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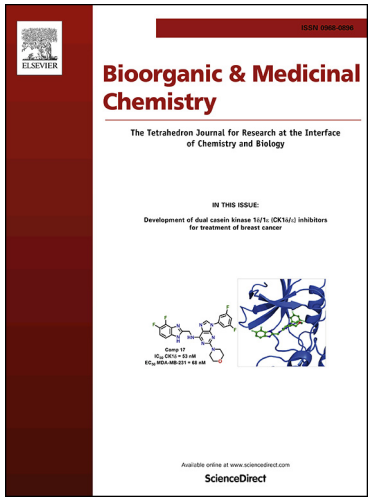

Development of selective inhibitors for the treatment of rheumatoid arthritis: (*R*)-3-(3-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile as a JAK1-selective inhibitor

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Graphical Abstract

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Development of selective inhibitors for the treatment of rheumatoid arthritis: (*R*)-3-(3-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile as a JAK1-selective inhibitor

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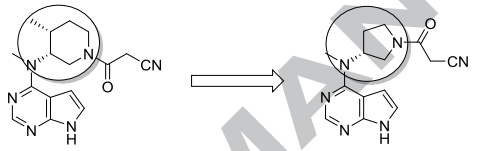
[+] *These two individuals contributed equally to this work.*



Tofacitinib

JAK1 IC_{50} = 2.0 nM
JAK2 IC_{50} = 9.9 nM
Selectivity Index = 5.0

6

JAK1 IC_{50} = 11 nM
JAK2 IC_{50} = 2.4×10^2 nM
Selectivity Index = 23

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Development of selective inhibitors for the treatment of rheumatoid arthritis: (*R*)-3-(3-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile as a JAK1-selective inhibitor

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[a] *Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 08876, South Korea*
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ABSTRACT

A series of 3(*R*)-aminopyrrolidine derivatives were designed and synthesized for JAK1-selective inhibitors through the modification of tofacitinib's core structure, (3*R*,4*R*)-3-amino-4-methylpiperidine. From the new core structures, we selected (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine as a scaffold for further SAR studies. From biochemical enzyme assays and liver microsomal stability tests, (*R*)-3-(3-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile (**6**) was chosen for further *in vivo* test through oral administration. Compound **6** showed improved selectivity for JAK1 compared to that of tofacitinib (IC_{50} 11, $2.4x10^2$, $2.8x10^3$, and $1.1x10^2$ nM for JAK1, JAK2, JAK3, and TYK2, respectively). In CIA and AIA model tests, compound **6** exhibited similar efficacy to tofacitinib citrate.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease related to inflammatory disorder that damages not only joints but also a wide variety of body systems.[1] Much effort has been concentrated in search of the RA's therapeutic targets and recently several targets[2,3] such as cytokines,[4,5] G-protein coupled receptors,[6] and kinases[7,8] have been identified. Several disease-modifying antirheumatic drugs (DMARDS)[9] have been used, however, they have been found to be inappropriate for long term use due to low therapeutic response and some side effects. Since then a few biologics such as etanercept, infliximab, and adalimumab have been introduced.[10] Though these biologics exhibit better efficacies than the synthetic ones, their use has been limited because of high cost, limited i.v. administration, etc.[11,12] Recently new therapeutic targets such as Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signal pathway[13] have been identified for RA treatment. Isolation of JAK kinases was first made in 1989[14] and their roles were discovered in 1994.[15]

Pfizer's tofacitinib, the first US FDA-approved oral rheumatoid arthritis drug, is believed to exhibit its efficacy through the inhibition of Janus kinases (JAKs).[16-18] Though it has shown distinguished therapeutic efficacy for patients who have not responded to the treatment of biologics such as adalimumab and etanercept, it has been known to exhibit some serious adverse effects.[19,20] They include anemia, liver toxicity, lipid level increase, etc., some of which may result from nonselective inhibition of JAK family enzymes. For example, anemia is believed to result from the inhibition of JAK2 isozyme.[21,22] Since tofacitinib is a pan-JAK inhibitor suppressing all JAK isozymes including JAK1, JAK2, JAK3 and TYK2, the need for a new selective inhibitor against a single JAK isozyme has surfaced recently.[23-32] Of the four isozymes, JAK1 has been focused as a selective target for treating rheumatoid arthritis because it can more effectively control the levels of the cytokines involved in the disease symptoms than the others.[33] The representative JAK1-selective inhibitors include filgotinib (GLPG0634),[26,34-41] upadacitinib (ABT-494),[42-46] solcitinib (GSK2586184),[35,47-50] itacitinib (INCB039110),[51-54] PF-04965842.[55-56] The most advanced drug candidate as a JAK1-selective inhibitor is Galapagos's filgotinib, and its phase II result provided the proof-of-concept in selective JAK1 inhibition.[24] Its phase III clinical trial started in 2016.[57-59]

However, from filgotinib's toxicological tests in rat and dog models, adverse effects in testes were reported.[60] Due to this

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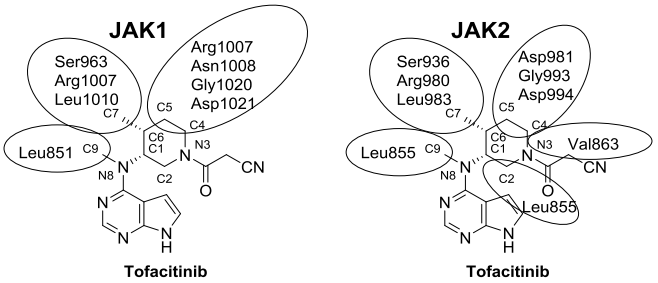


Figure 1. Interactions of tofacitinib with JAK1 or JAK2.

