Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 13 (2005) 4936–4951
Bioorganic & Medicinal Chemistry

Synthetic studies on novel Syk inhibitors. Part 1: Synthesis and structure–activity relationships of pyrimidine-5-carboxamide derivatives

Hiroyuki Hisamichi,[a,*] Ryo Naito,[a] Akira Toyoshima,[b] Noriyuki Kawano,[a] Atsushi Ichikawa,[a] Akiko Orita,[a] Masaya Orita,[a] Noritaka Hamada,[a] Makoto Takeuchi,[a] Mitsuaki Ohta[a] and Shin-ichi Tsukamoto[a]

[a]*Institute for Drug Discovery Research, Astellas Pharma Inc., 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan*
[b]*Department of Clinical Development, Astellas Pharma Inc., 3-17-1 Hasune, Itabashi, Tokyo 174-8612, Japan*

Received 3 March 2005; revised 17 May 2005; accepted 17 May 2005

Abstract—Spleen tyrosine kinase (Syk) is a non-receptor-type tyrosine kinase which mediates diverse responses in haematopoietic cells. Therefore, Syk is an attractive therapeutic target, and in a study of Syk inhibitors as potentially new therapeutic agents, we discovered the 4-anilinopyrimidine-5-carboxamides. Enzyme screening indicated that an aminoethylamino moiety at the 2-position of the pyrimidine ring was important for Syk inhibitory activity, and an investigation of the substituents at the 4-position revealed that an anilino moiety substituted at the meta position was preferred. These compounds showed high selectivity for Syk, compared to other kinases, such as ZAP-70, c-Src, and PKC, and exhibited good inhibitory activities against 5-HT release from RBL-cells. Among them, compound **9a** inhibited the passive cutaneous anaphylaxis reaction in mice, with an ID_{50} of 13 mg/kg following subcutaneous administration. These results suggest that our compounds are worthy of further evaluation as new anti-allergic agents.

1. Introduction

Protein tyrosine kinases (PTKs) are well known to be important in cellular signal transduction, and regulate cellular activation, proliferation, differentiation, and mitogenesis.[1] The PTKs can be classified into two types:[2] receptor-type PTKs, such as platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), and fibroblast growth factor (FGFR), all of which contain an extracellular ligand-binding domain; and non-receptor-type PTKs, such as c-Src, Lck, ZAP-70, and Syk, which have an intracellular location. Since abnormal activation of PTKs has been related to a number of diseases, PTK inhibitors are expected to have potential therapeutic utility.

Several natural product PTK inhibitors, including Erbstatin,[3] Lavendustin A,[4] and Piceatannol,[5] have been reported previously, and many studies of their derivatives have been carried out.[6,7] Recently, many types of PTK inhibitors have been reported, and work in this area has prospered. However, the main focus of this work has been inhibitors of receptor-type PTKs, and only a few studies have reported on non-receptor-type PTK inhibitors.

Among the non-receptor PTKs, spleen tyrosine kinase (Syk) is a 72 kDa cytoplasmic PTK that is expressed in B cells, mast cells, macrophages, and platelets.[8] This kinase is recruited to receptors, such as the B cell antigen receptor or FcεRI and plays an important role in signal transduction pathways in these cells.[9–11] Therefore, inhibition of Syk may result in the suppression of functions of these cells and be effective in the treatment of allergic or immunological disorders. Indeed, several series of a Syk inhibitor have been reported and it has been shown that antigen-induced responses are prevented by the inhibition of phosphorylation and activation of Syk.[12]

To discover a novel Syk inhibitor, we carried out high-throughput screening using our chemical library and discovered the 4-anilino-2-(2-aminoethylamino)pyrimidine-5-carboxamide derivative **9a** as a member of a

Keywords: Tyrosine kinase; Syk; ZAP-70; Pyrimidine.
* Corresponding author. Tel.: +81 0 29 863 6741; fax: +81 0 29 852 2971; e-mail: hiroyuki.hisamichi@jp.astellas.com

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.05.033

novel class of Syk inhibitors. In this paper, we report the synthesis and structure–activity relationships of pyrimidine-5-carboxamide derivatives as selective Syk inhibitors, as well as the pharmacological profile of compound **9a**.

2. Chemistry

As shown in Scheme 1, compound **4a**, which has no substituent at the 2-position, was synthesized starting from ethyl-4-chloropyrimidine-5-carboxylate **1a**.[13] The reaction of **1a** with 3-trifluoromethylaniline in toluene gave 4-anilinopyrimidine derivative **2a**. Hydrolysis of the carboethoxy group by 1 M NaOH afforded carboxylic acid **3a**, and condensation of the carboxylic acid with aqueous ammonia in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole gave the pyrimidine-5-carboxamide derivative **4a**. The 2-methyl derivative **4b** and 2-phenyl derivative **4c** were prepared from appropriate 4-chloropyrimidine derivatives (**1b** and **1c**)[14] by substitution with 3-trifluoromethylaniline, followed by hydrolysis and amidation.

Most of the pyrimidine-5-carboxamide derivatives with an amino group at the 2-position were prepared in five steps from ethyl 2,4-dichloropyrimidine-5-carboxylate **5**.[15] The synthetic route is shown in Scheme 2. The reaction of **5** with the appropriate anilines in MeCN or tetrahydrofuran (THF) gave the 4-anilinopyrimidine derivatives **6**. The substitution reaction proceeded selectively at the 4-position, and 2-anilino or 2,4-dianilino derivatives were not obtained. Hydrolysis of the carboethoxy group by 1 M NaOH provided the corresponding carboxylic acids **7**. Condensation with aqueous ammonia in the presence of EDCI and 1-hydroxybenzotriazole (HOBt) gave the corresponding pyrimidine-5-carboxamide derivatives **8**. Interestingly, displacement of the 2-chloro group to form a benzotriazol-1-yloxy group took place under these conditions and 2-chloro derivatives were obtained only in trace amounts. However, it was found that the benzotriazol-1-yloxy group served as a leaving group in the next reaction; displacement of this group by an amino group proceeded easily at room temperature and afforded the 2-aminopyrimidine derivatives **9**. In the case of reaction with diamines, such as ethylenediamine, about 10 equiv of diamines were used to ensure monosubstitution.

Commercially available ethyl-4-chloro-2-methylsulfanylpyrimidine-5-carboxylate **10** was also used as a starting material, especially for the preparation of 4-alkylamino derivatives, because the reaction of alkylamines, such as methylamine with **5** gave 2,4-dialkylamino derivatives as the major product (Scheme 3). The 4-alkylaminopyrimidine-5-carboxamides were obtained from **10** in a manner similar to that shown in Scheme 2, whereas the substitution of 2-methylsulfanyl groups by amino groups required a higher temperature, in contrast to the displacement of 2-benzotriazol-1-oxy groups.

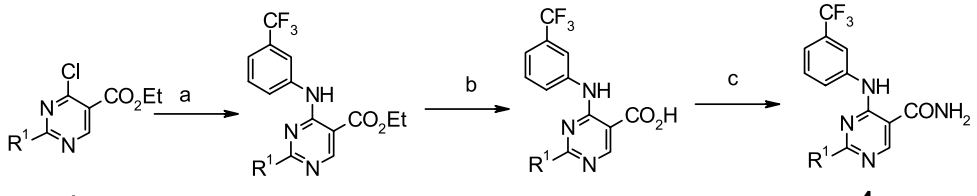
The 5-ester derivative **15** was prepared by a substitution reaction of **6a** with ethylenediamine and subsequent hydrolysis afforded the 5-carboxylic acid derivative **16** (see Scheme 4).

The chemical structures of the synthesized compounds were confirmed from spectroscopic data (^{1}H NMR, mass spectrometry) and elemental analysis.

3. Results and discussion

The compounds were evaluated for their ability to inhibit tyrosine phosphorylation of Band-3 peptide[16] by Syk and ZAP-70. These data are shown in Tables 1 and 2. Compounds showing potency in these assays were further investigated for their enzyme selectivity for Syk, compared to c-Src and several PKCs, and selected compounds were also evaluated for their inhibition of serotonin (5-HT) release in rat basophilic leukemia (RBL) cells.[17]

The 2-ethylenediaminopyrimidine derivative **9a**, which was discovered by high-throughput screening, exhibited good Syk inhibitory activity, with an IC_{50} value of 0.041 μM. In the first series of compounds, the effect of the 2-ethylenediamino group on Syk inhibitory activity was investigated. Compound **4a**, which has no substituent at the 2-position, did not show any Syk inhibitory



1

No.	R^1
1a	H
1b	Me
1c	Ph

2

No.	R^1
2a	H
2b	Me
2c	Ph

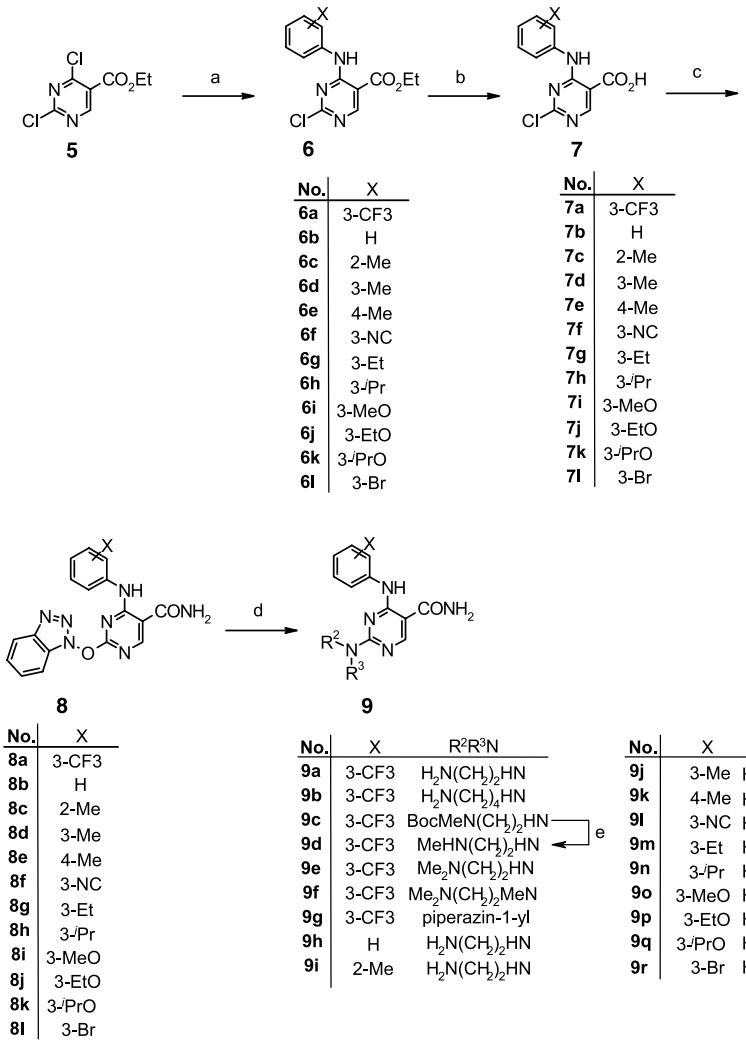
3

No.	R^1
3a	H
3b	Me
3c	Ph

4

No.	R^1
4a	H
4b	Me
4c	Ph

Scheme 1. Reagents: (a) $ArNH_2$, $^iPr_2NEt$, MeCN; (b) 1 M NaOH, MeOH; (c) aq NH_3, EDCI, HOBt, $ClCH_2CH_2Cl$ or DMF; (d) R_2R_3NH, MeCN; (e) 4 N HCl/AcOEt, EtOH.



No.	X
6a	3-CF3
6b	H
6c	2-Me
6d	3-Me
6e	4-Me
6f	3-NC
6g	3-Et
6h	3-iPr
6i	3-MeO
6j	3-EtO
6k	3-iPrO
6l	3-Br

No.	X
7a	3-CF3
7b	H
7c	2-Me
7d	3-Me
7e	4-Me
7f	3-NC
7g	3-Et
7h	3-iPr
7i	3-MeO
7j	3-EtO
7k	3-iPrO
7l	3-Br

No.	X
8a	3-CF3
8b	H
8c	2-Me
8d	3-Me
8e	4-Me
8f	3-NC
8g	3-Et
8h	3-iPr
8i	3-MeO
8j	3-EtO
8k	3-iPrO
8l	3-Br

No.	X	R^2R^3N
9a	3-CF3	$H_2N(CH_2)_2HN$
9b	3-CF3	$H_2N(CH_2)_4HN$
9c	3-CF3	$BocMeN(CH_2)_2HN$
9d	3-CF3	$MeHN(CH_2)_2HN$
9e	3-CF3	$Me_2N(CH_2)_2HN$
9f	3-CF3	$Me_2N(CH_2)_2MeN$
9g	3-CF3	piperazin-1-yl
9h	H	$H_2N(CH_2)_2HN$
9i	2-Me	$H_2N(CH_2)_2HN$

