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Structure–activity relationship studies of imidazo[1,2-*c*]pyrimidine derivatives as potent and orally effective Syk family kinases inhibitors

Akihito Hirabayashi *, Harunobu Mukaiyama, Hiroaki Kobayashi, Hiroaki Shiohara, Satoko Nakayama, Motoyasu Ozawa, Eiichi Tsuji, Keiji Miyazawa, Keiko Misawa, Hideki Ohnota, Masayuki Isaji

Central Research Laboratory, Kissei Pharmaceutical Company Ltd, 4365-1, Kashiwabara, Hotaka, Azumino-City, Nagano-Pref 399-8304, Japan

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ABSTRACT

Spleen tyrosine kinase (Syk) and zeta-associated protein kinase of 70 kDa (ZAP-70) are members of the Syk family and non-receptor-type protein tyrosine kinases, which play crucial roles in B- and T-cell activation. Therefore, a Syk family tyrosine kinases inhibitor would be a useful therapeutic agent for the treatment of various allergic disorders and autoimmune diseases. Previously, we reported that 1,2,4-triazolo[4,3-*c*]pyrimidine derivative **1** and 1,2,4-triazolo[1,5-*c*]pyrimidine derivative **2** showed strong inhibitory activities against Syk family kinases. These compounds also exhibited high-level suppression of IL-2 in cellular assays. However, their oral efficacies were poor in a mouse model of IL-2 production. To improve oral effectiveness, we investigated a new series of Syk family kinases inhibitors. We found that imidazo[1,2-*c*]pyrimidine derivatives potently inhibited the Syk family kinases. Among these agents, compound **9f** not only showed strong inhibitory activities against Syk and ZAP-70 kinases in vitro, but its oral administration resulted in the in vivo suppression of both the passive cutaneous anaphylaxis reaction and Concanavalin A-induced IL-2 production in a mouse model.

1. Introduction

Protein tyrosine kinases (PTKs) are important enzymes in cell signal transduction.[1] Syk family kinases comprise the cytosolic non-receptor tyrosine classes of Syk and ZAP-70.[2,3] Specifically, Syk is present in platelets, B lymphocytes, mast cells, basophils, neutrophils, dendritic cells, macrophages, and monocytes. Syk kinases also possess functional roles in immunoreceptor tyrosine-based activation motif (ITAM)-mediated signal transduction, which is expressed in various hematopoietic cell types. In contrast, ZAP-70 expression is restricted to T lymphocytes and natural killer (NK) cells. Syk family kinases play crucial roles in immune responses and are implicated in inflammatory and autoimmune diseases.[4–6] Furthermore, several Syk inhibitors were recently reported to be effective in the treatment of inflammatory and autoimmune diseases.[7–11] Therefore, Syk family tyrosine kinase inhibitors are candidate therapeutic agents for the treatment of various allergic and autoimmune disorders.

In the course of our studies aimed at developing a tyrosine kinase inhibitor,[12] we discovered that 1,2,4-triazolo[4,3-*c*]pyrimidine derivative **1** and 1,2,4-triazolo[1,5-*c*]pyrimidine derivative **2** showed strong inhibitory activities against Syk and ZAP-70 kinases in a cellular assay. However, despite the high potency of Syk family kinase inhibition in vitro, these derivatives exhibited poor oral effectiveness in suppressing IL-2 production in a mouse model (data not shown). We examined the physicochemical properties of compounds **1** and **2** and found that these derivatives exhibited low Caco-2 permeability and poor solubility in water. As these parameters are correlated with oral bioavailability,[13,14] we designed new imidazo[1,2-*c*]pyrimidine derivatives. Analyses of these new imidazo[1,2-*c*]pyrimidine derivatives indicated that compound **9f** possessed not only excellent in vitro inhibitory activity against Syk family kinases, but also oral efficacy in suppressing both the passive cutaneous anaphylaxis (PCA) reaction and Concanavalin (Con)A-induced IL-2 production in mice. In the present paper, we outline the development of this imidazo[1,2-*c*]pyrimidine derivative as a new Syk family kinase inhibitor.

2. Chemistry

As shown in Scheme 1, imidazo[1,2-*c*]pyrimidine derivatives were synthesized from 4,6-dichloro-2-methylsulfanylpyrimidine-5-carbonitrile **3**[15] in a four-step conversion. Compound **3** was treated with various aniline derivatives and Hünig's base in tetrahydrofuran (THF), to produce 4-anilinopyrimidine derivatives **4a–f**. The reaction of compounds **4a–f** with aqueous ammonia solution in *N,N*-dimethylformamide (DMF) generated 6-aminopyrimidine derivatives **5a–f**. Hydrolysis of the nitrile groups of **5a–f**

* Corresponding author. Tel.: +81 263 82 8820; fax: +81 263 82 8827.
E-mail address: akihito_hirabayashi@pharm.kissei.co.jp (A. Hirabayashi).

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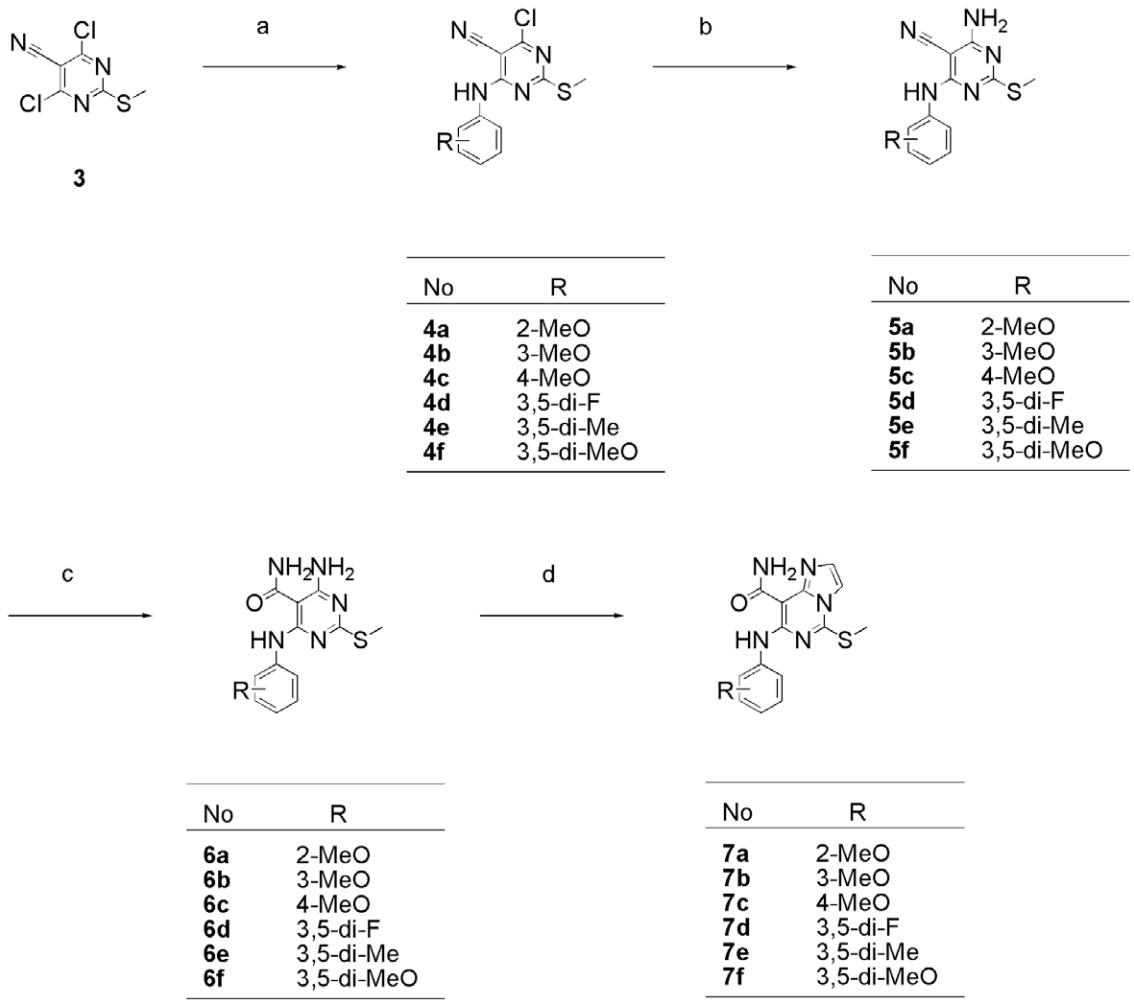


Scheme 1. Reagents: (a) RNH_2, *i*-Pr_2NEt, THF; (b) NH_4OH, DMF; (c) 30% H_2O_2, 5 M NaOH, DMSO, EtOH; (d) $ClCH_2CHO$, DMF.

under basic conditions afforded the corresponding carboxamides **6a–f**. The imidazo[1,2-*c*]pyrimidine derivatives **7a–f** were synthesized by treating carboxamide derivatives **6a–f** with chloroacetaldehyde in DMF. Compounds **8a–g** and **9a–f** were synthesized by substitution reactions of the appropriate commercially available amines in DMF or *N*-methylpyrrolidone (NMP), as shown in Scheme 2.

Scheme 3 summarizes the synthesis of 5-amino-2-phenylimidazo[1,2-*c*]pyrimidine-8-carboxamide derivatives **15a–c**. Compound **4f** was reacted with (*R*)-(−)-2-phenylglycinol to give the alcohol derivative **10**. Construction of 2,3-dihydroimidazo[1,2-*c*]pyrimidine core **11** was achieved by treating compound **10** with phosphorus oxychloride. Conversion of compound **11** to compounds **12a–c** was achieved by introducing various mono-Boc-protected diamino derivatives. Hydrolysis of the nitrile groups in **12a–c** under basic conditions yielded compounds **13a–c**. The absolute configuration of **13a** was confirmed by X-ray crystallography, as shown in Figure 1. The configuration of another diastereomer, **13b**, was determined based on X-ray crystallography of **13a**. The palladium-catalyzed oxidation of 2-phenyl-2,3-dihydroimidazo[1,2-*c*]pyrimidine derivatives **13a–c** afforded 2-phenylimidazo[1,2-*c*]pyrimidine derivatives **14a–c**. Finally, deprotection of the Boc group by treatment with hydrochloric acid or TFA of derivatives **14a–c** gave compounds **15a–c**. The chemical structures of the compounds thus prepared were determined from spectroscopic data (^{1}H NMR, mass spectrometry) and elemental analyses.

3. Binding model

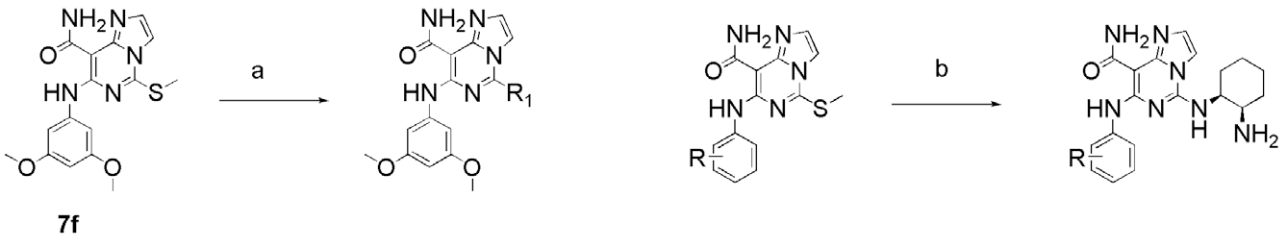
Due to the relatively high degrees of structural homology between Lck and ZAP-70 (or Syk) at their ATP-binding sites, we constructed a ZAP-70 homology model based on the previously published crystal structures of the activated Lck kinase domain (PDB code 1QPD)[16,17] using the full automatic modeling system (FAMS).[18] The binding modes of several compounds were examined using the ADAM software.[19]

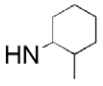
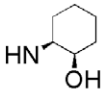
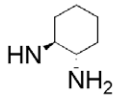
4. Results and discussion

4.1. SAR for Syk and ZAP-70 kinases

The Syk and ZAP-70 kinase inhibitory activities of the compounds synthesized in the present study were evaluated by coupled spectrophotometric enzyme assays (Tables 1–5).