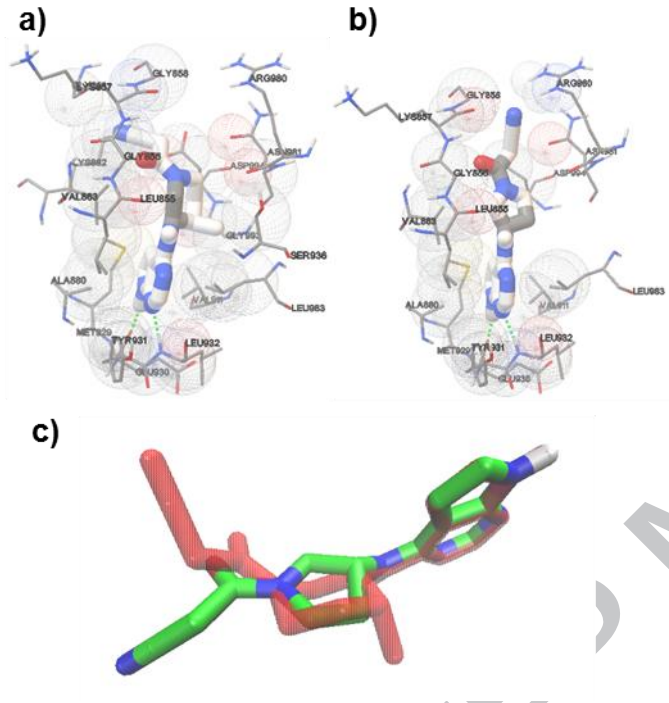
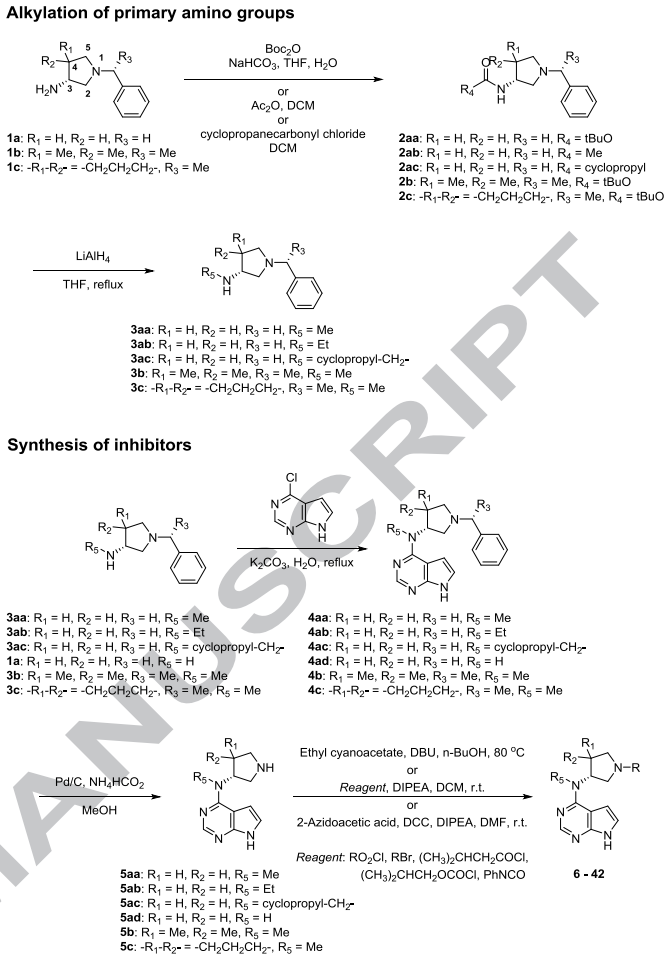


Figure 2. Docking simulation of a) tofacitinib and b) compound **6** at JAK2 (PDB ID: 3FUP) and c) an overlay of the lowest conformations of tofactinib (red color) and compound **6** at JAK2.

problem, the US FDA set lower maximum dosage in the case of male at the phase III clinical trials. In relation with these results, we would like to report our new JAK1 selective inhibitors aimed at resolving the toxicological issue. Our design principle was centered on searching for a JAK1-selective inhibitor possessing a substituted 3(*R*)-aminopyrrolidine moiety in place of the (3*R*,4*R*)-3-amino-4-methylpiperidine of tofacitinib. Here we describe our medicinal chemistry effort in the discovery of 3(*R*)-(3-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile as a more selective inhibitor against JAK1 over JAK2 than tofacitinib (IC_{50} 3.1 nM for JAK1 vs 4.2 nM for JAK2).[61]

2. Strategy

According to the tofacitinib's X-ray crystal structure reported by N. K. Williams *et al.*,[62] the interactions between the piperidine moiety of tofacitinib and each isozyme including JAK1 and JAK2 appear to be the basis for binding affinity differentiation (Figure 1). 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,



Scheme 1. Synthesis of substituted (*R*)-*N*-alkyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines.

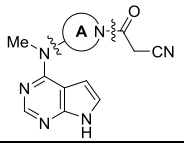
we hypothesized that changing the piperidine moiety of tofacitinib can alter the binding affinity with JAK2 more than that with JAK1.

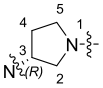
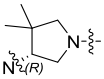
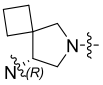
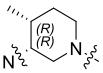
Based upon our hypothesis, we selected a pyrrolidine moiety in place of the piperidine of tofacitinib. A docking simulation using AutoDock 4.2 program[63-65] was performed to assess the effect of the pyrrolidine substitution at the piperidine site of the inhibitors (Figure 2). The estimated binding energies of tofacitinib and our representative compound **6** at JAK1 (PDB ID: 3EYG) were -8.10 and -7.50 kcal/mol, respectively. Besides, estimated binding energies of -8.98 and -7.93 kcal/mol, respectively, for tofacitinib and compound **6** were obtained in the case of JAK2 binding (PDB ID: 3FUP). Increased intermolecular energy of compound **6** with JAK2 appears to result from the absence of its interactions with Ser963 and Leu983 of JAK2. From the above result, we expected that compound **6** 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 **6**.

3. Synthesis

Three 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 (**1a**), (*R*)-4,4-dimethyl-1-((*R*)-1-phenylethyl)pyrrolidin-3-amine (**1b**), and (*R*)-6-((*R*)-1-

Table 1. The IC_{50} values of compounds **6 – 9** and tofacitinib 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 according to the substitution at the 4-position of the pyrrolidine ring.



Cmpd	A	IC_{50} (nM)		SI[a]
		JAK1	JAK2	
6		11	$2.4x10^2$	23
7		$2.6x10^2$	$5.9x10^3$	23
8		16	$4.5x10^2$	28
Tofacitinib		2.0	9.9	5.0

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

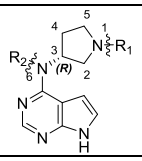
phenylethyl)-6-azaspiro[3.4]octan-8-amine (**1c**). Except for the commercially available (*R*)-3-amino-1-benzylpyrrolidine (**1a**), compounds **1b** and **1c** were synthesized according to published methods.[66,67] Scheme 1 shows a synthetic sequence leading to the pyrrrolidines **5aa – 5c**, from which a variety of derivatives (**6 – 42**) were prepared as potential JAK1 inhibitors: 1) the primary amino group of **1a-c** was protected from the reaction with di-*tert*-butyl dicarbonate, acetic anhydride, or cyclopropanecarbonyl chloride, 2) the *N*-*tert*-butoxycarbonyl-, *N*-acetyl- or *N*-cyclopropanecarbonyl- protected compounds **2aa – 2c** were treated with $LiAlH_4$ to yield alkylated amines **3aa – 3c**, 3) the alkylamines **3aa – 3c** and the unprotected amine **1a** were allowed to react with 6-chloro-7-deazapurine to produce compounds **4aa – 4c**, 4) hydrogenolysis using palladium on carbon and ammonium formate removed the benzyl group of **4aa – 4ad** or 1-phenylethyl moiety of **4b** and **4c**. The desired inhibitors **6 – 42** were obtained from **5aa – 5c** through amide coupling, sulfonylation, alkylation, carbonylation, etc.