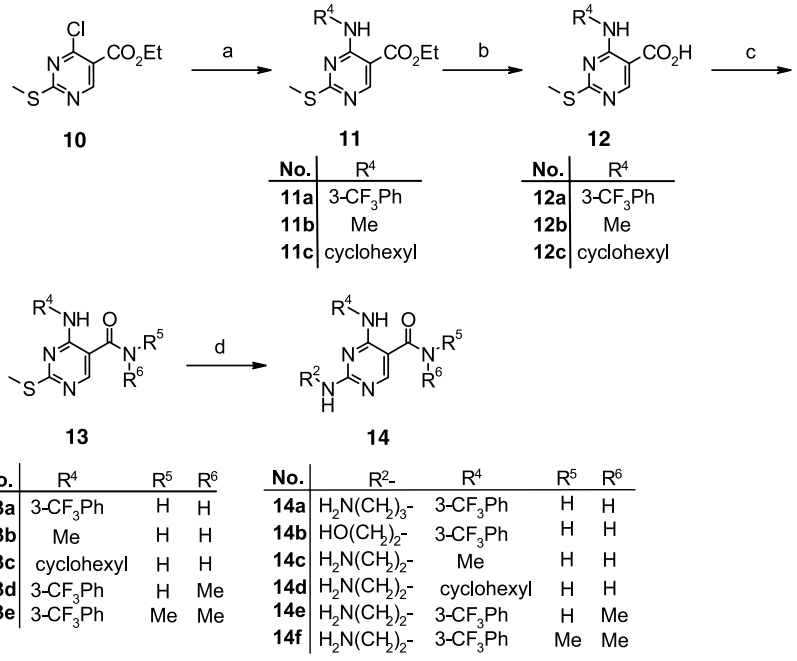
No.	X	R^2R^3N
9j	3-Me	$H_2N(CH_2)_2HN$
9k	4-Me	$H_2N(CH_2)_2HN$
9l	3-NC	$H_2N(CH_2)_2HN$
9m	3-Et	$H_2N(CH_2)_2HN$
9n	3-iPr	$H_2N(CH_2)_2HN$
9o	3-MeO	$H_2N(CH_2)_2HN$
9p	3-EtO	$H_2N(CH_2)_2HN$
9q	3-iPrO	$H_2N(CH_2)_2HN$
9r	3-Br	$H_2N(CH_2)_2HN$

Scheme 2. Reagents: (a) R^4NH_2, $^iPr_2NEt$, MeCN; (b) 1 M NaOH, MeOH; (c) R^5R^6NH, EDCI, HOBt, $ClCH_2CH_2Cl$ or DMF; (d) R^2NH_2, MeCN.

activity. Similarly, a lack of potency was observed for the 2-methyl and 2-phenyl derivatives, **4b** and **4c**. Compound **14b**, bearing a hydroxy group, was also less active than **9a**. For the *N*-methyl derivative **9d**, the activity was 4-fold less potent compared with **9a**, and the *N*,*N*-dimethyl derivative **9e** was less active than *N*-methyl derivative. Furthermore, introduction of a tertiary amino group at the 2-position strongly reduced the activity (**9f**), and conversion of the ethylenediamino group into a piperazinyl group (**9g**) also resulted in a large reduction in potency. These data suggest that not only the terminal primary amine but also the secondary amine at the 2-position of the pyrimidine ring is necessary for Syk inhibitory activity. The chain length of the diamino moiety at the 2-position has a strong influence on the Syk inhibitory activity; a marked difference was observed with a 2- or 4-carbon chain length (**9a** and **9b**) compared to a 3-carbon chain length (**14a**).

Subsequently, several 4-anilinopyrimidine-5-carboxamide derivatives were evaluated to examine the effect of varying the substituent in the anilino group. Although the 2′-Me and 4′-Me derivatives (**9i** and **9k**) showed inhibitory activities similar to those of the unsubstituted derivative **9h**, the 3′-Me derivative **9j** was about 10-fold more potent than **9h**. Based on the effectiveness of the 3′-substituent, we then synthesized and evaluated a series of compounds with meta substitution. Compounds with small alkyl groups at the 3′-position exhibited high potency, with 3′-alkyl derivatives being slightly more potent than 3′-alkoxy derivatives. Enlarging the substituents at the 3′-position decreased the activities of the 3′-alkyl derivatives. The 3′-bromo derivative **9r** was the most potent molecule in this series. On the other hand, **14c** and **14d**, which have substituents other than an anilino group at the 4-position of the pyrimidine ring, showed no activity. These structure–activity relationships associated with the anilino group are similar to those reported for other tyrosine kinase inhibitors, such as anilinoquinazoline derivatives[18] and anilinopyrrolopyrimidine derivatives,[19] which are reported to be competitors for ATP binding. These results suggest that our pyrimidine–carboxamide derivatives may interact with the ATP-binding site in a manner similar to other kinase inhibitors.[20]

Finally, the effect of the 5-carboxamide group was examined. The 5-carboxylate **15** and 5-carboxylic acid



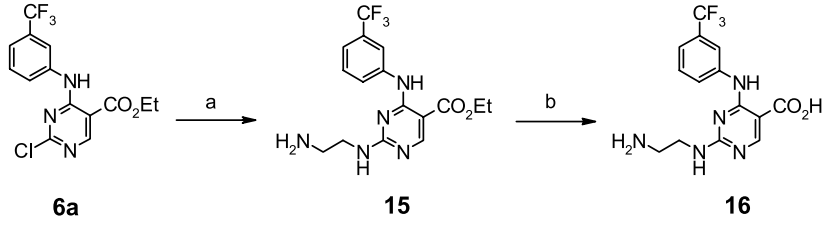
No.	R^4
11a	3-CF_3Ph
11b	Me
11c	cyclohexyl

No.	R^4
12a	3-CF_3Ph
12b	Me
12c	cyclohexyl

No.	R^4	R^5	R^6
13a	3-CF_3Ph	H	H
13b	Me	H	H
13c	cyclohexyl	H	H
13d	3-CF_3Ph	H	Me
13e	3-CF_3Ph	Me	Me

No.	R^2-	R^4	R^5	R^6
14a	$H_2N(CH_2)_3$-	3-CF_3Ph	H	H
14b	$HO(CH_2)_2$-	3-CF_3Ph	H	H
14c	$H_2N(CH_2)_2$-	Me	H	H
14d	$H_2N(CH_2)_2$-	cyclohexyl	H	H
14e	$H_2N(CH_2)_2$-	3-CF_3Ph	H	Me
14f	$H_2N(CH_2)_2$-	3-CF_3Ph	Me	Me

Scheme 3. Reagents: (a) EtONa, EtOH; (b) $POCl_3$; (c) 3-aminobenztrifluoride, $^iPr_2NEt$, MeCN; (d) 1 M NaOH, MeOH; (e) aq NH_3, EDCI, HOBt, $ClCH_2CH_2Cl$.



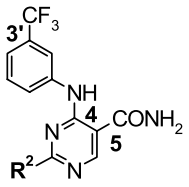
Scheme 4. Reagents: (a) ethylenediamine, MeCN; (b) 1 M NaOH, MeOH.

16 were both inactive. Furthermore, *N*-methyl carboxamide derivative **14e** and *N*,*N*-dimethyl carboxamide derivative **14f** were more than 100 times less potent than the primary carboxamide **9a**. The requirement of an interaction of the NH_2 moiety of the primary carboxamide with the receptor and steric limitations at this position in derivatives **14e** and **14f** may explain these weak activities. Because the 4-*N*-methyl-anilino and 4-phenylsulfanyl derivatives showed poor inhibitory activities (not shown in this paper), hydrogen bond formation between the 5-carboxamide and the NH group at the 4-position may be important for activity. The overall geometry of PTK catalytic domains may make inhibitor planarity a key factor for improved interaction of some classes of PTK inhibitors.[21] The carboxamide derivatives can form a pseudo-six-membered ring through formation of a hydrogen bond, resulting in a planar conformation, and X-ray crystallographic analysis of compound **9q** provided support for this proposal (Fig. 1).

To investigate the binding mode of our compounds, we built a three-dimensional (3D) model of the catalytic domain of Syk. The X-ray structure of Syk had not been reported,[22] and therefore we used the published X-ray structure of Lck as a template, since the Lck catalytic domain has high homology to the Syk kinase domain (Fig. 2).[1] Having built the 3D model of the Syk kinase domain, we assumed that the binding mode of our compounds to the domain was as shown in Figure 3. In this model, the 4-amino group and 5-carboxamide group act as a hydrogen bond donor and acceptor, respectively, to enhance binding at the ATP binding site. Specifically, the N–H of the 5-carboxamide group forms a hydrogen bond to the carbonyl group of Glu 449 and the carbonyl group of the inhibitor interacts with the N–H group of Ala 451. Furthermore, a third strong interaction with the enzyme is obtained by hydrogen bonding between the N–H of the 4-anilino group and the carbonyl group of Ala 451, while the 2-ethylenediamino group seems to mimic the triphosphate group of ATP. These interactions lead to a potent Syk inhibitory activity of our compounds.

Some of the compounds, which showed Syk inhibitory activities with IC_{50} values below 0.1 μM, were tested for their selectivity for Syk, compared to other kinases. Excellent selectivity was observed for all compounds,

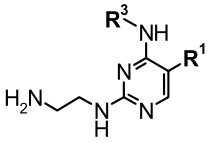
Table 1. Syk and ZAP-70 inhibitory activities of 4-(3-trifluoromethylanilino)pyrimidine derivatives



No.	R^2	IC_{50} (μM)[a]	
		Syk	ZAP-70
4a	H	>5	>5
4b	Me	>5	>5
4c	Ph	>5	>5
9a	$H_2N(CH_2)_2NH$	0.041	11.2
9b	$H_2N(CH_2)_4NH$	0.047	10.5
9d	$MeHN(CH_2)_2NH$	0.21	>10
9e	$Me_2N(CH_2)_2NH$	0.75	>10
9f	$Me_2N(CH_2)_2NMe$	>10	>10
9g	Piperazin-1-yl	>5	>5
14a	$H_2N(CH_2)_3NH$	0.23	>10
14b	$HO(CH_2)_2NH$	0.35	>10

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

Table 2. Syk and ZAP-70 inhibitory activities of 2-(2-aminoethylamino)pyrimidine derivatives



No.	R^1	R^3	IC_{50} (μM)[a]	
			Syk	ZAP-70
9a	$CONH_2$	3-CF_3–Ph	0.041	11.9
9h	$CONH_2$	Ph	0.28	13.2
9i	$CONH_2$	2-Me–Ph	0.23	>30
9j	$CONH_2$	3-Me–Ph	0.03	5.2
9k	$CONH_2$	4-Me–Ph	0.15	6.5
9l	$CONH_2$	3-NC–Ph	0.11	8.4
9m	$CONH_2$	3-Et–Ph	0.053	>10
9n	$CONH_2$	3-^{i}Pr–Ph	0.092	>10
9o	$CONH_2$	3-MeO–Ph	0.066	11.2
9p	$CONH_2$	3-EtO–Ph	0.081	>10
9q	$CONH_2$	3-^{i}PrO–Ph	0.072	>10
9r	$CONH_2$	3-Br–Ph	0.023	4.9
14c	$CONH_2$	Me	>10	>10
14d	$CONH_2$	Cyclohexyl	>10	>10
14e	CONHMe	3-CF_3–Ph	>10	>10
14f	$CONMe_2$	3-CF_3–Ph	>10	>10
15	CO_2Et	3-CF_3–Ph	>5.0	>5.0
16	CO_2H	3-CF_3–Ph	>5.0	>5.0

as shown in Table 3. These compounds also inhibited 5-HT release with IC_{50} values of 0.2–2.0 μM. Among them, compound **9r** showed the most potent activity, with an IC_{50} value of 0.27 μM. Although 3′-alkoxy derivatives showed potent Syk inhibitory activities, these compounds were less effective in the cellular

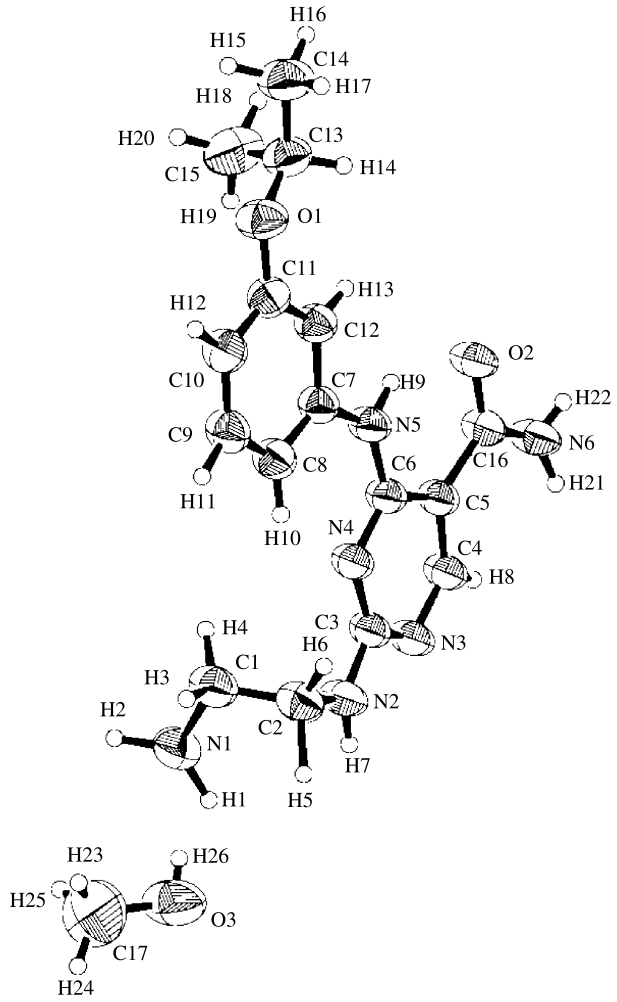


Figure 1. X-ray crystal structure of **9q**.

assay, which may be due to lack of penetration into the cells.

Finally, to investigate the anti-allergic activity of this series of compounds in vivo, compound **9a** was evaluated for its ability to inhibit the passive cutaneous anaphylaxis (PCA) reaction in mice. Compound **9a** was subcutaneously administered to mice 30 min before antigen challenge, and the effect was evaluated by measuring the amount of pigment in the blueing region. Compound **9a** inhibited the anaphylaxis reaction dose-dependently with an ID_{50} value of 13.2 mg/kg, as shown in Figure 4.