1,2,4-Triazolo[4,3-*c*]pyrimidine derivative **1** and 1,2,4-triazolo[1,5-*c*]pyrimidine derivative **2** showed good inhibitory activities against Syk and ZAP-70, being almost equivalent to the activities of staurosporine. However, these compounds were not orally effective in suppressing IL-2 production in the mouse model. In the analyses of the physicochemical properties of 1,2,4-triazolo[4,3-*c*]pyrimidine derivative **1** and 1,2,4-triazolo[1,5-*c*]pyrimidine derivative **2**, these compounds showed low Caco-2 permeability and poor solubility in water (Table 1). Compounds with high polar



No	R1
8a	NH_2
8b	$NHCH_2CH_2OH$
8c	$NHCH_2CH_2NH_2$
8d	$NHCH_2CH_2CH_2NH_2$
8e	
8f	
8g	

No	R
7a	2-MeO
7b	3-MeO
7c	4-MeO
7d	3,5-di-F
7e	3,5-di-Me
7f	3,5-di-MeO

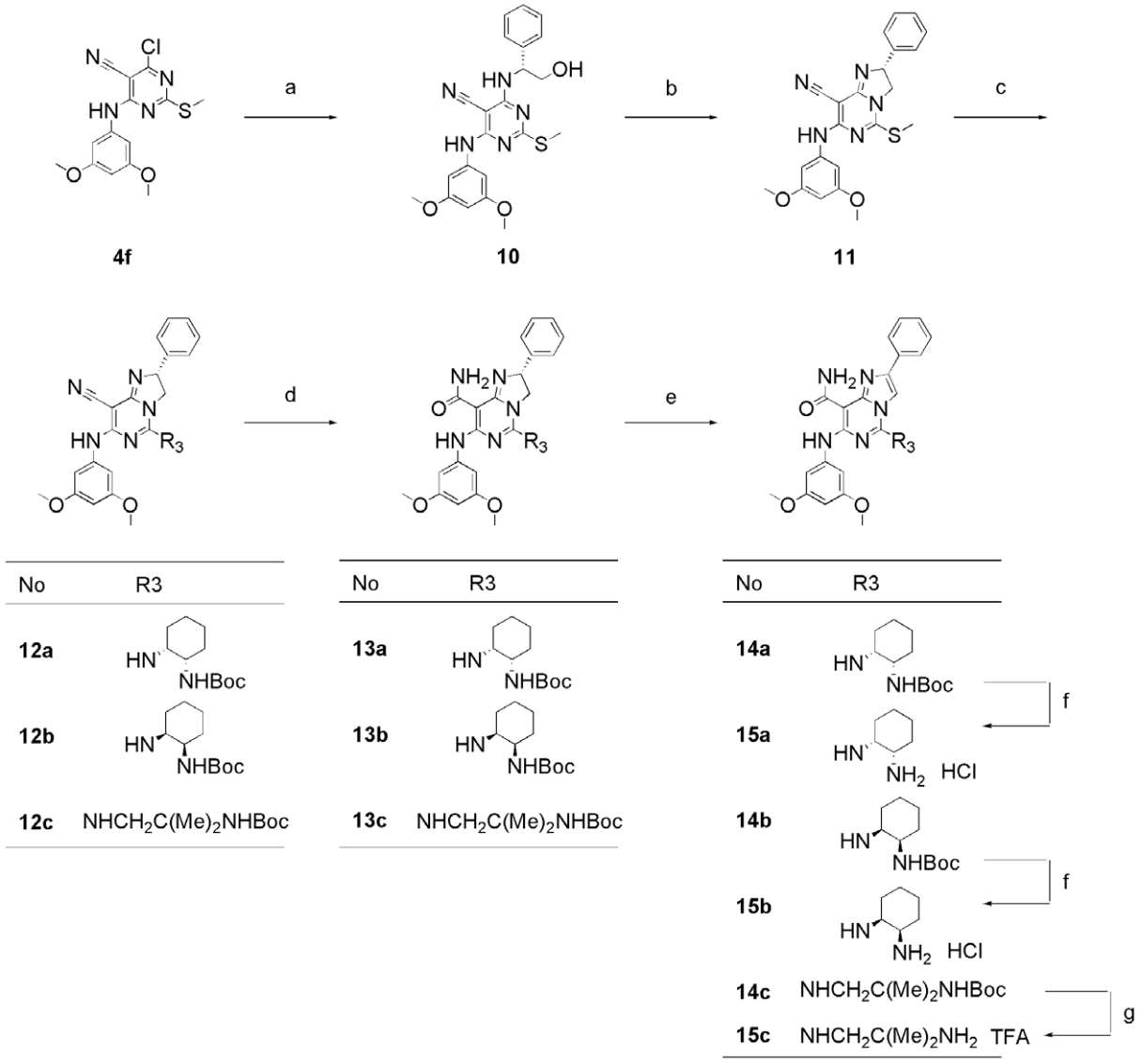
No	R
9a	2-MeO
9b	3-MeO
9c	4-MeO
9d	3,5-di-F
9e	3,5-di-Me
9f	3,5-di-MeO

Scheme 2. Reagents: (a) Commercially available amines, *i*-Pr_2NEt, DMF or NMP; (b) *cis*-1,2-cyclohexyldiamine, DMF.

surface areas (PSAs) tend to show low Caco-2 permeability and poor oral bioavailability.[20,21] We found that the PSA values of compounds **1–2** were comparatively high (141 for compound **1** and 139 for compound **2**). We designed imidazo[1,2-*c*]pyrimidine analogs of 1,2,4-triazolo[4,3-*c*]pyrimidine and 1,2,4-triazolo[1,5-*c*]pyrimidine derivatives with lower PSA values by reducing the number of nitrogen atom. The results of our previous studies on 1,2,4-triazolo[4,3-*c*]pyrimidine and 1,2,4-triazolo[1,5-*c*]pyrimidine derivatives[12] indicate that imidazo[1,2-*c*]pyrimidine derivatives retain similar pharmacophores with triazolopyrimidine analogs. Therefore, 5-aminoimidazo[1,2-*c*]pyrimidine derivatives would be expected to show strong inhibitory activities against Syk family kinases. We investigated the effects of substituents at the 5-position in the imidazo[1,2-*c*]pyrimidine skeleton (Table 2).

The simple amino derivative **8a** showed weak inhibitory activities against Syk and ZAP-70 kinase. For compound **8b**, the inhibitory effects on both Syk and ZAP-70 were slightly improved by the introduction of a hydroxyethylamino group, in comparison to compound **8a**. The 2-aminoethylamino analog **8c** exhibited enhanced Syk kinase inhibition, whereas **8c** showed only slightly improved ZAP-70 inhibitory activity. Both the Syk and ZAP-70 inhibitory activities were reduced in the 3-aminopropylamino derivative **8d**. We investigated several cyclohexylamino derivatives based on their abilities to fit in the sugar pocket. The 2-methylcyclohexylamino derivative **8e** exhibited moderate inhibitory activities against Syk and ZAP-70. Compound **8f**, which possesses a 2-hydroxycyclohexylamine at the 5-position, showed enhanced inhibition of both Syk and ZAP-70 in comparison to **8e**. Next, we examined the cyclohexyldiamino derivatives, which show excellent inhibitory effects on Syk family kinases in the 1,2,4-triazolo[4,3-*c*]pyrimidine state.[12b] The *trans*-cyclohexyldiamino derivative **8g** showed weak inhibitory activity against Syk family kinases and had completely lost the ZAP-70 kinase inhibitory activity. In contrast, *cis*-cyclohexyldiamino derivative **9f** showed excellent kinase inhibitory activity. This stereochemical preference was consistent with that found in the preceding triazole derivatives **1** and **2**.[12b] These findings indicate that the amino group in the cyclohexyl ring plays a crucial role in Syk and ZAP-70 inhibition in comparison to other substituents, such as the methyl and hydroxyl groups. Moreover, the configuration of the amino group in the cyclohexyl ring has an impact inhibitory activity against Syk family kinases.

Subsequently, we investigated the effects of varying substitutions at the 7-anilino groups of the imidazo[1,2-*c*]pyrimidines (Table 3). The 2-methoxy derivative **9a** showed no inhibitory effects on the Syk or ZAP-70 kinase (IC_{50} > 10 μM for both Syk and ZAP-70). The 3-methoxy derivative **9b** and 4-methyoxy derivative **9c** showed strong inhibitory effects against Syk kinase (IC_{50} = 0.030 and 0.092 μM for **9b** and **9c**, respectively) although they showed weak inhibitory activities against the ZAP-70 kinase (IC_{50} > 1.0 μM for both **9b** and **9c**) in comparison to Syk inhibition. The results for compounds **9b** and **9c** indicate that substitution in the *meta* position is superior to that in the *para* position. Therefore, we focused on the *meta* substituent and investigated 3,5-di-substituted derivatives. Compounds **9d–f** showed excellent inhibitory effects against Syk family kinases, especially the Syk kinase. Among these three derivatives, compound **9f** exhibited the greatest inhibitory potency against Syk family kinases (IC_{50} = 0.006 μM and 0.23 μM for Syk and ZAP-70, respectively). These findings indicate that substituents on the 7-anilino group influence inhibitory activity against Syk family kinase inhibition. Substitution at the *meta* and *para* positions is desirable for kinase inhibition. Substitution at the *meta* position was most favorable and di-substitution at the *meta* position in the 7-anilino-group contributed to a greater improvement in inhibition potency against Syk family kinases, as compared to a mono-substituent analog. Specifically, the 3,5-dimethoxy group was the most effective substituent for Syk family kinases inhibition as compared to 3,5-difluoro or 3,5-dimethyl



Scheme 3. Reagents: (a) (*R*)-(−)-2-Phenylglycinol, *i*-Pr_2NEt, DMF; (b) $POCl_3$; (c) diamine derivatives, DMF; Boc_2O, CH_2Cl_2; (d) 30% H_2O_2, 5 M NaOH, DMSO, EtOH; (e) O_2, 10% Pd–C, cyclohexene, EtOH, DMSO; (f) 4 M HCl–EtOAc; (g) TFA, CH_2Cl_2.

derivatives. Examination of the binding mode between ZAP-70 and compound **9f** revealed that the N–H group and the carbonyl group of 8-carbamoyl interacts with the carbonyl group of Glu415 and the N–H group of Ala417, respectively; the N–H group of aniline forms a hydrogen bond with the carbonyl group of Ala417; the imidazole core of compound **9f** occupies the gate keeper residue[22] to form a CH–π interaction with the methyl group of Val352; and the 7-anilino group of compound **9f** on the lipophilic plug[22] forms a CH–π interaction with the methylene groups of Leu344 and Gly420. Moreover, the cyclohexyldiamino group at the 5-position of the imidazo[1,2-*c*]pyrimidine core may contribute to the effective occupation of the sugar pocket (Fig. 2).[22] Conversely, substitution at the *ortho* position was undesirable. Several studies have reported that the conformation of the *ortho*-substituted diphenylaniline derivatives show twisting between the two aromatic rings.[23] We anticipated that twisting conformation between the two aromatic rings broke CH–π interaction with the methylene groups of Leu344 and Gly420 shown in Figure 2. This phenomenon may be responsible for the loss of Syk family kinase inhibitory activity of compound **9a**.

When examining the binding mode in the ZAP-70 homology model, we found a comparatively large space around the imidazole unit. In addition, Syk family kinase inhibition was increased by the introduction of a phenyl group at the 2-position.[12] To improve further the inhibitory activities against Syk and ZAP-70 kinases, we examined 2-phenylimidazo[1,2-*c*]pyrimidine-8-carboxamide derivatives (Table 4). The inhibitory effects of compound **15b** on ZAP-70 kinase were improved by introducing a phenyl group at the 2-position (IC_{50} = 0.041 μM). However, the Syk inhibitory activity was somewhat reduced (IC_{50} = 0.028 μM) compared to that of **9f** (IC_{50} = 0.006 μM). In contrast, compound **15a**, which is a diastereomer of compound **15b**, showed decreased potency for Syk family kinase inhibition. The 2-methylpropane-1,2-diamine derivative **15c** showed moderate inhibitory activities against Syk and ZAP-70 kinases, as compared to the cyclohexyldiamino derivative **15b**. A full large space was produced around the imidazole core by the introduction of a phenyl group (Fig. 3), such that this modification contributed to the inhibitory effect on ZAP-70. Contrary to our expectations, this conversion did not yield an inhibitory effect on Syk.

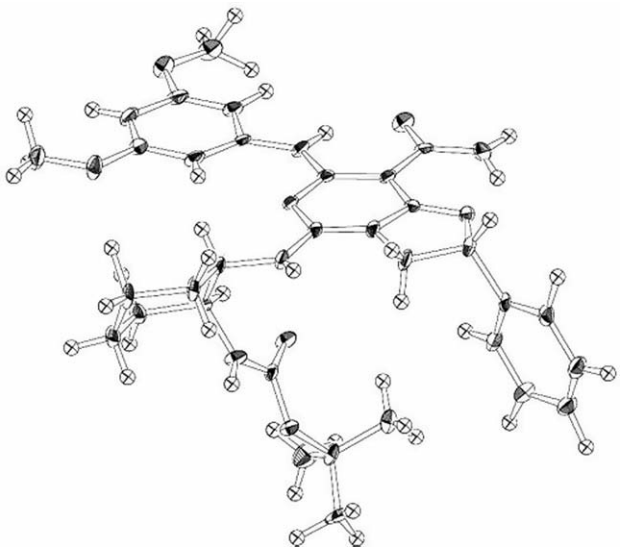
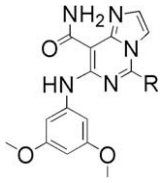


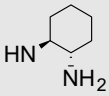
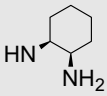
Figure 1. X-ray crystal structure of **13a**.