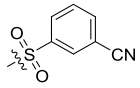
4. Results and discussions

4.1. Enzyme assay

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 **6 – 8** from the three parent pyrrolidine precursors, **5aa**, **5b**, and **5c**. 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 1). The unsubstituted inhibitor **6** exhibited an IC_{50} value of 11 nM for JAK1 and its selectivity index was 23, which was higher than that of tofacitinib (IC_{50} 2.0 nM and 9.9 nM for JAK1 and JAK2, repectively, n = 3). The dimethyl-substituted **7** was 23-fold less potent against JAK1 than that of compound **6**, however, the spirocyclic derivative **8** had similar levels of IC_{50}'s to compound **6**. After identifying the fact that the derivative **8** having (*R*)-6-azaspiro[3.4]octan-8-amine moiety showed the best selectivity of JAK1 over JAK2, we selected the scaffold derived from

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



Cmpd	R_1	R_2	IC_{50} (nM)		SI[a]
			JAK1	JAK2	
6		Me	11	$2.4x10^2$	23
9		Et	62	$1.3x10^3$	21
10			$1.6x10^2$	$1.7x10^3$	11
11		H	$5.1x10^2$	$1.9x10^3$	3.7
12		Me	4.1	57	14
13		Et	19	$1.1x10^2$	5.8
14			17	$1.4x10^3$	82

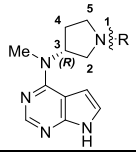
[a] SI: Selectivity Index = JAK2 IC_{50} / JAK1 IC_{50}

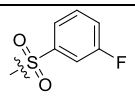
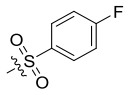
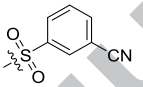
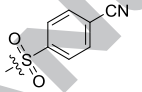
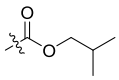
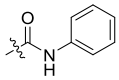
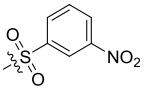
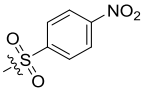
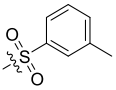
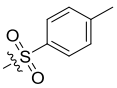
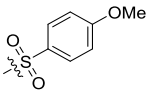
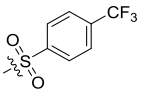
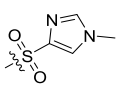
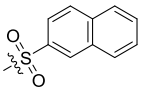
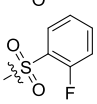
commercially available 3(*R*)-amino-1-benzylpyrrolidine (**1a**) for our structure-activity-relationship (SAR) studies involving many substituents at the pyrrolidine nitrogen because of the simple synthetic steps.

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 **11**, 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 2, we chose methyl group as R_2 and (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine as a scaffold for further SAR studies.

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 3). First, a comparison between amide and alkylamine groups of similar size (**6** vs **15**) 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 **20** 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 **6**, their inhibitions for JAK1 isozyme were less effective (**16**, **18**, and **19**). However, in the case of **20**, its inhibitory activity was similar to that of compound **6** although it has an *N*-phenyl side chain, which is larger than that of compound **6**. With compounds **6** and **17**, 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 (**16** vs **25**). Moreover, the arenesulfonamides (**12** and **27 – 40**) exhibited higher inhibitory activities than the sulfonamides having alkyl or heterocyclic groups (**21 – 26**). As for the substitutions at the benzene ring, inhibitors with substituents at *ortho*-position (**31** and **33**) showed lower

Table 3. 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.



Cmpd	R	IC_{50} (nM)		SI[a]	Cmpd	R	IC_{50} (nM)		SI[a]
		JAK1	JAK2				JAK1	JAK2	
6		11	$2.4x10^2$	23	**29**		14	53	3.8
15		53	$1.9x10^3$	36	**30**		11	$1.1x10^2$	10
16		$7.4x10^2$	$2.7x10^4$	36	**31**		52	$3.7x10^2$	7.1
17		10	$1.7x10^2$	17	**12**		4.1	57	14
18		70	$3.9x10^3$	56	**32**		11	$1.2x10^3$	$1.1x10^2$
19		$1.1x10^2$	$4.3x10^3$	39	**33**		$1.1x10^2$	$1.1x10^3$	10
20		22	$5.5x10^2$	25	**34**		1.9	18	9.5
21		70	$4.7x10^3$	67	**35**		3.6	89	25
22		$1.4x10^2$	$4.5x10^3$	32	**36**		29	$7.3x10^2$	25
23		79	$2.4x10^3$	30	**37**		19	$1.8x10^3$	95
24		$1.7x10^2$	$4.8x10^3$	28	**38**		26	$1.5x10^3$	58
25		34	$4.6x10^2$	14	**39**		9.5	$3.4x10^3$	$3.6x10^2$
26		$6.5x10^2$	$1.9x10^4$	29	**40**		13	$6.9x10^2$	53
27		28	$4.3x10^2$	15	**41**		66	$3.9x10^3$	59
28		25	$1.3x10^2$	5.2	**42**		$2.7x10^2$	$4.4x10^3$	16

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

Table 4. Liver microsomal stabilities of **6**, **12**, **34** and **39**.

Cmpd	% Remaining during 30 min			
	Human (%)	Dog (%)	Rat (%)	Mouse (%)
6	97.6	92.8	92.8	> 100
12	26.3	31.6	3.7	10.0
34	13.4	10.4	2.5	3.1
39	21.0	14.2	11.4	8.4

Table 5. *In vitro* ADME profiles of **6**.