4. Conclusion

In conclusion, we have discovered a series of anilinopyrimidine derivatives as Syk inhibitors and investigated the structure–activity relationships of these derivatives. An N–H group at the 2-position of the pyrimidine ring is necessary for Syk inhibitory activity, with ethylenediamino groups being the most favorable substituent. With regard to substituents at the 4-position, anilino groups were effective and formation of a hydrogen bond

```
                  *    *  *      ** * ** *  ***        **** **         *
LCK       KPWWEDEWEVPRETLKLV-ERLGAGQFGEVWMGYYNG---HTKVAVKSLKQ---GSMSPD  53
Syk       EEIRPKEVYLDRKLLTLEDKELGSGNFGTVKKGYYQMKKVVKTVAVKILKNEANDPALKD  60

            ***** * **     **       *      * *   *   *          *
LCK       AFLAEANLMKQLQHQRLVRLYAVVTQEPIYIITEYMENGSLVDFLKTPSGIKLTINKLLD  113
Syk       ELLAEANVMQQLDNPYIVRMIGICEAESWMLVMEMAELGPLNKYLQQNR—HVKDKNIIE   118

              *   **   ** *  **** * * *       ** ****        *   *    *
LCK       MAAQIAEGMAFIEERNYIHRDLRAANILVSDTLSCKIADFGLARLIE--DNEYTAREGAK  171
Syk       LVHQVSMGMKYLEESNFVHRDLAARNVLLVTQHYAKISDFGLSKALRADENYYKAQTHGK  178

             *  * *** ***  * ********* *  *    *  ** **   ***  ** * **
LCK       FPIKWTAPEAINYGTFTIKSDVWSFGILLTEIVTHGRIPYPGMTNPEVIQNLERGYRMVR  231
Syk       WPVKWYAPECINYYKFSSKSDVWSFGVLMWEAFSYGQKPYRGMKGSEVTAMLEKGERMGC  238

            *  ** * * ** ***    * **        *  **
LCK       PDNCPEELYQLMRLCWKERPEDRPTFDYLRSVLEDFFTAT                      271
Syk       PAGCPREMYDLMNLCWTYDVENRPGFAAVELRLRNYYYDV                      278
```

Figure 2. Sequence alignment of Syk (323–600) with Lck (231–501). In the sequences, an asterisk (*) indicates an identical amino acid.

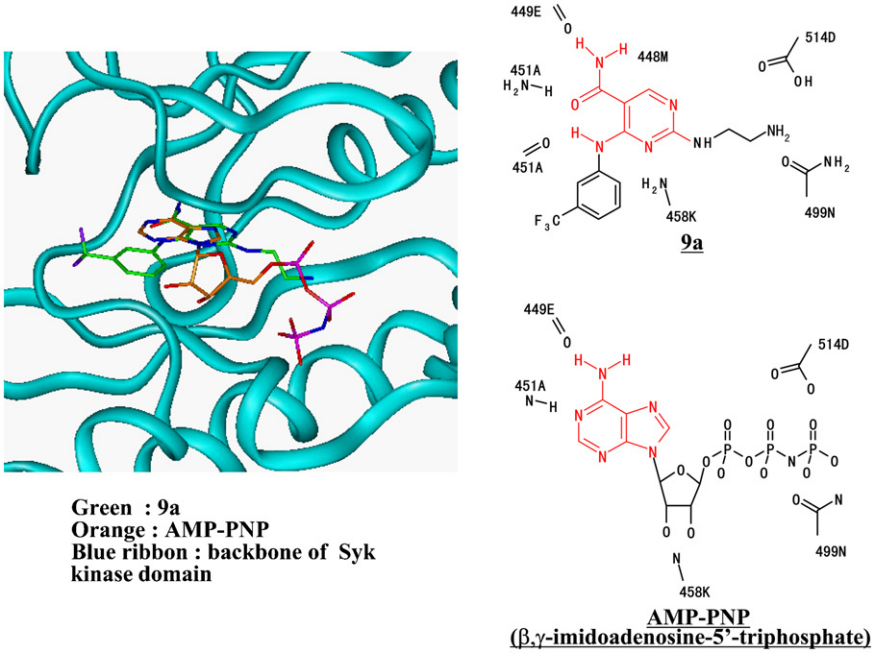


Figure 3. Binding model of **9a** to Syk.

Table 3. In vitro selectivity of Syk inhibition over other tyrosine kinases and 5-HT release inhibitory activity

No.	IC_{50} (μM)[a]					5-HT release inhibition IC_{50} (μM)[a]
	Syk	Itk	Btk	PKCε	PKCβ2	
9a	0.041	22.6	15.5	5.1	11	0.46
9b	0.047	4.0	2.9	1.5	16	1.45
9j	0.03	29.3	9.6	6	13	0.73
9m	0.053	nt[b]	nt	6.4	10	0.3
9n	0.092	nt	nt	7.9	9.5	0.45
9o	0.066	28.0	8.7	9	19	2.0
9p	0.081	23.1	10.7	8.5	27	0.93
9q	0.072	nt	nt	nt	nt	1.60
9r	0.023	nt	nt	2.8	0.69	0.27

[a] IC_{50} values were determined in duplicate.
[b] Not tested.

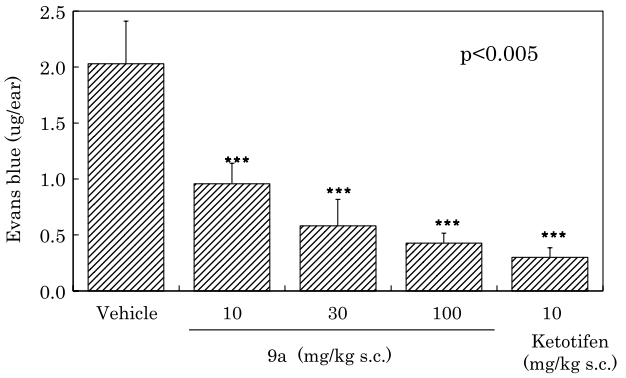


Figure 4. Effect of **9a** on the passive cutaneous anaphylaxis reaction in mice.

with the 5-carboxamide group may be very important for maintenance of inhibitor planarity, which will increase activity. All potent compounds showed high selectivity for Syk compared to other kinases and inhibited 5-HT release from RBL cells. Furthermore, one of the compounds was found to inhibit the PCA reaction in mice. These results suggest that potent Syk inhibitors may be useful for the treatment of allergic disorders. The results of further optimization and evaluation of this series of compounds will be reported elsewhere.

5. Experimental

5.1. Chemistry

^{1}H NMR spectra were measured with a JEOL EX400 (400 MHz) or GX500 (500 MHz) spectrometer; chemical shifts are expressed in δ units using tetramethylsilane as the standard (NMR peak description: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad peak). Some compounds with an amino group at the 2-position showed broad peaks in the ^{1}H NMR spectra, probably because of the presence of a conformational isomer. Therefore, the ^{1}H NMR spectra of selected compounds were measured at 80 °C to confirm the structure. Mass spectra were recorded with a Hitachi M-80 or a JEOL JMS-DX300 spectrometer. Column chromatography was carried out on silica gel (Wakogel C-200). Unless otherwise noted, all reagents and solvents obtained from commercial suppliers were used without further purification.

5.1.1. Ethyl-4-(3-trifluoromethylanilino) pyrimidine-5-carboxylate (2a). A mixture of ethyl-4-chloropyrimidine-5-carboxylate **1a** (680 mg, 3.6 mmol) and 3-trifluoromethylaniline (0.45 m, 3.6 mmol) in toluene (10 mL) was heated with reflux for 8 h. The mixture was concentrated and the residue was chromatographed on silica gel with elution using $CHCl_3$, to give 240 mg of **2a** (21%) as a yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.2 Hz), 4.45 (2H, q, J = 7.2 Hz), 7.40 (1H, d, J = 7.5 Hz), 7.50 (1H, t, J = 7.5 Hz), 7.88 (1H, d, J = 7.5 Hz), 8.06 (1H, s), 8.82 (1H, s), 9.04 (1H, s), 10.48 (1H, br s); FAB MS *m/e* $(M+H)^+$ 312.

5.1.2. Ethyl-2-methyl-4-(3-trifluoromethylanilino)pyrimidine-5-carboxylate (2b). Compound **2b** was prepared from ethyl-4-chloro-2-methylpyrimidine-5-carboxylate (**1b**) and *m*-aminobenztrifluoride in 97% yield as a yellow amorphous solid similarly: ^{1}H NMR ($CDCl_3$) δ 1.46 (3H, t, J = 7.2 Hz), 2.92 (3H, s), 4.51 (2H, q, J = 7.2 Hz), 7.58–7.65 (2H, m), 7.80–7.87 (1H, m), 7.99 (1H, s), 8.98 (1H, s), 11.27 (1H, br s); FAB MS *m/e* $(M+H)^+$ 326.

5.1.3. Ethyl-2-phenyl-4-(3-trifluoromethylanilino)pyrimidine-5-carboxylate hydrochloride (2c). Compound **2c** was prepared from ethyl-4-chloro-2-phenylpyrimidine-5-carboxylate (**1c**) and *m*-aminobenztrifluoride in 69% yield as a pale yellow amorphous solid similarly: ^{1}H NMR (DMSO-d_6) δ 1.39 (3H, t, J = 7.4 Hz), 4.42 (2H, q, J = 7.4 Hz), 7.48–7.63 (4H, m), 7.67 (1H, d, J = 7.6 Hz), 7.89 (1H, d, J = 8.8 Hz), 8.35 (2H, d, J = 7.6 Hz), 8.52 (1H, s), 9.07 (1H, s), 10.38 (1H, br s); FAB MS *m/e* $(M+H)^+$ 388.

5.1.4. 4-(3-Trifluoromethylanilino)pyrimidine-5-carboxylic acid (3a). A solution of **3a** (0.22 g, 0.71 mmol) in EtOH and 1 M NaOH was heated at 50 °C for 1 h. The solution was acidified with 1 M HCl and cooled to give a solid, which was collected by filtration and washed well with water and *n*-hexane to give 0.15 g of **3a** as a pale yellow amorphous powder: ^{1}H NMR ((DMSO-d_6) δ 7.49 (1H, d, J = 7.8 Hz), 7.62 (1H, t, J = 8.3 Hz), 7.92 (1H, br d, J = 8.3 Hz), 8.25 (1H, s), 8.83 (1H, s), 8.95 (1H, s), 10.68 (1H, br s); FAB MS *m/e* $(M+H)^+$ 284.

5.1.5. 2-Methyl-4-(3-trifluoromethylanilino)pyrimidine-5-carboxylic acid (3b). Compound **3b** was prepared in 77% yield as a pale yellow amorphous solid similarly: ^{1}H NMR (DMSO-d_6) 2.58 (3H, s), 7.46 (1H, d, J = 7.8 Hz), 7.60 (1H, t, J = 7.8 Hz), 7.88 (1H, br d, J = 8.3 Hz), 8.32 (1H, s), 8.85 (1H, s), 10.77 (1H, br s); FAB MS *m/e* $(M+H)^+$ 298.

5.1.6. 2-Phenyl-4-(3-trifluoromethylanilino)pyrimidine-5-carboxylic acid (3c). Compound **3c** was prepared in 95% yield as a colorless amorphous solid similarly: ^{1}H NMR (DMSO-d_6) 7.48–7.61 (4H, m), 7.64 (1H, t, J = 8.3 Hz), 7.84 (1H, br d, J = 8.3 Hz), 8.31–8.39 (2H, m), 8.60 (1H, s), 9.04 (1H, s), 11.28 (1H, br s); FAB MS *m/e* $(M+H)^+$ 360.

5.1.7. 4-(3-Trifluoromethylanilino)pyrimidine-5-carboxamide (4a). To a solution of **4a** (0.15 g, 0.53 mmol) in dimethylformamide (6 mL) were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.11 g, 18 mmol) and HOBt (0.8 g, 18 mmol). After stirring for 4 h at room temperature, NH_4OH (1 mL) was added and stirred for 14 h at room temperature. The solution was washed with water, satd NaCl, dried over anhydrous Na_2SO_4, and concentrated. The residue was chromatographed on silica gel with elution using $CHCl_3$–MeOH, to give 0.14 g of **4a** (94%) as a white powder: mp 201–203 °C (EtOH); ^{1}H NMR (DMSO-d_6) 5.98 (2H, br s), 7.39 (1H, d, J = 8.0 Hz), 7.49 (1H, t, J = 8.0 Hz), 7.86 (1H, br d, J = 8.6 Hz),

8.07 (1H, s), 8.65 (1H, s), 8.79 (1H, s), 11.00 (1H, br s); FAB MS *m/e* $(M+H)^+$ 283, Anal. Calcd for $C_{12}H_9N_4OF_3 \cdot 0.3H_2O$: C, 50.11; H, 3.36; N, 19.48; F, 19.82. Found: C, 50.06; H, 3.41; N, 19.10; F, 20.22.

5.1.8. 2-Methyl-4-(3-trifluoromethylanilino)pyrimidine-5-carboxamide (4b). Compound **4b** was prepared in 58% yield as a pale yellow solid similarly: mp 254–255 °C (H_2O–MeOH); ^1H NMR (DMSO-d_6) 2.67 (3H, s), 7.60 (1H, d, $J = 7.9$ Hz), 7.67 (1H, t, $J = 7.8$ Hz), 7.93 (1H, d, $J = 8.3$ Hz), 8.19 (2H, br s), 8.76 (1H, s), 9.11 (1H, s), 12.15 (1H, br s); FAB MS *m/e* $(M+H)^+$ 297. Anal. Calcd for $C_{13}H_{11}N_4OF_3 \cdot HCl$: C, 46.93; H, 3.64; N, 16.84; Cl, 10.66; F, 17.13. Found: C, 46.85; H, 3.62; N, 16.88; Cl, 10.57; F, 17.31.

5.1.9. 2-Phenyl-4-(3-trifluoromethylanilino)pyrimidine-5-carboxamide (4c). Compound **4c** was prepared in 57% yield as a pale yellow solid similarly: mp 233–235 °C (EtOH); ^1H NMR (DMSO-d_6) 5.90 (2H, br s), 7.39 (1H, d, $J = 7.8$ Hz), 7.47–7.55 (4H, m), 7.76 (1H, br d, $J = 7.5$ Hz), 8.32–8.47 (2H, m), 8.49 (1H, s), 8.74 (1H, s), 11.08 (1H, br s); FAB MS *m/e* $(M+H)^+$ 359. Anal. Calcd for $C_{18}H_{13}N_4OF_3$: C, 60.34; H, 3.66; N, 15.64; F, 15.91. Found: C, 60.09; H, 3.76; N, 15.68; F, 16.06.