4.2. Cellular assays and in vivo efficacy in several mouse models

Compounds **9d–f** and **15b** showed excellent kinase inhibitory activities. Therefore, we investigated their effects on IL-2 production in peripheral blood mononuclear cells (PBMCs) and a whole blood (WB) assay system, to evaluate the inhibitory effects on Syk family kinases. The results are summarized in Table 5. Although compounds **9d** and **9e** showed good Syk kinase inhibitory activities, they were less potent than staurosporine in both the PBMCs and WB assay. The 2-phenylimidazo[1,2-*c*]pyrimidine derivative **15b** was also less active than staurosporine in the cellular assay and in the WB system. In contrast, compound **9f** showed excellent inhibition in both the PBMCs and the WB assay. The IL-2-suppressive effect of compound **9f** was almost equivalent to that of staurosporine in our assay system. We then investigated the physicochemical properties of **9f** and **15b**. The Caco-2 permeability of compound **9f** (3.33×10^{-6} cm/s) was superior to that of compound **15b** (0.43×10^{-6} cm/s). We postulate that the higher Caco-2 permeability of **9f** contributes to its greater cellular potency in comparison with **15b**.

Table 2
Syk and ZAP-70 inhibitory activities of imidazo[1,2-*c*]pyrimidines

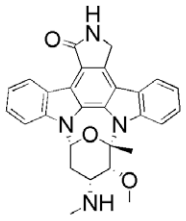


Compound	R	IC_{50}[a] (μM)	
		Syk	ZAP-70
8a	NH_2	7.1	10.0
8b	$NHCH_2CH_2OH$	4.3	1.9
8c	$NHCH_2CH_2NH_2$	0.23	3.5
8d	$NHCH_2CH_2CH_2NH_2$	1.5	12.4
8e		1.0	3.3
8f		0.72	1.8
8g		1.2	>10
9f		0.006	0.23

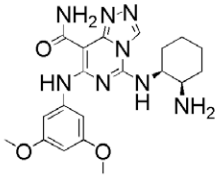
[a] The IC_{50} values were determined in duplicate.

Finally, we evaluated the in vivo effects of optimized Syk family kinase inhibitor **9f** on both the PCA reaction and ConA-induced IL-2 production in mice. Compound **9f** was administered orally to mice and showed a dose-dependent inhibitory effect on the anaphylaxis reaction, as shown in Figure 4. Moreover, compound **9f**, which was administered orally to mice, suppressed IL-2 production in a dose-dependent manner, as shown in Figure 5. These results indicate that compound **9f**, which strongly inhibits Syk and moderately inhibits ZAP-70, could improve diseases that involve both Syk and ZAP-70. The triazolopyrimidine derivative **1** did not suppress IL-2 production in mouse model (data not shown). We investigated the physicochemical properties and plasma concentrations of compounds **1**

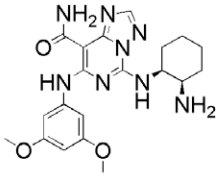
Table 1
Physicochemical properties of triazopyrimidine derivatives



Staurosporine



1

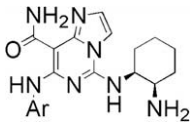


2

Compound	Solubility in water (μg/mL)	Caco2/Papp (10^{-6} cm/s)	IC_{50}[a] (μM)			
			Syk	ZAP-70	PBMC	WB
Staurosporine	NT[b]	NT[b]	0.003	0.053	0.016	0.10
1	351	0.01	0.009	0.12	0.046	0.44
2	42	0.33	0.004	0.33	0.076	0.48

[a] The IC_{50} values were determined in duplicate.
[b] NT, not tested.

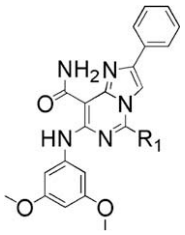
Table 3
Syk and ZAP-70 inhibitory activities of imidazo[1,2-*c*]pyrimidines

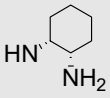
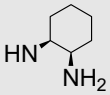


Compound	Ar	IC_{50}[a] (μM)	
		Syk	ZAP-70
9a	2-$MeOC_6H_4$	>10	>10
9b	3-$MeOC_6H_4$	0.030	1.7
9c	4-$MeOC_6H_4$	0.092	4.1
9d	3,5-Di-FC_6H_3	0.030	0.73
9e	3,5-Di-MeC_6H_3	0.006	0.48
9f	3,5-Di-$MeOC_6H_3$	0.006	0.23

[a] The IC_{50} values were determined in duplicate.

Table 4
Syk and ZAP-70 kinase inhibitory activities of 2-phenylimidazolo[1,2-*c*]pyrimidine derivatives



Compound	R1	IC_{50}[a] (μM)	
		Syk	ZAP-70
15a		0.38	0.60
15b		0.028	0.041
15c	$NHCH_2C(Me)_2NH_2$	0.84	0.16

[a] The IC_{50} values were determined in duplicate.

Table 5
Syk and ZAP-70 kinase inhibitory activities and the suppression of IL-2 production in PBMCs and a WB assay

Compound	IC_{50}[a] (μM)			
	Syk	ZAP-70	PBMC	WB
Staurosporine	0.003	0.053	0.016	0.10
9d	0.030	0.73	0.15	3.0
9e	0.006	0.48	0.20	1.0
9f	0.006	0.23	0.058	0.28
15b	0.028	0.041	0.20	2.0

[a] The IC_{50} values were determined in duplicate.

and **9f**, as summarized in Tables 6 and 7. Triazolopyrimidine derivative **1** showed low Caco-2 permeability and poor solubility in water. In contrast, imidazopyrimidine derivative **9f** showed higher Caco-2 permeability and better solubility in water than compound **1**. Furthermore, the PSA value for compound **9f** was lower than that for compound **1** (Table 6). With regard to their pharmacokinetic profiles, the plasma concentration of compound **9f** increased in a dose-dependent manner, while compound **1** was scarcely detected in the plasma after oral administration (Table 7). These results are

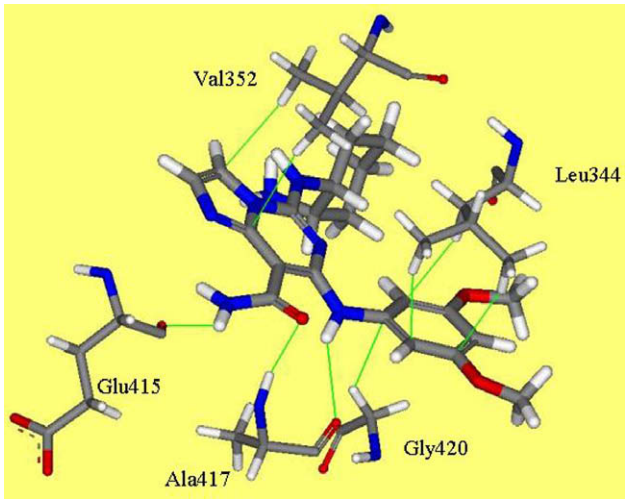


Figure 2. Docking mode of compound **9f** with ZAP-70.

in accordance with our initial hypothesis that Caco-2 permeability, PSA, and solubility in water affect oral efficacy. Therefore, we consider that improvements in its physicochemical properties contribute to the in vivo efficacy of compound **9f**.

5. Conclusions

We demonstrate that novel imidazo[1,2-*c*]pyrimidine derivatives are highly potent and orally effective Syk family kinase inhibitors. Among these agents, optimized compound **9f** is superior to 1,2,4-triazolo[4,3-*c*]pyrimidine derivative **1** with regard to physicochemical profile. Compound **9f** showed not only excellent in vitro kinase inhibitory activity, but also effectively suppressed both the PCA reaction and IL-2 production in a mouse model. We anticipate that the discovery of orally active inhibitors of Syk family kinases will contribute to the treatment of various allergic and immunologic disorders. We intend to continue the optimization and evaluation of these derivatives and will report further developments in due course.

6. Experimental

6.1. Chemistry

Melting points were taken on a Yanako MP-3S Micromelting point apparatus and were uncorrected. Infrared spectra were measured on a Nicolet 510 FT-IR spectrophotometer and were reported in reciprocal centimeters. Proton NMR spectra were recorded at 400 or 500 MHz with a Brucker AMX 400 or DRX 500 instrument, and chemical shifts were reported in parts per million (δ) downfield from tetramethylsilane as internal standard. The peak patterns were shown as the following abbreviations: br, broad; d, doublet; m, multiplet; s, singlet; t, triplet; q, quartet. The mass spectra (MS) were carried out with Thermo Quest FINNIGAN AQA electrospray ionization mass spectrometer. Elemental analyses were performed on an Elementar Vario EL analyzer (C, H, and N). Silica gel 60 F_{254} precoated plates on glass from Merck KgaA or aminopropyl silica gel (APS) precoated NH plates from Fuji Silysia Chemical Ltd were used for thin-layer chromatography (TLC). Flash or medium-pressure liquid chromatography (MPLC) was performed on silica gel BW-350 from Fuji Silysia Chemical Ltd or APS Daisogel IR-60 (particle size 25–40 μM) from Daiso Co., Ltd. All reagents and solvents were commercially available unless otherwise indicated.

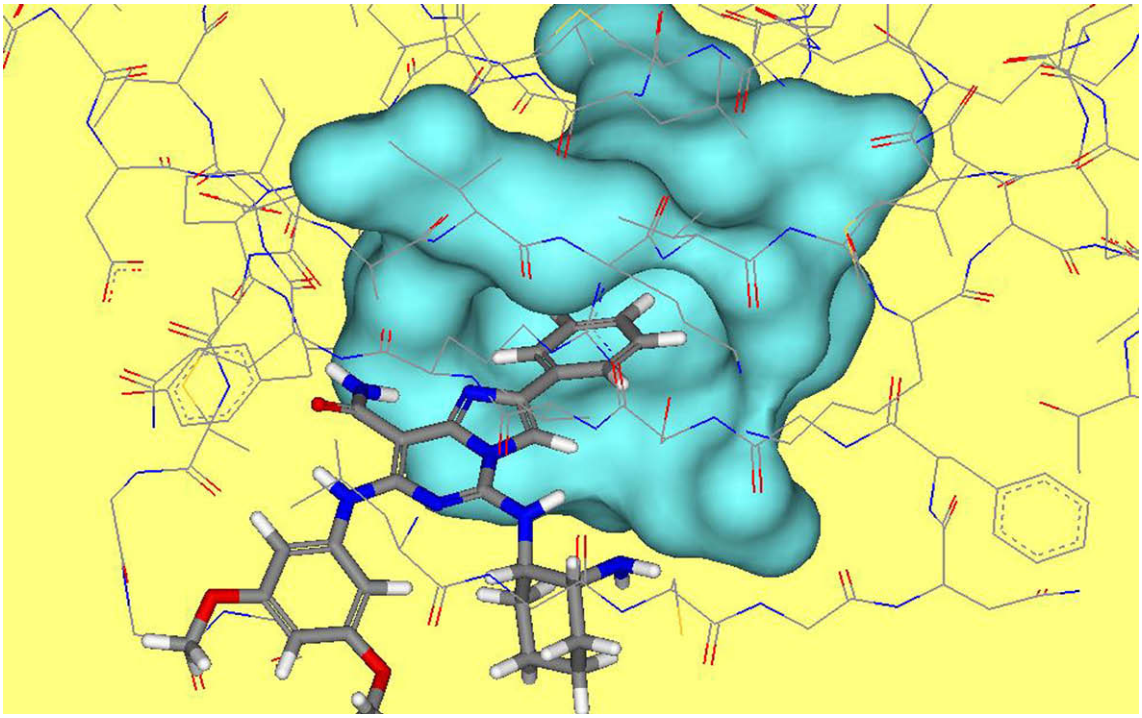


Figure 3. Docking mode of compound **15b** with ZAP-70.

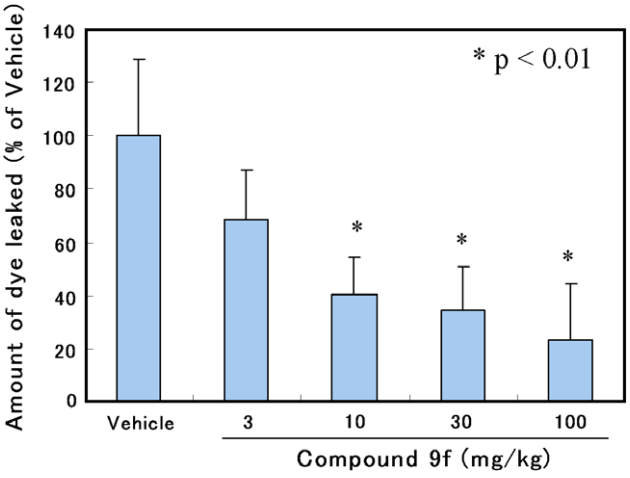


Figure 4. Suppressive effect of compound **9f** on the passive cutaneous anaphylaxis (PCA) reaction.

