR (500 MHz, CDCl$_3$) δ 12.14 (s, 1H), 8.72 (d, *J* = 15.1 Hz, 2H), 8.25 (d, *J* = 31.9 Hz, 1H), 7.41 (s, 2H), 7.11 (d, *J* = 12.7 Hz, 1H), 6.55 (d, *J* = 24.5 Hz, 1H), 5.46 (d, *J* = 111.2 Hz, 1H), 4.19 (dt, *J* = 26.7, 13.8 Hz, 2H), 3.64 (d, *J* = 12.1 Hz, 1H), 3.56 (ddd, *J* = 220.1, 10.6 Hz, 1H), 3.44 (d, *J* = 14.5 Hz, 3H), 1.04 (d, *J* = 6.5 Hz, 1H), 0.95 – 0.72 (m, 2H), 0.67 (s, 1H).

CARBON_01
Compound 19
LSM-97-91

^{13}C NMR (125 MHz, CDCl$_3$) δ 166.9, 157.5, 152.0, 150.4, 150.3, 143.5, 121.1, 120.7, 103.0, 101.7, 61.4, 57.2, 50.6, 33.4, 22.6, 16.6, 8.5.

(R)-3-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carbonyl)benzonitrile, 33

¹H NMR (400 MHz, CDCl₃) δ 12.28 (s, 1H), 8.27 (d, J = 23.4 Hz, 1H), 7.85 (s, 1H), 7.79 (d, J = 7.2 Hz, 1H), 7.76 – 7.64 (m, 1H), 7.62 – 7.46 (m, 1H), 7.11 (d, J = 5.6 Hz, 1H), 6.56 (d, J = 18.0 Hz, 1H), 5.46 (d, J = 82.3 Hz, 1H), 4.39 – 4.02 (m, 2H), 3.65 (t, J = 12.3 Hz, 1H), 3.58 (dd, J = 185.8, 10.4 Hz, 1H), 3.44 (d, J = 14.8 Hz, 3H), 1.05 (d, J = 10.3 Hz, 1H), 0.97 – 0.74 (m, 2H), 0.68 (s, 1H).

¹³C NMR (100 MHz, CDCl₃) δ 166.9, 157.5, 152.0, 150.2, 137.3, 133.5, 131.4, 130.8, 129.5, 120.8, 117.9, 112.8, 103.1, 101.7, 61.5, 54.5, 50.8, 33.4, 22.6, 16.6, 8.4.

*(R)-4-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carbonyl)benzonitrile, **34***

PROTON_01
Compound 21
LSM-97-74

¹H NMR (500 MHz, CDCl₃) δ 12.35 (s, 1H), 8.26 (d, *J* = 32.1 Hz, 1H), 7.78 – 7.67 (m, 2H), 7.64 (d, *J* = 6.7 Hz, 2H), 7.11 (d, *J* = 12.4 Hz, 1H), 6.55 (d, *J* = 25.3 Hz, 1H), 5.45 (d, *J* = 116.1 Hz, 1H), 4.19 (dt, *J* = 98.3, 17.9 Hz, 2H), 3.64 (d, *J* = 12.2 Hz, 1H), 3.55 (dd, *J* = 225.6, 10.4 Hz, 1H), 3.44 (d, *J* = 17.0 Hz, 3H), 1.03 (d, *J* = 6.0 Hz, 1H), 0.97 – 0.72 (m, 2H), 0.66 (s, 1H).

CARBON_01
Compound 21
LSM-97-74

¹³C NMR (125 MHz, CDCl₃) δ 167.4, 157.5, 152.0, 150.2, 140.3, 132.4, 127.8, 120.8, 118.0, 113.8, 103.0, 101.7, 61.5, 57.3, 50.7, 33.4, 22.6, 16.6, 8.5.

176

*(R)-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)(2-(trifluoromethyl)phenyl)methanone, **35***

PROTON_01
Compound 22
LSM-104-15

¹H NMR (400 MHz, CDCl₃) δ 12.19 (s, 1H), 8.30 (d, *J* = 32.9 Hz, 1H), 7.71 (dd, *J* = 13.4, 7.9 Hz, 1H), 7.66 – 7.56 (m, 1H), 7.52 (dt, *J* = 14.8, 7.6 Hz, 1H), 7.42 (d, *J* = 7.4 Hz, 1H), 7.09 (dd, *J* = 9.7, 3.5 Hz, 1H), 6.57 (dd, *J* = 18.0, 3.1 Hz, 1H), 5.69 – 5.31 (m, 1H), 4.07 (ddd, *J* = 19.1, 12.6, 7.8 Hz, 1H), 3.87 (dd, *J* = 163.0, 12.6 Hz, 1H), 4.18 – 3.35 (m, 1H), 3.45 (s, 3H), 3.31 (dd, *J* = 158.2, 10.8 Hz, 1H), 1.04 (dd, *J* = 11.7, 6.7 Hz, 1H), 0.94 – 0.68 (m, 2H), 0.68 – 0.56 (m, 1H).

CARBON_01
Compound 22
LSM-104-15

¹³C NMR (100 MHz, CDCl₃) δ 167.0, 157.5, 151.8, 150.0, 135.4 (d, *J* = 26.1 Hz), 132.3, 129.3 (d, *J* = 4.1 Hz), 127.1 (d, *J* = 12.0 Hz), 126.9 – 126.6 (m), 123.6 (q, *J* = 273.8 Hz), 120.7, 103.2, 101.9, 61.2, 56.6, 50.2, 33.3, 24.8, 16.8, 8.3.

177

(R)-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)(3-(trifluoromethyl)phenyl)methanone, **36**

PROTON_01
Compound 23
LSM-104-14-B

¹H NMR (400 MHz, CDCl₃) δ 11.67 (s, 1H), 8.27 (s, 1H), 7.82 (s, 1H), 7.71 (dd, *J* = 17.7, 7.8 Hz, 2H), 7.62 – 7.46 (m, 1H), 7.09 (s, 1H), 6.58 (d, *J* = 20.8 Hz, 1H), 5.48 (d, *J* = 77.8 Hz, 1H), 4.39 – 4.01 (m, 2H), 3.66 (t, *J* = 13.7 Hz, 1H), 3.59 (dd, *J* = 173.8, 10.5 Hz, 1H), 3.46 (d, *J* = 18.1 Hz, 3H), 1.05 (d, *J* = 10.5 Hz, 1H), 0.98 – 0.73 (m, 2H), 0.68 (s, 1H).

CARBON_01
Compound 23
LSM-104-14-B

¹³C NMR (100 MHz, CDCl₃) δ 167.8, 157.5, 151.4, 149.9, 136.8, 131.0 (q, *J* = 32.8 Hz), 130.4, 129.1, 126.9, 124.1, 123.6 (q, *J* = 273.0 Hz), 120.7, 103.4, 102.0, 61.5, 54.5, 50.7, 33.4, 22.8, 16.6, 8.4.

178

(R)-3-(7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)-3-thioxopropanenitrile, **37**

PROTON_01
Compound 24
CYC-96-60

1H NMR (400 MHz, CDCl3) δ 12.07 (s, 1H), 8.27 (d, *J* = 3.4 Hz, 1H), 7.20 – 7.08 (m, 1H), 6.57 (dd, *J* = 5.7, 3.7 Hz, 1H), 5.45 (tt, *J* = 186.7, 93.9 Hz, 1H), 4.42 – 4.25 (m, 1H), 4.20 (dd, *J* = 131.3, 13.0 Hz, 1H), 4.01 (dd, *J* = 162.4, 14.4 Hz, 1H), 3.98 – 3.85 (m, 2H), 3.90 (dd, *J* = 211.1, 11.8 Hz, 1H), 3.44 (t, *J* = 19.7 Hz, 3H), 1.16 – 1.00 (m, 1H), 0.97 – 0.74 (m, 3H).

CARBON_01
Compound 24
CYC-96-60

13C NMR (100 MHz, CDCl3) δ 184.8, 157.4, 152.0, 150.2, 121.0, 114.1, 103.2, 101.8, 62.3, 59.1, 57.0, 34.2, 33.7, 25.2, 16.9, 8.1.

179

Isobutyl (R)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxylate, 38

PROTON_01
Compound 25
LSM-97-72

¹H NMR (500 MHz, CDCl₃) δ 12.56 (s, 1H), 8.28 (s, 1H), 7.11 (s, 1H),
6.55 (s, 1H), 5.41 (s, 1H), 4.08 – 3.97 (m, 1H), 3.91 (d, *J* = 4.7 Hz, 2H),
3.85 – 3.66 (m, 2H), 3.42 (s, 3H), 3.39 – 3.29 (m, 1H), 2.02 – 1.87 (m, 1H),
1.01 (s, 1H), 0.94 (s, 6H), 0.75 (d, *J* = 9.4 Hz, 3H).

CARBON_01
Compound 25
LSM-97-72

¹³C NMR (125 MHz, CDCl₃) δ 157.7, 154.9, 152.0, 150.3, 120.6, 102.9, 101.8, 71.4, 61.0, 54.6, 51.1, 33.0,
28.0, 23.6, 19.1, 16.5, 8.1.

(R)-N-Butyl-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, **39**

^{1}H NMR (400 MHz, CDCl$_3$) δ 12.31 (s, 1H), 8.26 (s, 1H), 7.10 (s, 1H), 6.54 (s, 1H), 5.38 (d, *J* = 5.5 Hz, 1H), 4.46 (s, 1H), 3.98 (dd, *J* = 10.4, 7.6 Hz, 1H), 3.71 (dd, *J* = 31.5, 10.3 Hz, 2H), 3.41 (s, 3H), 3.33 (d, *J* = 9.8 Hz, 1H), 3.26 (d, *J* = 5.1 Hz, 2H), 1.50 (dt, *J* = 14.5, 7.2 Hz, 2H), 1.34 (td, *J* = 14.5, 7.2 Hz, 2H), 0.99 (d, *J* = 7.7 Hz, 1H), 0.92 (t, *J* = 7.2 Hz, 3H), 0.85 (s, 1H), 0.75 (s, 2H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 156.6, 151.5, 150.0, 120.7, 102.9, 101.9, 61.0, 54.4, 51.1, 40.4, 33.1, 32.5, 24.1, 20.0, 16.7, 13.8, 8.1.

(R)-N-Cyclohexyl-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, **40**

PROTON_01
Compound 27
CYC-96-79

^{1}H NMR (400 MHz, CDCl$_3$) δ 12.25 (s, 1H), 8.28 (s, 1H), 7.10 (d, J = 2.1 Hz, 1H), 6.56 (s, 1H), 5.39 (d, J = 5.8 Hz, 1H), 4.18 (d, J = 7.6 Hz, 1H), 3.97 (dd, J = 10.8, 7.5 Hz, 1H), 3.66 (t, J = 10.8 Hz, 2H), 3.53 (dd, J = 163.5, 9.8 Hz, 2H), 3.43 (s, 3H), 1.97 (d, J = 11.4 Hz, 2H), 1.70 (d, J = 9.8 Hz, 2H), 1.60 (d, J = 12.8 Hz, 1H), 1.36 (td, J = 14.2, 2.5 Hz, 2H), 1.22 – 1.04 (m, 3H), 1.03 – 0.93 (m, 1H), 0.76 (s, 3H).

CARBON_01
Compound 27
CYC-96-79

^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 155.9, 151.5, 150.0, 120.6, 103.0, 101.9, 61.0, 54.4, 51.1, 49.2, 34.1, 33.1, 25.6, 25.0, 24.0, 16.7, 8.1.

(R)-7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-phenyl-5-azaspiro[2.4]heptane-5-carboxamide, **41**

PROTON_01
Compound 28
CYC-96-73

^{1}H NMR (400 MHz, CDCl$_3$) δ 11.63 (s, 1H), 8.29 (s, 1H), 7.44 (d, *J* = 7.2 Hz, 2H), 7.29 (s, 2H), 7.09 (s, 1H), 7.02 (t, *J* = 7.2 Hz, 1H), 6.52 (d, *J* = 45.2 Hz, 2H), 5.43 (s, 1H), 4.11 (s, 1H), 3.84 (dd, *J* = 21.3, 10.0 Hz, 2H), 3.68 – 3.25 (m, 1H), 3.46 (s, 3H), 1.04 (d, *J* = 8.6 Hz, 1H), 0.95 – 0.69 (m, 3H).

CARBON_01
Compound 28
CYC-96-73

^{13}C NMR (100 MHz, CDCl$_3$) δ 157.5, 153.7, 150.9, 149.6, 138.8, 128.9, 123.1, 120.7, 119.7, 103.1, 102.2, 61.2, 54.6, 51.4, 33.3, 24.0, 16.8, 8.2.

(R)-N-(4-Fluorophenyl)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, **42**

PROTON_01
Compound 29
LSM-104-24

¹H NMR (400 MHz, DMSO-d6) δ 12.03 (s, 1H), 8.41 – 8.28 (m, 1H), 8.18 (s, 1H), 7.52 (dd, *J* = 6.9, 5.2 Hz, 2H), 7.26 (s, 1H), 7.07 (t, *J* = 7.9 Hz, 2H), 6.72 (s, 1H), 5.20 (s, 1H), 4.03 (dd, *J* = 11.1, 7.4 Hz, 1H), 3.80 (t, *J* = 10.6 Hz, 2H), 3.49 (s, 1H), 3.37 (s, 3H), 0.95 (d, *J* = 10.2 Hz, 1H), 0.84 (d, *J* = 13.6 Hz, 2H), 0.68 (d, *J* = 10.1 Hz, 1H).