5.1.10. Ethyl-2-chloro-4-(3-trifluoromethylanilino)pyrimidine-5-carboxylate (6a). A mixture of ethyl-2,4-dichloropyrimidine-5-carboxylate (4.86 g, 22.0 mmol), 3-aminobenztrifluoride (3.0 mL, 24.2 mmol), and diisopropylethylamine (3.82 mL, 22.0 mmol) in CH_3CN (50 mL) was heated with reflux for 1 h. The mixture was diluted with AcOEt and washed successively with water, saturated aqueous NaCl, dried over Na_2SO_4, and concentrated. The residue was filtered and washed with hexane to give 6.66 g of **6a** (88%) as a pale yellow amorphous solid: ^1H NMR ($CDCl_3$) δ 1.37 (3H, t, $J = 7.5$ Hz), 4.40 (2H, q, $J = 7.5$ Hz), 7.55 (1H, d, $J = 7.5$ Hz), 7.65 (1H, t, $J = 7.5$ Hz), 7.87 (1H, d, $J = 7.5$ Hz), 8.13 (1H, s), 8.84 (1H, s), 10.38 (1H, br s); FAB MS *m/e* $(M+H)^+$ 346.

The following analogues were prepared in a similar manner.

5.1.11. Ethyl-4-anilino-2-chloropyrimidine-5-carboxylate (6b). Compound **6b** was prepared from ethyl-2,4-dichloropyrimidine-5-carboxylate (**5**) and aniline in 81% yield as a colorless amorphous solid similarly: ^1H NMR ($CDCl_3$) δ 1.44 (3H, t, $J = 7.4$ Hz), 4.43 (2H, q, $J = 7.4$ Hz), 7.18 (1H, t, $J = 7.5$ Hz), 7.40 (1H, t, $J = 7.5$ Hz), 7.67 (1H, d, $J = 7.5$ Hz), 8.83 (1H, s), 10.45 (1H, br s); FAB MS *m/e* $(M+H)^+$ 278.

5.1.12. Ethyl-2-chloro-4-(2-methylanilino)pyrimidine-5-carboxylate (6c). Compound **6c** was prepared from ethyl-2,4-dichloropyrimidine-5-carboxylate (**5**) and *o*-toluidine in 86% yield as a pale gray amorphous powder similarly: ^1H NMR ($CDCl_3$) δ 1.43 (3H, t, $J = 7.4$ Hz), 2.35 (3H, s), 4.43 (2H, q, $J = 7.4$ Hz), 7.11–7.15 (1H, m), 7.20–7.29 (2H, m), 7.98 (1H, d, $J = 8.3$ Hz), 8.80 (1H, s), 10.28 (1H, br s); FAB MS *m/e* $(M+H)^+$ 292.

5.1.13. Ethyl-2-chloro-4-(3-methylanilino)pyrimidine-5-carboxylate (6d). Compound **6d** was prepared from ethyl-2,4-dichloropyrimidine-5-carboxylate (**5**) and *m*-toluidine in 82% yield as a colorless amorphous powder similarly: ^1H NMR ($CDCl_3$) δ 1.43 (3H, t, $J = 7.0$ Hz), 2.38 (3H, s), 4.42 (2H, q, $J = 7.0$ Hz), 6.99 (1H, d, $J = 7.6$ Hz), 7.26–7.30 (1H, m), 7.40 (1H, s), 7.53–7.55 (1H, m), 8.85 (1H, s), 10.39 (1H, br s); FAB MS *m/e* $(M+H)^+$ 292.

5.1.14. Ethyl-2-chloro-4-(4-methylanilino)pyrimidine-5-carboxylate (6e). Compound **6e** was prepared from ethyl-2,4-dichloropyrimidine-5-carboxylate (**5**) and *p*-toluidine in 77% yield as a pale yellow amorphous powder similarly: ^1H NMR ($CDCl_3$) δ 1.43 (3H, t, $J = 7.2$ Hz), 2.35 (3H, s), 4.42 (2H, q, $J = 7.2$ Hz), 7.19 (2H, d, $J = 8.4$ Hz), 7.52 (2H, d, $J = 8.4$ Hz), 8.80 (1H, s), 10.35 (1H, br s); FAB MS *m/e* $(M+H)^+$ 292.

5.1.15. Ethyl-2-chloro-4-(3-cyanoanilino)pyrimidine-5-carboxylate (6f). Compound **6f** was prepared from ethyl-2, 4-dichloropyrimidine-5-carboxylate (**5**) and 3-aminobenzonitrile in 95% yield as a colorless amorphous powder similarly: ^1H NMR ($CDCl_3$) δ 1.45 (3H, t, $J = 7.3$ Hz), 4.45 (2H, q, $J = 7.3$ Hz), 7.44–7.52 (2H, m), 7.86–7.89 (1H, m), 8.08–8.12 (1H, m), 8.89 (1H, s), 10.62 (1H, br s); FAB MS *m/e* $(M+H)^+$ 303.

5.1.16. Ethyl-2-chloro-4-(3-ethylanilino)pyrimidine-5-carboxylate (6g). Compound **6g** was prepared from ethyl-2,4-dichloropyrimidine-5-carboxylate (**5**) and *m*-ethylaniline in 61% yield as a pale yellow amorphous powder similarly: ^1H NMR ($CDCl_3$) δ 1.36 (3H, t, $J = 7.4$ Hz), 1.42 (3H, t, $J = 7.4$ Hz), 2.67 (2H, q, $J = 7.4$ Hz), 4.42 (2H, q, $J = 7.4$ Hz), 7.01 (1H, br d, $J = 8.3$ Hz), 7.29 (1H, t, $J = 7.8$ Hz), 7.42 (1H, s), 7.55 (1H, dd, $J = 8.3$, 1.4 Hz), 8.80 (1H, s), 10.39 (1H, br s); FAB MS *m/e* $(M+H)^+$ 306.

5.1.17. Ethyl-2-chloro-4-(3-isopropylanilino)pyrimidine-5-carboxylate (6h). Compound **6h** was prepared from ethyl-2,4-dichloropyrimidine-5-carboxylate (**5**) and *m*-isopropylaniline in 45% yield as a pale yellow amorphous powder similarly: ^1H NMR ($CDCl_3$) δ 1.28 (6H, d, $J = 6.8$ Hz), 1.44 (3H, t, $J = 7.3$ Hz), 2.93 (1H, qq, $J = 6.8$ Hz), 4.43 (2H, q, $J = 7.3$ Hz), 7.05 (1H, br d, $J = 7.8$ Hz), 7.32 (1H, t, $J = 7.8$ Hz), 7.45–7.48 (1H, m), 7.54–7.58 (1H, m), 8.82 (1H, s), 10.40 (1H, br s); FAB MS *m/e* $(M+H)^+$ 320.

5.1.18. Ethyl-2-chloro-4-(3-methoxyanilino)pyrimidine-5-carboxylate (6i). Compound **6i** was prepared from ethyl-2, 4-dichloropyrimidine-5-carboxylate (**5**) and *m*-methoxyaniline in 87% yield as a colorless amorphous powder similarly: ^1H NMR ($CDCl_3$) δ 1.43 (3H, t, $J = 7.3$ Hz), 3.85 (3H, s), 4.43 (2H, q, $J = 7.3$ Hz), 6.73 (1H, ddd, $J = 8.3$, 2.4, 1.0 Hz), 7.15–7.19 (1H, m), 7.26–7.30 (1H, m), 7.43 (1H, t, $J = 2.4$ Hz), 8.83 (1H, s), 10.45 (1H, br s); FAB MS *m/e* $(M+H)^+$ 308.

5.1.19. Ethyl-2-chloro-4-(3-ethoxyanilino)pyrimidine-5-carboxylate (6j). Compound **6j** was prepared from ethyl-2, 4-dichloropyrimidine-5-carboxylate (**5**) and

m-ethoxyaniline as a pale yellow amorphous powder. This compound was used for next reaction without purification.

5.1.20. Ethyl-2-chloro-4-(3-isopropoxyanilino)pyrimidine-5-carboxylate (6k). Compound **6k** was prepared from ethyl-2,4-dichloropyrimidine-5-carboxylate (**5**) and *m*-isopropoxyaniline in 66% yield as a pale yellow amorphous powder similarly: ^{1}H NMR ($CDCl_3$) δ 1.38 (6H, d, J = 6.3 Hz), 1.43 (3H, t, J = 7.3 Hz), 4.43 (2H, q, J = 7.3 Hz), 4.58 (1H, qq, J = 6.3 Hz), 6.69–6.73 (1H, m), 7.09–7.14 (1H, m), 7.25 (1H, t, J = 7.8 Hz), 7.42 (1H, t, J = 2.0 Hz), 8.82 (1H, s), 10.42 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 336.

5.1.21. Ethyl-4-(3-bromoanilino)-2-chloropyrimidine-5-carboxylate (6l). Compound **6l** was prepared from ethyl-2, 4-dichloropyrimidine-5-carboxylate (**5**) and *m*-bromoaniline in 100% yield as a pale yellow amorphous powder similarly: ^{1}H NMR ($CDCl_3$) δ 1.36 (3H, t, J = 7.4 Hz), 4.39 (2H, q, J = 7.4 Hz), 7.32–7.42 (2H, m), 7.57–7.62 (1H, m), 7.98 (1H, s), 8.83 (1H, s), 10.27 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 356, 358.

5.1.22. 2-Chloro-4-(3-trifluoromethylanilino)pyrimidine-5-carboxylic acid (7a). A solution of **6a** (6.22 g, 18 mmol) in MeOH and 1 M NaOH was heated at 50 °C for 1 h. The solution was acidified with 1 M HCl and cooled to give a solid, which was collected by filtration and washed well with water and *n*-hexane to give 5.48 g of **7a** as a colorless amorphous powder: ^{1}H NMR (DMSO-d_6) δ 7.54 (1H, d, J = 7.5 Hz), 7.65 (1H, t, J = 7.5 Hz), 7.88 (1H, br d, J = 8.4 Hz), 8.15 (1H, s), 8.82 (1H, s), 10.72 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 318.

The following analogues were prepared in a similar manner.

5.1.23. 4-Anilino-2-chloropyrimidine-5-carboxylic acid (7b). Compound **7b** was prepared in 79% yield as a pale yellow amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 7.16–7.24 (1H, m), 7.33–7.46 (2H, m), 7.61–7.67 (1H, m), 8.78 (1H, s), 10.57 (1H, br s); FAB MS *m*/*e* $(M-H)^-$ 248.

5.1.24. 2-Chloro-4-(2-methylanilino)pyrimidine-5-carboxylic acid (7c). Compound **7c** was prepared in 99% yield as a pale ivory amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 2.27 (3H, s), 7.14–7.18 (1H, m), 7.27–7.38 (2H, m), 7.81 (1H, d, J = 7.9 Hz), 8.77 (1H, s), 10.44 (1H, br s); FAB MS *m*/*e* $(M-H)^-$ 262.

5.1.25. 2-Chloro-4-(3-methylanilino)pyrimidine-5-carboxylic acid (7d). Compound **7d** was prepared in 99% yield as a pale yellow amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 2.29 (3H, s), 6.89 (1H, d, J = 6.9 Hz), 7.22–7.25 (1H, m), 7.37 (1H, s), 7.54 (1H, d, J = 6.9 Hz), 8.68 (1H, s), 12.82 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 264.

5.1.26. 2-Chloro-4-(4-methylanilino)pyrimidine-5-carboxylic acid (7e). Compound **7e** was prepared in 100% yield as an ivory amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 2.31 (3H, s), 7.22 (2H, d, J = 8.3 Hz), 7.50 (2H, d, J = 8.3 Hz), 8.75 (1H, s), 10.49 (1H, br s); FAB MS *m*/*e* $(M-H)^-$ 262.

5.1.27. 2-Chloro-4-(3-cyanoanilino)pyrimidine-5-carboxylic acid (7f). Compound **7f** was prepared in 100% yield as a colorless amorphous powder similarly: ^{1}H NMR (DMSO) δ 7.49–7.52 (1H, m), 7.57 (1H, t, J = 7.8 Hz), 7.82–7.85 (1H, m), 8.23–8.24 (1H, m), 8.64 (1H, s), 14.10 (1H, br s); FAB MS *m*/*e* $(M-H)^-$ 273.

5.1.28. 2-Chloro-4-(3-ethylanilino)pyrimidine-5-carboxylic acid (7g). Compound **7g** was prepared in 98% yield as a pale yellow amorphous powder similarly: ^{1}H NMR (DMSO-d_6) 1.21 (3H, t, J = 7.8 Hz), 2.63 (2H, q, J = 7.8 Hz), 7.05 (1H, br d, J = 7.8 Hz), 7.32 (1H, t, J = 7.8 Hz), 7.43 (1H, br s), 7.52 (1H, br d, J = 7.8 Hz), 8.77 (1H, s), 10.56 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 278.

5.1.29. 2-Chloro-4-(3-isopropylanilino)pyrimidine-5-carboxylic acid (7h). Compound **7h** was prepared in 100% yield as a pale yellow amorphous powder similarly: ^{1}H NMR (DMSO-d_6): δ 1.23 (6H, d, J = 6.8 Hz), 2.93 (1H, qq, J = 6.8 Hz), 7.07 (1H, br d, J = 7.8 Hz), 7.32 (1H, t, J = 7.4 Hz), 7.47–7.52 (2H, m), 8.76 (1H, s), 10.69 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 292.

5.1.30. 2-Chloro-4-(3-methoxyanilino)pyrimidine-5-carboxylic acid (7i). Compound **7i** was prepared in 72% yield as a colorless amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 3.77 (3H, s), 6.67 (1H, br d, J = 8.0 Hz), 7.16 (1H, d, J = 8.0 Hz), 7.27 (1H, t, J = 8.0 Hz), 7.42 (1H, br s), 8.66 (1H, s), 12.93 (1H, br s); FAB MS *m*/*e* $(M-H)^-$ 278.