Plasma protein binding (% bound)	
Human	Rat
14.7	17.7

Plasma stability (% remaining)			
Human		Rat	
30 min	120 min	30 min	120 min
96.3	99.8	> 100	> 100

Caco-2 permeability ($\times10^{-5}$ cm/sec)		
P_{app}, A to B	P_{app}, B to A	Efflux ratio
0.38	0.77	2.02

CYP_{450} inhibition (activity % at 10 μM)				
1A2	2C9	2C19	2D6	3A4
89.3	> 100	91.8	> 100	98.2

inhibition than the *meta*- or *para*-counterparts, presumably due to steric interaction with JAK1 except for the fluorine substitution cases (**28** – **30**). In the case of the selectivity for JAK1 over JAK2, the inhibitors with substitution at *para*-position (**30**, **32**, and **35**) showed 2.5 to 7.9-fold improved JAK1 selectivity compared to those having *meta*-substitutions. Consequently, compounds **32** and **39** were the most selective for JAK1 over JAK2.

4.2. In vitro ADME

Since we aimed to develop a drug candidate that can be administered orally for rheumatoid arthritis, we tested some selected compounds (**6**, **12**, **34** and **39**) for liver microsomal stabilities in several species to predict the liver first-pass effect. Remaining percentages of the three compounds were measured after 30 min incubation with human, dog, rat and mouse liver microsomes (LM). As shown in Table 4, the amide **6** showed good stabilities (over 90% remaining) against LM of all species, but the benzenesulfonamides **12**, **34** and **39** showed low stabilities (below 30% remaining) in almost all the species so that the LM stability screening for the other benzenesulfonamide derivatives. Therefore, compound **6**, of which the cyanoacetamide group at the nitrogen of pyrrolidine moiety composes the structure of tofacitinib, was selected for *in vivo* efficacy tests.

In addition, we performed *in vitro* ADME tests such as plasm protein binding, plasma stability, Caco-2 permeability, and CYP_{450} inhibition for the selected compound **6**. It showed low human plasma protein bound percentage of 14.7%, which comes from the low lipophilicity of compound **6**. In human and rat plasma stability tests, compound **6** displayed high plasma stability in both tests. Compound **6** showed moderate permeability in Caco-2 permeability test like filgotinib (filgotinib's P_{app}, A to B = $0.37\text{x}10^{-5}$ cm/sec).[26] However, unlike filgotinib (filgotinib's efflux ratio = 16.5), compound **6** did not seem to be heavily influenced by efflux mechanism. Investigation of compound **6** against five major CYP_{450} isozymes at 10 μM concentration exhibited minimal degrees of inhibition.

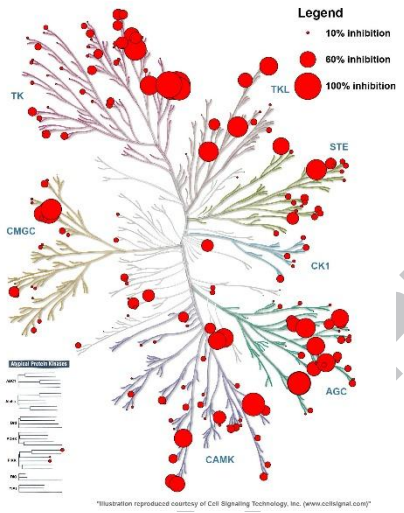


Figure 3. The kinome tree of **6** against 345 kinases at the 10 μM concentration drawn by the web accessible KinMap program.

4.3. Kinase profiling and human ether-a-go-go related gene (hERG) potassium channel assay

Inhibitors targeting ATP-binding site of a kinase can inhibit other kinases because it generally resembles the structure of dephosphorylated ATP. Therefore, we carried out kinase profiling for compound **6** against 345 kinases. At 10 μM concentration, 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. This result indicates that the selectivity of compound **6** may be superior to tofacitinib's one, which inhibited 26 kinases over 90% at the same concentration, as reported by D. C. Borie and colleages.[17] Additionally, we identified that compound **6** exhibited IC_{50} values of $2.8\text{x}10^{3}$ and $1.1\text{x}10^{2}$ nM's for JAK3 and TYK2, respectively, which are two other important targets for pan-JAK inhibitors.

For the prediction for cardiotoxicity of compound **6**, hERG assay was performed at HEK293 cell with the automated patch clamp method. Compound **6** showed IC_{50} value of 93 μM. When filgotinib, competitive JAK1-selective inhibitor, was carried out under the same condition, it showed the IC_{50} value of 85 μM.

*4.4. Pharmacokinetics of **6***

To address oral bioavailability of compound **6**, 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 compound **6** 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*.[68] and R. Weaver *et al*.[69]

In the case of oral administration at 10 mg/kg dosage in male Sprague Dawley rats, compound **6** showed 2.1 hours of half-life ($t_{1/2}$), $4.3\text{x}10^{3}$ ng·h/mL of area under curve from 0 to infinite ($AUC_{0\rightarrow inf}$), $1.9\text{x}10^{3}$ ng/mL of maximum concetration (C_{max}), and 0.30 hour of the time to reach the maximum concentration (T_{max}). 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} = $2.4\text{x}10^{3}$ ng/mL, T_{max} = 0.31 h), compound **6** surpassed tofacitinib with $AUC_{0\rightarrow inf}$ value of $2.8\text{x}10^{3}$ ng·h/mL on drug exposure.[70] In comparison

Table 6. Pharmacokinetic profiles of **6**.

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)	$1.9x10^3$		$1.9x10^3$		$1.0x10^3$	
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)	$6.5x10^3$	$2.2x10^3$	$4.3x10^3$	$9.5x10^2$	$2.1x10^3$	$6.8x10^2$
$AUC_{0\rightarrow t}$ (ng·h/mL)	$6.4x10^3$	$2.1x10^3$	$4.1x10^3$	$9.3x10^2$	$1.9x10^3$	$5.1x10^2$
MRT (h)	3.1	2.4	2.9	0.9	3.1	1.4
F (%)	$1.8x10^2$		$2.2x10^2$		$1.9x10^2$	

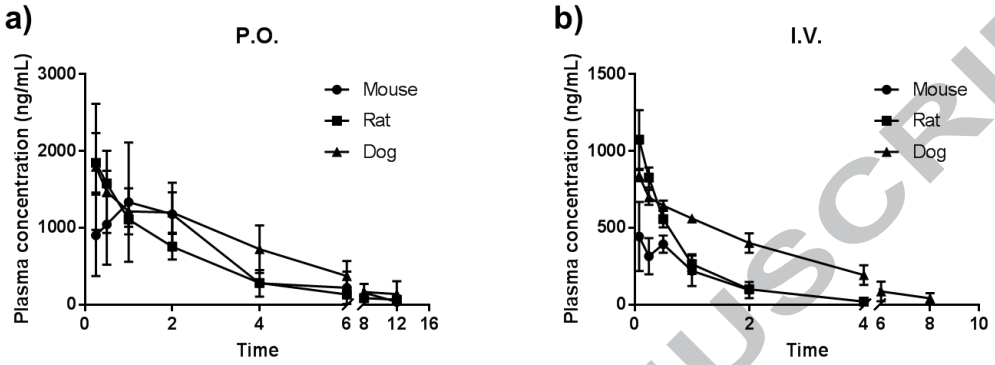


Figure 4. Plasma concentrations after a) oral administration and b) intravenous injection of **6** in Beagle dogs, Sprague-Dawley rats, and ICR mice.