CARBON_01
Compound 29
LSM-104-24

¹³C NMR (100 MHz, DMSO-d6) δ 157.7 (d, *J* = 237.8 Hz), 154.2, 149.9, 148.6, 137.1 (d, *J* = 2.4 Hz), 122.2, 121.5 (d, *J* = 7.6 Hz), 115.2 (d, *J* = 22.0 Hz), 102.9, 102.5, 61.6, 54.5, 51.5, 33.6, 24.2, 16.5, 8.2.
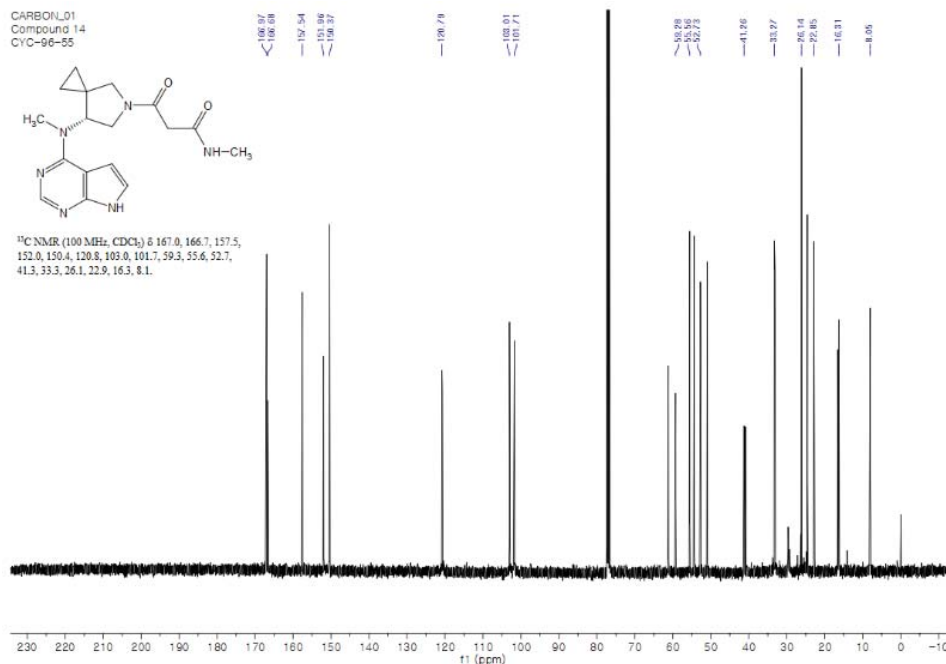
184

(R)-N-(2,4-Dichlorophenyl)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, **43**

PROTON_01
Compound 30
LSM-104-19

¹H NMR (400 MHz, CDCl₃) δ 12.01 (s, 1H), 8.28 (d, *J* = 8.9 Hz, 2H), 7.40 – 7.31 (m, 1H), 7.22 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.13 (d, *J* = 2.6 Hz, 1H), 6.82 (s, 1H), 6.59 (s, 1H), 5.53 (d, *J* = 6.3 Hz, 1H), 4.16 (dd, *J* = 10.9, 7.5 Hz, 1H), 3.87 (dd, *J* = 23.7, 10.4 Hz, 2H), 3.48 (s, 3H), 3.42 (s, 1H), 1.08 (d, *J* = 10.3 Hz, 1H), 0.84 (s, 3H).

CARBON_01
Compound 30
LSM-104-19

¹³C NMR (100 MHz, CDCl₃) δ 157.6, 152.6, 151.5, 150.0, 134.4, 128.3, 127.9, 127.4, 122.3, 121.2, 120.7, 103.0, 102.0, 60.7, 54.4, 51.1, 33.2, 24.1, 16.8, 8.2.

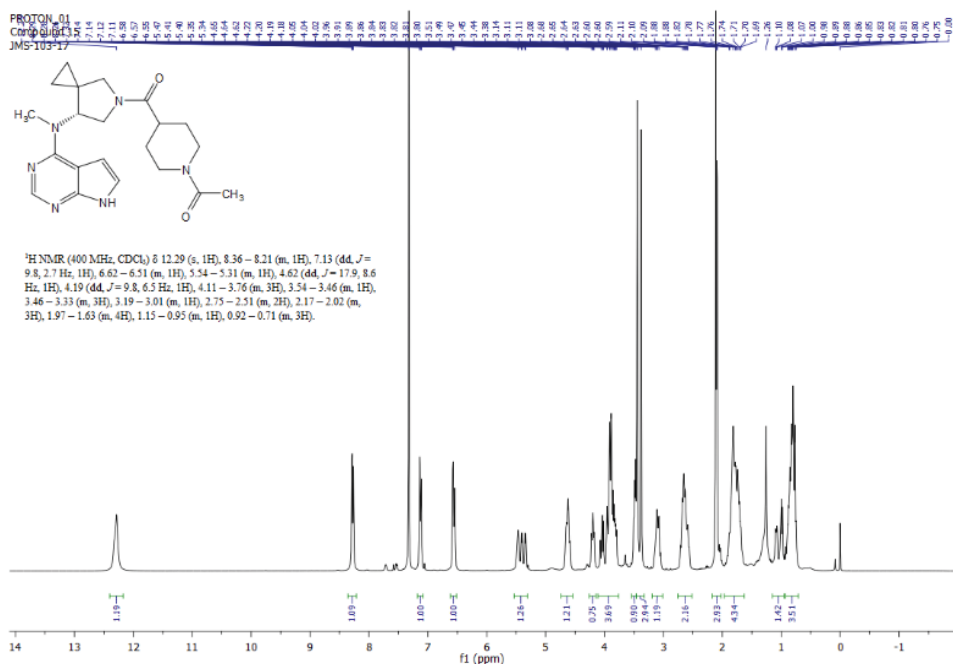*(R)-N-(3,4-Dichlorophenyl)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, 44*

PROTON_01
Compound 31
JMS-103-20

¹H NMR (400 MHz, CDCl₃) δ 11.85 (d, *J* = 18.4 Hz, 1H), 8.24 (s, 1H), 7.64 (s, 1H), 7.26 (d, *J* = 4.1 Hz, 2H), 7.07 (d, *J* = 2.2 Hz, 1H), 6.72 (d, *J* = 12.8 Hz, 1H), 6.52 (d, *J* = 2.7 Hz, 1H), 5.37 (d, *J* = 4.8 Hz, 1H), 4.05 (dd, *J* = 10.6, 7.0 Hz, 1H), 3.79 (dd, *J* = 25.3, 10.5 Hz, 2H), 3.40 (s, 4H), 1.00 (s, 1H), 0.82 (d, *J* = 42.1 Hz, 3H).

CARBON_01
Compound 31
JMS-103-20

¹³C NMR (100 MHz, CDCl₃) δ 157.6, 153.2, 151.8, 150.3, 138.5, 132.4, 130.2, 125.9, 121.2, 120.6, 118.9, 103.0, 101.9, 61.0, 54.6, 51.5, 33.2, 23.8, 16.8, 8.2.

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(R)-N-(2,5-Dichlorophenyl)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, 45

PROTON_01
Compound 32
JMS-103-21

^{1}H NMR (400 MHz, CDCl$_3$) δ 12.32 (s, 1H), 8.36 (d, J = 53.7 Hz, 2H), 7.12 (s, 1H), 7.07 (dd, J = 124.5, 8.5 Hz, 2H), 6.88 (s, 1H), 6.57 (s, 1H), 5.53 (d, J = 5.8 Hz, 1H), 4.24 – 4.07 (m, 1H), 3.87 (dd, J = 21.7, 10.3 Hz, 2H), 3.48 (s, 1H), 3.46 (s, 3H), 1.07 (d, J = 8.7 Hz, 1H), 0.83 (s, 3H).

CARBON_01
Compound 32
JMS-103-21

^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 152.4, 152.1, 150.3, 136.5, 133.5, 129.3, 122.9, 120.7, 120.2, 119.9, 103.0, 101.8, 60.6, 54.4, 51.0, 33.2, 24.1, 16.7, 8.1.

(R)-N-(2,3-Dichlorophenyl)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, 46

PROTON_01
Compound 33
CYC-96-82

¹H NMR (400 MHz, CDCl₃) δ 12.36 (s, 1H), 8.45 – 8.16 (m, 2H), 7.23 – 7.06 (m, 3H), 6.97 (s, 1H), 6.58 (s, 1H), 5.54 (d, J = 5.8 Hz, 1H), 4.17 (dd, J = 10.8, 7.6 Hz, 1H), 3.88 (dd, J = 19.3, 10.4 Hz, 2H), 3.50 (s, 1H), 3.47 (s, 3H), 1.07 (d, J = 8.0 Hz, 1H), 0.86 (d, J = 18.6 Hz, 3H).

CARBON_01
Compound 33
CYC-96-82

¹³C NMR (100 MHz, CDCl₃) δ 157.6, 152.6, 151.7, 150.0, 137.3, 132.3, 127.7, 123.7, 120.8, 120.4, 118.4, 103.1, 101.9, 60.7, 54.4, 51.1, 33.2, 24.2, 16.7, 8.2.

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(R)-N-(3-Chloro-4-methylphenyl)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, 47

^{1}H NMR (400 MHz, CDCl$_3$) δ 12.26 (s, 1H), 8.24 (s, 1H), 7.49 (s, 1H), 7.20 (d, J = 6.6 Hz, 1H), 7.13 – 6.98 (m, 2H), 6.87 (s, 1H), 6.49 (s, 1H), 5.36 (s, 1H), 4.04 (s, 1H), 3.79 (d, J = 8.2 Hz, 2H), 3.42 (d, J = 32.5 Hz, 1H), 3.36 (s, 3H), 2.25 (s, 3H), 0.97 (d, J = 8.3 Hz, 1H), 0.72 (s, 3H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 157.5, 153.8, 151.5, 150.0, 137.9, 134.1, 130.7, 130.3, 120.7, 120.5, 118.4, 103.0, 101.9, 61.0, 54.5, 51.3, 33.2, 23.9, 19.3, 16.6, 8.1.

(R)-N-([1,1'-Biphenyl]-2-yl)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carboxamide, **48**

Feb19-2014
Compound 35
CYC-96-83
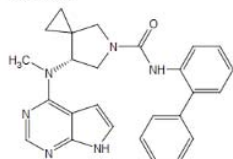
¹H NMR (400 MHz, CDCl₃) δ 11.27 (s, 1H), 8.24 (s, 1H), 8.20 (d, J = 8.3 Hz, 1H), 7.51 – 7.30 (m, 6H), 7.21 (d, J = 7.2 Hz, 1H), 7.10 (dd, J = 9.2, 5.5 Hz, 2H), 6.55 (d, J = 2.9 Hz, 1H), 6.38 (s, 1H), 5.34 (d, J = 6.4 Hz, 1H), 3.82 (dd, J = 10.9, 7.5 Hz, 1H), 3.64 (d, J = 10.0 Hz, 1H), 3.48 (d, J = 10.1 Hz, 1H), 3.38 (s, 3H), 3.20 (d, J = 9.9 Hz, 1H), 1.04 – 0.95 (m, 1H), 0.78 – 0.59 (m, 3H).
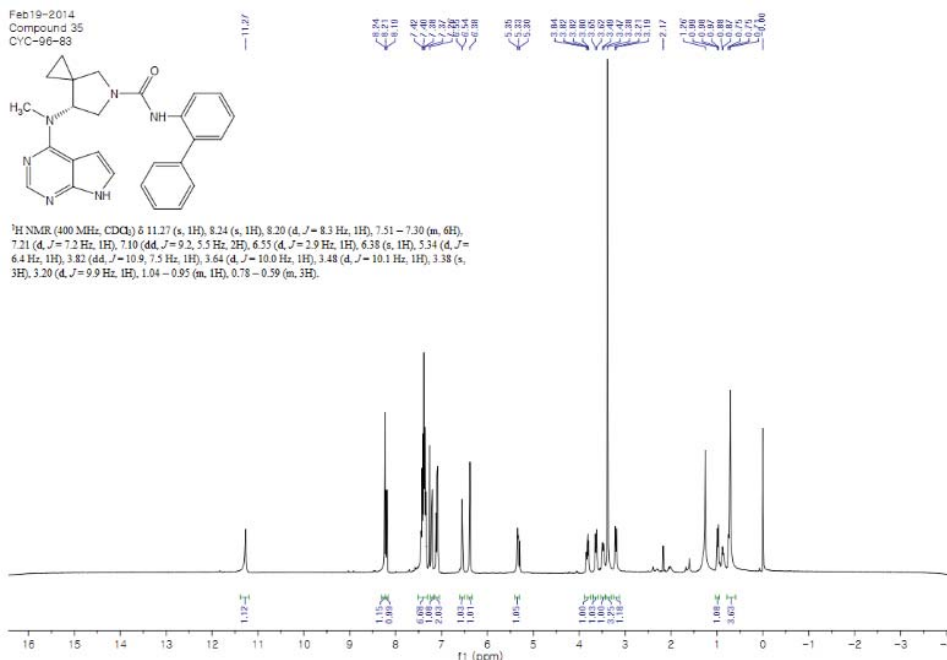
CARBON_01
Compound 35
CYC-96-82
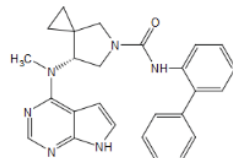
¹³C NMR (100 MHz, CDCl₃) δ 157.4, 153.4, 149.6, 148.6, 138.5, 135.8, 131.5, 129.6, 129.2, 129.1, 128.5, 128.0, 123.0, 120.9, 120.6, 102.5, 61.1, 54.2, 50.9, 31.9, 22.7, 16.8, 8.1.