5.1.31. 2-Chloro-4-(3-ethoxyanilino)pyrimidine-5-carboxylic acid (7j). Compound **7j** was prepared in 61% yield as a yellow amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 1.35 (3H, t, J = 6.8 Hz), 4.05 (2H, q, J = 6.8 Hz), 6.75 (1H, dd, J = 8.3, 2.4 Hz), 7.13 (1H, br d, J = 8.3 Hz), 7.29 (1H, t, J = 8.3 Hz), 7.33–7.36 (1H, m), 8.78 (1H, s), 10.56 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 294.

5.1.32. 2-Chloro-4-(3-isopropoxyanilino)pyrimidine-5-carboxylic acid (7k). Compound **7k** was prepared in 97% yield as a pale yellow amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 1.30 (6H, d, J = 6.3 Hz), 4.61 (1H, qq, J = 6.3 Hz), 6.77 (1H, dd, J = 8.3, 2.4 Hz), 7.05–7.09 (1H, m), 7.27 (1H, t, J = 8.3 Hz), 7.39 (1H, m), 8.75 (1H, s), 11.07 (1H, br s); FAB MS *m*/*e* $(M+H)^+$ 308.

5.1.33. 4-(3-Bromoanilino)-2-chloropyrimidine-5-carboxylic acid (7l). Compound **7l** was prepared in 100% yield as a pale yellow amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 7.36–7.39 (2H, m), 7.58–7.62 (1H, m), 8.00 (1H, br s), 8.80 (1H, s), 10.64 (1H, br s); FAB MS *m*/*e* $(M-H)^-$ 326, 328.

5.1.34. 2-(1*H*-Benzotriazol-1-yloxy)-4-(3-trifluoromethylanilino)pyrimidine-5-carboxamide (8a). To a suspension

of **7a** (5.41 g, 17 mmol) in dichloroethane (60 mL) were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (3.45 g, 18 mmol) and HOBt (2.43 g, 18 mmol). After stirring for 30 min at room temperature, NH_4OH (10 mL) was added and stirred for 1 h at room temperature. The solution was washed with water, satd NaCl, dried over anhydrous Na_2SO_4, and concentrated. The residue was chromatographed on silica gel with elution using $CHCl_3$, to give 3.7 g of **8a** (52%) as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 7.19 (1H, t, J = 7.8 Hz), 7.29 (1H, br d, J = 8.7 Hz), 7.36 (1H, d, J = 7.8 Hz), 7.54 (1H, t, J = 7.5 Hz), 7.63 (1H, t, J = 7.5 Hz), 7.67 (1H, br s), 7.81 (1H, d, J = 8.4 Hz), 7.96 (1H, br s), 8.19 (1H, d, J = 8.4 Hz), 8.41 (1H, br s), 8.86 (1H, s), 11.79 (1H, br s); FAB MS m/e $(M+H)^+$ 416.

The following analogues were prepared in a similar manner.

5.1.35. 4-Anilino-2-(1*H*-benzotriazol-1-yloxy)pyrimidine-5-carboxamide (8b). Compound **8b** was prepared in 34% yield as a colorless amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 6.91–7.00 (5H, m), 7.52–7.60 (1H, m), 7.62–7.69 (1H, m), 7.82 (1H, d, J = 8.3 Hz), 7.90 (1H, br s), 8.23 (1H, d, J = 8.3 Hz), 8.39 (1H, br s), 8.87 (1H, s), 11.64 (1H, br s); FAB MS m/e $(M+H)^+$ 348.

5.1.36. 2-(1*H*-Benzotriazol-1-yloxy)-4-(2-methylanilino)-pyrimidine-5-carboxamide (8c). Compound **8c** was prepared in 57% yield as an ivory amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 2.18 (3H, s), 6.59–6.62 (1H, m), 6.88–6.92 (1H, m), 6.97 (1H, d, J = 8.3 Hz), 7.12 (1H, d, J = 7.3 Hz), 7.53–7.57 (1H, m), 7.62–7.66 (1H, m), 7.80 (1H, d, J = 8.3 Hz), 7.88 (1H, br s), 8.20 (1H, d, J = 8.3 Hz), 8.39 (1H, br s), 8.86 (1H, s), 11.59 (1H, br s); FAB MS m/e $(M+H)^+$ 362.

5.1.37. 2-(1*H*-Benzotriazol-1-yloxy)-4-(3-methylanilino)-pyrimidine-5-carboxamide (8d). Compound **8d** was prepared in 50% yield as a pale yellow amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 2.08 (3H, s), 6.72–6.90 (4H, m), 7.51–7.57 (1H, m), 7.61–7.67 (1H, m), 7.79–7.83 (1H, m), 7.91 (1H, br s), 8.18–8.23 (1H, m), 8.40 (1H, br s), 8.87 (1H, s), 11.67 (1H, br s); FAB MS m/e $(M+H)^+$ 362.

5.1.38. 2-(1*H*-Benzotriazol-1-yloxy)-4-(4-methylanilino)-pyrimidine-5-carboxamide (8e). Compound **8e** was prepared in 55% yield as a pale yellow amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 2.19 (3H, s), 6.79 (2H, d, J = 8.3 Hz), 6.83 (2H, d, J = 8.3 Hz), 7.57 (1H, t, J = 7.8 Hz), 7.65 (1H, t, J = 7.8 Hz), 7.81 (1H, d, J = 8.3 Hz), 7.88 (1H, br s), 8.24 (1H, d, J = 8.8 Hz), 8.32 (1H, br s), 8.84 (1H, s), 11.56 (1H, br s); FAB MS m/e $(M+H)^+$ 362.

5.1.39. 2-(1*H*-Benzotriazol-1-yloxy)-4-(3-cyanoanilino)-pyrimidine-5-carboxamide (8f). Compound **8f** was prepared in 18% yield as a yellow amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 7.17 (1H, t, J = 7.8 Hz), 7.31–7.34 (1H, m), 7.44–7.46 (1H, m), 7.51–7.55 (1H, m), 7.61–7.65 (2H, m), 7.79 (1H, d, J = 8.3 Hz), 7.97 (1H, br s), 8.18 (1H, d, J = 8.3 Hz), 8.42 (1H, br s), 8.88 (1H, s), 11.75 (1H, br s); FAB MS m/e $(M-H)^-$ 371.

5.1.40. 2-(1*H*-Benzotriazol-1-yloxy)-4-(3-ethylanilino)pyrimidine-5-carboxamide (8g). Compound **8g** was prepared similarly and used for next reaction without purification.

5.1.41. 2-(1*H*-Benzotriazol-1-yloxy)-4-(3-isopropylanilino)-pyrimidine-5-carboxamide (8h). Compound **8h** was prepared similarly and used for next reaction without purification.

5.1.42. 2-(1*H*-Benzotriazol-1-yloxy)-4-(3-methoxyanilino)-pyrimidine-5-carboxamide (8i). Compound **8i** was prepared in 34% yield as a yellow amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 3.86 (3H, s), 6.78 (1H, dd, J = 8.6, 2.2 Hz), 7.12–7.16 (1H, m), 7.32 (1H, t, J = 8.1 Hz), 7.59 (1H, t, J = 7.5 Hz), 7.71 (1H, t, J = 2.2 Hz), 7.76 (1H, t, J = 7.8 Hz), 7.98–8.00 (3H, m), 8.52 (1H, d, J = 8.6 Hz), 9.03 (1H, s), 11.54 (1H, br s); FAB MS m/e $(M+H)^+$ 378.

5.1.43. 2-(1*H*-Benzotriazol-1-yloxy)-4-(3-ethoxyanilino)-pyrimidine-5-carboxamide (8j). Compound **8j** was prepared in 35% yield as a pale yellow amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 1.35 (3H, t, J = 6.8 Hz), 4.05 (2H, q, J = 6.8 Hz), 6.53–6.60 (2H, m), 6.80 (1H, t, J = 2.5 Hz), 6.85 (1H, t, J = 7.8 Hz), 7.54 (1H, t, J = 7.3 Hz), 7.64 (1H, t, J = 7.3 Hz), 7.80 (1H, d, J = 8.3 Hz), 7.90 (1H, br s), 8.19 (1H, d, J = 8.3 Hz), 8.40 (1H, br s), 8.83 (1H, s), 11.53 (1H, br s); FAB MS m/e $(M+H)^+$ 392.

5.1.44. 2-(1*H*-Benzotriazol-1-yloxy)-4-(3-isopropoxyanilino)pyrimidine-5-carboxamide (8k). Compound **8k** was prepared in 55% yield as a pale yellow amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 1.23 (6H, d, J = 5.8 Hz), 4.50 (1H, qq, J = 5.8 Hz), 6.53 (1H, dd, J = 7.3, 1.9 Hz), 6.58 (1H, dd, J = 7.3, 2.0 Hz), 6.82–6.86 (2H, m), 7.51–7.57 (1H, m), 7.61–7.67 (1H, m), 7.80 (1H, d, J = 8.3 Hz), 7.54 (1H, t, J = 7.3 Hz), 7.64 (1H, t, J = 7.3 Hz), 7.80 (1H, d, J = 8.3 Hz), 7.90 (1H, br s), 8.20 (1H, d, J = 8.3 Hz), 8.37 (1H, br s), 8.80 (1H, s), 11.62 (1H, br s); FAB MS m/e $(M+H)^+$ 406.

5.1.45. 2-(1*H*-Benzotriazol-1-yloxy)-4-(3-bromoanilino)-pyrimidine-5-carboxamide (8l). Compound **8l** was prepared in 80% yield as a pale yellow amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 6.91–7.20 (2H, m), 7.19 (1H, dt, J = 7.3, 1.4 Hz), 7.49 (1H, t, J = 1.9 Hz), 7.51–7.57 (1H, m), 7.64 (1H, t, J = 7.3 Hz), 7.81 (1H, d, J = 8.3 Hz), 7.95 (1H, br s), 8.21 (1H, d, J = 8.3 Hz), 8.41 (1H, br s), 8.86 (1H, s), 11.74 (1H, br s); FAB MS m/e $(M-H)^-$ 423, 425.

5.1.46. 2-(2-Aminoethylamino)-4-(3-trifluoromethylanilino)-pyrimidine-5-carboxamide dihydrochloride (9a). To a suspension of **8a** (3.49 g, 8.4 mmol) in CH_3CN (50 mL) was added ethylenediamine (7.35 mL, 110 mmol) and stirred for 30 min at room temperature. The mixture was diluted with water and extracted with AcOEt. The organic

layer was dried over anhydrous Na_2SO_4, and evaporated to give 2.80 g of **9a** (98%) as a colorless solid. **9a** was converted into the hydrochloride and recrystallized from water–EtOH to give 1.76 g of **9a** as a colorless powder: mp 270–272 °C (dec.); ^{1}H NMR (DMSO-d_6, 80 °C) δ 3.04 (2H, br s), 3.68 (2H, m), 7.08 (1H, br s), 7.48 (1H, br d, J = 7.8 Hz), 7.64 (1H, br t, J = 7.8 Hz), 7.75–8.40 (3H, m), 8.06 (1H, br s), 8.79 (1H, s), 12.09 (1H, br s); FAB MS *m/e* $(M+H)^+$ 341. Anal. Calcd for $C_{14}H_{15}N_6OF_3 \cdot 2HCl$: C, 40.69; H, 4.15; N, 20.34; Cl, 17.16; F, 13.79. Found: C, 40.69; H, 4.11; N, 20.32; Cl, 16.87; F, 13.64.

The following analogues were prepared similarly.

5.1.47. 2-(4-Aminobutylamino)-4-(3-trifluoromethylanilino)pyrimidine-5-carboxamide (9b). Compound **9b** was prepared from compound **8a** and 1,4-diaminobutane in 33% yield as a colorless powder similarly: mp 198–200 °C (AcOEt–EtOH); ^{1}H NMR (DMSO-d_6) 1.38 (2H, m), 1.57 (2H, m), 2.52 (2H, m), 3.31 (2H, m), 7.35 (2H, m), 7.50–8.30 (4H, m), 8.60 (0.8H, s), 8.62 (0.2H, s), 11.77 (0.2H, br s), 11.95 (0.8H, br s); FAB MS *m/e* $(M+H)^+$. Anal. Calcd for $C_{16}H_{19}N_6OF_3 \cdot 0.25C_2H_6O.1.5H_2O$: C, 50.38; H, 5.64; N, 21.36; F, 14.49. Found: C. 50.40; H, 5.66; N, 21.14; F, 14.64.

5.1.48. *tert*-Butyl-(2-{[5-(aminocarbonyl)-4-(3-trifluoromethylanilino)pyrimidin-2-yl]amino}ethyl)methylcarbamate (9c). Compound **9c** was prepared from compound **8a** and *tert*-butyl (2-aminoethyl)methylcarbamate similarly. This compound was used for next reaction without purification.

5.1.49. 2-[(2-Methylaminoethyl)amino]-4-(3-trifluoromethylanilino)pyrimidine-5-carboxamide (9d). To a suspension of **9c** (0.32 g, 0.7 mmol) in EtOH (10 mL) was added 4 N HCl/AcOEt (1 mL) and stirred for 1 day at room temperature. The solution was evaporated and diluted with $CHCl_3$–2-PrOH (3:1 by volume). The mixture was washed with saturated aqueous Na_2CO_3, H_2O, dried over anhydrous Na_2SO_4, and evaporated. The residue was chromatographed on silica gel with elution using $CHCl_3$–MeOH–aq NH_3, to give 0.17 g of **9d** (30%) as a colorless solid: mp 194–196 °C (EtOH–H_2O); ^{1}H NMR (DMSO-d_6) δ 1.74 (1H, br s), 2.28 (3H, s), 2.68 (2H, t, J = 5.9 Hz), 3.39 (2H, q, J = 5.9 Hz), 7.25–7.45 (2H, m), 7.50–7.57 (2H, m), 7.66 (1H, d, J = 8.3 Hz), 7.80–8.20 (1H, m), 8.54 (1H, s), 8.60 (1H, s), 11.78 (0.3H, br s), 11.94 (0.7H, br s); FAB MS *m/e* $(M+H)^+$ 355. Anal. Calcd for $C_{15}H_{17}N_6OF_3$: C, 50.85; H, 4.84; N, 23.72; F, 16.09. Found: C, 50.59; H, 4.77; N, 23.55; F, 16.37.