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 slightly lower drug exposure ($AUC_{0\rightarrow t}$ = $1.7x10^3$ ng·h/mL) than compound **6**,[26] although direct comparison with filgotinib and **6** is impossible because of their different oral administration dosages. Compound **6** showed a superior drug exposure to tofacitinib ($AUC_{0\rightarrow inf}$ = $2.3x10^3$ ng·h/mL)[70] in the PK study in male beagle dogs at 5 mg/kg dosage. However, PK profiles of compound **6** in dogs are inferior to the reported values of filgotinib, which features 5.2 hours of half-life ($t_{1/2}$) and $1.4x10^4$ ng·h/mL of $AUC_{0\rightarrow t}$.[26]

*4.5. In vivo efficacy of **6***

To identify *in vivo* efficacies in rheumatoid arthritis models, we employed collagen-induced arthritis (CIA) in DBA/1J mouse[71] and adjuvant-induced arthritis (AIA) in Lewis rat.[72] In mouse CIA model, following indexes were used to evaluate the efficacy of compound **6**: clinical arthritis score, paw volume, serum concentration of IL-6 and TNF-α, bone surface/volume ratio, histopathological semiquantitative score of ankle joint, thickness of ankle joint, thickness of articular surface cartilage and inflammatory cell infiltration in ankle joint (Figure 5). In clinical arthritis score, treatment with compound **6** (100 mg/kg/day) showed more potent inhibition of arthritis symptom than a JAK1-selective inhibitor filgotinib treatment (100 mg/kg/day) and showed similar arthritic score of tofacitinib citrate treatment (50 mg/kg/day) at day 8 to 11 (Figure 5a). However, its symptom relief level was nearly identical to those of other drugs at day 18. Treatment with compound **6** resulted in significant improvements of all other evaluation criteria compared to vehicle treatment. Furthermore, the ameliorating effects of compound **6** treatment were even better than filgotinib or tofacitinib citrate in serum cytokine concentration, bone surface/volume ratio, histopathological features and inflammatory cell infiltration. In a rat AIA model, clinical arthritis score and paw thickness were assessed to evaluate efficacy (Figure 6). Treatment with compound **6** (20 mg/kg/day) significantly attenuated arthritis symptoms to a similar extent as filgotinib (20 mg/kg/day) treatment (Figure 6a) and significantly reduced paw swelling to a similar extent as tofacitinib citrate (10 mg/kg/day) treatment (Figure 6b).

5. Conclusions

From our SAR studies based on substituted pyrrolidine-containing JAK1 selective inhibitors, we have identified our lead compound, (*R*)-3-(3-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile (**6**) as a potential rheumatoid arthritis drug candidate. Its inhibitory activity and selectivity for JAK1 over other JAK's was based on the replacement of the piperidine moiety of tofacitinib by a pyrrolidine moiety. Compound **6** showed desirable selectivity index for JAK1 over JAK2, JAK3 and TYK2, which may lead to decreased side effects. Its human liver microsomal stability was identified to withstand the liver first-pass. Also, we have shown that compound **6** has improved efficacies compared to tofacitinib for treating rheumatoid arthritis in CIA and AIA models, albeit in a somewhat increased dose because of decreased inhibition of JAK isozymes, especially JAK2 and JAK3 by compound **6** than tofacitinib at the same serum concentration. Furthermore, compound **6** surpassed filgotinib, a JAK1-selective inhibitor in phase III, on many *in vivo* efficacy factors, although compound **6** has similar drug exposure through oral administration to filgotinib. In summary, compound **6** has desirable physicochemical properties and efficacy as an oral JAK1-selective inhibitor and these findings suggest that compound **6** can be a good candidate for the treatment of rheumatoid arthritis.

6. Experimental Section

6.1. Docking simulation

The initial structures of ligands, tofacitinib and compound **6**, were obtained through the optimization with Gaussian 03 package[73] with Hatree-Fock method at 6-31G basis set level. JAK1 (PDB ID: 3EYG) and JAK2 (PDB ID: 3FUP) were prepared with removing waters and other ligands from only chain A. Rigid docking simulations were performed with AutoDock 4.2 and AutoDockTools 1.5.6.[63] The grid box composed of 60x60x60 points with spacing of 0.375 angstrom. The Lamarckian genetic algorithm was used as search method for best docking