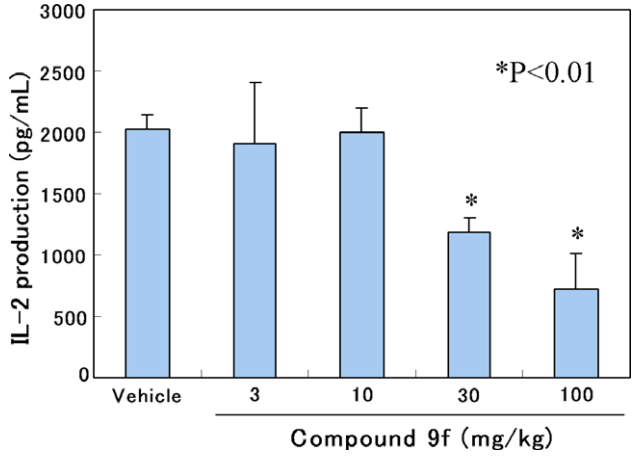


Figure 5. Suppressive effect of compound **9f** on ConA-induced IL-2 production in mice.

6.1.1. 4-Chloro-6-(2-methoxyphenylamino)-2-methylsulfanylpyrimidine-5-carbonitrile (4a)

To a mixture of 4,6-dichloro-2-methylsulfanylpyrimidine-5-carbonitrile **3** (0.660 g, 3.00 mmol) and *o*-anisidine (0.377 g, 3.06 mmol) in THF (10 mL) was added diisopropylethylamine (0.549 mL, 3.15 mmol) at 0 °C and the mixture was stirred overnight at room temperature. Water was added to the mixture and resulting precipitates were collected by suction filtration. The precipitates were washed with water and dried in vacuo to give **4a** (0.920 g, quant.) as white solid: mp 190–192 °C (MeOH–THF); IR (KBr) 2214, 1615, 1549, 1463, 1423, 1355, 1261, 1116 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.32 (3H, s), 3.79 (3H, s), 6.95–7.05 (1H, m), 7.11 (1H, d, J = 7.5 Hz), 7.27–7.32 (1H, m), 7.42 (1H, d, J = 7.5 Hz), 9.76 (1H, br s); MS *m/z*: 307 $(M+H)^+$. Anal. Calcd for $C_{13}H_{11}ClN_4OS$: C, 50.90; H, 3.61; N, 18.26. Found: C, 50.71; H, 3.56; N, 18.11.

6.1.2. 4-Chloro-6-(3-methoxyphenylamino)-2-methylsulfanylpyrimidine-5-carbonitrile (4b)

The title compound was prepared from **3** and *m*-anisidine in the same manner as described for **4a**, and was obtained as white solid (92%): mp 234–235 °C (MeOH–THF); IR (KBr) 2223, 1616, 1555, 1464, 1404, 1261 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.42 (3H, s), 3.75 (3H, s), 6.78 (1H, dd, J = 7.0, 1.0 Hz), 7.14–7.19 (2H, m), 7.28 (1H, t, J = 8.0 Hz), 10.13 (1H, br s); MS *m/z*: 307 $(M+H)^+$. Anal. Calcd for $C_{13}H_{11}ClN_4OS$: C, 50.90; H, 3.61; N, 18.26. Found: C, 50.80; H, 3.58; N, 18.21.

6.1.3. 4-Chloro-6-(4-methoxyphenylamino)-2-methylsulfanylpyrimidine-5-carbonitrile (4c)

The title compound was prepared from **3** and *p*-anisidine in the same manner as described for **4a**, and was obtained as white solid (94%): mp 195–197 °C (MeOH–THF); IR (KBr) 2219, 1617, 1561,

Table 6
Physicochemical properties and cellular potencies of compounds **1** and **9f**

Compound	Solubility in water (μg/mL)	PSA	Caco2/Papp (10^{-6} cm/s)	IC_{50}[a] (μM)			
				Syk	ZAP-70	PBMC	WB
1	351	141	0.01	0.009	0.12	0.046	0.44
9f	930	128	3.33	0.006	0.23	0.058	0.28

[a] The IC_{50} values were determined in duplicate.

Table 7
Plasma concentrations of compounds **1** and **9f** after oral administration

Applied dose (mg/kg)	Plasma concentration (ng/mL)	
	1	**9f**
3	ND[a]	40
10	28.4	191
30	26.5	770
100	45.2	4031

[a] ND, not detected.

1512, 1466, 1437, 1250 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.38 (3H, s), 3.76 (3H, s), 6.94 (2H, d, J = 9.0 Hz), 7.41 (2H, d, J = 9.0 Hz), 10.08 (1H, br s); MS m/z: 307 $(M+H)^+$. Anal. Calcd for $C_{13}H_{11}ClN_4OS$: C, 50.90; H, 3.61; N, 18.26. Found: C, 50.92; H, 3.63; N, 18.26.

6.1.4. 4-Chloro-6-(3,5-difluorophenylamino)-2-methylsulfanylpyrimidine-5-carbonitrile (4d)

The title compound was prepared from **3** and 3,5-difluoroaniline in the same manner as described for **4a**, and was obtained as white solid (92%): mp 203–204 °C (MeOH–THF); IR (KBr) 2221, 1630, 1562, 1410, 1123 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.47 (3H, s), 7.02–7.10 (1H, m), 7.41 (2H, dd, J = 8.5 Hz, 2.0 Hz), 10.38 (1H, br s); MS m/z: 313 $(M+H)^+$. Anal. Calcd for $C_{12}H_7ClF_2N_4S{\cdot}0.2H_2O$: C, 45.56; H, 2.36; N, 17.71. Found: C, 45.60; H, 2.26; N, 17.68.

6.1.5. 4-Chloro-6-(3,5-dimethylphenylamino)-2-methylsulfanylpyrimidine-5-carbonitrile (4e)

The title compound was prepared from **3** and 3,5-dimethylaniline in the same manner as described for **4a**, and was obtained as white solid (98%): mp 180–182 °C (MeOH–THF); IR (KBr) 2223, 1589, 1559,1540,1405 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.26 (6H, s), 2.41 (3H, s), 6.84 (1H, br s), 7.18 (2H, br s), 10.07 (1H, br s); MS m/z: 305 $(M+H)^+$. Anal. Calcd for $C_{14}H_{13}ClN_4S$: C, 55.17; H, 4.30; N, 18.38. Found: C, 54.95; H, 4.26; N, 18.24.

6.1.6. 4-Chloro-6-(3,5-dimethoxylphenylamino)-2-methylsulfanylpyrimidine-5-carbonitrile (4f)

The title compound was prepared from **3** and 3,5-dimethoxyaniline in the same manner as described for **4a**, and was obtained as white solid (91%): mp 214–216 °C (MeOH–THF); IR (KBr) 2225, 1584, 1540, 1481, 1404, 1158 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.45 (3H, s), 3.73 (6H, s), 6.35 (1H, t, J = 2.0 Hz), 6.83 (2H, d, J = 2.0 Hz), 10.05 (1H, br s); MS m/z: 355 $(M+H)^+$. Anal. Calcd for $C_{14}H_{13}ClN_4O_2S$: C, 49.93; H, 3.89; N, 16.64. Found: C, 49.93; H, 3.99; N, 16.49.

6.1.7. 4-Amino-6-(2-methoxyphenylamino)-2-methylsulfanylpyrimidine-5-carboxamide (6a)

To a stirred suspension of **4a** (0.337 g, 1.10 mmol) in DMF (2.4 mL) was added 28% ammonia solution (0.6 mL) and the mixture was heated for 4 h at 60 °C. The mixture was poured into water and resulting residue was collected by suction filtration to give **5a** as white solid. To a suspension of crude **5a** in DMSO (2.5 mL) and EtOH (2.5 mL) were added 5 M NaOH solution (1.10 mL, 5.50 mmol) and 30% H_2O_2 solution (0.625 mL, 5.50 mmol) at 0 °C and the mixture was stirred for 30 min at room temperature. The mixture was poured into water (ca. 100 mL) and resulting precipitates were collected by suction filtration. The precipitates were washed with water and were suspended in EtOAc. The mixture was heated at 110 °C for 1 h and resulted precipitates were collected by glass filter to give **6a** (0.105 g, 31%) as white solid: mp 285–287 °C (MeOH–THF); IR (KBr) 1653, 1589, 1575, 1539, 1533, 1375, 1298, 1272 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.44 (3H, s), 3.84 (3H, s), 6.76 (2H, br s), 6.87–7.04 (3H, m), 7.55 (2H, br s), 8.09 (1H, d, J = 8.0 Hz), 10.16 (1H, s); MS m/z: 306 $(M+H)^+$. Anal. Calcd for $C_{13}H_{15}N_5O_2S{\cdot}0.2CH_4O$: C, 50.85; H, 5.11; N, 22.46. Found: C, 50.85; H, 4.90; N, 22.11.

6.1.8. 4-Amino-6-(3-methoxyphenylamino)-2-methylsulfanylpyrimidine-5-carboxamide (6b)

The title compound was prepared from **4b** in the same manner as described for **6a**, and was obtained as white solid (48%): mp 219–221 °C (MeOH–THF); IR (KBr) 1576, 1539, 1517, 1300 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.43 (3H, s), 3.73 (3H, s), 6.52–6.60 (1H, m), 6.85 (2H, br s), 7.00–7.06 (1H, m), 7.14–7.21 (1H, m), 7.31–7.36 (1H, m), 7.55 (2H, br s), 9.88 (1H, br s); MS m/z: 306 $(M+H)^+$. Anal. Calcd for $C_{13}H_{15}N_5O_2S{\cdot}0.3H_2O$: C, 50.24; H, 5.06; N, 22.54. Found: C, 50.34; H, 4.77; N, 22.35.

6.1.9. 4-Amino-6-(4-methoxyphenylamino)-2-methylsulfanylpyrimidine-5-carboxamide (6c)

The title compound was prepared from **4c** in the same manner as described for **6a**, and was obtained as white solid (46%): mp 246–248 °C (MeOH–THF); IR (KBr) 1576, 1521, 1506, 1300, 1250 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.37 (3H, s), 3.72 (3H, s), 6.65–6.90 (4H, m), 7.30–7.55 (4H, m), 9.69 (1H, br s); MS m/z: 306 $(M+H)^+$. Anal. Calcd for $C_{13}H_{15}N_5O_2S{\cdot}0.42CH_4O$: C, 50.52; H, 5.27; N, 21.97. Found: C, 50.12; H, 4.88; N, 21.82.

6.1.10. 4-Amino-6-(3,5-difluorophenylamino)-2-methylsulfanylpyrimidine-5-carboxamide (6d)

The title compound was prepared from **4d** in the same manner as described for **6a**, and was obtained as white solid (50%): mp 274–276 °C (MeOH–THF); IR (KBr) 1586, 1576, 1538, 1525, 1477, 1297, 1182 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.42 (3H, s), 6.60–7.18 (3H, m), 7.20–7.90 (4H, m), 9.90 (1H, br s); MS m/z: 312 $(M+H)^+$. Anal. Calcd for $C_{12}H_{11}F_2N_5OS{\cdot}0.72H_2O$ $0.1CH_4O$: C, 44.37; H, 3.95; N, 21.38. Found: C, 44.77; H, 3.55; N, 20.98.

6.1.11. 4-Amino-6-(3,5-dimethylphenylamino)-2-methylsulfanylpyrimidine-5-carboxamide (6e)

The title compound was prepared from **4e** in the same manner as described for **6a**, and was obtained as white solid (40%): mp 260–262 °C (MeOH–THF); IR (KBr) 1575, 1538, 1520, 1293 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.23 (6H, s), 2.43 (3H, s), 6.64 (1H, br s), 6.81 (2H, br s), 7.24 (2H, br s), 7.54 (2H, br s), 9.88 (1H, s); MS m/z: 304 $(M+H)^+$. Anal. Calcd for $C_{14}H_{17}N_5OS{\cdot}0.3H_2O$: C, 54.46; H, 5.74; N, 22.68. Found: C, 54.73; H, 5.51; N, 22.69.

6.1.12. 4-Amino-6-(3,5-dimethoxyphenylamino)-2-methylsulfanylpyrimidine-5-carboxamide (6f)

The title compound was prepared from **4f** in the same manner as described for **6a**, and was obtained as white solid (46%): mp 232–234 °C (MeOH–THF); IR (KBr) 1583, 1529, 1482, 1471, 1151 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.44 (3H, s), 3.72 (6H, s), 6.15 (1H, t, J = 2.0 Hz), 6.83 (2H, d, J = 2.0 Hz), 6.85 (2H, s), 7.56 (2H, s), 9.87 (1H, s); MS m/z: 336 $(M+H)^+$. Anal. Calcd for $C_{14}H_{17}N_5O_3S{\cdot}0.5H_2O$: C, 48.83; H, 5.27; N, 20.34. Found: C, 48.63; H, 4.95; N, 20.31.