5.1.50. 2-[(2-Dimethylaminoethyl)amino]-4-(3-trifluoromethylanilino)pyrimidine-5-carboxamide (9e). Compound **9e** was prepared from compound **8a** and *N*,*N*-dimethylethylenediamine in 71% yield as a colorless powder similarly: mp 185–187 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 2.15 (6H, s), 2.44 (2H, br t, J = 6.4 Hz), 3.37–3.47 (2H, m), 7.33 (1H, br s), 7.36 (1H, d, J = 7.4 Hz), 7.45 (1H × 0.7, m), 7.53 (1H, t, J = 7.8 Hz), 7.69 (0.7H, br d, J = 7.8 Hz), 7.58–8.25 (1.9H, m), 8.48 (0.7H, s), 8.60 (0.7H, s), 8.64 (0.3H, s), 11.78 (0.3H, br s), 11.94 (0.7H, br s); FAB MS *m/e* $(M+H)^+$ 369. Anal. Calcd for $C_{16}H_{19}N_6OF_3 \cdot 0.2H_2O$: C, 51.67; H, 5.26; N, 22.59; F, 15.32. Found: C, 51.58; H, 5.17; N, 22.66; F, 15.09.

5.1.51. 2-[(2-Dimethylaminoethyl)methylamino]-4-(3-trifluoromethylanilino)pyrimidine-5-carboxamide (9f). Compound **9f** was prepared from compound **8a** and *N*,*N*,*N*′-trimethylethylenediamine in 51% yield similarly: mp 153–157 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 2.14 (3H, br s), 2.25 (3H, br s), 2.43–2.60 (2H, m), 3.16 (3H, s), 3.71 (0.8H, br s), 3.79 (2H, br s), 7.36 (1H, d, J = 7.8 Hz), 7.54 (1H, br t, J = 7.8 Hz), 7.60–8.20 (3H, m), 8.24 (0.4H, br s), 8.62 (0.6H, br s), 8.68 (1H,s), 11.86 (0.4H, br s), 11.90 (0.6H, br s); FAB MS *m/e* $(M+H)^+$ 383. Anal. Calcd for $C_{17}H_{21}N_6OF_3 \cdot 0.7H_2O$: C, 51.69; H, 5.72; N, 21.28; F, 14.43. Found: C, 51.47; H, 5.44; N, 21.24; F, 14.75.

5.1.52. 2-(Piperazin-1-yl)-4-(3-trifluoromethylanilino)pyrimidine-5-carboxamide (9g). Compound **9g** was prepared from compound **8a** and piperazine in 59% yield as a colorless powder similarly: mp 207–209 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 2.74 (4H, br s), 3.72 (4H, br s), 7.33–7.45 (1H, m), 7.37 (1H, d, J = 6.8 Hz), 7.55 (1H, t, J = 8.3 Hz), 7.59 (1H, d, J = 8.3 Hz), 7.99 (1H, br s), 8.46 (1H, s), 8.68 (1H, s), 11.80 (1H, s); FAB MS *m/e* $(M+H)^+$ 367. Anal. Calcd for $C_{16}H_{17}N_6OF_3 \cdot 0.2H_2O$: C, 51.95; H, 4.74; N, 22.72; F, 15.41. Found: C, 52.20; H, 4.71; N, 22.61; F, 15.44.

5.1.53. 2-(2-Aminoethylamino)-4-anilinopyrimidine-5-carboxamide (9h). Compound **9h** was prepared from compound **8b** and ethylenediamine in 46% yield as a colorless powder similarly: mp 173–175 °C (EtOH); ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.75 (2H, t, J = 6.0 Hz), 3.33 (2H, q, J = 6.0 Hz), 7.00 (1H, t, J = 7.3 Hz), 7.31 (2H, t, J = 7.3 Hz), 7.70 (2H, d, J = 7.3 Hz), 8.56 (1H, s), 11.44 (1H, br s); FAB MS *m/e* $(M+H)^+$ 273. Anal. Calcd for $C_{13}H_{16}N_6O \cdot 0.5H_2O$: C, 55.50; H, 6.09; N, 29.87. Found: C, 55.61; H, 5.86; N, 29.84.

5.1.54. 2-(2-Aminoethylamino)-4-(2-methylanilino)pyrimidine-5-carboxamide (9i). Compound **9i** was prepared from compound **8c** and ethylenediamine in 71% yield as a colorless powder similarly: mp185–187 °C (EtOAc–EtOH); ^{1}H NMR (DMSO-d_6, 80 °C) δ 1.52 (2H, br s), 2.28 (3H, s), 2.72 (2H, t, J = 6.0 Hz), 3.29 (2H, q, J = 6.0 Hz), 6.94–7.29 (6H, m), 8.28 (1H, d, J = 7.6 Hz), 8.56 (1H, s), 11.26 (1H, br s); FAB MS *m/e* $(M+H)^+$ 287. Anal. Calcd for $C_{14}H_{18}N_6O \cdot 0.25H_2O$: C, 57.82; H, 6.41; N, 28.90. Found: C, 57.65; H, 6.19; N, 28.94.

5.1.55. 2-(2-Aminoethylamino)-4-(3-methylanilino)pyrimidine-5-carboxamide (9j). Compound **9j** was prepared from compound **8d** and ethylenediamine in 33% yield as a colorless powder similarly: mp 185–188 °C (EtOAc–EtOH); ^{1}H NMR (DMSO-d_6, 80 °C): 1.44 (2H, br s), 2.30 (3H, s), 2.75 (2H, t, J = 6.4 Hz), 3.33 (2H, td, J = 6.4, 5.8 Hz), 6.83 (1H, d, J = 7.8 Hz), 7.08 (1H, br s), 7.18 (1H, t, J = 7.8 Hz), 7.29 (2H, br s),

7.40–7.70 (2H, m), 8.54 (1H, s), 11.39 (1H, br s); FAB MS *m/e* $(M+H)^+$ 287. Anal. Calcd for $C_{14}H_{18}N_6O\cdot 0.2H_2O$: C, 58.00; H, 6.40; N, 28.99. Found: C, 57.93; H, 6.39; N, 28.84.

5.1.56. 2-(2-Aminoethylamino)-4-(4-methylanilino)pyrimidine-5-carboxamide (9k). Compound **9k** was prepared from compound **8e** and ethylenediamine in 92% yield similarly: mp 191–193 °C (EtOAc–EtOH); ^{1}H NMR (DMSO-d_6, 80 °C) δ 1.54 (2H, br s), 2.27 (3H, s), 2.74 (2H, t, J = 6.4 Hz), 3.32 (2H, q, J = 6.4 Hz), 7.04 (1H, br s), 7.11 (2H, d, J = 8.4 Hz), 7.28 (2H, br s), 7.56 (2H, d, J = 8.4 Hz), 8.53 (1H, s), 11.32 (1H, br s); FAB MS *m/e* $(M+H)^+$ 287. Anal. Calcd for $C_{14}H_{18}N_6O\cdot 0.4H_2O$: C, 57.28; H, 6.46; N, 28.63. Found: C, 57.46; H, 6.33; N, 28.61.

5.1.57. 2-(2-Aminoethylamino)-4-(3-cyanolanilino)pyrimidine-5-carboxamide (9l). Compound **9l** was prepared from compound **8f** and ethylenediamine in 66% yield as a colorless powder similarly: mp 192–194 °C (2-PrOH); ^{1}H NMR (DMSO-d_6, 80 °C) δ 1.45 (2H, br s), 2.76 (2H, t, J = 6.0 Hz), 3.36 (2H, q, J = 6.0 Hz), 7.33 (1H, br s), 7.40–7.41 (3H, m), 7.49 (1H, t, J = 8.0 Hz), 7.81 (1H, d, J = 8.5 Hz), 8.37 (1H, s), 8.60 (1H, s), 11.71 (1H, br s); FAB MS *m/e* $(M+H)^+$ 298. Anal. Calcd for $C_{14}H_{15}N_7O.1.\ 2H_2O\cdot 0.25C_3H_7O$: C, 53.09; H, 5.78; N, 29.38. Found: C, 52.96; H, 5.54; N, 29.25.

5.1.58. 2-(2-Aminoethylamino)-4-(3-ethylanilino)pyrimidine-5-carboxamide (9m). Compound **9m** was prepared from compound **8g** and ethylenediamine in 29% yield as a colorless powder similarly: mp 149–151 °C (MeOH); ^{1}H NMR (DMSO-d_6, 80 °C) δ 1.20 (3H, t, J = 7.6 Hz), 1.49 (2H, br s), 2.61 (2H, q, J = 7.6 Hz), 2.75 (2H, t, J = 6.4 Hz), 3.34 (2H, q, J = 6.4 Hz), 6.86 (1H, d, J = 7.6 Hz), 7.07 (1H, br s), 7.20 (1H, t, J = 7.6 Hz), 7.29 (1H, br s), 7.44–7.60 (2H, m), 8.54 (1H, s), 11.40 (1H, br s); FAB MS *m/e* $(M+H)^+$ 301; Anal. Calcd for $C_{15}H_{20}N_6O\cdot 0.5H_2O$: C, 58.24; H, 6.84; N, 27.17. Found: C, 58.13; H, 6.76; N, 27.02.

5.1.59. 2-(2-Aminoethylamino)-4-(3-isopropylanilino)pyrimidine-5-carboxamide (9n). Compound **9n** was prepared from compound **8h** and ethylenediamine in 9% yield as a yellow powder similarly: mp 160–162 °C (MeOH); ^{1}H NMR (DMSO-d_6, 80 °C) δ 1.22 (6H, d, J = 6.8 Hz), 1.29 (2H, br s), 2.74 (2H, t, J = 6.4 Hz), 2.89 (1H, qq, J = 6.8 Hz), 3.34 (2H, q, J = 6.4 Hz), 6.80 (1H, d, J = 8.0 Hz), 7.05 (1H, br s), 7.21 (1H, t, J = 8.0 Hz), 7.29 (1H, br s), 7.42–7.62 (2H, m), 8.54 (1H, s), 11.41 (1H, br s); FAB MS *m/e* $(M+H)^+$ 315. Anal. Calcd for $C_{16}H_{22}N_6O\cdot 0.5H_2O$: C, 59.42; H, 7.17; N, 25.99. Found: C, 59.39; H, 7.39; N, 26.02.

5.1.60. 2-(2-Aminoethylamino)-4-(3-methoxyanilino)pyrimidine-5-carboxamide (9o). Compound **9o** was prepared from compound **8i** and ethylenediamine in 70% yield as a pale yellow powder similarly: mp 142–145 °C (EtOH); ^{1}H NMR (DMSO-d_6, 80 °C) δ 1.37 (2H, br s), 2.74 (2H, t, J = 6.4 Hz), 3.34 (2H, q, J = 6.4 Hz), 3.86 (3H, s), 6.78 (1H, dd, J = 8.6, 2.2 Hz), 7.12–7.16 (1H, m), 7.32 (1H, t, J = 8.1 Hz), 7.59 (1H, t, J = 7.5 Hz), 7.71 (1H, t, J = 2.2 Hz), 7.76 (1H, t, J = 7.8 Hz), 7.98–8.00 (3H, m), 8.52 (1H, d, J = 8.6 Hz), 9.03 (1H, s), 11.54 (1H, br s); FAB MS *m/e* $(M+H)^+$ 303. *Anal.* Calcd for $C_{14}H_{18}N_6O_2\cdot 0.7H_2O$: C, 53.39; H, 6.21; N, 26.68. Found: C, 53.55; H, 6.33; N, 26.42.

5.1.61. 2-(2-Aminoethylamino)-4-(3-ethoxyanilino)pyrimidine-5-carboxamide (9p). Compound **9p** was prepared from compound **8j** and ethylenediamine in 63% yield as a pale yellow powder similarly: mp 176–178 °C (MeOH); ^{1}H NMR (DMSO-d_6, 80 °C) δ 1.33 (3H, t, J = 6.8 Hz), 1.48 (2H, br s), 2.75 (2H, t, J = 6.4 Hz), 3.34 (2H, q, J = 6.4 Hz), 4.05 (2H, q, J = 6.8 Hz), 6.57 (1H, dd, J = 8.0, 2.0 Hz), 7.05–7.15 (2H, m), 7.18 (1H, t, J = 8.0 Hz), 7.31 (1H, br s), 7.49 (1H, br s), 8.55 (1H, br s), 11.47 (1H, br s); FAB MS *m/e* $(M+H)^+$ 317; Anal. Calcd for $C_{15}H_{20}N_6O_2\cdot 1.1H_2O$: C, 53.59; H, 6.66; N, 25.00. Found: C, 53.63; H, 6.38; N, 24.73.

5.1.62. 2-(2-Aminoethylamino)-4-(3-isopropoxyanilino)-pyrimidine-5-carboxamide (9q). Compound **9q** was prepared from compound **8k** and ethylenediamine in 72% yield similarly: mp 143–145 °C (MeOH); ^{1}H NMR (DMSO-d_6, 80 °C) δ 1.27 (6H, d, J = 6.0 Hz), 1.31 (2H, br s), 2.74 (2H, t, J = 6.4 Hz), 3.33 (2H, q, J = 6.4 Hz), 4.58 (1H, qq, J = 6.0 Hz), 6.57 (1H, br d, J = 8.4 Hz), 7.02–7.14 (2H, m), 7.17 (1H, t, J = 8.4 Hz), 7.22–7.38 (2H, m), 7.40–7.48 (1H, m), 8.54 (1H, s), 11.45 (1H, br s); FAB MS *m/e* $(M+H)^+$ 331. Anal. Calcd for $C_{16}H_{22}N_6O_2$: C, 58.17; H, 6.71; N, 25.44. Found: C, 58.34; H, 6.52; N, 25.14.