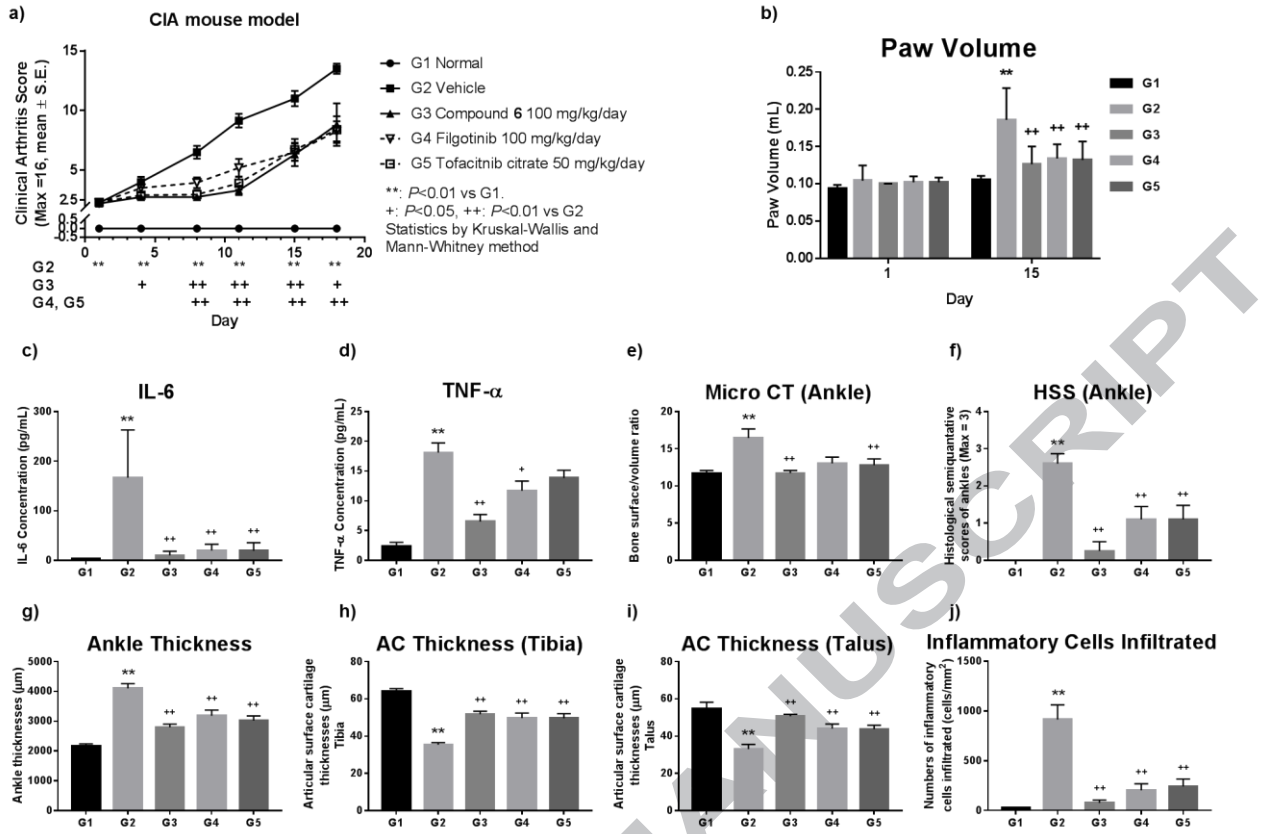


Figure 5. Effects of **6** treatment on collagen-induced arthritis in DBA/1J mice: a) the clinical arthritis scores for 18 days, b) the volumes of right hind paws on days 1 and 15, c-d) the concentrations of IL-6 and TNF-α, respectively, at the serums sampled after autopsy, e) the bone surface/volume ratios of right hind ankle joints measured by micro-CT, f) the histopathological semiquantitative scores of right hind ankle joints, g) the right hind ankle joint thicknesses, h-i) the articular surface cartilage thicknesses (tibia and talus) in right hind ankle joints, and j) 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).

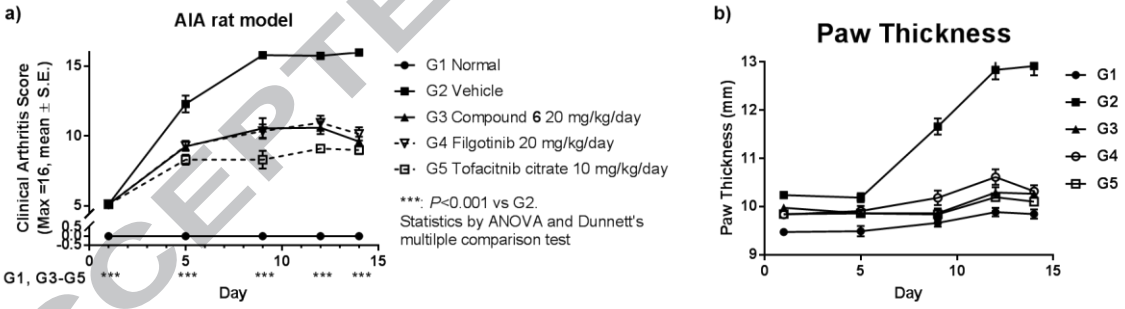


Figure 6. Effects of **6** treatment on adjuvant-induced arthritis in Lewis rats: a) the clinical arthritis scores and b) the volumes of right hind paws. The data were measured twice per week for 14 days.

conformations. The other options were default settings at AutoDockTools 1.5.6.

6.2. Chemistry

All reagents for the syntheses were obtained from commercially available sources and used without any further purification. Except for the commercially available (*R*)-3-amino-1-benzylpyrrolidine (**1a**) and (*R*)-6-((*R*)-1-phenylethyl)-6-azaspiro[3.4]octan-8-amine (**1c**)[66,67] were synthesized according to published methods. The detailed synthetic procedures of (*R*)-4,4-dimethyl-1-((*R*)-1-phenylethyl)pyrrolidin-3-amine (**1b**) was provided in Supporting information. 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) analyses. 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 from JASCO's P-1030 Polarimeter.

6.2.1. Synthesis of tert-butyl (R)-(1-benzylpyrrolidin-3-yl)carbamate, ***2aa***

Sodium bicarbonate (5.92 g, 70.5 mmol) in 118 mL of deionized water was added to (3*R*)-(+)-benzylaminopyrrolidine **1a** (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 educed pressure and the residue was extracted with dichloromethane three times. The 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). $[\alpha]_D$ +2.5^o (*c* 0.620, $CHCl_3$).

In the cases of **2b** and **2c**, the desired products were synthesized from (*R*)-4,4-dimethyl-1-((*R*)-1-phenylethyl)pyrrolidin-3-amine (**1b**) and (*R*)-6-((*R*)-1-phenylethyl)-6-azaspiro[3.4]octan-8-amine (**1c**), respectively, instead of (3*R*)-(+)-benzylaminopyrrolidine **1a** according to the aforementioned process (vide supra).

6.2.1.1. tert-Butyl ((R)-4,4-dimethyl-1-((R)-1-phenylethyl)pyrrolidin-3-yl)carbamate, ***2b***

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). $[\alpha]_D$ +7.4^o (*c* 0.153, $CHCl_3$).

6.2.1.2. tert-Butyl ((R)-6-((R)-1-phenylethyl)-6-azaspiro[3.4]octan-8-yl)carbamate, ***2c***

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^o (*c* 0.387, $CHCl_3$).

In the cases of **2ab** and **2ac**, 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).

6.2.1.3. (R)-N-(1-Benzylpyrrolidin-3-yl)acetamide, ***2ab***

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^o (*c* 0.410, $CHCl_3$).

6.2.1.4. (R)-N-(1-Benzylpyrrolidin-3-yl)cyclopropanecarboxamide, ***2ac***

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^o (*c* 0.397, $CHCl_3$).

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

A *tert*-butyl (*R*)-(1-benzylpyrrolidin-3-yl)carbamate **2aa** (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 ^{o}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 ^{o}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, $CDCl_3$) 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 **3ab** to **3c**, the desired products were synthesized from **2ab** – **2c**, respectively, instead of (*R*)-(1-benzylpyrrolidin-3-yl)carbamate **2aa** according to the aforementioned process (vide supra).