(R)-N-(3,5-Bis(trifluoromethyl)phenyl)-7-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptane-5-carbothioamide, **49**
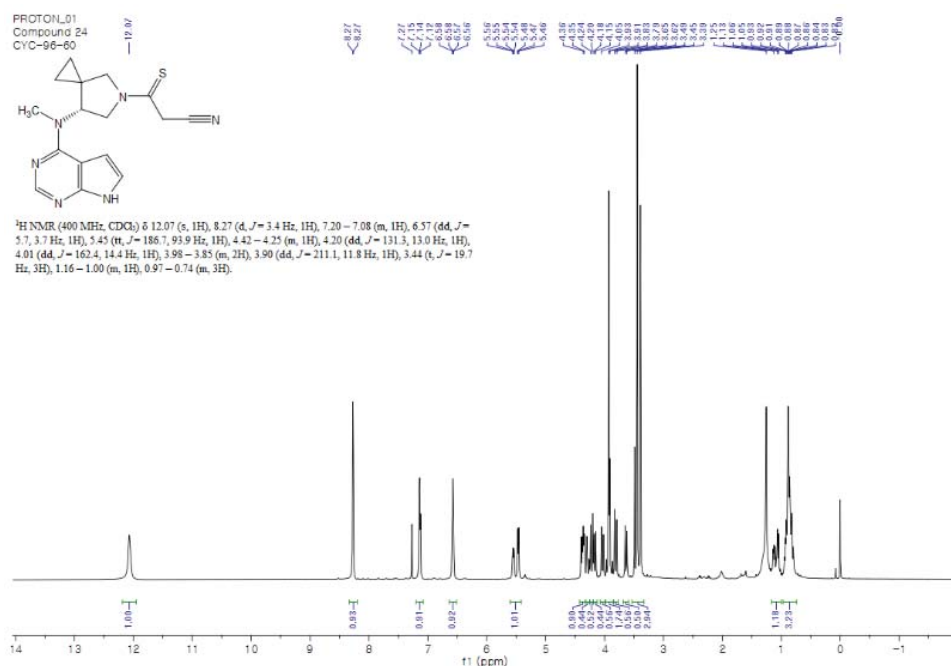
PROTON_01
Compound 36
CYC-96-80

^{1}H NMR (400 MHz, CDCl$_3$) δ 12.02 (s, 1H), 8.27 (s, 1H), 7.97 (s, 2H), 7.69 (s, 1H), 7.62 (s, 1H), 7.08 (s, 1H), 6.55 (s, 1H), 5.42 (s, 1H), 4.30 (s, 1H), 4.15 (s, 2H), 3.72 (s, 1H), 3.43 (s, 3H), 1.03 (s, 1H), 0.83 (s, 3H).

CARBON_01
Compound 36
CYC-96-80

^{13}C NMR (100 MHz, CDCl$_3$) δ 177.8, 157.5, 151.4, 149.7, 140.7, 131.6 (q, *J* = 33.6 Hz), 124.8, 123.0 (q, *J* = 272.9 Hz), 120.9, 118.6, 103.4, 102.0, 61.0, 55.3, 33.4, 29.7, 23.3, 16.8, 8.0.

(R)-N-(5-(Ethylsulfonyl)-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **50**

PROTON_01
Compound 37
LSM-97-66

¹H NMR (500 MHz, CDCl₃) δ 12.42 (s, 1H), 8.26 (s, 1H), 7.12 (s, 1H), 6.56 (s, 1H), 5.55 (s, 1H), 3.92 (t, *J* = 9.1 Hz, 1H), 3.68 (d, *J* = 9.9 Hz, 2H), 3.48 (s, 3H), 3.33 (d, *J* = 9.8 Hz, 1H), 3.14 – 3.03 (m, 2H), 1.42 (t, *J* = 7.0 Hz, 3H), 1.03 (d, *J* = 9.9 Hz, 1H), 0.77 (d, *J* = 11.9 Hz, 2H), 0.73 (d, *J* = 10.9 Hz, 1H).

CARBON_01
Compound 37
LSM-97-66

¹³C NMR (125 MHz, CDCl₃) δ 157.7, 152.0, 150.2, 120.7, 103.0, 101.8, 60.5, 55.9, 52.4, 44.2, 33.5, 24.4, 15.4, 9.2, 7.9.

(R)-N-(5-(Isopropylsulfonyl)-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **51**

PROTON_01
Compound 38
LSM-97-76

¹H NMR (500 MHz, CDCl₃) δ 12.17 (s, 1H), 8.26 (s, 1H), 7.11 (s, 1H), 6.57 (s, 1H), 5.51 (s, 1H), 3.99 (t, J = 8.2 Hz, 1H), 3.71 (d, J = 8.7 Hz, 2H), 3.48 (s, 3H), 3.38 (d, J = 9.6 Hz, 1H), 3.28 (d, J = 4.9 Hz, 1H), 1.40 (s, 6H), 1.03 (d, J = 8.7 Hz, 1H), 0.85 – 0.69 (m, 3H).

CARBON_01
Compound 38
LSM-97-76

¹³C NMR (125 MHz, CDCl₃) δ 157.6, 151.9, 150.3, 120.5, 102.9, 101.8, 60.6, 56.4, 53.4, 52.9, 33.5, 24.4, 16.6, 15.5, 8.9.

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(R)-N-Methyl-N-(5-(propylsulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **52**

PROTON_01
Compound 39
LSM-104-09-1

¹H NMR (400 MHz, CDCl₃) δ 12.17 (s, 1H), 8.29 (s, 1H), 7.12 (d, *J* = 3.3 Hz, 1H), 6.59 (s, 1H), 5.65 – 5.46 (m, 1H), 3.91 (dd, *J* = 10.9, 7.6 Hz, 1H), 3.77 – 3.61 (m, 2H), 3.49 (s, 3H), 3.32 (d, *J* = 9.8 Hz, 1H), 3.11 – 2.94 (m, 2H), 1.91 (dd, *J* = 15.4, 7.6 Hz, 2H), 1.09 (t, *J* = 7.4 Hz, 3H), 1.07 – 0.98 (m, 1H), 0.76 (dt, *J* = 11.3, 9.9 Hz, 3H).

CARBON_01
Compound 39
LSM-104-09-1

¹³C NMR (100 MHz, CDCl₃) δ 157.7, 151.8, 150.1, 120.7, 103.1, 101.9, 60.5, 55.8, 52.3, 51.3, 33.6, 24.4, 17.0, 15.4, 13.1, 9.2.

(R)-N-Methyl-N-(5-(phenylsulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **53**

PROTON_01
Compound 40
LSM-97-68

¹H NMR (500 MHz, CDCl₃) δ 12.22 (s, 1H), 8.11 (s, 1H), 7.75 (s, 2H), 7.56 (s, 1H), 7.48 (s, 2H), 7.00 (s, 1H), 6.42 (s, 1H), 5.34 (s, 1H), 3.51 (s, 2H), 3.43 (d, J = 8.4 Hz, 1H), 3.24 (s, 3H), 2.99 (d, J = 8.3 Hz, 1H), 0.79 (s, 1H), 0.64 (s, 1H), 0.52 (s, 2H).

CARBON_01
Compound 40
LSM-97-68

¹³C NMR (125 MHz, CDCl₃) δ 157.6, 152.0, 150.2, 135.1, 133.0, 129.1, 127.9, 120.6, 102.9, 101.8, 59.9, 56.0, 52.7, 33.3, 23.9, 14.5, 9.6.

*(R)-N-(5-((2-Fluorophenyl)sulfonyl)-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **54***

PROTON_01
Compound 41
LSM-104-05-1

¹H NMR (400 MHz, CDCl₃) δ 11.84 (s, 1H), 8.21 (s, 1H), 7.91 (t, J = 7.3 Hz, 1H), 7.68 – 7.56 (m, 1H), 7.39 – 7.20 (m, 2H), 7.09 (d, J = 3.4 Hz, 1H), 6.54 (d, J = 3.0 Hz, 1H), 5.47 (d, J = 5.4 Hz, 1H), 3.82 (dd, J = 10.9, 7.8 Hz, 1H), 3.77 – 3.67 (m, 1H), 3.48 (dd, J = 160.2, 9.9 Hz, 2H), 3.41 (s, 3H), 0.98 – 0.89 (m, 1H), 0.76 (dt, J = 13.1, 6.5 Hz, 1H), 0.72 – 0.61 (m, 2H).

CARBON_01
Compound 41
LSM-104-05-1

¹³C NMR (100 MHz, CDCl₃) δ 159.1 (d, J = 255.8 Hz), 157.6, 151.7, 150.2, 135.2 (d, J = 8.4 Hz), 131.5, 125.0 (d, J = 14.8 Hz), 124.5 (d, J = 3.8 Hz), 120.6, 117.3 (d, J = 22.0 Hz), 102.9, 102.0, 60.3, 55.7, 52.3, 33.4, 24.2, 14.9, 9.3.

(R)-N-(5-((3-Fluorophenyl)sulfonyl)-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 55

PROTON_01
Compound 42
LSM-104-03

^{1}H NMR (400 MHz, CDCl$_3$) δ 11.90 (s, 1H), 8.20 (s, 1H), 7.67 – 7.62 (m, 1H), 7.56 (dt, *J* = 13.4, 6.6 Hz, 2H), 7.36 (ddd, *J* = 10.0, 5.3, 1.5 Hz, 1H), 7.10 (d, *J* = 3.5 Hz, 1H), 6.53 (d, *J* = 3.4 Hz, 1H), 5.44 (t, *J* = 5.0 Hz, 1H), 3.62 (d, *J* = 5.2 Hz, 2H), 3.37 (s, 3H), 3.33 (dd, *J* = 185.2, 9.6 Hz, 2H), 0.96 – 0.85 (m, 1H), 0.77 (dd, *J* = 10.2, 4.9 Hz, 1H), 0.69 – 0.54 (m, 2H).

CARBON_01
Compound 42
LSM-104-03

^{13}C NMR (100 MHz, CDCl$_3$) δ 162.5 (d, *J* = 252.1 Hz), 157.6, 151.7, 150.1, 137.4 (d, *J* = 6.5 Hz), 130.9 (d, *J* = 7.7 Hz), 123.6 (d, *J* = 3.4 Hz), 120.6, 120.2 (d, *J* = 21.2 Hz), 115.1 (d, *J* = 24.1 Hz), 102.9, 101.9, 60.0, 56.0, 52.8, 33.4, 23.9, 14.6, 9.7.

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(R)-N-(5-((4-Fluorophenyl)sulfonyl)-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **56**

PROTON_01
Compound 43
LSM-97-81

¹H NMR (500 MHz, CDCl₃) δ 12.06 (s, 1H), 8.11 (d, *J* = 3.9 Hz, 1H), 7.78 (ddd, *J* = 8.8, 4.6, 2.0 Hz, 2H), 7.22 – 7.11 (m, 2H), 7.02 (d, *J* = 3.5 Hz, 1H), 6.44 (d, *J* = 3.4 Hz, 1H), 5.35 (s, 1H), 3.51 (d, *J* = 5.1 Hz, 2H), 3.44 (d, *J* = 9.6 Hz, 1H), 3.28 (s, 3H), 2.98 (d, *J* = 9.6 Hz, 1H), 0.87 – 0.77 (m, 1H), 0.71 – 0.61 (m, 1H), 0.60 – 0.48 (m, 2H).

CARBON_01
Compound 43
LSM-97-81

¹³C NMR (100 MHz, CDCl₃) δ 165.3 (d, *J* = 255.3 Hz), 157.6, 151.9, 150.2, 131.4, 130.5 (d, *J* = 9.3 Hz), 120.6, 116.4 (d, *J* = 22.5 Hz), 102.9, 101.9, 60.0, 56.0, 52.7, 33.4, 23.9, 14.6, 9.7.

(R)-2-((7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)sulfonyl)benzonitrile, 57

PROTON_01
Compound 44
LSM-97-80

¹H NMR (500 MHz, CDCl₃) δ 12.05 (s, 1H), 8.12 (s, 1H), 8.00 (d, J = 7.5 Hz, 1H), 7.83 (d, J = 7.3 Hz, 1H), 7.66 (dt, J = 22.3, 7.4 Hz, 2H), 7.02 (d, J = 3.1 Hz, 1H), 6.45 (d, J = 3.0 Hz, 1H), 5.41 (d, J = 4.8 Hz, 1H), 3.74 (dd, J = 10.9, 7.7 Hz, 1H), 3.68 (d, J = 9.8 Hz, 1H), 3.61 (dd, J = 11.0, 2.7 Hz, 1H), 3.32 (s, 3H), 3.29 (d, J = 9.8 Hz, 1H), 0.88 (t, J = 7.0 Hz, 1H), 0.72 (dd, J = 8.7, 5.5 Hz, 1H), 0.69 – 0.57 (m, 2H).

CARBON_01
Compound 44
LSM-97-80

¹³C NMR (100 MHz, CDCl₃) δ 157.6, 151.9, 150.2, 140.0, 135.6, 133.0, 132.9, 130.3, 120.7, 116.4, 110.9, 103.0, 101.8, 60.3, 56.1, 52.6, 33.5, 24.1, 14.9, 9.3.

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*(R)-3-((7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)sulfonyl)benzonitrile, **58***

PROTON_01
Compound 45
LSM-97-79
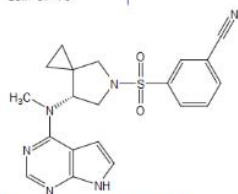
¹H NMR (500 MHz, CDCl₃) δ 12.03 (s, 1H), 8.19 (s, 1H), 8.14 (s, 1H), 8.06 (d, J = 7.9 Hz, 1H), 7.91 (d, J = 7.7 Hz, 1H), 7.70 (t, J = 7.8 Hz, 1H), 7.11 (d, J = 2.8 Hz, 1H), 6.52 (d, J = 2.8 Hz, 1H), 5.40 (s, 1H), 3.65 (t, J = 5.1 Hz, 2H), 3.60 (d, J = 9.8 Hz, 1H), 3.36 (s, 3H), 3.10 (d, J = 9.7 Hz, 1H), 0.90 (dd, J = 9.8, 4.4 Hz, 1H), 0.83 – 0.74 (m, 1H), 0.73 – 0.58 (m, 2H).
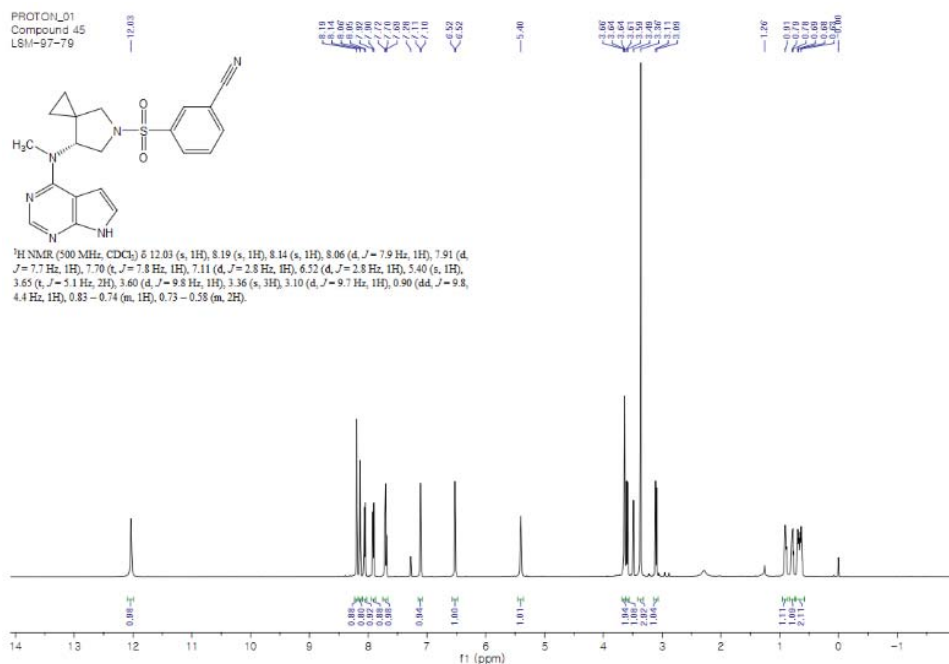
CARBON_01
Compound 45
LSM-97-79
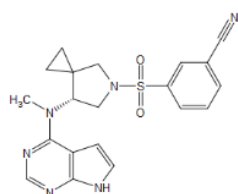
¹³C NMR (100 MHz, CDCl₃) δ 157.5, 151.9, 150.2, 137.5, 136.0, 131.6, 131.2, 130.2, 120.7, 117.1, 113.8, 103.0, 101.8, 60.1, 56.1, 52.8, 33.5, 23.8, 14.8, 9.6.