5.1.63. 2-(2-Aminoethylamino)-4-(3-bromoanilino)pyrimidine-5-carboxamide (9r). Compound **9r** was prepared from compound **8l** and ethylenediamine in 71% yield similarly: mp 194–196 °C (EtOAc–EtOH); ^{1}H NMR (DMSO-d_6, 80 °C) δ 1.37 (2H, br s), 2.76 (2H, t, J = 6.4 Hz), 3.33 (2H, q, J = 6.4 Hz), 7.15–7.20 (1H, m), 7.20–7.25 (1H, m), 7.25 (1H, t, J = 6.0 Hz), 7.37 (2H, br s), 7.49 (1H, br s), 8.20 (1H, br s), 8.58 (1H, s), 11.61 (1H, br s); FAB MS *m/e* $(M+H)^+$ 351, 353. Anal. Calcd for $C_{13}H_{15}N_6OBr\cdot 0.25H_2O$: C, 43.90; H, 4.39; N, 23.63; Br, 22.46. Found: C, 43.84; H, 4.18; N, 23.44; Br, 22.19.

5.1.64. Ethyl-2-methylsulfanyl-4-(3-trifluoromethylanilino)pyrimidine-5-carboxylate (11a). Compound **11a** was prepared from ethyl-4-chloro-2-methylsulfanylpyrimidine-5-carboxylate (**10**) and *m*-aminobenztrifluoride according to the same procedure as that for **2a** in 45% yield as a pale brown amorphous powder similarly: ^{1}H NMR ($CDCl_3$) δ 1.42 (3H, t, J = 6.9 Hz), 2.57 (3H, s), 4.41 (2H, q, J = 6.9 Hz), 7.39 (1H, br d, J = 7.8 Hz), 7.47 (1H, t, J = 7.8 Hz), 7.65 (1H, br d, J = 7.8 Hz), 8.33 (1H, s), 8.82 (1H, s), 10.62 (1H, br s); FAB MS *m/e* $(M+H)^+$ 358.

5.1.65. Ethyl-4-methylamino-2-methylsulfanylpyrimidine-5-carboxylate (11b). Compound **11b** was prepared from ethyl-4-chloro-2-methylsulfanylpyrimidine-5-carboxylate (**10**) and methylamine in 84% yield as a colorless

amorphous powder similarly: ^{1}H NMR ($CDCl_3$) δ 1.37 (3H, t, J = 7.2 Hz), 2.53 (3H, s), 3.09 (3H, s), 4.33 (2H, q, J = 6.9 Hz), 8.17 (1H, br s), 8.51 (1H, s); FAB MS *m/e* $(M+H)^+$ 228.

5.1.66. Ethyl-4-cyclohexylamino-2-methylsulfanylpyrimidine-5-carboxylate (11c). Compound **11c** was prepared from ethyl-4-chloro-2-methylsulfanylpyrimidine-5-carboxylate (**10**) and cyclohexylamine. This compound was used for next reaction without purification.

5.1.67. 2-Methylsulfanyl-4-(3-trifluoromethylanilino) pyrimidine-5-carboxylic acid (12a). Compound **12a** was prepared according to the same procedure as that for **3a** in 79% yield as a pale brown amorphous powder: ^{1}H NMR (DMSO-d_6) δ 2.51 (3H, s), 7.48 (1H, d, J = 8.1 Hz), 7.61 (1H, t, J = 7.8 Hz), 7.79 (1H, d, J = 8.1 Hz), 8.39 (1H, s), 8.78 (1H, s), 10.72 (1H, br s); FAB MS *m/e* $(M+H)^+$ 330.

5.1.68. 4-Methylamino-2-methylsulfanylpyrimidine-5-carboxylic acid (12b). Compound **12b** was prepared in 94% yield as a colorless amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 2.98 (3H, s), 8.41 (1H, br s), 8.50 (1H, s); FAB MS *m/e* $(M+H)^+$ 200.

5.1.69. 4-Cyclohexylamino-2-methylsulfanylpyrimidine-5-carboxylic acid (12c). Compound **12c** was prepared similarly. This compound was used for next reaction without purification.

5.1.70. 2-Methylsulfanyl-4-(3-trifluoromethylanilino)-pyrimidine-5-carboxamide (13a). Compound **13a** was prepared from compound **12a** according to the same procedure as that for **4a** in 91% yield as a pale yellow amorphous powder: ^{1}H NMR (DMSO-d_6) δ 2.52 (3H, s), 7.44 (1H, d, J = 6.9 Hz), 7.60 (1H, t, J = 7.5 Hz), 7.72 (1H, d, J = 7.5 Hz), 7.81 (1H, br s), 8.34 (1H, s), 8.42 (1H, s), 8.77 (1H, s), 10.72 (1H, br s); FAB MS *m/e* $(M+H)^+$ 329.

5.1.71. 4-Methylamino-2-methylsulfanylpyrimidine-5-carboxamide (13b). Compound **13b** was prepared in 43% yield as a colorless amorphous powder similarly: ^{1}H NMR (DMSO-d_6) δ 2.49 (3H, s), 2.93 (3H, s), 7.40 (1H, br s), 7.99 (1H, br s), 8.48 (1H, s), 8.92 (1H, br s); FAB MS *m/e* $(M+H)^+$ 199.

5.1.72. 4-Cyclohexylamino-2-methylsulfanylpyrimidine-5-carboxamide (13c). Compound **13c** was prepared in 72% as a colorless amorphous powder yield similarly: ^{1}H NMR (DMSO-d_6) δ 1.20–1.44 (5H, m), 1.50–1.74 (3H, m), 1.83–1.95 (2H, m), 2.44 (3H, s), 3.90–4.03 (1H, m), 7.42 (1H, br s), 8.01 (1H, br s), 8.49 (1H, s), 9.13 (1H, br d, J = 7.6 Hz); FAB MS *m/e* $(M+H)^+$ 267.

5.1.73. *N*-Methyl-2-methylsulfanyl-4-(3-trifluoromethylanilino)pyrimidine-5-carboxamide (13d). Compound **13d** was prepared from compound **12a** and methylamine in 97% yield as a colorless amorphous solid similarly: ^{1}H NMR (DMSO-d_6) δ 2.51 (3H, s), 2.82 (3H, d, J = 4.4 Hz), 7.44 (1H, d, J = 7.9 Hz), 7.59 (1H, t, J = 7.8 Hz), 7.73 (1H, d, J = 7.8 Hz), 8.42 (1H, s), 8.69 (1H, s), 8.82 (1H, br d, J = 4.4 Hz), 11.59 (1H, br s); FAB MS *m/e* $(M+H)^+$ 343.

5.1.74. *N*,*N*-Dimethyl-2-methylsulfanyl-4-(3-trifluoromethylanilino)pyrimidine-5-carboxamide (13e). Compound **13e** was prepared from compound **12a** and dimethylaminehydrochloride in 100% yield as a colorless amorphous powder similarly. ^{1}H NMR (DMSO-d_6) δ 2.43 (3H, s), 3.00 (6H, s), 7.42 (1H, d, J = 7.8 Hz), 7.57 (1H, t, J = 7.8 Hz), 7.84 (1H, d, J = 7.8 Hz), 8.21 (1H, s), 8.23 (1H, s), 9.37 (1H, br s); FAB MS *m/e* $(M+H)^+$ 357.

5.1.75. 2-(3-Aminopropylamino)-4-(3-trifluoromethylanilino)pyrimidine-5-carboxamide (14a). Compound **14a** was prepared from compound **13a** and 1,3-diaminopropane according to the same procedure as that for **9a** in 76% yield as a colorless powder: mp 185–189 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) 1.58–1.72 (2H, m), 2.58–2.69 (2H, m), 3.30–3.46 (2H, m), 7.35 (1H, d, J = 7.6 Hz), 7.54 (1H, t, J = 7.6 Hz), 7.25–8.30 (5H, m), 8.61 (0.8H, s), 8.63 (0.2H, s), 11.78 (0.2H, br s), 11.96 (0.8H, br s); FAB MS *m/e* $(M+H)^+$. Anal. Calcd for $C_{15}H_{17}N_6OF_3 \cdot 1.5H_2O$: C, 47.24; H, 5.29; N, 22.04; F, 14.95. Found: C, 47.75; H, 5.02; N, 21.61; F, 14.95.

5.1.76. 2-(2-Hydroxyethylamino)-4-(3-trifluoromethylanilino)pyrimidine-5-carboxamide (14b). Compound **14b** was prepared from compound **13a** and 2-aminoethanol in 67% yield as a colorless powder similarly: mp 216–219 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 3.35–3.44 (2H, m), 3.50–3.62 (2H, m), 4.67 (0.7H, br t, J = 4.8 Hz), 4.70–4.78 (0.3H, m), 7.35 (1H, d, J = 7.3 Hz), 7.25–8.15 (5.3H, m), 8.53 (0.7H, s), 8.61 (0.7H, s), 8.63 (0.3H, s), 11.79 (0.3H, br s), 11.93 (0.7H, br s); FAB MS *m/e* $(M+H)^+$ 342. Anal. Calcd for $C_{14}H_{14}N_5O_2F_3 \cdot 0.5H_2O$: C, 48.00; H, 4.32; N, 19.99; F, 16.27. Found: C, 47.78; H, 4.27; N, 19.79; F, 16.09.

5.1.77. 2-(2-Aminoethylamino)-4-methylaminopyrimidine-5-carboxamide (14c). Compound **14c** was prepared from compound **13b** and ethylenediamine in 28% yield similarly: mp 136–138 °C (AcOEt); ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.72 (2H, t, J = 6.4 Hz), 2.88 (3H, s), 3.30 (2H, q, J = 6.4 Hz), 6.70 (1H, br s), 6.98 (1H, br s), 8.33 (1H, s), 8.72 (1H, br s); FAB MS *m/e* $(M+H)^+$ 311. Anal. Calcd for $C_8H_{14}N_6O \cdot 0.75H_2O$: C, 42.94; H, 6.98; N, 37.56. Found: C, 43.01; H, 6.88; N, 37.56.

5.1.78. 2-(2-Aminoethylamino)-4-cyclohexylaminopyrimidine-5-carboxamide (14d). Compound **14d** was prepared from compound **13c** and ethylenediamine in 47% yield similarly: mp 161–164 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ 1.10–1.72 (8H, m), 1.76–1.95 (2H, m), 2.60–2.70 (2H, m), 3.17–3.27 (2H, m), 3.28–3.36 (2H, m), 3.86–3.97 (1H, m), 6.88 (1H, br s), 7.06 (1H, br s), 7.32–7.80 (1H, br s), 8.34 (1H, s), 8.90–9.07 (1H, m); FAB MS *m/e* $(M+H)^+$ 279. Anal. Calcd for $C_{13}H_{22}N_6O \cdot 0.5H_2O$: C, 54.34; H, 8.07; N, 29.25. Found: C, 54.59; H, 7.97; N, 29.16.

5.1.79. 2-(2-Aminoethylamino)-*N*-methyl-4-(3-trifluoromethylanilino)pyrimidine-5-carboxamide (14e). Compound

14e was prepared from compound **13d** and ethylenediamine in 80% yield as a pale yellow solid similarly: mp 175–176 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ 1.69 (2H, br s), 2.72 (2H, t, J = 5.9 Hz), 2.77 (3H, d, J = 4.4 Hz), 3.27–3.36 (2H, m), 7.35 (1H, d, J = 7.3 Hz), 7.50–7.70 (2H, m), 8.39 (1H, m), 8.55 (1H, s), 8.59 (1H, s), 11.81 (1H, br s); FAB MS *m/e* $(M+H)^+$ 355. Anal. Calcd for $C_{15}H_{17}N_6OF_3 \cdot 0.75H_2O$: C, 48.98; H, 5.07; N, 22.85; F, 15.49. Found: C, 48.75; H, 4.75; N, 22.70; F, 15.55.

5.1.80. 2-(2-Aminoethylamino)-*N*,*N*-dimethyl-4-(3-trifluoromethylanilino)pyrimidine-5-carboxamide (14f). Compound **14f** was prepared from compound **13e** and ethylenediamine in 58% yield similarly: mp 115–116 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 1.48 (2H, br s), 2.72 (2H, t, J = 5.9 Hz), 3.01 (6H, s), 3.29 (2H, q, J = 5.9 Hz), 7.34 (1H, d, J = 7.8 Hz), 7.52 (1H, t, J = 7.9 Hz), 7.70–7.80 (1H, m), 8.10 (1H, s), 8.40–8.50 (1H, m), 9.60 (1H, br s); FAB MS *m/e* $(M+H)^+$ 369. Anal. Calcd for $C_{16}H_{19}N_6OF_3 \cdot 0.75H_2O$: C, 50.32; H, 5.41; N, 22.01; F, 14.92. Found: C, 50.52; H, 5.23; N, 22.22; F, 14.90.

5.1.81. Ethyl-2-(2-aminoethylamino)-4-(3-trifluoromethylanilino)pyrimidine-5-carboxylate (15). Compound **15** was prepared from compound **6a** and ethylenediamine in 64% yield similarly: mp 147–151 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ 1.34 (3H, t, J = 7.3 Hz), 3.01 (2H, br q, J = 5.4 Hz), 3.53–3.66 (2H, m), 4.34 (2H, q, J = 7.3 Hz), 7.44 (0.3H, d, J = 7.8 Hz), 7.51 (0.7H, d, J = 7.8 Hz), 7.58 (0.3H, t, J = 7.8 Hz), 7.65 (0.7H, t, J = 7.8 Hz), 7.88–8.35 (5H, m), 8.68 (0.3H, s), 8.70 (0.7H, s), 10.39 (0.3H, br s), 10.59 (0.7H, br s); FAB MS *m/e* $(M+H)^+$ 370. Anal. Calcd for $C_{16}H_{18}N_5O_2F_3 \cdot 2HCl$: C, 43.45; H, 4.56; N, 15.84; Cl, 16.03; F, 12.89. Found: C, 43.33; H, 4.55; N, 15.81; Cl, 16.04; F, 13.09.