6.1.13. 7-(2-Methoxyphenylamino)-5-methylsulfanylimidazo-[1,2-*c*]pyrimidine-8-carboxamide (7a)

To a solution of **6a** (0.095 g, 0.311 mmol) in DMF (1 mL) was added 40% chloroacetaldehyde solution (0.183 mL, 0.933 mmol) and the mixture was heated for 6 h at 60 °C. Resulting precipitates were collected by glass filter and washed with water to give **7a** (0.050 g, 49%) as yellowish solid. mp 244–246 °C (MeOH–THF); IR (KBr) 1659, 1610, 1572, 1480, 1312, 1233 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.77 (3H, s), 3.87 (3H, s), 6.94–6.99 (1H, m), 7.02–7.09 (2H, m), 7.49 (1H, d, J = 2.0 Hz), 7.67–7.72 (1H, br), 7.70 (1H, d, J = 2.0 Hz), 8.22 (1H, dd, J = 8.0, 2.0 Hz), 9.56 (1H, d, J = 2.5 Hz), 12.24 (1H, s); MS m/z: 330 $(M+H)^+$. Anal. Calcd for $C_{15}H_{15}N_5O_2S{\cdot}0.2H_2O$: C, 54.10; H, 4.66; N, 21.03. Found: C, 54.21; H, 4.60; N, 20.93.

6.1.14. 7-(3-Methoxyphenylamino)-5-methylsulfanylimidazo-[1,2-*c*]pyrimidine-8-carboxamide (7b)

The title compound was prepared from **6b** in the same manner as described for **7a**, and was obtained as white solid (66%): mp 190–192 °C (MeOH–THF); IR (KBr) 1657, 1595, 1573, 1480, 1465, 1310, 1237, 1158 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.78 (3H, s), 3.77 (3H, s), 6.66 (1H, dd, J = 8.0, 2.0 Hz), 7.11 (1H, d, J = 8.0 Hz), 7.20–7.30 (2H, m), 7.51 (1H, d, J = 1.5 Hz), 7.73 (1H, d, J = 1.5 Hz), 7.88 (1H, br s), 9.61 (1H, br s), 12.29 (1H, s); MS m/z: 330 $(M+H)^+$. Anal. Calcd for $C_{15}H_{15}N_5O_2S$: C, 54.70; H, 4.59; N, 21.26. Found: C, 54.63; H, 4.56; N, 21.07.

6.1.15. 7-(4-Methoxyphenylamino)-5-methylsulfanylimidazo-[1,2-*c*]pyrimidine-8-carboxamide (7c)

The title compound was prepared from **6c** in the same manner as described for **7a**, and was obtained as white solid (70%): mp 221–223 °C (MeOH–THF); IR (KBr) 1607, 1576, 1508, 1487, 1363, 1261 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.65–2.71 (3H, m), 3.70–3.77 (3H, m), 6.90–6.98 (2H, m), 7.46–7.53 (3H, m), 7.68–7.71 (1H, m), 7.80 (1H, br s), 9.55 (1H, br s), 11.95–12.05 (1H, m); MS m/z: 330 $(M+H)^+$. Anal. Calcd for $C_{15}H_{15}N_5O_2S$: C, 54.70; H, 4.59; N, 21.26. Found: C, 54.57; H, 4.56; N, 21.16.

6.1.16. 7-(3,5-Difluorophenylamino)-5-methylsulfanylimidazo-[1,2-*c*]pyrimidine-8-carboxamide (7d)

The title compound was prepared from **6d** in the same manner as described for **7a**, and was obtained as white solid (80%): mp 295–297 °C (MeOH–THF); IR (KBr) 1657, 1608, 1587, 1476, 1363, 1285, 1235, 1146 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.81 (3H, s), 6.87–6.92 (1H, m), 7.40–7.45 (2H, m), 7.56 (1H, d, J = 1.5 Hz), 7.80 (1H, d, J = 1.5 Hz), 8.03 (1H, d, J = 3.0 Hz), 9.63 (1H, d, J = 3.0 Hz), 12.50 (1H, s); MS m/z: 336 $(M+H)^+$. Anal. Calcd for $C_{14}H_{11}F_2N_5OS$: C, 50.14; H, 3.31; N, 20.88. Found: C, 49.79; H, 3.28; N, 20.68.

6.1.17. 7-(3,5-Dimethylphenylamino)-5-methylsulfanylimidazo-[1,2-*c*]pyrimidine-8-carboxamide (7e)

The title compound was prepared from **6e** in the same manner as described for **7a**, and was obtained as white solid (65%): mp 252–254 °C (MeOH–THF); IR (KBr) 1652, 1613, 1582, 1480, 1324, 1236, 1096 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.27 (6H, s), 2.79 (3H, s), 6.72 (1H, s), 7.27 (2H, s), 7.49 (1H, d, J = 2.0 Hz), 7.72 (1H, d, J = 2.0 Hz), 7.86 (1H, d, J = 3.0 Hz), 9.60 (1H, d, J = 3.0 Hz), 12.20 (1H, s); MS m/z: 328 $(M+H)^+$. Anal. Calcd for $C_{16}H_{17}N_5OS$: C, 58.70; H, 5.23; N, 21.39. Found: C, 58.65; H, 5.21; N, 21.30.

6.1.18. 7-(3,5-Dimethoxylphenylamino)-5-methylsulfanyl-imidazo[1,2-*c*]pyrimidine-8-carboxamide (7f)

The title compound was prepared from **6f** in the same manner as described for **7a**, and was obtained as white solid (65%): mp 232–233 °C (MeOH–THF); IR (KBr) 1658, 1578, 1480, 1236, 1157 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.80 (3H, s), 3.76 (6H, s), 6.25 (1H, t, J = 2.0 Hz), 6.80 (2H, d, J = 2.0 Hz), 7.51 (1H, d, J = 1.5 Hz), 7.74 (1H, d, J = 1.5 Hz), 7.90 (1H, d, J = 3.0 Hz), 9.63 (1H, d, J = 3.0 Hz), 12.32 (1H, s); MS m/z: 360 $(M+H)^+$. Anal. Calcd for $C_{16}H_{17}N_5O_3S$: C, 53.47; H, 4.77; N, 19.49. Found: C, 53.45; H, 4.82; N, 19.54.

6.1.19. 5-Amino-7-(3,5-dimethoxyphenylamino)imidazo[1,2-*c*]pyrimidine-8-carboxamide (8a)

A mixture of **7f** (0.150 g, 0.417 mmol) and 28% ammonia solution (0.5 mL) in NMP (1 mL) was heated overnight at 100 °C. The mixture was poured into water and extracted with EtOAc. Organic phase was washed with water and brine, sequentially, dried over anhydrous $MgSO_4$, and evaporated in vacuo. Resulting residue was purified by column chromatography on silica gel with hexane/EtOAc = 1:2 to give **8a** (0.049 g, 38%) as pale green crystal: mp 274–276 °C (MeOH–THF); IR (KBr) 1595, 1508, 1481, 1150 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.76 (6H, s), 6.17 (1H, t, J = 2.0 Hz), 6.86 (2H, d, J = 2.0 Hz), 7.33 (1H, d, J = 2.0 Hz), 7.39 (1H, d, J = 3.5 Hz), 7.80 (1H, d, J = 2.0 Hz), 8.17 (2H, br s), 9.52 (1H, d, J = 3.5 Hz), 12.40 (1H, s); MS m/z: 329 $(M+H)^+$. Anal. Calcd for $C_{15}H_{16}N_6O_3$: C, 54.87; H, 4.91; N, 25.60. Found: C, 54.88; H, 5.09; N, 25.21.

6.1.20. 7-(3,5-Dimethoxyphenylamino)-5-(2-hydroxyethyl-amino)imidazo[1,2-*c*]pyrimidine-8-carboxamide (8b)

The title compound was prepared from **7f** and 2-hydroxyethylamine in the same manner as described for **8a**, and was obtained as white solid (13%): mp 261–263 °C (MeOH–THF); IR (KBr) 1616, 1593, 1550, 1507 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.61–3.70 (4H, m), 3.75 (6H, s), 4.90 (1H, t, J = 5.0 Hz), 6.16 (1H, t, J = 2.0 Hz), 6.86 (2H, d, J = 2.0 Hz), 7.34 (1H, d, J = 1.5 Hz), 7.41 (1H, d, J = 3.0 Hz), 7.93 (1H, d, J = 1.5 Hz), 8.46 (1H, s), 9.55 (1H, d, J = 3.0 Hz), 12.52 (1H, s); MS m/z: 373 $(M+H)^+$. Anal. Calcd for $C_{17}H_{20}N_6O_4$: C, 54.83; H, 5.41; N, 22.57. Found: C, 54.56; H, 5.43; N, 22.43.

6.1.21. 5-(2-Aminoethylamino)-7-(3,5-dimethoxyphenyl-amino)imidazo[1,2-*c*]pyrimidine-8-carboxamide (8c)

The title compound was prepared from **7f** and 2-aminoethylamine in the same manner as described for **8a**, and was obtained as white solid (46%): mp 221–223 °C (MeOH–THF); IR (KBr) 1668, 1595, 1502, 1478, 1372 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.85 (2H, t, J = 6.0 Hz), 3.25–3.45 (3H, br), 3.56 (2H, t, J = 6.0 Hz), 3.76 (6H, s), 6.17 (1H, t, J = 2.0 Hz), 6.89 (2H, d, J = 2.0 Hz), 7.34 (1H, d, J = 1.5 Hz), 7.41 (1H, d, J = 3.0 Hz), 7.90 (1H, d, J = 1.5 Hz), 9.54 (1H, d, J = 3.0 Hz), 12.51 (1H, s); MS m/z: 372 $(M+H)^+$. Anal. Calcd for $C_{17}H_{21}N_7O_3{\cdot}1.0C_5H_9NO$: C, 56.16; H, 6.43; N, 23.81. Found: C, 55.57; H, 6.35; N, 23.66.

6.1.22. 5-(3-Aminopropylamino)-7-(3,5-dimethoxyphenyl-amino)imidazo[1,2-*c*]pyrimidine-8-carboxamide (8d)

The title compound was prepared from **7f** and 1,3-diaminopropane in the same manner as described for **8a**, and was obtained as white solid (24%): mp 241–242 °C (MeOH–THF); IR (KBr) 3390, 3282, 1691, 1594, 1553, 1503, 1480, 1458, 1239, 1149 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.82–1.86 (2H, m), 3.20 (2H, q, J = 7.5 Hz), 3.50–3.70 (2H, m), 3.75 (6H, s), 6.18 (1H, t, J = 2.0 Hz), 6.86 (2H, d, J = 2.0 Hz), 7.34 (1H, d, J = 2.0 Hz), 7.40 (1H, d, J = 4.5 Hz), 7.85 (1H, d, J = 2.0 Hz), 7.95–8.05 (2H, m), 8.31 (1H, br s), 9.53 (1H, d, J = 4.5 Hz), 12.49 (1H, s); MS m/z: 386 $(M+H)^+$. Anal. Calcd for $C_{18}H_{23}N_7O_3{\cdot}1.0CH_4O$: C, 54.66; H, 5.52; N, 23.49. Found: C, 54.79; H, 5.69; N, 23.31.

6.1.23. 7-(3,5-Dimethoxyphenylamino)-5-(2-methylcyclohexylamino)imidazo[1,2-*c*]pyrimidine-8-carboxamide (8e)

The title compound was prepared from **7f** and 2-methylcyclohexylamine in the same manner as described for **8a**, and was obtained as white solid (22%): mp 187–188 °C (MeOH–THF); IR (KBr) 3404, 2931, 1595, 1497, 1480, 1378, 1291, 1239, 1203, 1151, 1067 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 0.92 (3H, d, J = 8.0 Hz), 1.08–2.01 (9H, m), 3.74–3.84 (1H, m), 3.76 (6H, s), 6.21 (1H, t, J = 2.0 Hz), 6.83 (2H, d, J = 2.0 Hz), 7.34 (1H, d, J = 1.5 Hz), 7.36 (1H, d, J = 4.5 Hz), 7.60–8.17 (2H, m), 9.52 (1H, d, J = 4.5 Hz), 12.41 (1H, s); MS m/z: 425 $(M+H)^+$. Anal. Calcd for $C_{22}H_{28}N_6O_3$: C, 62.25; H, 6.65; N, 19.80. Found: C, 61.87; H, 6.61; N, 19.65.