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

Yield: 1.61 g (94.0%). ^{1}H NMR (400 MHz, $CDCl_3$) 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).

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

Yield: 1.68 g (64.0%). ^{1}H NMR (400 MHz, $CDCl_3$) 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).

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

Yield: 238 mg (97.9%). ^{1}H NMR (400 MHz, $CDCl_3$) 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).

6.2.2.4. (R)-N-Methyl-6-((R)-1-phenylethyl)-6-azaspiro[3.4]octan-8-amine, ***3c***

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).

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

A solution of (*R*)-1-benzyl-*N*-methylpyrrolidin-3-amine **3aa** (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. The 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 **4ab**, **4ac**, **4ad**, **4b**, and **4c**, the desired products were synthesized from **3ab**, **3ac**, **1a**, **3b**, and **3c**, respectively, instead of (*R*)-1-benzyl-*N*-methylpyrrolidin-3-amine **3aa** according to the aforementioned process (vide supra).

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

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).

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

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).

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

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).

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

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).

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

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).

6.2.4. Synthesis of (R)-N-methyl-N-(pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***5aa***

A (*R*)-*N*-(1-benzylpyrrolidin-3-yl)-*N*-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **4aa** (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 °C 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)-7*H*-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 **5ab** to **5c**, the desired products were synthesized from **4ab – 4c**, respectively, instead of (*R*)-*N*-(1-benzylpyrrolidin-3-yl)-*N*-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **4aa** according to the aforementioned process (vide supra).

6.2.4.1. (R)-N-Ethyl-N-(pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***5ab***

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).

6.2.4.2. (R)-N-(Cyclopropylmethyl)-N-(pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***5ac***

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).

6.2.4.3. (R)-N-(Pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***5ad***

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).

6.2.4.4. (R)-N-(4,4-Dimethylpyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***5b***

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).

6.2.4.5. (R)-N-Methyl-N-(6-azaspiro[3.4]octan-8-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***5c***

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).

6.2.5. Syntheses of (R)-3-(3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile, ***6***

To an (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **5aa** (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 $C_{14}H_{17}N_6O$: 285.1464. Obsd: 285.1452. $[\alpha]_D$ +42.6° (*c* 1.00, $CHCl_3$).

In the cases of **7**, **8**, **9**, and **10**, the desired products were synthesized from **5b**, **5c**, **5ab**, and **5ac**, respectively, instead of (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **5aa** according to the aforementioned process (vide supra).

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

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 $C_{16}H_{21}N_6O$: 313.1777. Obsd: 313.1772. $[\alpha]_D$ -8.93° (*c* 0.864, $CHCl_3$).

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

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 $C_{17}H_{21}N_6O$: 325.1777. Obsd: 325.1770. $[\alpha]_D$ +7.04° (*c* 0.557, $CHCl_3$).

6.2.5.3. (R)-3-(3-(Ethyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile, ***9***

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. $[\alpha]_D$ +78.5° (*c* 1.09, DMSO).

6.2.5.4. (R)-3-(3-((Cyclopropylmethyl)(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)-3-oxopropanenitrile, ***10***

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. $[\alpha]_D$ +24.5° (*c* 1.08, $CHCl_3$).

6.2.6. Synthesis of (R)-3-((3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, ***12***

To an (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **5aa** (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. $[\alpha]_D$ -45.7° (*c* 0.530, $CHCl_3$).

In the cases of **11**, **13**, and **14**, the desired products were synthesized from **5ad**, **5ab**, and **5ac**, respectively, instead of (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **5aa** according to the aforementioned process (vide supra).

6.2.6.1. (R)-3-((3-((7H-Pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, ***11***

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. $[\alpha]_D$ -27.4° (*c* 1.09, DMSO).

6.2.6.2. (R)-3-((3-(Ethyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, ***13***

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 $C_{19}H_{21}N_6O_2S$: 397.1447. Obsd: 397.1442. $[\alpha]_D$ -63.5° (*c* 0.568, $CHCl_3$).

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

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 $C_{21}H_{23}N_6O_2S$: 423.1603. Obsd: 423.1598. $[\alpha]_D$ -31.5° (*c* 1.49, $CHCl_3$).

6.2.7. Synthesis of (R)-3-(3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)propanenitrile, ***15***

To an (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **5aa** (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. $[\alpha]_D$ +35.3° (*c* 1.07, $CHCl_3$).

In the cases of compound **16**, the desired products were synthesized through substitution reactions with *n*-butyl bromide instead of 3-bromopropionitrile according to the aforementioned process (vide supra).

6.2.7.1. (R)-N-(1-Butylpyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***16***

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. $[\alpha]_D$ +10.6° (*c* 3.42, DMSO).

6.2.8. Synthesis of (R)-2-azido-1-(3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)ethan-1-one, ***17***

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 **5aa** (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 $C_{13}H_{17}N_8O$: 301.1525. Obsd: 301.1522. $[\alpha]_D$ +33.2° (*c* 0.753, $CHCl_3$).

6.2.9. Synthesis of (R)-3-methyl-1-(3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)butan-1-one, ***18***

To an (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **5aa** (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 $C_{16}H_{24}N_5O$: 302.1981. Obsd: 302.1977. $[\alpha]_D$ +29.6° (*c* 1.47, $CHCl_3$).

6.2.10. Synthesis of isobutyl (R)-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidine-1-carboxylate, ***19***

To an (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **5aa** (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 $C_{16}H_{24}N_5O_2$: 318.1930. Obsd: 318.1924. $[\alpha]_D$ +23.7° (*c* 0.550, $CHCl_3$).

6.2.11. Synthesis of (R)-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)-N-phenylpyrrolidine-1-carboxamide, ***20***

To an (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **5aa** (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 $C_{18}H_{21}N_6O$: 337.1777. Obsd: 337.1772. $[\alpha]_D$ +43.6° (*c* 2.44, DMSO).

6.2.12. Synthesis of (R)-N-methyl-N-(1-(methylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***21***

To an (*R*)-*N*-methyl-*N*-(pyrrolidin-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **5aa** (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. $[\alpha]_D$ +23.0° (*c* 1.20, DMSO).

In the cases from **22** to **42**, 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, 3-cyanobenzenesulfonyl chloride, 4-cyanobenzenesulfonyl chloride, 2-nitrobenzenesulfonyl chloride, 3-nitrobenzenesulfonyl chloride, 4-nitrobenzenesulfonyl chloride, 3-toluenesulfonyl chloride, 4-methoxybenzenesulfonyl chloride, 4-toluenesulfonyl 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).