5.1.82. 2-(2-Aminoethylamino)-4-(3-trifluoromethylanilino)pyrimidine-5-carboxylic acid (16). Compound **16** was prepared according to the same procedure as that for **3** in 72% yield as a colorless solid: mp 248–249 °C (H_2O–EtOH); ^{1}H NMR (DMSO-d_6) δ 3.07 (2H, m), 3.20–3.50 (2H, m), 3.55 (2H, m), 7.12 (1H, br s), 7.23 (1H, d, J = 7.8 Hz), 7.46 (1H, t, J = 7.8 Hz), 7.50–8.50 (3H, m), 7.74 (1H, m), 8.48 (1H, m), 13.48 (1H, br s); FAB MS *m/e* $(M+H)^+$ 342. Anal. Calcd for $C_{14}H_{14}N_5O_2F_3 \cdot 0.2H_2O$: C, 48.76; H, 4.21; N, 20.31; F, 16.53. Found: C, 48.71; H, 4.17; N, 20.41; F, 16.52.

5.2. Biology

5.2.1. Kinase assay. The genes encoding human Syk, ZAP-70, Itk, and Btk were subcloned into pFastBac1 vector (GIBCO) in which FLAG-tag was incorporated or pFastBac HT vector (GIBCO). Recombinant baculoviruses were obtained by using Bac-to-Bac system (GIBCO). Sf-9 insect cells (American Type Culture Collection) were infected with the recombinant viruses and grown in spinner cultures. FLAG- or 6xHis-tagged proteins were partially purified by M2-agarose affinity gel (SIGMA) or TALON metal affinity resin (CLONTECH). Recombinant human PKCβ2 and PKCε were purchased from Calbiochem. Biotinylated substrate peptides were as follows: a peptide from human Band 3 (MEELQDDYEDMMEENL) for Syk and ZAP-70 assays; a peptide from human SLP-76 (GEDDGDYESP NEEEE) for Itk and Btk assays. In the cases of PKCβ2 and PKCε , PKC 'pseudosubstrate' peptide (ERMRPR KRQGSVRRRV) was used.

Kinase activity was measured by using a scintillation proximity assay (SPA) system. SPA is a system that has been developed by Amersham making use of a phenomenon in which scintillation occurs when a molecule having radioactivity is in proximity to the surface of plastic beads having a scintillant included therein. These beads are coated in advance with streptoavidin to which the biotin moiety of substrate peptide is bound. A 2 μM portion of DMSO solution of each compound to be tested was added to each well containing 50 μl of a reaction solution [composition: 20–200 ng of recombinant kinase, 50 mM Tris–HCl (pH 7–8), 10 mM $MgCl_2$ or $MnCl_2$, 50 mM NaCl, 1 mM DTT, optimum concentration of the substrate peptide, and 0.1 μCi [$\gamma-^{33}$P]ATP (10 mCi/mL, Amersham)]. This was prepared in Optiplate™ (PACKARD) and allowed to stand at room temperature for 1 h to effect tyrosine phosphorylation. The reaction was terminated by adding PBS containing 0.25 mg SPA beads, 50 μM ATP, 5 mM EDTA, and 1% Triton X-100 in an amount of 150 μl per well. The plate was sealed, stirred, allowed to stand at room temperature for 15 min, and then centrifuged at 1500 rpm for 3 min to effect precipitation of the SPA beads. Radioactivity of each well was measured using TOP COUNT (PACKARD), and the tyrosine phosphorylation activity by the kinases was calculated.

5.2.2. 5-HT release assay. This test was carried out in accordance with the method reported by Collado-Escobar et al. RBL-2H3 cells were preincubated with tritium-labeled 5-HT at 37 °C overnight, followed by priming with dinitrophenyl (DNP)-specific monoclonal IgE antibody. The cells were reseeded in 96-well plates and incubated with or without compounds, followed by stimulation with DNP conjugated with bovine serum albumin (DNP–BSA, 0.1 μg/mL). Aliquots of culture supernatant were added to MicroScinti-20 and its radioactivity was measured.

5.2.3. 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 24hr, each mouse was challenged by injecting a mixture of DNP-conjugated bovine serum albumin and 200 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 both ears and the amount of dye from the blueing region was measured. Test compounds or vehicle alone as a control were subcutaneously administered to the mice 30 min before the antigen challenge. The dye in the tissues was extracted with formamide and colorimetrically determined at 620 nm. A value obtained by subtracting the dye content of the left ear from the dye content of

the right ear was used as the amount of dye leaked into the tissues by the PCA reaction. The PCA inhibition ratio by the test compound was calculated based on the following equation. In the formula, CA: amount of dye leaked into the sensitized right ear at the time of administration of the vehicle alone, CB: amount of dye leaked into the unsensitized left ear at the time of administration of vehicle alone, XA: amount of dye leaked into the sensitized right ear at the time of administration of the compound to be tested, and XB: amount of dye leaked into the unsensitized left ear at the time of administration of the compound to be tested. Inhibition ratio (%) = {(CA-CB)-(XA-XB)} × 100/(CA-CB)

5.2.4. Single-crystal X-ray diffraction analysis of compound 9q. A colorless prismatic crystal of compound **9q** methanolate, $C_{16}H_{22}N_6O_2{\cdot}CH_3OH$ (F.W. = 362.43, 0.50 mm × 0.35 mm × 0.30 mm), was mounted on a glass fiber. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated CuKα radiation and a rotating anode generator. 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 $58.26 < 2\theta < 60.17$ °, corresponded to a primitive monoclinic cell ($P2_1/n$, For $Z = 4$) 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 298 ± 1 K using the ω-2θ scan technique to a maximum 2θ value of 154.5°.

Of the 4309 reflections which were collected, 4173 were unique ($R_{int} = 0.023$). The intensities of three representative reflection were measured after every 150 reflections. No decay correction was applied. The linear absorption coefficient, μ, for CuKα radiation is 7.1 cm^{-1}. An empirical absorption correction based on azimuthal scans of several reflections was applied, which resulted in transmission factors ranging from 0.89 to 1.00. A correction for secondary extinction was applied (coefficient = 4.25786×10^{-6}).

The structure was solved by direct methods[23] and expanded using Fourier techniques.[24] The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement[25] was based on 2225 observed reflections ($I > 3.00\sigma(I)$, $2\theta < 154.51°$) and 237 variable parameters, and converged (largest parameter shift was 0.00 times its esd) with unweighted and weighted agreement factors of: $R = 0.066$, $R_w = 0.111$, respectively. The goodness of fit[26] was 1.34. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.22 and −0.24 e$^-$/Å^3, respectively. Neutral atom scattering factors were taken from Cromer and Waber.[27] The values for the mass attenuation coefficients are those of Creagh and Hubbel.[28] All calculations were performed using the teXsan[29] crystallographic software package of Molecular Structure Corporation.

5.2.5. Molecular modeling and docking study. A 3D model of the catalytic domain of the human Syk has been constructed using a program, MOE[30] (Chemical Computing Group Inc., Montreal, Quebec), with the crystal structure of LCK[31] (PDB code: 3lck) as a template. To remove side-chain close contacts, the obtained model was finally refined using the Tripos force field's minimization[32] in which the backbone of the protein was held fixed. Docking studies of compound **9a** and AMP-PNP with modeled catalytic domain of Syk were performed using a program, GOLD.[33] The 10 independent genetic algorithms (GA) in which a maximum number of 100,000 GA operations were performed on a single population of 100 individuals were calculated. Operator weights for crossover, mutation, and migration were set to 95, 95, and 10, respectively.

Acknowledgments

The authors deeply acknowledge Dr. Nobuaki Taniguchi and Dr. Kiyohiro Samizu for useful discussion and helpful support for preparing the manuscript, and are also grateful to the staff of the Division of Analytical Science Laboratories for the elemental analysis and spectral measurements.

References and notes

1. Shlessinger, J. *Cell* **2000**, *103*, 211–225.
2. Hanks, S. K.; Quinn, A. M.; Hunter, T. *Science* **1988**, *241*, 42–52.
3. Nakamura, H.; Iitaka, Y.; Imoto, M.; Isshiki, K.; Naganawa, H.; Takeuchi, T.; Umezawa, H. J. *Antibiotics* **1986**, *39*, 314–315.
4. Onoda, T.; Inuma, H.; Sasaki, Y.; Hamada, M.; Isshiki, K.; Naganawa, H.; Takeuchi, T. *J. Nat. Prod.* **1989**, *52*, 1252–1257.
5. Geahlen, R. L.; McLaughlin, J. L. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 241–245.
6. Mu, F.; Coffing, S. L.; Riese, D. J., II; Geahlen, R. L.; Pinard, P. V.; Hamel, E.; Johnson, J.; Cushman, M. *J. Med. Chem.* **2001**, *44*, 441–452.
7. Mark, S. S.; Irena, S.; Frank, H.; Ivan, D. H.; Nir, O.; Aqlexander, L.; Ferrence, R. B. *J. Med. Chem.* **1993**, *36*, 3010–3014.
8. Sada, K.; Takano, T.; Yanagi, S.; Yamamura, H. *J. Biochem.* **2001**, *130*, 177–186.
9. Nishizumi, H. *Clin. Immunol.* **1996**, *28*, 1530–1536.
10. Sumita, K.; Sakaguchi, N. *Clin. Immunol.* **1998**, *30*, 601–608.
11. Kihara, H.; Morita, Y. *Clin. Immunol.* **1996**, *28*, 565–571.
12. (a) Moriya, K.; Rivera, J.; Odom, S.; Sakura, Y.; Muramoto, K.; Yoshiuchi, T.; Miyamoto, M.; Yamada, K. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12539–12544; (b) Lai, J.; Cox, Y.; Patel, Q.; Sadiq, P.; Aldous, D. J.; Thurairatnam, S.; Smith, S.; Wheeler, D.; Jagpal, S.; Parveen, S.; Fenton, G.; Harrison, T. K. P.; McCarthy, C.; Bamborough, P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3111–3114; (c) Cywin, C. L.; Zhao, B. P.; McNeil, D. W.; Hrapchak, M.; Prokopowicz, A. S., III; Goldberg, D. R.; Morwick, T. M.; Gao, A.; Jakes, S.; Kashem, M.; Magolda, R. L.; Soll, R. M.; Player, M. R.; Bobko, M. A.; Rinker, J.; DesJarlais, R. L.; Winters, M. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1415–1418.
13. Bredereck, H.; Effenberger, F.; Schweizer, E. H. *Chem. Ber.* **1962**, *95*, 803–809.

14. Palanki, M. S. S.; Erdman, P. E.; Manning, A. M.; Ow, A.; Ransone, L. J.; Spooner, C.; Suto, C.; Suto, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1645–1648.
15. Smith, H. V.; Christensen, B. E. *J. Org. Chem.* **1955**, *20*, 829–838.
16. Harrison, M. L.; Issacson, C. C.; Burg, D.; Geahlen, R. L.; Low, P. S. *J. Biol. Chem.* **1994**, *269*, 955–959.
17. Collado-Escobar, D.; Ali, H.; Beaven, M. A. *J. Immunol.* **1990**, *144*, 3449–3457.
18. Alexander, J. B.; Hairong, Z.; Donna, R. C.; Gordon, W. R.; Amy, M. H. D.; Hollis, S.; David, W. F.; Alan, J. K.; William, A. D. *J. Med. Chem.* **1996**, *39*, 267–276.
19. Peter, M. T.; Pascal, F.; Helmut, M.; Elisabeth, B.; Thomas, M.; Nicholas, L. *J. Med. Chem.* **1996**, *39*, 2285–2292.
20. One of this series of compounds has been shown to be the ATP competitor. Ichikawa, A.; Orita, A.; Hisamichi, H.; Orita, M.; Ohtsuka, T.; Ball, E.; Nakai, E.; Katoh, M.; Furuichi K. Sixth World Congress on Inflammation, Vancouver, 2003
21. Mark, S. S.; Irena, S.; Ivan, D. H.; Ferrence, R. B. *J. Med. Chem.* **1993**, *36*, 3015–3020.
22. Recently, the X-ray structure of the unphosphorylated form of Syk: Atwell, S.; Adams, J. M.; Badger, J.; Buchanan, M. D.; Feil, I. K.; Froning, K. J.; Gao, X.; Hendle, J.; Keegan, K.; Leon, B. C.; Müller-Dieckmann, H. J.; Nienaber, V. L.; Noland, B. W.; Post, K.; Rajashankar, K. R.; Ramos, A.; Russell, M.; Burley, S. K.; Buchanan, S. G. *J. Biol. Chem.* **2004**, *279*, 55827–55832.
23. Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, M.; Giacovazzo, C.; Guagliardi, A.; Polidori, G. *J. Appl. Crystallogr.* **1994**, *27*, 435.
24. Beurskens, P. T.; Admiraal, G.; Beurskens, G.; Bosman, W. P.; de Gelder, R.; Israel, R.; Smits, J. M. M. The DIRDIF-94 program system. Technical Report of the Crystallography Laboratory University of Nijmegen, The Netherlands, 1994.
25. Least-squares: function minimized: $\sum w(|F_o| - |F_c|)^2$ where $w = 1/[\sigma^2(F_o)] = [\sigma_c^2(F_o) + p^2F_o^2/4]^{-1}$, $\sigma_c(F_o)$ = esd based on counting statistics, p = p-factor (0.1270).
26. Standard deviation of an observation of unit weight: $[\sum w(|F_o| - |F_c|)^2(N_o - N_v)]^{1/2}$, where: N_o = number of observations, N_v = number of variables.
27. Cromer, D. T.; Waber, J. T. In *International Tables for X-ray Crystallography*; The Kynoch Press: Birmingham, England, 1974; Vol. IV, Table 2.2 A.
28. Creagh, D. C; Hubbell, J. H. In *International Tables for Crystallography*; Wilson, A. J. C., Ed.; Kluwer Academic: Boston, 1992; Vol C, Table 4.2.4.3, 200–206.
29. teXsan: Crystal Structure Analysis Package, Molecular Structure Corporation (1985&1999).
30. MOE revision 2003.02; Chemical Computing Group: Montreal, 2003.
31. Hubbard, S. R. *EMBO J.* **1997**, *16*, 5572–5581.
32. SYBYL revision 6.8, Tripos: St. Louis, 2002
33. Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. *J. Mol. Biol.* **1997**, *267*, 727–748.