(R)-4-((7-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-5-azaspiro[2.4]heptan-5-yl)sulfonyl)benzonitrile, **59**

Jul28-2017
Compound 46
LSM-20-63

¹H NMR (400 MHz, CDCl₃) δ 10.59 (s, 1H), 8.18 (s, 1H), 8.00 – 7.90 (m, 2H), 7.89 – 7.81 (m, 2H), 7.08 (dd, *J* = 3.5, 1.8 Hz, 1H), 6.52 (d, *J* = 2.4 Hz, 1H), 5.36 (dd, *J* = 5.7, 4.4 Hz, 1H), 3.65 (s, 1H), 3.64 (d, *J* = 1.9 Hz, 1H), 3.59 (d, *J* = 9.7 Hz, 1H), 3.34 (s, 3H), 3.10 (d, *J* = 9.7 Hz, 1H), 0.91 (ddd, *J* = 10.2, 6.2, 4.0 Hz, 1H), 0.82 – 0.75 (m, 1H), 0.73 – 0.59 (m, 2H).

CARBON_01
Compound 46
LSM-20-63

¹³C NMR (100 MHz, CDCl₃) δ 157.5, 151.9, 150.2, 134.0, 132.9, 128.3, 120.8, 117.2, 116.7, 103.0, 101.8, 60.2, 56.0, 52.9, 33.5, 23.8, 14.9, 9.5.

(R)-N-Methyl-N-(5-((2-nitrophenyl)sulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 60

PROTON_01
Compound 47
JMS-103-24

¹H NMR (400 MHz, CDCl₃) δ 12.38 (s, 1H), 8.23 (s, 1H), 8.00 (d, *J* = 7.3 Hz, 1H), 7.69 (dd, *J* = 16.0, 7.9 Hz, 2H), 7.62 (d, *J* = 7.3 Hz, 1H), 7.10 (s, 1H), 6.53 (s, 1H), 5.50 (d, *J* = 6.2 Hz, 1H), 4.03 – 3.87 (m, 1H), 3.75 (d, *J* = 9.6 Hz, 2H), 3.39 (s, 3H), 3.38 – 3.27 (m, 1H), 0.96 (d, *J* = 5.5 Hz, 1H), 0.91 – 0.59 (m, 3H).

CARBON_01
Compound 47
JMS-103-24

¹³C NMR (100 MHz, CDCl₃) δ 157.6, 152.0, 150.2, 148.4, 133.9, 131.6, 130.8, 124.1, 120.7, 103.0, 101.8, 60.4, 55.9, 52.6, 33.4, 24.2, 15.1, 9.2.

(R)-N-Methyl-N-(5-((3-nitrophenyl)sulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **61**

PROTON_01
Compound 48
JMS-103-25

¹H NMR (400 MHz, CDCl₃) δ 10.49 (s, 1H), 8.67 (d, J = 1.4 Hz, 1H), 8.49 (d, J = 8.2 Hz, 1H), 8.16 (d, J = 3.4 Hz, 2H), 7.78 (td, J = 8.1, 3.3 Hz, 1H), 7.06 (s, 1H), 6.52 (s, 1H), 5.47 – 5.32 (m, 1H), 3.79 – 3.57 (m, 3H), 3.37 (d, J = 3.2 Hz, 3H), 3.14 (d, J = 9.7 Hz, 1H), 0.90 (dd, J = 6.2, 3.2 Hz, 1H), 0.84 – 0.74 (m, 1H), 0.74 – 0.58 (m, 2H).

CARBON_01
Compound 48
JMS-103-25

¹³C NMR (100 MHz, CDCl₃) δ 157.5, 152.0, 150.6, 148.4, 138.1, 133.2, 130.5, 127.4, 122.7, 120.3, 102.8, 102.1, 60.1, 56.1, 52.9, 33.6, 23.8, 14.8, 9.6.

(R)-N-Methyl-N-(5-((4-nitrophenyl)sulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 62

PROTON_01
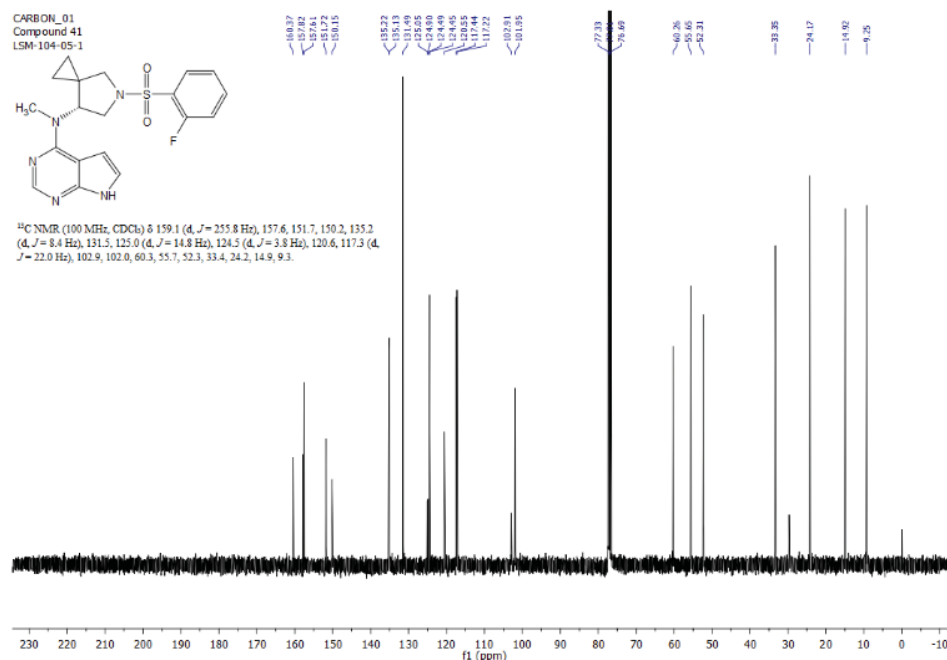Compound 49
JMS-103-26

¹H NMR (400 MHz, CDCl₃) δ 11.61 (s, 1H), 8.50 – 8.30 (m, 2H), 8.19 (d, *J* = 3.4 Hz, 1H), 8.02 (s, 2H), 7.09 (s, 1H), 6.50 (s, 1H), 5.36 (d, *J* = 3.5 Hz, 1H), 3.67 (s, 2H), 3.65 – 3.56 (m, 1H), 3.36 (d, *J* = 3.5 Hz, 3H), 3.19 – 3.05 (m, 1H), 0.83 – 0.75 (m, 1H), 0.67 (dd, *J* = 14.9, 9.8 Hz, 3H).

CARBON_01
Compound 49
JMS-103-26

¹³C NMR (100 MHz, CDCl₃) δ 157.5, 152.0, 150.3, 150.2, 141.6, 128.9, 124.3, 120.6, 103.0, 101.9, 60.2, 56.1, 52.9, 33.5, 23.8, 14.9, 9.5.
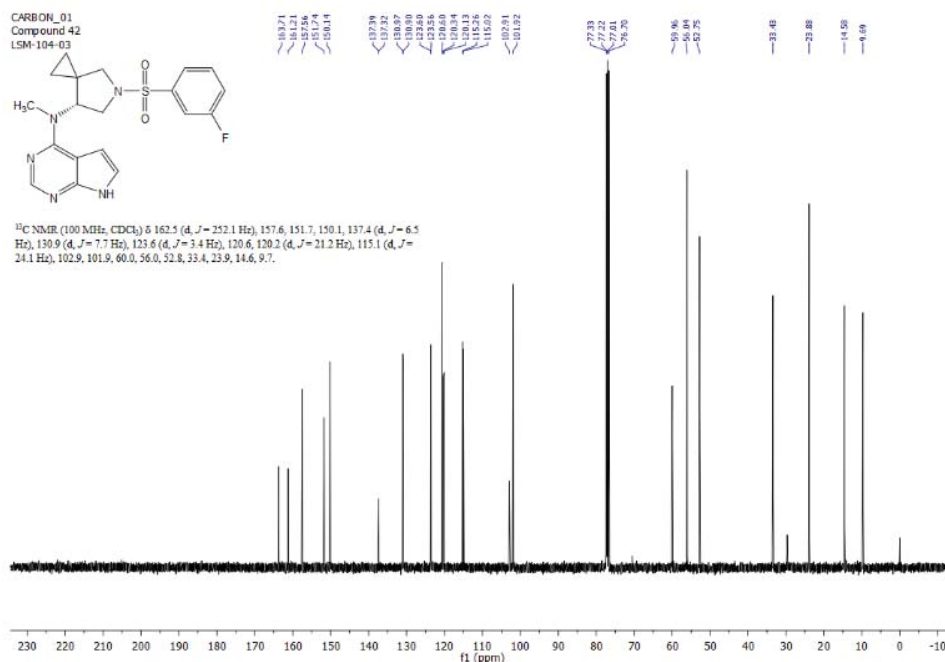
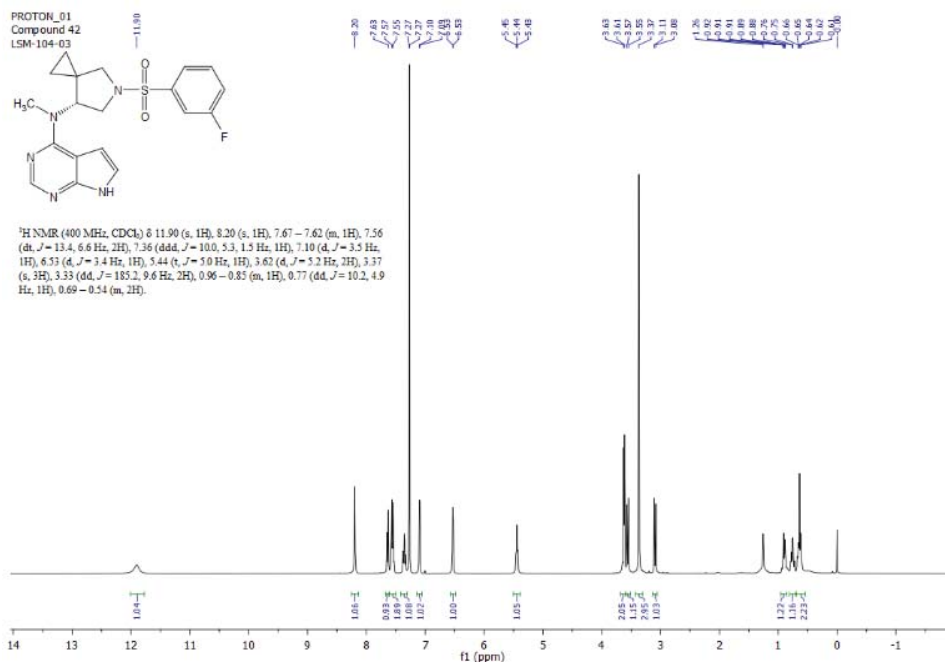*(R)-N-Methyl-N-(5-(m-tolylsulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine,* **63**

PROTON_01
Compound 50
LSM-104-53

¹H NMR (400 MHz, CDCl₃) δ 12.57 (s, 1H), 8.22 (d, J = 4.7 Hz, 1H), 7.65 (s, 2H), 7.43 (s, 2H), 7.10 (s, 1H), 6.50 (s, 1H), 5.41 (s, 1H), 3.61 (s, 2H), 3.52 (dd, J = 9.3, 4.4 Hz, 1H), 3.33 (d, J = 4.3 Hz, 3H), 3.08 (dd, J = 9.3, 4.5 Hz, 1H), 2.42 (d, J = 4.3 Hz, 3H), 0.87 (d, J = 4.6 Hz, 1H), 0.75 (d, J = 9.6 Hz, 1H), 0.61 (d, J = 2.9 Hz, 2H).