6.1.24. *cis*-7-(3,5-Dimethoxyphenylamino)-5-(2-hydroxycyclohexylamino)imidazo[1,2-*c*]pyrimidine-8-carboxamide (8f)

The title compound was prepared from **7f** and *cis*-2-hydroxycyclohexylamine in the same manner as described for **8a**, and was obtained as white solid (22%): mp 261–262 °C (MeOH–THF); IR (KBr) 3405, 2935, 1594, 1544, 1502, 1476, 1378, 1240, 1150 cm^{-1}; ^1H NMR (DMSO-d_6) δ:1.25–2.00 (8 H, m), 3.75 (6H, s), 4.03–4.15 (2H, m), 4.80 (1H, d, J = 5.5 Hz), 6.16 (1H, t, J = 2.0 Hz), 6.79 (2H, d, J = 2.0 Hz), 7.31 (1H, d, J = 2.0 Hz), 7.37 (1H, d, J = 4.5 Hz), 7.85 (1H, d, J = 9.5 Hz), 8.14 (1H, d, J = 2.0 Hz), 9.54 (1H, d, J = 4.5 Hz), 12.37 (1H, s); MS m/z: 427 $(M+H)^+$. Anal. Calcd for $C_{21}H_{26}N_6O_4{\cdot}0.2H_2O$: C, 58.64; H, 6.19; N, 19.54. Found: C, 58.96; H, 6.23; N, 19.19.

6.1.25. *trans*-5-(2-Aminocyclohexylamino)-7-(3,5-dimethoxyphenylamino)imidazo[1,2-*c*]pyrimidine-8-carboxamide (8g)

The title compound was prepared from **7f** and *trans*-cyclohexyldiamine in the same manner as described for **8a**, and was obtained as white solid (37%): mp 213–215 °C (MeOH–THF); IR (KBr) 1598, 1499, 1457, 1240, 1147 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.05–1.41 (4H, m), 1.65–2.10 (4H, m), 2.71–2.78 (1H, m), 3.30–3.35 (3H, br), 3.75–3.83 (1H, m), 3.76 (6H, s), 6.20 (1H, t, J = 2.0 Hz), 6.84 (2H, d, J = 2.0 Hz), 7.33 (1H, d, J = 1.5 Hz), 7.38 (1H, d, J = 3.5 Hz), 7.96 (1H, d, J = 1.5 Hz), 9.54 (1H, d, J = 3.5 Hz), 12.41 (1H, s); MS m/z: 426 $(M+H)^+$. Anal. Calcd for $C_{21}H_{27}N_7O_3{\cdot}0.8H_2O$: C, 57.34; H, 6.55; N, 22.29. Found: C, 57.34; H, 6.25; N, 21.92.

6.1.26. *cis*-5-(2-Aminocyclohexylamino)-7-(2-methoxyphenylamino)imidazo[1,2-*c*]pyrimidine-8-carboxamide (9a)

The title compound was prepared from **7a** in the same manner as described for **8a**, and was obtained as white solid (16%): mp 212–214 °C (MeOH–THF); IR (KBr) 1608, 1502, 1480, 1382, 1233 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.30–1.95 (8H, m), 3.26–3.34 (3H, br), 3.35–3.38 (1H, m), 3.85 (3H, s), 4.06–4.13 (1H, m), 6.90 (1H, dt, J = 8.0, 1.5 Hz), 6.97 (1H, dt, J = 8.0, 1.5 Hz), 7.30 (1H, dd, J = 8.0, 1.5 Hz), 7.19 (1H, d, J = 3.5 Hz), 7.32 (1H, d, J = 2.0 Hz), 8.07 (1H, d, J = 2.0 Hz), 8.38 (1H, dd, J = 8.0, 1.5 Hz), 9.49 (1H, d, J = 3.5 Hz), 12.35 (1H, s); MS m/z: 396 $(M+H)^+$. Anal. Calcd for $C_{20}H_{25}N_7O_2{\cdot}0.7H_2O$: 58.87; H, 6.52; N, 24.03. Found: C, 59.16; H, 6.40; N, 23.63.

6.1.27. *cis*-5-(2-Aminocyclohexylamino)-7-(3-methoxyphenylamino)imidazo[1,2-*c*]pyrimidine-8-carboxamide (9b)

The title compound was prepared from **7b** in the same manner as described **8a**, and obtained as white solid (26%): mp 188–190 °C (MeOH–THF); IR (KBr) 1598, 1497, 1374, 1238, 1150 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.26–1.97 (8H, m), 3.25–3.38 (4H, m), 3.77 (3H, s), 4.07–4.15 (1H, m), 6.61 (1H, d, J = 8.0 Hz), 7.15 (1H, d, J = 7.5 Hz), 7.21 (1H, t, J = 7.5 Hz), 7.26 (1H, s), 7.32 (1H, br s), 7.39 (1H, br s), 8.10 (1H, s), 9.53 (1H, s), 12.39 (1H, s); MS m/z: 396 $(M+H)^+$. Anal. Calcd for $C_{20}H_{25}N_7O_2$ $0.7H_2O$: C, 58.87; H, 6.52; N, 24.03. Found: C, 59.19; H, 6.42; N, 23.65.

6.1.28. *cis*-5-(2-Aminocyclohexylamino)-7-(4-methoxyphenylamino)imidazo[1,2-*c*]pyrimidine-8-carboxamide (9c)

The title compound was prepared from **7c** in the same manner as described **8a**, and obtained as white solid (8.3%): mp 213–214 °C (MeOH–THF); IR (KBr) 1601, 1549, 1506, 1387, 1294, 1237 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.25–1.93 (8H, m), 3.26–3.38 (4H, m), 3.74 (3H, s), 3.98–4.05 (1H, m), 6.89 (2H, d, J = 9.0 Hz), 7.29 (1H, d, J = 1.5 Hz), 7.32 (1H, d, J = 3.5 Hz), 7.50 (2H, d, J = 9.0 Hz), 8.06 (1H, d, J = 1.5 Hz), 9.47 (1H, d, J = 3.5 Hz), 12.14 (1H, s); MS m/z: 396 $(M+H)^+$. Anal. Calcd for $C_{20}H_{25}N_7O_2$ $0.3H_2O$: C, 59.92; H, 6.44; N, 24.46. Found: C, 60.21; H, 6.41; N, 24.13.

6.1.29. *cis*-5-(2-Aminocyclohexylamino)-7-(3,5-difluorophenylamino)imidazo[1,2-*c*]pyrimidine-8-carboxamide (9d)

The title compound was prepared from **7d** in the same manner as described for **8a**, and was obtained as white solid (21%): mp 240–241 °C (MeOH–THF); IR (KBr) 1601, 1501, 1475, 1449, 1377, 1238, 1145 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.28–1.98 (8H, m), 3.20–3.45 (3H, br), 3.36–3.41 (1H, m), 4.05–4.11 (1H, m), 6.75–6.84 (1H, m), 7.36 (1H, d, J = 1.5 Hz), 7.37–7.41 (2H, m), 7.54 (1H, d, J = 3.0 Hz), 8.15 (1H, d, J = 1.5 Hz), 9.56 (1H, d, J = 3.0 Hz), 12.74 (1H, s); MS m/z: 402 $(M+H)^+$. Anal. Calcd for $C_{19}H_{21}F_2N_7O{\cdot}0.2CH_4O$: C, 56.55; H, 5.39; N, 24.04. Found: C, 56.53; H, 5.30; N, 23.66.

6.1.30. *cis*-5-(2-Aminocyclohexylamino)-7-(3,5-dimethylphenylamino)imidazo[1,2-*c*]pyrimidine-8-carboxamide (9e)

The title compound was prepared from **7e** in the same manner as described for **8a**, and was obtained as white solid (16%): mp 188–189 °C (MeOH–THF); IR (KBr) 1616, 1558, 1539, 1499, 1375 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.22–1.94 (8H, m), 2.28 (6H, s), 3.26–3.38 (4H, m), 4.11–4.16 (1H, m), 6.66 (1H, s), 7.23 (2H, s), 7.31 (1H, s), 7.37 (1H, d, J = 3.0 Hz), 8.10 (1H, s), 9.54 (1H, d, J = 3.0 Hz), 12.35 (1H, s); MS m/z: 394 $(M+H)^+$. Anal. Calcd for $C_{21}H_{27}N_7O{\cdot}0.3CH_4O$: C, 63.47; H, 7.05; N, 24.32. Found: C, 63.64; H, 6.91; N, 24.05.

6.1.31. *cis*-5-(2-Aminocyclohexylamino)-7-(3,5-dimethoxylphenylamino)imidazo[1,2-*c*]pyrimidine-8-carboxamide (9f)

The title compound was prepared from **7f** in the same manner as described for **8a**, and was obtained as white solid (37%): mp 198–200 °C (MeOH–THF); IR (KBr) 1596, 1540, 1497, 1240, 1154 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.26–1.96 (8H, m), 3.26–3.40 (4H, m), 3.75 (6H, s), 4.11–4.18 (1H, m), 6.21 (1H, s), 6.80 (2H, d, J = 2.0 Hz), 7.33 (1H, s), 7.40 (1H, s), 8.11 (1H, s), 9.54 (1H, s), 12.38 (1H, s); MS m/z: 426 $(M+H)^+$. Anal. Calcd for $C_{21}H_{27}N_7O_3{\cdot}1.0H_2O$: C, 56.87; H, 6.59; N, 22.11. Found: C, 56.76; H, 6.30; N, 21.87.

6.1.32. 4-(3,5-Dimethoxyphenylamino)-6-((*R*)-2-hydroxy-1-phenylethylamino)-2-methylsulfanylpyrimidine-5-carbonitrile (10)

A mixture of compound **4f** (0.914 g, 2.71 mmol), (*R*)-(−)-2-amino-2-phenylethanol (0.391 g, 2.85 mmol) and diisopropylethylamine (0.520 mL, 2.99 mmol) in DMF (10 mL) was stirred at 50 °C for 3 h. Water was added to the mixture and extracted with EtOAc. The organic phase was washed with water and brine, sequentially, dried over anhydrous $MgSO_4$, and evaporated in vacuo. Resulting residue was purified by column chromatography on silica gel with hexane/EtOAc = 1:1 to give **10** (0.876 g, 74%) as yellowish solid: mp 141–144 °C (MeOH); α_D^{28} −34.78° (c 0.23, MeOH); IR (KBr) 2195, 1591, 1560, 1487, 1437, 1155 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.31 (3H, s), 3.65–3.84 (2H, m), 3.70 (6H, s), 5.04 (1H, t, J = 6.0 Hz), 5.21–5.28 (1H, m), 6.21 (1H, t, J = 2.0 Hz), 6.82 (2H, d, J = 2.0 Hz), 7.20–7.40 (5H, m), 7.70 (1H, d, J = 7.0 Hz), 9.14 (1H, s); MS m/z: 438 $(M+H)^+$. Anal. Calcd for $C_{22}H_{23}N_5O_3S{\cdot}0.1H_2O$: C, 60.14; H, 5.32; N, 15.94. Found: C, 59.92; H, 5.26; N, 15.91.

6.1.33. (*R*)-7-(3,5-Dimethoxyphenylamino)-5-methylsulfanyl-2-phenyl-2,3-dihydroimidazo[1,2-*c*]pyrimidine-8-carbonitrile (11)

A solution of compound **10** (0.697 g, 1.59 mmol) in phosphorus oxychloride (6 mL) was stirred at 60 °C for 3 h. The mixture was poured into ice-cold water and neutralized with $NaHCO_3$ powder. The mixture was extracted with EtOAc. The organic phase was wished with water and brine, sequentially, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The residue was triturated from Et_2O and resulting precipitates were collected by suction filtration to give **11** (0.622 g, 93%) as yellowish solid: mp 207–209 °C (MeOH–THF); α_D^{28} −76.30° (*c* 0.54, MeOH); IR (KBr) 2195, 1645, 1588, 1489, 1405, 1205, 1154 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.47 (3H, s), 3.55–3.62 (1H, m), 3.71 (6H, s), 4.36 (1H, t, *J* = 10.0 Hz), 5.26 (1H, dd, *J* = 10.0 Hz, 2.5 Hz), 6.26 (1H, t, *J* = 2.0 Hz), 6.70 (2H, d, *J* = 2.0 Hz), 7.25–7.39 (5H, m), 9.43 (1H, s); MS *m/z*: 420 $(M+H)^+$. Anal. Calcd for $C_{22}H_{21}N_5O_2S{\cdot}0.15CH_4O$: C, 62.70; H, 5.13; N, 16.50. Found: C, 63.06; H, 5.20; N, 16.14.

6.1.34. *tert*-Butyl {(1*S*,2*R*)-2-[(*R*)-8-cyano-7-(3,5-dimethoxyphenylamino)-2-phenyl-2,3-dihydroimidazo[1,2-*c*]pyrimidin-5-ylamino]-cyclohexyl}carbamate (12a) and *tert*-butyl {(1*R*,2*S*)-2-[(*R*)-8-cyano-7-(3,5-dimethoxyphenylamino)-2-phenyl-2,3-dihydroimidazo[1,2-*c*]pyrimidin-5-ylamino]cyclohexyl}carbamate (12b)

To a suspension of compound **11** (1.260 g, 3.00 mmol) in DMF (12 mL) was added *cis*-1,2-diaminocyclohexane (1.80 mL, 15.00 mmol) and the mixture was stirred for 5 h at 50 °C. The mixture was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, sequentially, dried over Na_2SO_4, and evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (10 mL) and added di-*tert*-butyldicarbonate (0.655 g, 3.00 mmol), then stirred overnight at room temperature. The mixture was purified by column chromatography on silica gel with hexane/EtOAc = 1:4 → MeOH/EtOAc = 1:50 → 1:30 to give **12a** (0.454 g, 26%) as white solid and **12b** (0.580 g, 33%) as white solid.