6.2.12.1. (R)-N-Methyl-N-(1-((trifluoromethyl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***22***

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 $C_{12}H_{15}F_3N_5O_2S$: 350.0899. Obsd: 350.0893. $[\alpha]_D$ +19.4° (*c* 2.79, $CHCl_3$).

6.2.12.2. (R)-N-(1-(Ethylsulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***23***

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 $C_{13}H_{20}N_5O_2S$: 310.1338. Obsd: 310.1335. $[\alpha]_D$ +13.1° (*c* 1.24, $CHCl_3$).

6.2.12.3. (R)-N-(1-(Isopropylsulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***24***

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 $C_{14}H_{22}N_5O_2S$: 324.1494. Obsd: 324.1487. $[\alpha]_D$ +18.2° (*c* 0.950, $CHCl_3$).

6.2.12.4. (R)-N-Methyl-N-(1-(propylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***25***

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 $C_{14}H_{22}N_5O_2S$: 324.1494. Obsd: 324.1488. $[\alpha]_D$ +11.3° (*c* 1.52, $CHCl_3$).

6.2.12.5. (R)-N-Methyl-N-(1-((1-methyl-1H-imidazol-4-yl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***26***

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 $C_{15}H_{20}N_7O_2S$: 362.1399. Obsd: 362.1393. $[\alpha]_D$ +12.5° (*c* 0.477, DMSO).

6.2.12.6. (R)-N-Methyl-N-(1-(phenylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***27***

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, $CHCl_3$).

6.2.12.7. (R)-N-(1-((2-Fluorophenyl)sulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***28***

Yield: 96.8 mg (80.6%). 99.4% purity by HPLC. ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (100 MHz, $CDCl_3$) 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, $CHCl_3$).

6.2.12.8. (R)-N-(1-((3-Fluorophenyl)sulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***29***

Yield: 101 mg (84.0%). 100% purity by HPLC. ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (100 MHz, $CDCl_3$) 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, $CHCl_3$).

6.2.12.9. (R)-N-(1-((4-Fluorophenyl)sulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***30***

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 $C_{17}H_{19}FN_5O_2S$: 376.1243. Obsd: 376.1237. $[\alpha]_D$ -35.9° (*c* 0.670, $CHCl_3$).

6.2.12.10. (R)-2-((3-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, ***31***

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 $C_{18}H_{19}N_6O_2S$: 383.1290. Obsd: 383.1287. $[\alpha]_D$ -12.6° (*c* 2.34, $CHCl_3$).

6.2.12.11. (R)-4-((3-(Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)pyrrolidin-1-yl)sulfonyl)benzonitrile, ***32***

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. $[\alpha]_D$ -10.7° (*c* 2.72, DMSO).

6.2.12.12. (R)-N-Methyl-N-(1-((2-nitrophenyl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***33***

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. $[\alpha]_D$ +12.6° (*c* 2.17, $CHCl_3$).

6.2.12.13. (R)-N-Methyl-N-(1-((3-nitrophenyl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***34***

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. $[\alpha]_D$ -41.1° (*c* 1.24, $CHCl_3$).

6.2.12.14. (R)-N-Methyl-N-(1-((4-nitrophenyl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***35***

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 $C_{17}H_{19}N_6O_4S$: 403.1188. Obsd: 403.1185. $[\alpha]_D$ -63.5° (*c* 0.568, $CHCl_3$).

6.2.12.15. (R)-N-Methyl-N-(1-(m-tolylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***36***

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 $C_{18}H_{22}N_5O_2S$: 372.1494. Obsd: 372.1495. $[\alpha]_D$ -39.6° (*c* 3.34, $CHCl_3$).

6.2.12.16. (R)-N-Methyl-N-(1-tosylpyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***37***

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, $CHCl_3$).

6.2.12.17. (R)-N-(1-((4-Methoxyphenyl)sulfonyl)pyrrolidin-3-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***38***

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, $CHCl_3$).

6.2.12.18. (R)-N-Methyl-N-(1-((4-(trifluoromethyl)phenyl)sulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***39***

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, $CHCl_3$).

6.2.12.19. (R)-N-Methyl-N-(1-(naphthalen-2-ylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***40***

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 $C_{21}H_{22}N_5O_2S$: 408.1494. Obsd: 408.1495. $[\alpha]_D$ -40.3° (*c* 3.97, $CHCl_3$).

6.2.12.20. (R)-N-Methyl-N-(1-(piperidin-1-ylsulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***41***

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 $C_{16}H_{25}N_6O_2S$: 365.1760. Obsd: 365.1755. $[\alpha]_D$ +13.7° (*c* 3.06, $CHCl_3$).

6.2.12.21. (R)-N-Methyl-N-(1-(morpholinosulfonyl)pyrrolidin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine, ***42***

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 $C_{15}H_{23}N_6O_3S$: 367.1552. Obsd: 367.1547. $[\alpha]_D$ +12.9° (*c* 1.53, $CHCl_3$).

6.3. In vitro enzyme assays and in vitro ADME tests

All enzyme inhibition assay results were obtained by commercially available KinaseProfiler™ services (Eurofins Scientific, UK) 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 345 kinases at the 10 μM concentration for **6** was drawn by KinMap web accessible tool.[74] All *in vitro* ADME 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.

6.4. 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. The free base form of compound **6** clearly dissolved in 10% ethanol and 90% PEG400 with 1 mL/kg dose volume for intravenous administration. Blood samplings were performed at 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after intravenous administration. For oral administration, compound **6** was suspended in corn oil with 5 mL/kg dose volume. Animals were fasted for 16 hours before oral administration but had free access to water. Blood samplings were performed at 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after oral administration. 20 μL of the sampled plasma was diluted with 180 μL of acetonitrile containing 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 (2.1 x 100 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).

6.5. 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 emulsion of 1:1 mixture of type II collagen (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 emulsion of type II collagen and incomplete Freund's adjuvant. The emulsions were prepared according to manufacturer's instruction.[75] 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 4 treatment groups (n = 10 each) and same-aged naïve mice were assigned to a normal group (n = 6). 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. 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 the following 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 not 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.[76] 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-6 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 the following criteria where 0 = normal; 1 = infiltration of inflammatory cells; 2 = synovial hyperplasia and pannus formation; and 3 = bone erosion and destruction.[77] 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 $P < 0.05$.

6.6. Rat adjuvant-induced arthritis

AIA was induced in SPF Lewis LEW/SsNSlc rats (Japan SLC Inc., Japan). After 2 weeks of acclimation, 10 weeks 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 4 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). 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 $P < 0.05$.

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