CARBON_01
Compound 50
LSM-104-53

¹³C NMR (100 MHz, CDCl₃) δ 157.6, 151.9, 150.1, 139.3, 134.9, 133.8, 129.0, 128.2, 125.0, 120.7, 102.9, 101.8, 60.0, 56.0, 52.8, 33.3, 23.9, 21.4, 14.4, 9.7.
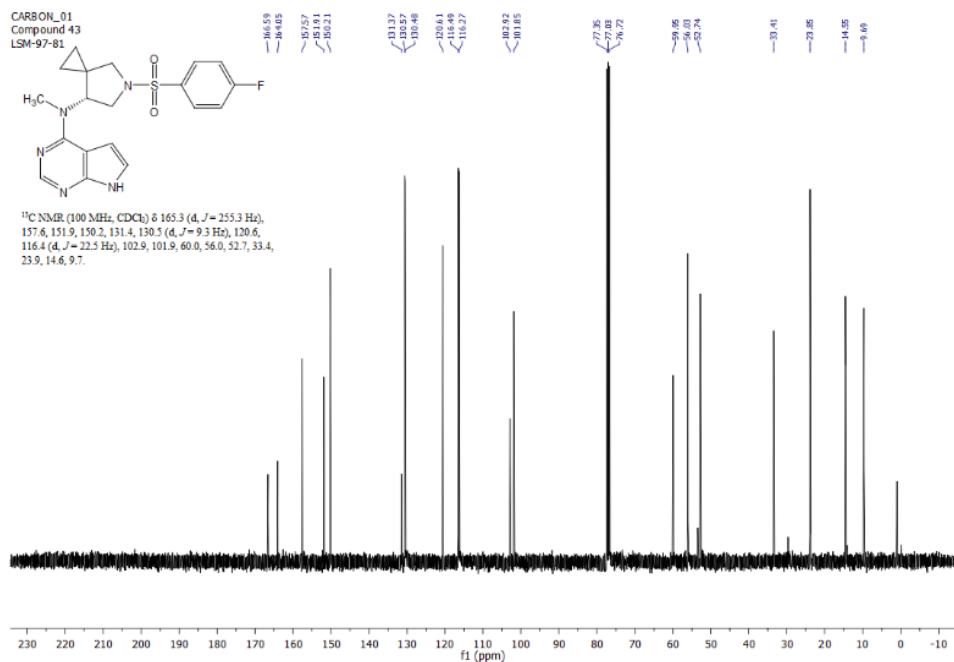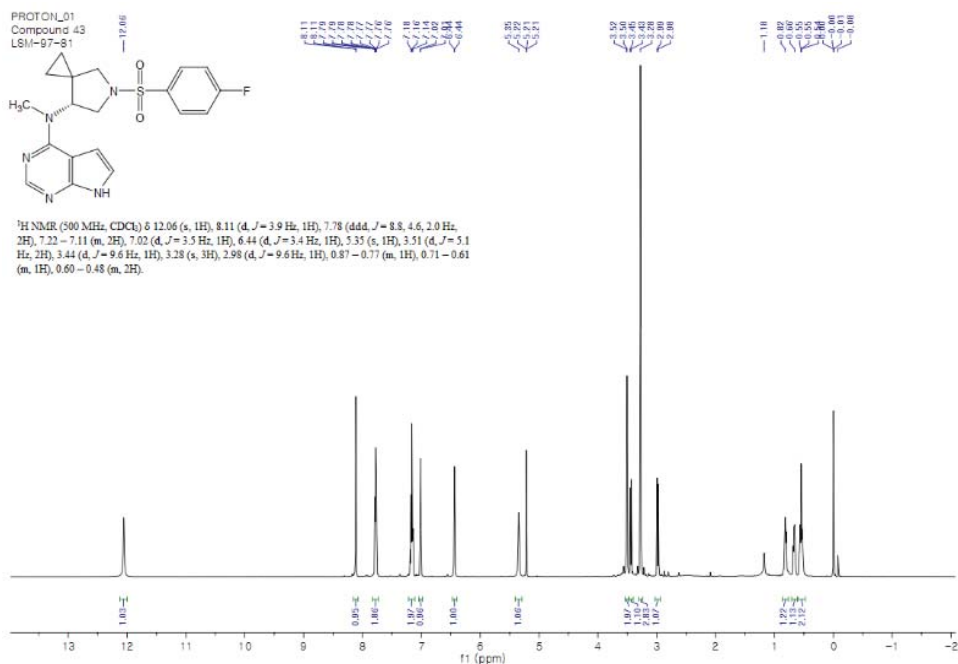
205

(R)-N-(5-((4-Methoxyphenyl)sulfonyl)-5-azaspiro[2.4]heptan-7-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **64**

PROTON_01
Compound 51
LSM-104-06-2

¹H NMR (400 MHz, CDCl₃) δ 11.94 (s, 1H), 8.19 (s, 1H), 7.78 (d, *J* = 8.8 Hz, 2H), 7.09 (d, *J* = 3.4 Hz, 1H), 7.02 (d, *J* = 8.8 Hz, 2H), 6.53 (d, *J* = 3.1 Hz, 1H), 5.43 (t, *J* = 4.7 Hz, 1H), 3.89 (s, 3H), 3.56 (d, *J* = 4.9 Hz, 2H), 3.36 (s, 3H), 3.27 (dd, *J* = 174.5, 9.5 Hz, 2H), 0.95 – 0.84 (m, 1H), 0.78 – 0.68 (m, 1H), 0.62 (t, *J* = 7.5 Hz, 2H).

CARBON_01
Compound 51
LSM-104-06-2

¹³C NMR (100 MHz, CDCl₃) δ 163.2, 157.6, 151.7, 150.2, 130.1, 126.6, 120.5, 114.2, 102.9, 102.0, 59.9, 56.0, 55.6, 52.7, 33.4, 23.9, 14.3, 9.9.

(R)-N-Methyl-N-(5-((4-(trifluoromethyl)phenyl)sulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **65**

PROTON_01
Compound 52
LSM-104-07

^{1}H NMR (400 MHz, CDCl$_3$) δ 11.77 (s, 1H), 8.23 (s, 1H), 7.90 (dd, J = 54.7, 8.2 Hz, 4H), 7.10 (d, J = 3.1 Hz, 1H), 6.54 (s, 1H), 5.41 (dd, J = 6.0, 3.1 Hz, 1H), 3.65 (dd, J = 15.1, 4.8 Hz, 2H), 3.37 (s, 3H), 3.34 (dd, J = 191.6, 9.6 Hz, 2H), 0.99 – 0.87 (m, 1H), 0.82 – 0.72 (m, 1H), 0.71 – 0.57 (m, 2H).

CARBON_01
Compound 52
LSM-104-07

^{13}C NMR (100 MHz, CDCl$_3$) δ 157.5, 151.5, 149.9, 139.1, 134.7 (q, J = 33.1 Hz), 128.3, 126.3 (q, J = 3.6 Hz), 123.2 (q, J = 272.9 Hz), 120.7, 103.1, 102.0, 60.2, 56.0, 52.8, 33.5, 23.9, 14.7, 9.7.

*(R)-N-Methyl-N-(5-(naphthalen-2-ylsulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **66***

PROTON_01
Compound 53
LSM-104-43

¹H NMR (400 MHz, CDCl₃) δ 12.58 (s, 1H), 8.42 (s, 1H), 8.19 (d, *J* = 1.7 Hz, 1H), 7.96 (d, *J* = 8.1 Hz, 2H), 7.93 – 7.77 (m, 2H), 7.62 (dt, *J* = 16.4, 7.3 Hz, 2H), 7.06 (s, 1H), 6.43 (s, 1H), 5.39 (s, 1H), 3.67 (d, *J* = 3.8 Hz, 2H), 3.36 (dd, *J* = 171.8, 9.6 Hz, 2H), 3.30 (s, 3H), 0.81 (d, *J* = 7.0 Hz, 1H), 0.71 (d, *J* = 4.6 Hz, 1H), 0.57 (t, *J* = 6.5 Hz, 2H).
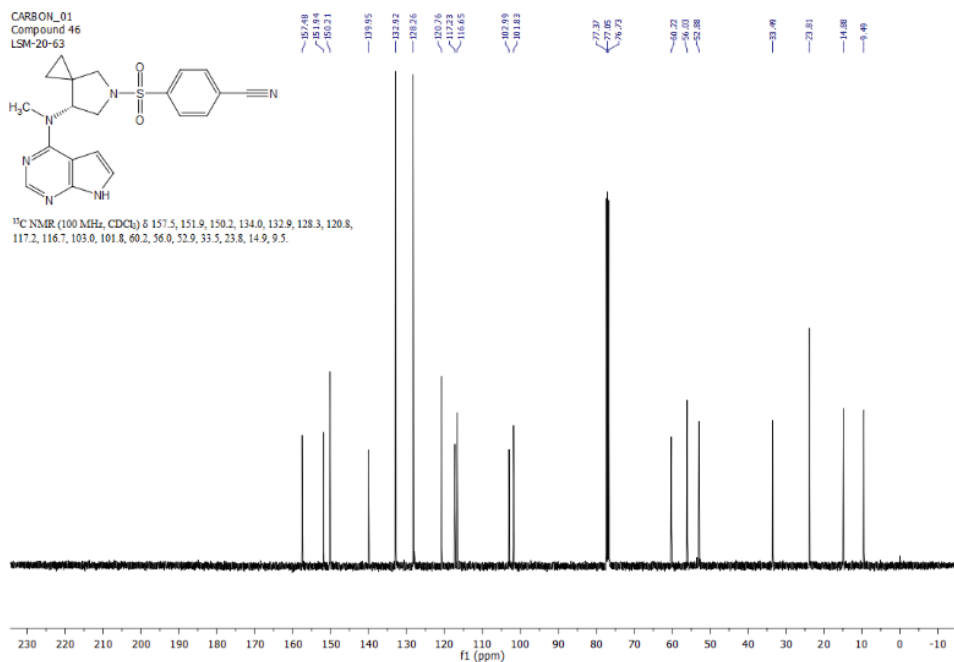

CARBON_01
Compound 53
LSM-104-43

¹³C NMR (100 MHz, CDCl₃) δ 157.5, 151.9, 150.1, 134.9, 132.3, 132.1, 129.3, 129.2, 128.9, 127.9, 127.6, 123.1, 120.7, 102.9, 101.8, 60.0, 56.1, 52.9, 33.4, 23.9, 14.5, 9.7.

(R)-N-Methyl-N-(5-(piperidin-1-ylsulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 67

PROTON_01
Compound 54
LSM-104-35

^{1}H NMR (400 MHz, CDCl$_3$) δ 11.36 (s, 1H), 8.25 (s, 1H), 7.09 (s, 1H), 6.58 (s, 1H), 5.56 (d, J = 5.8 Hz, 1H), 3.92 – 3.76 (m, 1H), 3.54 (d, J = 9.5 Hz, 2H), 3.48 (s, 4H), 3.28 (d, J = 4.5 Hz, 4H), 1.61 (dd, J = 26.6, 3.9 Hz, 6H), 1.03 (d, J = 9.7 Hz, 1H), 0.76 (d, J = 9.9 Hz, 2H), 0.69 (d, J = 9.9 Hz, 1H).

CARBON_01
Compound 54
LSM-104-35

^{13}C NMR (100 MHz, CDCl$_3$) δ 157.7, 152.0, 150.6, 120.3, 102.8, 102.0, 60.2, 56.6, 52.9, 47.2, 33.5, 25.5, 24.0, 23.8, 15.1, 9.5.

(R)-N-Methyl-N-(5-(morpholinosulfonyl)-5-azaspiro[2.4]heptan-7-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **68**

PROTON_01
Compound 55
LSM-104-40-1

^{1}H NMR (400 MHz, CDCl$_3$) δ 12.33 (s, 1H), 8.27 (d, *J* = 2.8 Hz, 1H), 7.13 (s, 1H), 6.57 (s, 1H), 5.63 – 5.51 (m, 1H), 3.90 (ddd, *J* = 10.6, 7.6, 2.7 Hz, 1H), 3.75 (dd, *J* = 5.8, 3.1 Hz, 4H), 3.65 – 3.55 (m, 2H), 3.48 (d, *J* = 2.8 Hz, 3H), 3.37 – 3.22 (m, 5H), 1.04 (d, *J* = 9.6 Hz, 1H), 0.85 – 0.75 (m, 2H), 0.75 – 0.65 (m, 1H).

CARBON_01
Compound 55
LSM-104-40-1

^{13}C NMR (100 MHz, CDCl$_3$) δ 157.7, 152.0, 150.3, 120.7, 102.9, 101.8, 66.4, 60.2, 56.8, 53.1, 46.4, 33.5, 24.0, 15.3, 9.3.

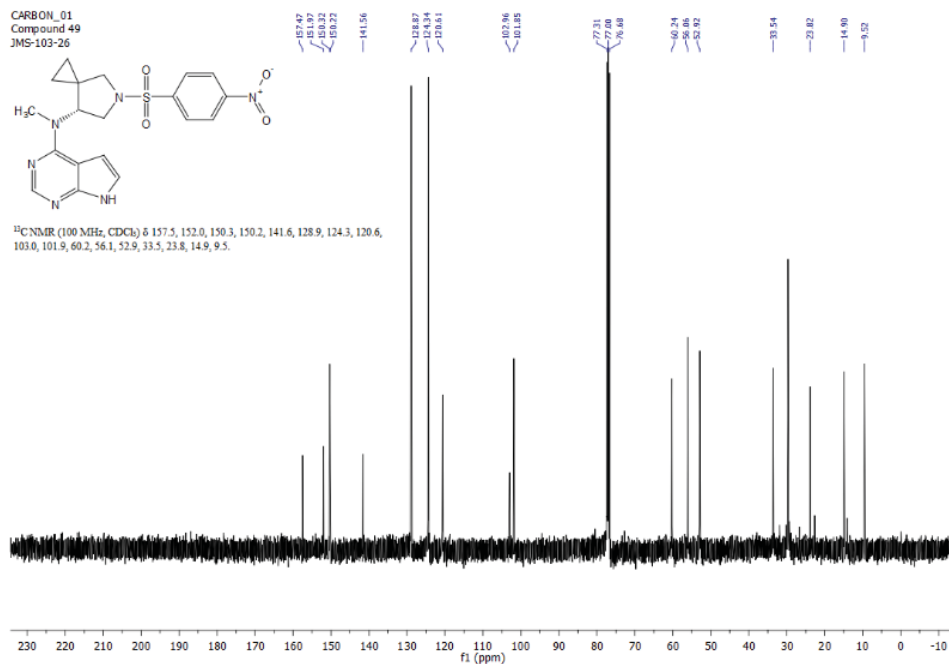*(R)-3-(3-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)propanenitrile,* **69**

PROTON_01
Compound 17
LSM-113-18

¹H NMR (400 MHz, CDCl₃) δ 12.32 (s, 1H), 8.32 (s, 1H), 7.10 (d, *J* = 3.4 Hz, 1H), 6.58 (d, *J* = 3.3 Hz, 1H), 5.73 (s, 1H), 3.42 (s, 3H), 3.06 (t, *J* = 7.1 Hz, 1H), 2.94 (dd, *J* = 9.9, 3.1 Hz, 1H), 2.83 (m, 1H), 2.72 (m, 1H), 2.64 (t, *J* = 9.2 Hz, 1H), 2.56 (t, *J* = 6.8 Hz, 2H), 2.34 (m, 2H), 1.93 (dt, *J* = 13.0, 10.0 Hz, 1H).