*Physicochemical data of **12a**:* mp 163–164 °C (MeOH–THF); α_D^{28} −65.22° (*c* 0.23, MeOH); IR (KBr) 2196, 1591, 1538, 1506, 1457, 1204, 1155 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.18–1.39 (2H, m), 1.40 (9H, s), 1.41–1.83 (6H, m), 2.08–2.18 (1H, m), 3.45 (1H, dd, *J* = 10.0, 2.0 Hz), 3.77 (6H, s), 3.78–3.82 (1H, m), 3.96–4.02 (1H, m), 4.23 (1H, t, *J* = 10.0 Hz), 4.83 (1H, d, *J* = 5.5 Hz), 5.43 (1H, dd, *J* = 10.0 Hz, 2.0 Hz), 6.22 (1H, t, *J* = 2.0 Hz), 6.68 (2H, d, *J* = 2.0 Hz), 6.92 (1H, s), 7.20-7.25 (1H, m), 7.31–7.36 (4H, m); MS *m/z*: 586 $(M+H)^+$. Anal. Calcd for $C_{32}H_{39}N_7O_4$: C, 65.62; H, 6.71; N, 16.74. Found: C, 65.39; H, 6.72; N, 16.84.

*Physicochemical data of **12b**:* mp 202–203 °C (MeOH–THF); α_D^{28} −127.41° (*c* 0.27, MeOH); IR (KBr) 2195, 1591, 1528, 1506, 1457, 1204, 1153 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.42 (9H, s), 1.43–1.82 (8H, m), 2.10–2.18 (1H, m), 3.64–3.69 (1H, m), 3.77 (6H, s), 3.78–3.82 (1H, m), 3.91–3.96 (1H, m), 4.00 (1H, t, *J* = 10.5 Hz), 4.81 (1H, d, *J* = 5.0 Hz), 5.38 (1H, dd, *J* = 10.5 Hz, 2.0 Hz), 6.22 (1H, t, *J* = 2.0 Hz), 6.69 (2H, d, *J* = 2.0 Hz), 6.92 (1H, s), 7.27–7.38 (5H, m); MS *m/z*: 586 $(M+H)^+$. Anal. Calcd for $C_{32}H_{39}N_7O_4{\cdot}0.8H_2O$: C, 64.04; H, 6.82; N, 16.34. Found: C, 64.13; H, 6.82; N, 16.26.

6.1.35. *tert*-Butyl {(1*S*,2*R*)-2-[(*R*)-8-carbamoyl-7-(3,5-dimethoxyphenylamino)-2-phenyl-2,3-dihydroimidazo[1,2-*c*]pyrimidin-5-ylamino]cyclohexyl}carbamate (13a)

To a solution of compound **12a** (0.454 g, 0.755 mmol) in DMSO (6 mL) and EtOH (3 mL) was added 5 M NaOH solution (1.09 mL, 5.43 mmol) and 30% H_2O_2 solution (0.615 mL, 5.43 mmol), then the mixture was stirred overnight at room temperature. Water (1 mL) was added and the mixture was adjusted at pH 11 with 1 M HCl solution. The obtained pale yellow precipitate was collected by filtration and washed with H_2O. The solid was dried at 50 °C and was purified by column chromatography on APS with EtOAc to give **13a** (0.332 g, 71%) as white solid: mp 210–211 °C (EtOAc); α_D^{28} −83.08° (*c* 0.13, MeOH); IR (KBr) 1692, 1630, 1595, 1529, 1497, 1456, 1202, 1154 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.20–1.42 (2H, m), 1.41 (9H, s), 1.52–1.82 (6H, m), 2.18–2.26 (1H, m), 3.44 (1H, dd, *J* = 9.5, 2.0 Hz), 3.76 (6H, s), 3.84–3.92 (1H, m), 3.98–4.04 (1H, m), 4.25 (1H, t, *J* = 10.5 Hz), 4.85 (1H, d, *J* = 5.5 Hz), 5.20–5.40 (1H, br), 5.43 (1H, dd, *J* = 10.5, 2.0 Hz), 6.17 (1H, t, *J* = 2.0 Hz), 6.85 (2H, d, *J* = 2.0 Hz), 6.84–6.93 (1H, br), 7.26–7.36 (5H, m), 12.56 (1H, s); MS *m/z*: 604 $(M+H)^+$. Anal. Calcd for $C_{32}H_{41}N_7O_5$: C, 63.29; H, 6.87; N, 16.14. Found: C, 63.04; H, 6.80; N, 16.14.

6.1.36. *tert*-Butyl {(1*R*,2*S*)-2-[(*R*)-8-carbamoyl-7-(3,5-dimethoxyphenylamino)-2-phenyl-2,3-dihydroimidazo[1,2-*c*]pyrimidin-5-ylamino]cyclohexyl}carbamate (13b)

The title compound was prepared from **12b** in the same manner as described for **13a**, and was obtained as white solid (64%): mp 248–249 °C (EtOAc); α_D^{28} −98.67° (*c* 0.15, MeOH); IR (KBr) 1715, 1623, 1597, 1524, 1496, 1457, 1163 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.20–1.41 (2H, m), 1.42 (9H, s), 1.52–1.81 (6H, m), 2.18–2.25 (1H, m), 3.61–3.68 (1H, m), 3.76 (6H, s), 3.86–3.98 (2H, m), 4.02 (1H, t, *J* = 10.5 Hz), 4.79–4.85 (1H, m), 5.23–5.28 (1H, m), 5.38 (1H, dd, *J* = 10.5 Hz, 2.0 Hz), 6.17 (1H, t, *J* = 2.0 Hz), 6.85 (2H, d, *J* = 2.0 Hz), 7.26–7.36 (5H, m), 10.03 (1H, s), 12.58 (1H, s); MS *m/z*: 604 $(M+H)^+$. Anal. Calcd for $C_{32}H_{41}N_7O_5{\cdot}0.5C_4H_8O_2$: C, 63.04; H, 7.00; N, 15.13. Found: C, 62.72; H, 6.99; N, 14.90.

6.1.37. *tert*-Butyl {(1*S*,2*R*)-2-[8-carbamoyl-7-(3,5-dimethoxyphenylamino)-2-phenylimidazo[1,2-*c*]pyrimidin-5-ylamino]-cyclohexyl}carbamate (14a)

To a mixture of compound 13a (0.170 g, 0.282 mmol) and 10% Pd–C (56.5% water containing reagent, 0.040 g) in DMSO (1 mL) and EtOH (1 mL) was added cyclohexene (0.086 mL, 0.845 mmol) and was stirred overnight at 100 °C under oxygen atmosphere. Catalyst was removed by filtration through Celite and the filtrate was dissolved in EtOAc. The mixture was washed with water and brine, sequentially, dried over anhydrous $MgSO_4$, and evaporated in vacuo. Resulting residue was purified by column chromatography on silica gel with hexane/EtOAc = 1:1 to give 14a (0.060 g, 35%) as white solid: mp 224–225 °C (EtOAc); α_D^{28} +12.17° (*c* 0.23, MeOH); IR (KBr) 1685, 1602, 1498, 1458, 1236, 1202, 1153 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.30–1.55 (2H, m), 1.58 (9H, s), 1.60–1.92 (6H, m), 3.79 (6H, s), 4.01–4.17 (2H, m), 4.98 (1H, d, *J* = 6.0 Hz), 5.55 (1H, s), 6.18 (1H, t, *J* = 2.0 Hz), 6.92 (2H, d, *J* = 2.0 Hz), 7.28–7.34 (1H, m), 7.41 (2H, t, *J* = 8.0 Hz), 7.55 (1H, s), 7.87 (2H, d, *J* = 8.0 Hz), 8.19 (1H, s), 10.10 (1H, s), 12.18 (1H, s); MS *m/z*: 602 $(M+H)^+$. Anal. Calcd for $C_{32}H_{39}N_7O_5$: C, 63.88; H, 6.53; N, 16.30. Found: C, 63.58; H, 6.54; N, 16.02.

6.1.38. *tert*-Butyl {(1R,2S)-2-[8-carbamoyl-7-(3,5-dimethoxyphenylamino)-2-phenylimidazo[1,2-*c*]pyrimidin-5-ylamino]-cyclohexyl}carbamate (14b)

The title compound was prepared from **13b** in the same manner as described for **14a**, and was obtained **14b** as white solid (33%): mp 222–224 °C (EtOAc); α_D^{28} −25.00° (*c* 0.20, MeOH); IR (KBr) 1685, 1599, 1497, 1458, 1380, 1235, 1202, 1152 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.29–1.55 (2H, m), 1.58 (9H, s), 1.61–1.92 (6H, m), 3.79 (6H, s), 4.01–4.17 (2H, m), 4.79–4.85 (1H, m), 5.55 (1H, s), 6.18 (1H, t, *J* = 2.0 Hz), 6.92 (2H, d, *J* = 2.0 Hz), 7.28–7.34 (1H, m), 7.41 (2H, t, *J* = 8.0 Hz), 7.55 (1H, s), 7.87 (2H, d, *J* = 8.0 Hz), 8.17 (1H, s), 10.10 (1H, s), 12.18 (1H, s); MS *m/z*: 602 $(M+H)^+$. Anal. Calcd for $C_{32}H_{39}N_7O_5{\cdot}0.2H_2O$: C, 63.49; H, 6.56; N, 16.19. Found: C, 63.24; H, 6.47; N, 16.03.

6.1.39. *tert*-Butyl {2-[8-Carbamoyl-7-(3,5-dimethoxyphenylamino)-2-phenylimidazo[1,2-*c*]pyrimidin-5-ylamino]-1,1-dimethyl-ethyl}-carbamate (14c)

A suspension of compound **11** (0.126 g, 0.300 mmol) and 2-methylpropane-1,2-diamine (0.157 mL, 1.500 mmol) in DMF (4 mL) was stirred for 4 h at 60 °C. The mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (8 mL) and added di-*tert*butyldicarbonate (0.066 g, 0.300 mmol) and stirred overnight at room temperature. The mixture was purified by column chromatography on APS with EtOAc → MeOH/EtOAc = 1:10 to give **12c** (0.094 g, 56%) as yellowish amorphous. The title compound was prepared from **12c** in the same manner as described for **13a** and **14a**, and was obtained **14c** as white solid (15%, three steps yield): mp 210–212 °C (EtOAc); IR (KBr) 1617, 1596, 1559, 1457, 1204, 1151 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.45 (6H, s), 1.51 (9H, s), 2.98 (2H, s), 3.77 (1H, d, J = 5.0 Hz), 3.79 (6H, s), 4.87 (1H, s), 5.58 (1H, s), 6.18 (1H, t, J = 2.0 Hz), 6.97 (1H, d, J = 2.0 Hz), 7.31–7.35 (1H, m), 7.43 (2H, t, J = 8.0 Hz), 7.61 (1H, s), 7.90 (2H, d, J = 8.0 Hz), 8.17 (1H, s), 10.10 (1H, s), 12.24 (1H, s); MS *m/z*: 576 $(M+H)^+$. Anal. Calcd for $C_{30}H_{37}N_7O_5$: C, 62.59; H, 6.48; N, 17.03. Found: C, 62.29; H, 6.46; N, 16.88.

6.1.40. 5-((1*R*,2*S*)-2-Aminocyclohexylamino)-7-(3,5-dimethoxyphenylamino)-2-phenylimidazo[1,2-*c*]pyrimidine-8-carboxamide (15a)

To a mixture of compound **14a** (0.035 mg, 0.0582 mmol) in MeOH (1 mL) was added 4 M HCl–EtOAc (2 mL). After being stirred for 1 h at room temperature, volatiles were evaporated in vacuo and to the residue was added EtOAc. Resulting precipitates were collected by suction filtration and dried under reduced pressure to give **15a** (0.027 mg, 86%) as yellowish solid: mp 279–280 °C (EtOAc); α_D^{28} +1.90° (*c* 0.21, DMSO); IR (KBr) 1599, 1500, 1375, 1238, 1203, 1155 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.38–2.06 (8H, m), 3.77 (6H, s), 3.79–3.92 (2H, m), 4.31–4.39 (1H, m), 6.25 (1H, t, J = 2.0 Hz), 6.78 (1H, d, J = 2.0 Hz), 7.36 (1H, t, J = 8.0 Hz), 7.49 (2H, t, J = 8.0 Hz), 7.55 (1H, s), 7.88 (2H, d, J = 8.0 Hz), 8.02 (4H, br s), 8.70 (1H, s), 9.67 (1H, br s), 12.42 (1H, s); MS *m/z*: 502 $(M+H)^+$. Anal. Calcd for $C_{27}H_{31}N_7O_3 \cdot 2.0HCl$: C, 56.45; H, 5.79; N, 17.07. Found: C, 56.57; H, 5.98; N, 16.80.