CARBON_01
Compound 17
LSM-113-18

¹³C NMR (100 MHz, CDCl₃) δ 157.6, 151.8, 150.6, 120.4, 118.8, 103.1, 102.1, 57.2, 54.4, 53.7, 50.8, 32.4, 29.3, 17.7.

(R)-N-(1-Butylpyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **70**

¹H NMR (400 MHz, DMSO-d6) δ 11.69 (s, 1H), 8.11 (s, 1H), 7.11 (d, *J* = 3.1 Hz, 1H), 6.58 (d, *J* = 3.2 Hz, 1H), 5.53 (dt, *J* = 15.1, 7.7 Hz, 1H), 3.25 (s, 4H), 3.08 (m, 2H), 2.81 (m, 3H), 2.16 (m, 1H), 1.95 (m, 1H), 1.50 (dt, *J* = 15.2, 7.4 Hz, 2H), 1.25 (dq, *J* = 14.5, 7.3 Hz, 2H), 0.82 (t, *J* = 7.3 Hz, 3H).

¹³C NMR (100 MHz, DMSO-d6) δ 156.7, 151.6, 150.4, 121.1, 102.5, 101.5, 54.5, 54.3, 53.7, 52.9, 32.7, 28.2, 27.1, 19.7, 13.6.

(R)-2-Azido-1-(3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)ethan-1-one, **71**

¹H NMR (400 MHz, CDCl₃) δ 11.97 (d, *J* = 32.0 Hz, 1H), 8.34 (d, *J* = 1.9 Hz, 1H), 7.15 (dd, *J* = 6.5, 3.6 Hz, 1H), 6.59 (s, 1H), 5.75 (m, 1H), 3.92 (m, 3H), 3.79 (dd, *J* = 19.2, 10.6 Hz, 1H), 3.57 (tt, *J* = 11.9, 8.2 Hz, 2H), 3.34 (m, 3H), 2.21 (m, 2H).

¹³C NMR (100 MHz, CDCl₃) δ 166.2, 157.8, 152.3, 150.7, 121.1, 103.6, 101.8, 55.0, 51.3, 46.7, 44.6, 32.3, 26.7.

(R)-3-Methyl-1-(3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)butan-1-one, 72

¹H NMR (400 MHz, CDCl₃) δ 11.67 (s, 1H), 8.38 (s, 1H), 7.14 (s, 1H), 6.60 (s, 1H), 5.72 (m, 1H), 3.81 (m, 2H), 3.49 (m, 2H), 3.34 (d, *J* = 11.5 Hz, 3H), 2.18 (m, 4H), 1.50 (d, *J* = 35.8 Hz, 1H), 0.94 (d, *J* = 44.9 Hz, 6H).

¹³C NMR (100 MHz, CDCl₃) δ 171.6, 157.7, 151.9, 150.2, 121.1, 103.5, 101.7, 54.9, 47.8, 45.4, 43.8, 32.1, 29.7, 25.5, 22.8.

*Isobutyl (R)-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidine-1-carboxylate, **73***

PROTON_01
Compound 19
CYC-112-88

¹H NMR (400 MHz, CDCl₃) δ 11.83 (s, 1H), 8.33 (s, 1H), 7.11 (d, J = 2.8 Hz, 1H), 6.58 (d, J = 3.3 Hz, 1H), 5.72 (d, J = 6.3 Hz, 1H), 3.90 (d, J = 6.5 Hz, 2H), 3.76 (d, J = 9.2 Hz, 1H), 3.69 (m, 1H), 3.45 (m, 2H), 3.33 (s, 3H), 2.15 (dd, J = 21.9, 12.3 Hz, 2H), 1.95 (d, J = 6.1 Hz, 1H), 0.94 (d, J = 6.2 Hz, 6H).

CARBON_01
Compound 19
CYC-112-88

¹³C NMR (100 MHz, CDCl₃) δ 157.9, 155.5, 152.0, 150.6, 120.8, 103.4, 102.0, 71.5, 54.7, 46.8, 44.8, 32.0, 28.2, 19.2, 9.5.

(R)-3-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-phenylpyrrolidine-1-carboxamide, **74**

PROTON_01
Compound 22
LSM-113-19

¹H NMR (400 MHz, DMSO-d6) δ 11.72 (s, 1H), 8.22 (s, 1H), 8.18 (s, 1H), 7.54 (d, *J* = 8.1 Hz, 2H), 7.23 (t, *J* = 7.8 Hz, 2H), 7.18 (d, *J* = 2.3 Hz, 1H), 6.92 (t, *J* = 7.2 Hz, 1H), 6.64 (d, *J* = 3.1 Hz, 1H), 5.56 (m, 1H), 3.75 (m, 1H), 3.67 (m, 1H), 3.45 (m, 2H), 3.25 (s, 3H), 2.16 (m, 2H).

CARBON_01
Compound 22
LSM-113-19

¹³C NMR (100 MHz, DMSO-d6) δ 157.1, 154.1, 151.8, 150.6, 140.5, 128.3, 121.7, 121.1, 119.5, 102.6, 101.6, 54.2, 46.8, 44.4, 31.6, 27.6.

(R)-N-Methyl-N-(1-(methylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **75**

¹H NMR (400 MHz, DMSO-d6) δ 11.70 (s, 1H), 8.15 (d, *J* = 1.3 Hz, 1H), 7.17 (d, *J* = 2.9 Hz, 1H), 6.62 (d, *J* = 2.7 Hz, 1H), 5.58 (m, 1H), 3.52 (t, *J* = 9.0 Hz, 1H), 3.46 (m, 1H), 3.31 (dd, *J* = 17.4, 8.7 Hz, 1H), 3.23 (dd, *J* = 10.0, 4.4 Hz, 4H), 2.98 (d, *J* = 1.3 Hz, 3H), 2.14 (m, 2H).

¹³C NMR (100 MHz, DMSO-d6) δ 157.0, 151.8, 150.5, 121.1, 102.6, 101.5, 54.2, 48.4, 46.3, 33.4, 31.8, 27.9.

PROTON_01
Compound 24
LSM-104-36

^{1}H NMR (400 MHz, CDCl$_3$) δ 12.77 (s, 1H), 8.37 (d, J = 8.8 Hz, 1H), 7.18 (m, 1H), 6.59 (m, 1H), 5.84 (m, 1H), 3.89 (m, 2H), 3.62 (m, 1H), 3.53 (m, 1H), 3.36 (d, J = 8.7 Hz, 3H), 2.29 (dd, J = 17.0, 8.6 Hz, 2H).

CARBON_01
Compound 24
LSM-104-36

^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 152.2, 150.3, 121.3, 120.4 (q, J = 323.8 Hz), 103.7, 101.6, 54.7, 48.9, 47.7, 32.4, 28.5.

PROTON_01
Compound 23
LSM-104-26

^{1}H NMR (400 MHz, CDCl$_3$) δ 11.30 (s, 1H), 8.34 (s, 1H), 7.13 (s, 1H), 6.61 (s, 1H), 5.80 (m, 1H), 3.70 (dd, *J* = 19.4, 10.1 Hz, 2H), 3.43 (d, *J* = 10.3 Hz, 2H), 3.38 (s, 3H), 3.08 (q, *J* = 7.3 Hz, 2H), 2.28 (s, 1H), 2.19 (m, 1H), 1.43 (t, *J* = 7.3 Hz, 3H).

CARBON_01
Compound 25
LSM-104-26

^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 151.6, 149.9, 121.1, 103.8, 102.0, 54.9, 48.6, 46.6, 44.6, 29.8, 28.8, 8.1.

(R)-N-(1-(Isopropylsulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **78**

¹H NMR (400 MHz, CDCl₃) δ 12.23 (s, 1H), 8.35 (s, 1H), 7.14 (d, *J* = 3.2 Hz, 1H),
6.59 (d, *J* = 2.6 Hz, 1H), 5.78 (m, 1H), 3.75 (m, 2H), 3.46 (m, 2H), 3.36 (s, 3H),
3.28 (dt, *J* = 13.6, 6.8 Hz, 1H), 2.26 (m, 1H), 2.16 (m, 1H), 1.41 (d, *J* = 6.8 Hz, 6H).

¹³C NMR (100 MHz, CDCl₃) δ 157.8, 152.2, 150.6, 120.9, 103.5, 101.9, 55.0, 53.6, 49.0, 47.2, 32.3, 28.9, 16.8.

(R)-N-Methyl-N-(1-(propylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **79**

PROTON_01
Compound 27
LSM-113-16

¹H NMR (400 MHz, CDCl₃) δ 12.36 (s, 1H), 8.34 (s, 1H), 7.15 (s, 1H), 6.58 (s, 1H), 5.78 (m, 1H), 3.67 (m, 2H), 3.41 (d, J = 9.3 Hz, 2H), 3.36 (s, 3H), 3.01 (m, 2H), 2.27 (s, 1H), 2.19 (m, 1H), 1.90 (dd, J = 14.5, 7.2 Hz, 2H), 1.09 (t, J = 7.1 Hz, 3H).

CARBON_01
Compound 27
LSM-113-16

¹³C NMR (100 MHz, CDCl₃) δ 157.7, 152.1, 150.5, 121.0, 103.5, 101.8, 54.8, 51.5, 48.5, 46.5, 32.3, 28.7, 17.1, 13.3.

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(R)-N-Methyl-N-(1-((1-methyl-1H-imidazol-4-yl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 80

PROTON_01
Compound 28
JMS-103-55

¹H NMR (400 MHz, DMSO-d6) δ 11.70 (s, 1H), 8.10 (s, 1H), 7.89 (s, 2H), 7.16 (s, 1H), 6.52 (s, 1H), 5.31 (dd, J = 15.6, 7.8 Hz, 1H), 3.74 (s, 3H), 3.50 (m, 2H), 3.27 (m, 2H), 3.12 (s, 3H), 1.99 (dd, J = 15.6, 8.0 Hz, 2H).

CARBON_01
Compound 28
JMS-103-55

¹³C NMR (100 MHz, DMSO-d6) δ 156.8, 151.6, 150.3, 140.2, 135.8, 126.1, 121.2, 102.4, 101.4, 54.2, 48.7, 46.9, 33.6, 31.4, 27.7.

(R)-N-Methyl-N-(1-(phenylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **81**

PROTON_01
Compound 29
LSM-104-31

¹H NMR (400 MHz, CDCl₃) δ 12.18 (s, 1H), 8.26 (s, 1H), 7.87 (dd, *J* = 7.0, 1.4 Hz, 2H), 7.65 (m, 1H), 7.58 (m, 2H), 7.10 (d, *J* = 3.0 Hz, 1H), 6.50 (d, *J* = 2.9 Hz, 1H), 5.60 (m, 1H), 3.64 (dd, *J* = 12.1, 5.7 Hz, 1H), 3.45 (dd, *J* = 13.6, 5.3 Hz, 1H), 3.35 (m, 1H), 3.27 (d, *J* = 2.1 Hz, 3H), 3.12 (dt, *J* = 16.6, 4.7 Hz, 1H), 2.17 (ddd, *J* = 12.3, 10.0, 7.7 Hz, 1H), 2.05 (m, 1H).

CARBON_01
Compound 29
LSM-104-31

¹³C NMR (100 MHz, CDCl₃) δ 157.6, 152.1, 150.5, 135.8, 133.1, 129.3, 127.9, 120.9, 103.4, 101.8, 54.4, 49.3, 47.2, 32.2, 28.5.

(R)-N-(1-((2-Fluorophenyl)sulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **82**

PROTON_01
Compound 30
JMS-103-33

¹H NMR (400 MHz, CDCl₃) δ 12.58 (s, 1H), 8.29 (s, 1H), 7.91 (t, *J* = 6.8 Hz, 1H), 7.60 (d, *J* = 5.0 Hz, 1H), 7.27 (m, 2H), 7.11 (s, 1H), 6.51 (s, 1H), 5.68 (m, 1H), 3.73 (d, *J* = 8.2 Hz, 1H), 3.63 (t, *J* = 9.1 Hz, 1H), 3.40 (m, 1H), 3.34 (d, *J* = 8.5 Hz, 1H), 3.28 (s, 3H), 2.20 (s, 1H), 2.10 (dd, *J* = 19.9, 9.1 Hz, 1H).

CARBON_01
Compound 30
JMS-103-33

¹³C NMR (100 MHz, CDCl₃) δ 160.3, 157.7, 157.5, 152.0, 150.3, 135.2 (d, *J* = 8.4 Hz), 131.4, 125.4 (d, *J* = 14.9 Hz), 124.6 (d, *J* = 3.6 Hz), 121.0, 117.4 (d, *J* = 22.0 Hz), 102.5 (d, *J* = 172.3 Hz), 54.5, 48.4, 46.6, 32.1, 28.5.

*(R)-N-(1-((3-Fluorophenyl)sulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **83***

¹H NMR (400 MHz, CDCl₃) δ 12.64 (s, 1H), 8.27 (s, 1H), 7.65 (d, *J* = 7.5 Hz, 1H), 7.55 (dd, *J* = 14.7, 6.6 Hz, 2H), 7.33 (m, 1H), 7.11 (d, *J* = 2.8 Hz, 1H), 6.49 (d, *J* = 2.9 Hz, 1H), 5.59 (dd, *J* = 14.9, 7.4 Hz, 1H), 3.63 (t, *J* = 7.3 Hz, 1H), 3.47 (t, *J* = 9.3 Hz, 1H), 3.33 (dd, *J* = 10.2, 6.6 Hz, 1H), 3.26 (s, 3H), 3.13 (dd, *J* = 16.7, 9.1 Hz, 1H), 2.15 (m, 1H), 2.06 (m, 1H).