6.1.41. 5-((1*S*,2*R*)-2-Aminocyclohexylamino)-7-(3,5-dimethoxyphenylamino)-2-phenylimidazo[1,2-*c*]pyrimidine-8-carboxamide (15b)

The title compound was prepared from **14b** in the same manner as described for **15a,** and was obtained **15b** as yellowish solid (54%): mp 280–281 °C (EtOAc); α_D^{28} +5.22° (*c* 0.23, DMSO); IR (KBr) 1653, 1600, 1560, 1507, 1155 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.38–2.06 (8H, m), 3.77 (6H, s), 3.78–3.86 (2H, m), 4.34 (1H, br s), 4.35 (1H, br s), 6.25 (1H, t, J = 2.0 Hz), 6.78 (1H, d, J = 2.0 Hz), 7.36 (1H, t, J = 8.0 Hz), 7.49 (2H, t, J = 8.0 Hz), 7.55 (1H, s), 7.88 (2H, d, J = 8.0 Hz), 8.01 (4H, br s), 8.70 (1H, s), 9.65 (1H, br s), 12.42 (1H, s); MS *m/z*: 502 $(M+H)^+$. Anal. Calcd for $C_{27}H_{31}N_7O_3 \cdot 2.0HCl$: C, 56.45; H, 5.79; N, 17.07. Found: C, 56.34; H, 5.88; N, 16.83.

6.1.42. 5-(2-Amino-2-methylpropylamino)-7-(3,5-dimethoxyphenylamino)-2-phenylimidazo[1,2-*c*]pyrimidine-8-carboxamide (15c)

To a mixture of compound **14c** (0.016 g, 0.028 mmol) in CH_2Cl_2 (0.5 mL) was added TFA (0.2 mL) and the mixture was stirred for 1 h at room temperature. Volatiles were evaporated in vacuo and to the residue was added EtOAc. Resulting precipitates were collected by suction filtration and dried under reduced pressure to give **15c** (0.012 g, 73%) as yellowish solid: mp 252–254 °C (EtOAc); IR (KBr) 1599, 1559, 1507, 1203, 1154 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.34 (6H, s), 3.77 (6H, s), 3.78–3.84 (2H, m), 6.24 (1H, s), 6.84 (2H, s), 7.37 (1H, t, J = 8.0 Hz), 7.49 (2H, t, J = 8.0 Hz), 7.56 (1H, s), 7.87 (2H, d, J = 8.0 Hz), 8.02 (3H, br s), 8.53 (1H, s), 8.61 (1H, br s), 9.63 (1H, br s), 12.46 (1H, s); MS *m/z*: 476 $(M+H)^+$. Anal. Calcd for $C_{25}H_{29}N_7O_3 \cdot C_2HF_3O_2 \cdot 0.1H_2O$: C, 54.83; H, 5.15; N, 16.58. Found: C, 54.55; H, 5.39; N, 16.91.

6.2. Molecular modeling

The 3D coordinates of the ZAP-70 receptor model were constructed based on the published crystal structure of an activated Lck kinase domain[16,17] (PDB code: 1QPD) using the program FAMS.[17] The structure of staurosporine was extracted from the X-ray structure of LCK (PDB code: 1QPD) and manually docked into the ATP-binding site of the ZAP-70 receptor model using QUANTA2000[24] (Accelrys Inc., San Diego, CA, USA). All docking calculations were performed using the program ADAM.[18]

6.3. Biology

6.3.1. Intracellular ZAP-70 kinase inhibition assay

The kinase domain of human ZAP-70 kinase (Leu325-Ala619) was cloned to NdeI and XhoI sites of pET-19b expression vector (Novagen Inc.) by PCR amplification from human thymus Marathon-Ready™ cDNA (Clontech Inc.). The ZAP-70 kinase domain binding with pET-19b His-tag gene at 5' region was integrated into pFASTBAC1 vector of the BAC-TO-BAC™ (Gibco-BRL Inc.) baculovirus expression system. The transfected virus was obtained by transfecting Sf-9 cell (Invitrogen Inc.) with pFASTBAC1 containing His-tag-fused ZAP kinase domain described above. High Five™ baculocells, which was infected with this transfected virus, were recovered and these cells were dissolved by ultrasonication. After soluble fraction being separated by centrifuge, the supernatant was added to TALON™ metal affinity resin (Clontech Inc.) and to this resin was adsorbed His-tag-fused protein of ZAP-70 kinase domain. The resin was washed several times and extracted His-tag-fused protein of ZAP-70 kinase domain by imidazole containing a buffer. A coupled spectrophotometric assay was used wherein ADP generated by ZAP-70 kinase was converted to ATP by pyruvate kinase (PK), with concomitant production of pyruvate from phosphoenolpyruvate (PEP). LDH reduces pyruvate to lactate by oxidizing NADH. NADH depletion was monitored at 340 nm using a microplate reader (Spectra Max 250, Molecular Device) at 30 °C for 20 min. Reactions were performed at 30 °C in 100 mM Hepes buffer (pH 7.6), containing 20 mM $MgCl_2$, and 10% glycerol, and started by adding ATP. PK (150 μg/mL), LDH (500 μg/mL), PEP (2.5 mM), and NADH (150 μM) were added in large excess. Addition of 100 μM ZAP-70 optimal peptide substrate (peptide sequence: AEEEIYGEFEAKKKK, Sawady, Tokyo), allowed measurement of kinase activity.

6.3.2. Intracellular Syk kinase inhibition assay

The kinase domain of human Syk kinase (Met343–Asn635) was cloned to NdeI and XhoI sites of pET-19b expression vector (Novagen Inc.) by PCR amplification from human thymus Marathon-Ready™ cDNA (Clontech Inc.). The Syk kinase domain binding with pET-19b His-tag gene at 5′ region was integrated to pFASTBAC1 vector of the BAC-TO-BAC™ baculovirus expression system (Gibco-BRL Inc.). The transfected virus was obtained by transfecting Sf-9 cell (Invitrogen Inc.) with pFASTBAC vector containing His-tag-fused Syk kinase domain described above. High Five™ baculocells, which was infected with this transfected virus, were recovered and dissolved by ultrasonication. After soluble fraction being separated by centrifuge, the supernatant was

mixed with TALON™ metal affinity resin (Clontech Inc.) and to this resin was adsorbed His-tag fused protein of Syk kinase domain. The resin was washed several times and extracted His-tag fused protein of Syk kinase domain by imidazole containing a buffer. A coupled spectrophotometric assay was used wherein ADP generated by Syk kinase was converted to ATP by pyruvate kinase (PK), with concomitant production of pyruvate from phosphoenolpyruvate (PEP). LDH reduces pyruvate to lactate by oxidizing NADH. NADH depletion was monitored at 340 nm using a microplate reader (Spectra Max 250, Molecular Device) at 30 °C for 20 min. Reactions were performed at 37 °C in 100 mM Hepes buffer (pH 7.6), containing 40 mM $MgCl_2$ and 10% glycerol, and started by adding ATP. PK (150 μg/mL), LDH (50 μg/mL), PEP (2.5 mM), and NADH (200 μM) were added in large excess. Addition of 100 μM Syk optimal peptide (peptide sequence: AEEEIYGEFEAKKKK, Sawady, Tokyo) allowed measurement of kinase activity.

6.3.3. Peripheral blood mononuclear cells (PBMCs) and whole blood (WB)

6.3.3.1. Preparation of PBMCs. Heparinized human peripheral blood was obtained from healthy donors. PBMCs were isolated by the Ficoll-Hypaque gradient density method as described previously. Blood cells were diluted with PBS buffer, and then centrifuged in a Ficoll-Hypaque discontinuous gradient at 1500 rpm for 30 min. The PBMC layers were collected and washed with cold distilled water and 10× Hanks' buffer saline solution (HBSS) to remove red blood cells. The cells were resuspended to a concentration of 2×10^6 cells/mL inAIM-V medium (Invitrogen) containing100 U/mL penicillin and 100 μg/mL streptomycin.

6.3.3.2. Determination of IL-2 production by PBMCs and WB. PBMCs or WB were cultured with 10 μg/mL PHA alone or in combination with varying concentrations of compounds in 5% CO_2-air humidified atmosphere at 37 °C for 24 h. The cell supernatants were then collected and assayed for IL-2 concentrations by the enzyme-linked immunoassay (ELISA). The ELISA used here are not reported to exhibit detectable cross-reactivity with the other cytokines.

6.3.4. Passive cutaneous anaphylaxis (PCA) assays

ICR mice were passively sensitized by subcutaneously injecting anti-dinitrophenyl (DNP)-coupled IgE under the right ear pinna, while lightly anesthetizing with ether. After 48 h, each mouse was challenged by injecting a mixture of DNP-conjugated bovine serum albumin and 250 μL of 0.5% Evans blue solution via the tail vein to induce passive cutaneous anaphylaxis. Thirty minutes after the challenge, the mice were sacrificed to take ears and the amount of dye from the blueing region was measured. Test compounds or vehicle alone as a control were orally administered to the mice 1 h before the antigen challenge. The dye in the tissues was extracted with acetone and colorimetrically determined at 620 nm. The PCA reaction (% of vehicle) by the test compound was calculated based on the following equation. In the formula, *A*: amount of dye leaked into the sensitized ear at the time of administration of the vehicle alone; *B*: amount of dye leaked into the unsensitized ear at the time of administration of vehicle alone; *C*: amount of dye leaked into the sensitized ear at the time of administration of the compound to be tested. Amount of dye leaked (% of vehicle) = $\{(A-B)-(C-B)\} \times 100/(A-B)$.

6.3.5. ConA-induced IL-2 levels in vivo

In initial experiments, the kinetics of IL-2 production following intravenous injection of ConA (20 mg/kg) was analyzed, and the time point with maximum plasma IL-2 concentration was chosen to study the effect of compounds. One hour after oral or subcutaneous administration of compounds, ConA (20 mg/kg) was intravenously injected. Groups of mice ($n = 4$) were euthanized at 2 h following ConA treatment. Plasma was collected and analyzed for IL-2 levels by ELISA. The ELISA used here are not reported to exhibit detectable cross-reactivity with the other cytokines.

6.3.6. Single-crystal X-ray diffraction analysis of compound 13a

A colorless prismatic crystal of compound [**13a**], [**13a** $C_{32}H_{41}N_7O_5$] (FW = [603.71]) having approximate dimensions of $0.5 \times 0.2 \times 0.2$ mm was mounted on a nylon loop (Hampton Research, Aliso Viejo, CA, USA). All measurements were made on an R-AXIS V imaging plate detector (Rigaku, Tokyo, Japan) with synchrotron radiation at SPring-8 BL32B2 beam line (Hyogo, Japan). Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 25 carefully centered reflections in the range $58.26 < 2h < 60.17°$, corresponded to a primitive triclinic cell (*P*1, for Z = 1) with dimensions: $a = 17.772(3)$ Å, $b = 10.812(3)$ Å, $c = 10.286(3)$ Å, $V = 1976.2(7)$ Å 3, the calculated density: 1.22 g/cm^3. The data were collected at a temperature of −170 ± 1.0 °C at a maximum resolution of 0.86 Å ($\lambda = 0.71$ Å). The low-temperature experiment was performed using Paratone-N-coated rapidly cooled crystals on the tip of a copper pin. Of the 32,817 reflections that were collected, 32,452 were unique (*Rint* = 0.032). Data were collected and processed using the CrystalClear program (Version. 1.3.5; Rigaku). The structure was solved by direct methods[25] and expanded using Fourier techniques.[26] The non-hydrogen atoms were refined anisotropically. All hydrogen atoms were detected using difference Fourier techniques, and their positions were fixed in the course of refinement. The final cycle of full-matrix least-squares refinement[27] was based on 3087 independent reflections and 440 variable parameters, and converged (largest parameter shift was 1.69 times its esd) with unweighted and weighted agreement factors of $R = 0.066$ and $R_w = 0.111$, respectively. The goodness of fit[28] was 1.34. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.30 and −0.27 e$^-$/Å^3, respectively. Neutral atoms scattering factors were taken from Cromer and Weber,[29] and the values for the mass attenuation coefficients are those of Creagh and Hubbel.[30] All calculations were performed using the teXsan[31] crystallographic software package of the Molecular Structure Corporation (The Woodlands, TX, USA).

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30. Creagh, D. C.; Hubbell, J. H.. In *In International Tables for Crystallography*; Wilson, A. J. C., Ed.; Kluwer Academic: Boston, MA, 1992; Vol. C, pp 200–206. Table 4.2.4.3.
31. teXsan: Crystal Structure Analysis Package. Molecular Structure Corporation (1995 and 2004).