¹³C NMR (100 MHz, CDCl₃) δ 163.8, 161.3, 157.4, 152.0, 150.2, 137.9 (d, *J* = 6.5 Hz), 131.1 (d, *J* = 7.7 Hz), 123.5 (d, *J* = 3.2 Hz), 121.0 (s), 120.2 (d, *J* = 21.2 Hz), 115.0 (d, *J* = 24.1 Hz), 102.5 (d, *J* = 172.4 Hz), 54.3, 49.1, 47.0, 32.1, 28.3.

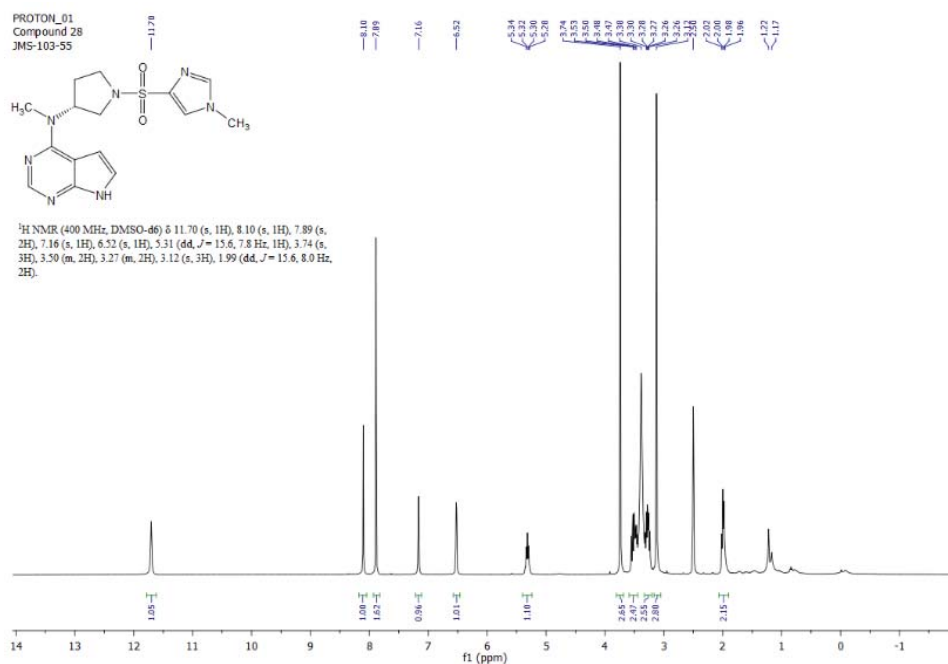*(R)-N-(1-((4-Fluorophenyl)sulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine,* **84**

PROTON_01
Compound 30
JMS-103-27

^{1}H NMR (400 MHz, CDCl$_3$) δ 11.08 (s, 1H), 8.24 (s, 1H), 7.89 (dd, *J* = 8.4, 5.1 Hz, 2H), 7.26 (dd, *J* = 9.2, 7.5 Hz, 2H), 7.09 (d, *J* = 2.7 Hz, 1H), 6.52 (d, *J* = 2.9 Hz, 1H), 5.61 (m, 1H), 3.65 (t, *J* = 7.5 Hz, 1H), 3.38 (dt, *J* = 10.1, 6.9 Hz, 2H), 3.31 (d, *J* = 8.7 Hz, 3H), 3.11 (m, 1H), 2.18 (d, *J* = 4.7 Hz, 1H), 2.08 (dd, *J* = 14.9, 6.4 Hz, 1H).

CARBON_01
Compound 32
JMS-103-27

^{13}C NMR (100 MHz, CDCl$_3$) δ 166.8, 164.2, 157.6, 152.1, 150.5, 132.1, 130.6 (d, *J* = 9.6 Hz), 120.9, 116.6 (d, *J* = 22.5 Hz), 90.4, 54.4, 49.2, 47.2, 32.3, 28.5.

(R)-2-((3-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, **85**

PROTON_01
Compound 33
JMS-103-29

¹H NMR (400 MHz, CDCl₃) δ 12.29 (s, 1H), 8.28 (d, *J* = 3.7 Hz, 1H), 8.11 (dd, *J* = 7.4, 2.8 Hz, 1H), 7.91 (m, 1H), 7.74 (m, 2H), 7.12 (s, 1H), 6.55 (s, 1H), 5.73 (d, *J* = 6.8 Hz, 1H), 3.80 (t, *J* = 8.8 Hz, 1H), 3.67 (td, *J* = 10.1, 3.5 Hz, 1H), 3.43 (m, 2H), 3.32 (d, *J* = 3.5 Hz, 3H), 2.22 (ddd, *J* = 18.3, 11.1, 4.5 Hz, 2H).

CARBON_01
Compound 33
JMS-103-29

¹³C NMR (100 MHz, CDCl₃) δ 157.6, 152.1, 150.4, 140.7, 135.7, 133.2, 133.0, 130.4, 121.0, 116.5, 110.8, 103.5, 101.8, 54.6, 48.7, 47.2, 32.3, 28.5.

(R)-4-((3-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, **86**

PROTON_01
Compound 34
JMS-103-35

¹H NMR (400 MHz, DMSO-d6) δ 11.66 (s, 1H), 8.09 (m, 2H), 8.03 (d, *J* = 3.0 Hz, 1H), 7.98 (dd, *J* = 4.7, 3.8 Hz, 2H), 7.09 (d, *J* = 1.3 Hz, 1H), 6.43 (s, 1H), 5.24 (m, 1H), 3.44 (m, 2H), 3.17 (ddd, *J* = 18.1, 10.9, 5.2 Hz, 2H), 3.06 (d, *J* = 2.8 Hz, 3H), 1.96 (m, 2H).

CARBON_01
Compound 34
JMS-103-35

¹³C NMR (100 MHz, DMSO-d6) δ 156.8, 151.7, 150.4, 139.8, 133.6, 128.2, 121.1, 117.7, 115.6, 102.6, 101.4, 54.1, 48.7, 46.8, 31.7, 27.6.

(R)-N-Methyl-N-(1-((2-nitrophenyl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 87

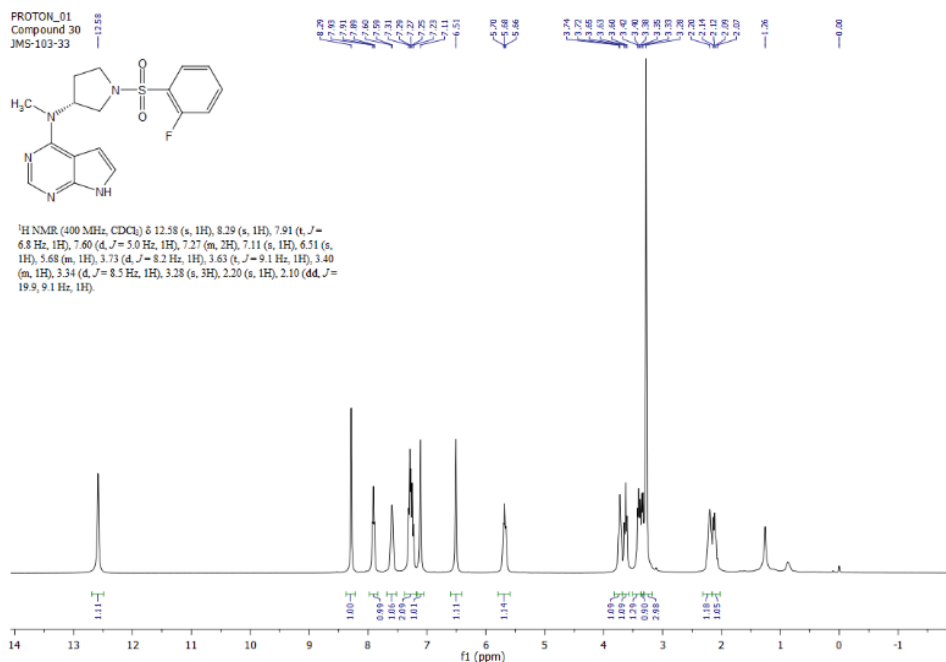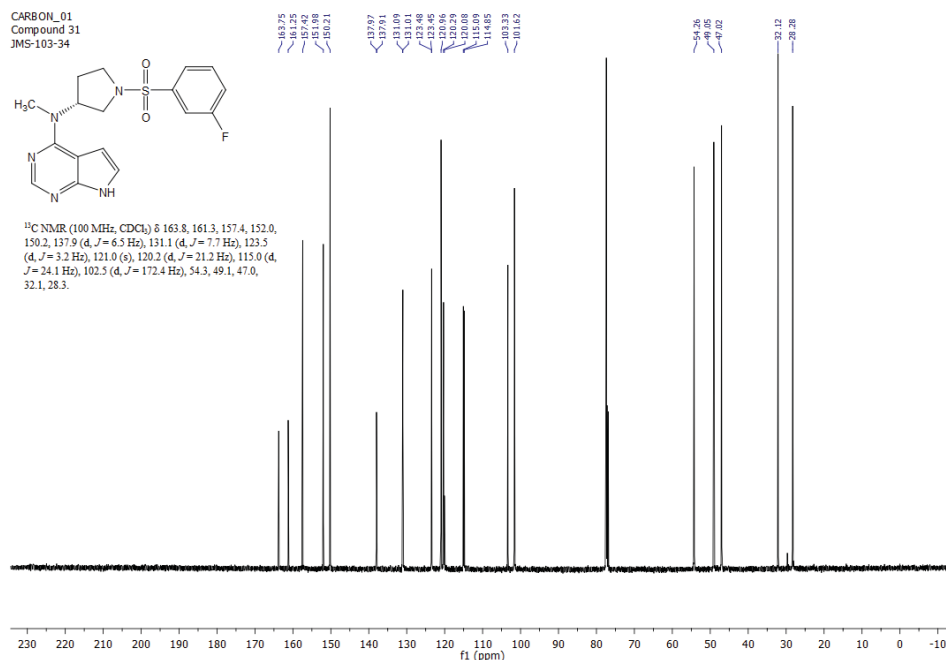PROTON_01
Compound 35
JMS-103-30

^{1}H NMR (400 MHz, CDCl$_3$) δ 11.92 (s, 1H), 8.30 (s, 1H), 8.05 (dd, J = 7.3, 1.7 Hz, 1H), 7.72 (m, 2H), 7.64 (dd, J = 7.5, 1.6 Hz, 1H), 7.12 (d, J = 3.2 Hz, 1H), 6.56 (d, J = 3.3 Hz, 1H), 5.75 (dd, J = 15.9, 7.8 Hz, 1H), 3.76 (m, 2H), 3.46 (ddd, J = 17.0, 9.8, 7.2 Hz, 2H), 3.32 (s, 3H), 2.26 (m, 1H), 2.18 (m, 1H).

CARBON_01
Compound 35
JMS-103-30

^{13}C NMR (100 MHz, CDCl$_3$) δ 157.7, 152.2, 150.6, 148.5, 133.9, 131.8, 131.6, 131.1, 124.2, 120.9, 103.5, 101.9, 54.7, 48.6, 47.0, 32.3, 28.7.

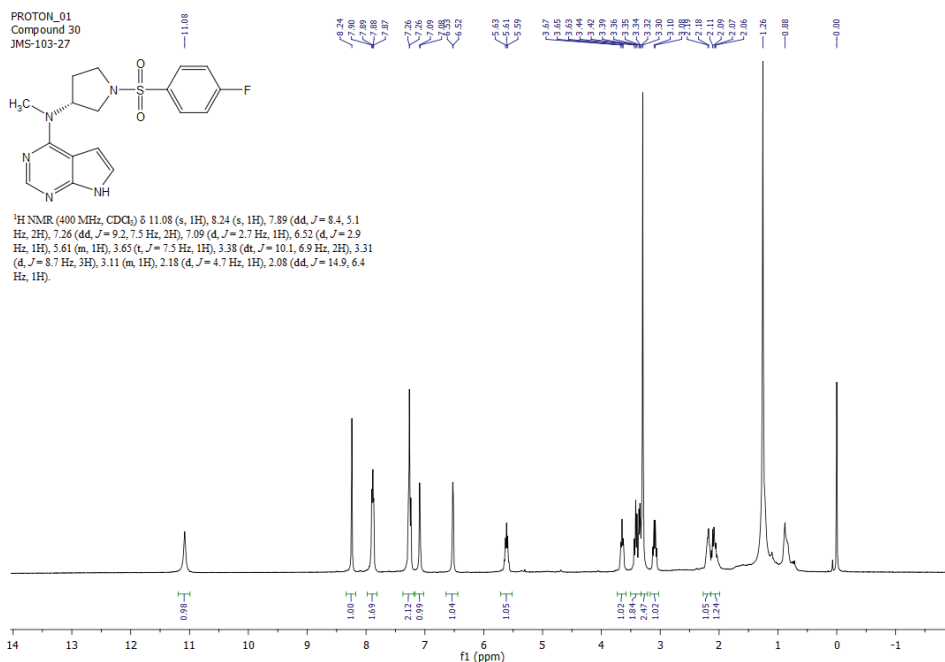*(R)-N-Methyl-N-(1-((3-nitrophenyl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine,* **88**

PROTON_01
Compound 34
JMS-103-31

^{1}H NMR (400 MHz, CDCl$_3$) δ 10.63 (s, 1H), 8.70 (s, 1H), 8.50 (d, J = 7.9 Hz, 1H), 8.22 (s, 1H), 8.19 (d, J = 8.1 Hz, 1H), 7.80 (t, J = 7.9 Hz, 1H), 7.08 (s, 1H), 6.54 (s, 1H), 5.60 (m, 1H), 3.73 (t, J = 7.5 Hz, 1H), 3.50 (dd, J = 11.6, 7.1 Hz, 1H), 3.40 (m, 1H), 3.31 (s, 3H), 3.18 (dd, J = 16.8, 9.2 Hz, 1H), 2.15 (m, 2H).

CARBON_01
Compound 36
JMS-103-31

^{13}C NMR (100 MHz, CDCl$_3$) δ 157.6, 152.2, 150.5, 148.6, 138.7, 133.2, 130.7, 127.5, 122.8, 121.0, 103.5, 101.9, 54.4, 49.1, 47.2, 32.5, 28.4.

(R)-N-Methyl-N-(1-((4-nitrophenyl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **89**

PROTON_01
Compound 37
JMS-103-32

¹H NMR (400 MHz, CDCl₃) δ 10.99 (s, 1H), 8.42 (d, *J* = 8.6 Hz, 2H), 8.22 (s, 1H), 8.05 (d, *J* = 8.6 Hz, 2H), 7.09 (d, *J* = 2.1 Hz, 1H), 6.52 (d, *J* = 2.6 Hz, 1H), 5.59 (dt, *J* = 15.6, 7.9 Hz, 1H), 3.70 (m, 1H), 3.51 (m, 1H), 3.38 (dd, *J* = 10.2, 7.0 Hz, 1H), 3.30 (s, 3H), 3.18 (dd, *J* = 16.9, 9.2 Hz, 1H), 2.15 (m, 2H).

CARBON_01
Compound 37
JMS-103-32

¹³C NMR (100 MHz, CDCl₃) δ 157.6, 152.2, 150.9, 150.5, 142.3, 129.0, 124.6, 120.7, 103.5, 102.1, 54.5, 49.1, 47.2, 32.5, 28.4.

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(R)-N-Methyl-N-(1-(m-tolylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **90**

PROTON_01
Compound 38
LSM-104-47

¹H NMR (400 MHz, CDCl₃) δ 12.57 (s, 1H), 8.27 (s, 1H), 7.66 (d, *J* = 9.2 Hz, 2H), 7.44 (m, 2H), 7.10 (d, *J* = 3.0 Hz, 1H), 6.48 (d, *J* = 2.7 Hz, 1H), 5.58 (dt, *J* = 14.9, 7.4 Hz, 1H), 3.62 (t, *J* = 7.5 Hz, 1H), 3.44 (m, 1H), 3.33 (dd, *J* = 10.2, 6.4 Hz, 1H), 3.25 (s, 3H), 3.10 (dd, *J* =

CARBON_01
Compound 38
LSM-104-47

¹³C NMR (100 MHz, CDCl₃) δ 157.4, 152.0, 150.2, 139.4, 135.5, 133.8, 129.0, 128.1, 124.9, 120.9, 103.3, 101.6, 54.3, 49.2, 47.1, 32.0, 28.4, 21.4.

(R)-N-Methyl-N-(1-tosylpyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **91**

PROTON_01
Compound 40
JMS-103-36

^{1}H NMR (400 MHz, CDCl$_3$) δ 12.57 (s, 1H), 8.26 (s, 1H), 7.73 (d, *J* = 6.8 Hz, 2H), 7.35 (d, *J* = 7.1 Hz, 2H), 7.10 (s, 1H), 6.48 (s, 1H), 5.58 (m, 1H), 3.61 (d, *J* = 7.6 Hz, 1H), 3.41 (t, *J* = 9.0 Hz, 1H), 3.31 (dd, *J* = 8.7, 6.2 Hz, 1H), 3.25 (s, 3H), 3.08 (dd, *J* = 16.3, 7.7 Hz, 1H), 2.44 (s, 3H), 2.13 (s, 1H), 2.04 (dd, *J* = 18.2, 9.9 Hz, 1H).

CARBON_01
Compound 40
JMS-103-36

^{13}C NMR (100 MHz, CDCl$_3$) δ 157.4, 151.9, 150.2, 143.8, 132.5, 129.8, 127.8, 120.8, 103.2, 101.6, 54.2, 49.2, 47.0, 32.0, 28.3, 21.5.

(R)-N-(1-((4-Methoxyphenyl)sulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **92**

PROTON_01
Compound 39
JMS-103-38

¹H NMR (400 MHz, CDCl₃) δ 12.56 (s, 1H), 8.27 (s, 1H), 7.79 (d, *J* = 6.6 Hz, 2H), 7.10 (s, 1H), 7.02 (d, *J* = 6.6 Hz, 2H), 6.48 (s, 1H), 5.58 (s, 1H), 3.87 (s, 3H), 3.59 (t, *J* = 8.5 Hz, 1H), 3.40 (t, *J* = 9.0 Hz, 1H), 3.31 (d, *J* = 6.3 Hz, 1H), 3.25 (s, 3H), 3.08 (m, 1H), 2.14 (s, 1H), 2.03 (m, 1H).

CARBON_01
Compound 39
JMS-103-38

¹³C NMR (100 MHz, CDCl₃) δ 163.1, 157.4, 151.9, 150.2, 129.9, 127.1, 120.8, 114.3, 103.2, 101.6, 55.6, 54.2, 49.2, 47.0, 32.0, 28.3.

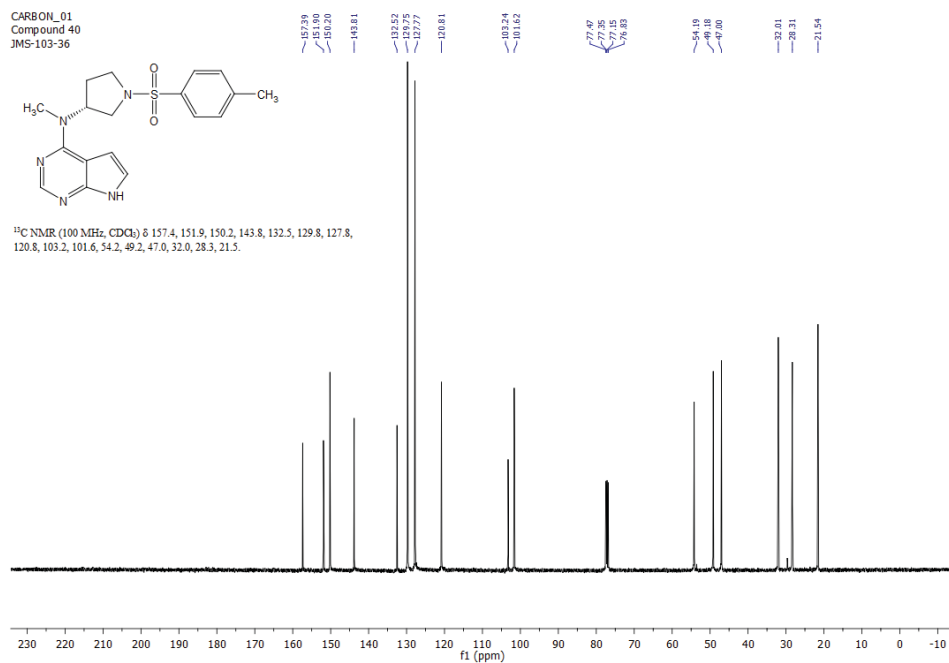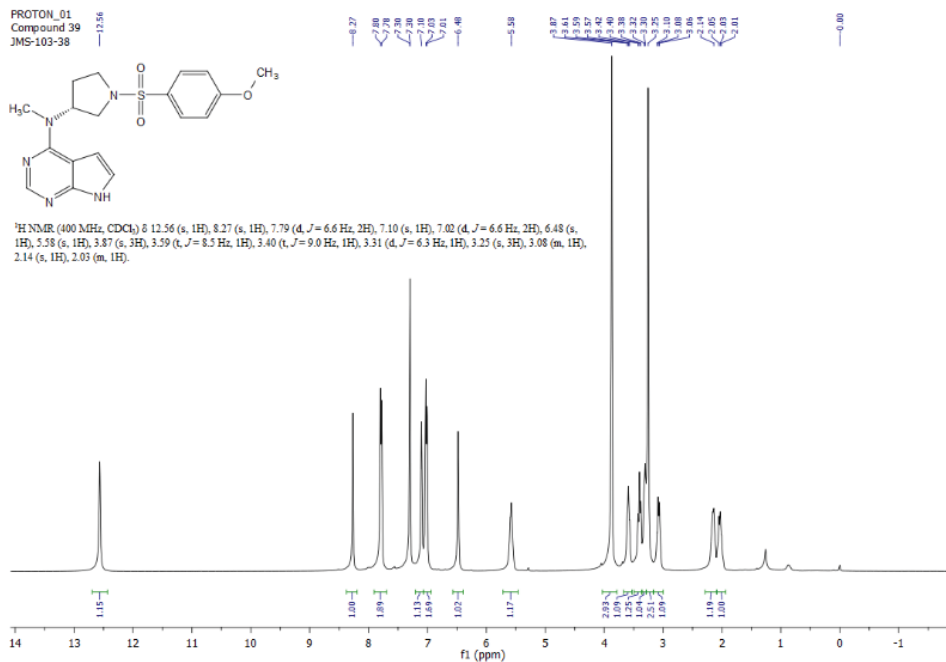*(R)-N-Methyl-N-(1-((4-(trifluoromethyl)phenyl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, 93*

PROTON_01
Compound 41
JMS-103-37

^{1}H NMR (400 MHz, DMSO-d6) δ 11.71 (s, 1H), 8.05 (m, 5H), 7.12 (s, 1H), 6.48 (s, 1H), 5.28 (dd, *J* = 14.4, 7.2 Hz, 1H), 3.51 (s, 1H), 3.43 (dd, *J* = 18.2, 8.2 Hz, 3H), 3.22 (m, 2H), 3.12 (m, 3H), 2.04 (m, 2H).

CARBON_01
Compound 41
JMS-103-37

^{13}C NMR (100 MHz, DMSO-d6) δ 157.2, 152.1, 150.8, 140.0, 133.2 (q, *J* = 32.4 Hz), 128.9, 127.0 (d, *J* = 3.1 Hz), 123.9 (q, *J* = 272.7 Hz), 121.5, 102.9, 101.7, 54.5, 49.1, 47.2, 32.1, 28.0.

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PROTON_01
Compound 42
LSM-104-44

¹H NMR (400 MHz, CDCl₃) δ 12.57 (s, 1H), 8.43 (s, 1H), 8.21 (s, 1H), 7.97 (m, 2H), 7.91 (d, *J* = 7.9 Hz, 1H), 7.85 (d, *J* = 8.6 Hz, 1H), 7.61 (td, *J* = 15.0, 7.0 Hz, 2H), 7.02 (d, *J* = 2.5 Hz, 1H), 6.39 (d, *J* = 2.6 Hz, 1H), 5.52 (m, 1H), 3.68 (m, 1H), 3.50 (t, *J* = 9.4 Hz, 1H), 3.37 (dd, *J* = 9.9, 6.7 Hz, 1H), 3.20 (s, 3H), 3.16 (m, 1H), 2.10 (ddd, *J* = 18.9, 8.3, 6.1 Hz, 1H), 2.00 (m, 1H).

CARBON_01
Compound 42
LSM-104-44

¹³C NMR (100 MHz, CDCl₃) δ 157.4, 151.9, 150.2, 134.9, 132.8, 132.2, 129.4, 129.2, 129.1, 128.9, 127.9, 127.6, 123.0, 120.8, 103.2, 101.6, 54.3, 49.2, 47.1, 32.0, 28.3.

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(R)-N-Methyl-N-(1-(piperidin-1-ylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **95**

PROTON_01
Compound 43
LSM-104-34

¹H NMR (400 MHz, CDCl₃) δ 12.65 (s, 1H), 8.36 (s, 1H), 7.15 (s, 1H), 6.58 (s, 1H), 5.78 (m, 1H), 3.60 (m, 2H), 3.35 (s, 3H), 3.34 (m, 2H), 3.26 (d, *J* = 4.7 Hz, 4H), 2.28 (dd, *J* = 9.6, 7.4 Hz, 1H), 2.13 (m, 1H), 1.64 (d, *J* = 4.2 Hz, 4H), 1.56 (d, *J* = 3.4 Hz, 2H).

CARBON_01
Compound 43
LSM-104-34

¹³C NMR (100 MHz, CDCl₃) δ 157.6, 152.0, 150.4, 120.9, 103.3, 101.7, 77.5, 77.2, 76.9, 54.6, 49.4, 47.3, 47.1, 32.0, 28.5, 25.5, 23.8.

(R)-N-Methyl-N-(1-(morpholinosulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, **96**

PROTON_01
Compound 44
LSM-104-39

^{1}H NMR (400 MHz, CDCl$_3$) δ 12.37 (s, 1H), 8.35 (s, 1H), 7.15 (s, 1H), 6.58 (s, 1H), 5.79 (m, 1H), 3.76 (m, 4H), 3.65 (m, 2H), 3.38 (m, 2H), 3.36 (s, 3H), 3.28 (m, 4H), 2.27 (dt, *J* = 10.4, 8.5 Hz, 1H), 2.16 (m, 1H).

CARBON_01
Compound 44
LSM-104-39

^{13}C NMR (100 MHz, CDCl$_3$) δ 157.7, 152.2, 150.5, 121.0, 103.5, 101.8, 66.5, 54.7, 49.4, 47.6, 46.5, 32.2, 28.6.

국문초록

핵심 뼈대구조로서 (*R*)-*N*-메틸-*N*-(5-아자스피로[2.4]헵탄-7-일)-7*H*-피롤로[2,3-*d*]피리미딘-4-아민을 이용하여, JAK1 선택적 억제제인 (*R*)-3-(7-(메틸(7*H*-피롤로[2,3-*d*]피리미딘-4-일)아미노)-5-아자스피로[2.4]헵탄-5-일)-3-옥소프로판니트릴 [**(*R*)-6c**]을 발굴하였다. 이 화합물의 구조적 설계는 토파시티닙의 7-디아자퓨린과 5-아자스피로[2.4]헵탄-7-아민의 조합을 기반으로 하였다. 화합물 **(*R*)-6c**은 JAK1의 IC$_{50}$가 8.5 nM이었고, JAK2에 대해 JAK1 선택성 지수는 48배였다. 선도물질로서 화합물 **(*R*)-6c**의 최적화를 위해 세포기반 분석, 인간 전혈 시험, 시험관 수준 ADME, hERG, 인산화효소 프로파일링, 및 약동학 시험을 진행하였다. 마우스 및 랫트 생체 시험을 통해, 화합물 **(*R*)-6c**의 CIA 및 AIA 모델에서의 효력을 확인하였다.

Key words: JAK 억제제, 류마티스 관절염, JAK1-선택적, 콜라겐-유도성 관절염 마우스 모델, adjuvant-유동성 관절염 랫트 모델