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Novel 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives as potent and orally active STAT6 inhibitors

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ABSTRACT

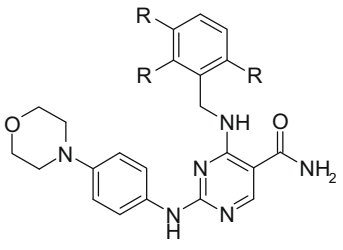
Signal transducers and activators of transcription 6 (STAT6) is an important transcription factor in interleukin (IL)-4 signaling pathway and a key regulator of the type 2 helper T (Th2) cell immune response. Therefore, STAT6 may be an excellent therapeutic target for allergic conditions, including asthma and atopic diseases. Previously, we reported 4-aminopyrimidine-5-carboxamide derivatives as STAT6 inhibitors. To search for novel STAT6 inhibitors, we synthesized fused bicyclic pyrimidine derivatives and identified a 7*H*-pyrrolo[2,3-*d*]pyrimidine derivative as a STAT6 inhibitor. Optimization of the pyrrolopyrimidine derivatives led to identification of 2-[4-(4-{[7-(3,5-difluorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-amino}phenyl)piperazin-1-yl]acetamide (**24**, AS1810722) which showed potent STAT6 inhibition and a good CYP3A4 inhibition profile. Compound **24** also inhibited in vitro Th2 differentiation without affecting type 1 helper T (Th1) cell differentiation and eosinophil infiltration in an antigen-induced mouse asthmatic model after oral administration.

1. Introduction

Peripheral T helper (Th) cells are classified into two subsets, type 1 helper T (Th1) and type 2 helper T (Th2), based on cytokine production and functional ability.[1] Th1 cells produce Th1 cytokines such as interferon-γ (IFN-γ), interleukin (IL)-2, and tumor necrosis factor-β for enhancement of cellular immunity for elimination of intracellular pathogens. In contrast, Th2 cells produce Th2 cytokines such as IL-4, IL-5, IL-10 and IL-13 for modulation of humoral immunity for protection against parasites and allergens. The immune system maintains a proper balance between Th1 and Th2 responses, and Th1/Th2 polarization is thought to play an important role in the pathogenesis of allergic and autoimmune diseases.[2]

Differentiation of naive Th cells into Th2 cells is induced by antigen stimulation of T cell receptors in the presence of IL-4. Signal transducers and activators of transcription 6 (STAT6) is strongly associated with the IL-4 signaling pathway and plays an important role in Th2 differentiation.[3] In STAT6-deficient mice, Th cells are unable to differentiate into Th2 cells, and B cells cannot switch immunoglobulin (Ig) production to IgG1 and IgE.[4] In addition, antigen-induced airway hyperresponsiveness and eosinophil infiltration are significantly decreased.[5] These findings indicate that STAT6 might be an excellent therapeutic target for allergic diseases, such as asthma and atopic diseases.

Previously, we reported several 4-benzylaminopyrimidine-5-carboxamide derivatives as STAT6 inhibitors (Fig. 1).[6] Among these compounds, **1b** (YM-341619, AS161672) inhibited Th2 differentiation and suppressed antigen-induced eosinophil accumulation in the lungs of mice and rats after oral administration.[7] In the further studies of STAT6 inhibitors, we explored new lead compounds for identification of a novel scaffold (based on **1a**), since this is



1a R=H
1b (YM-341619, AS1617612) R=F

Figure 1. Structures of 4-benzylamino-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide derivatives.

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important in drug discovery. In these studies, we focused on the carboxamide group and the amino group of the benzylamine moiety, which could form a pseudo-six-membered ring through an intramolecular hydrogen bond between the oxygen of the carboxamide and the hydrogen of the amine. We hypothesized that the bicyclic system formed by pyrimidine and the pseudo ring might be an important factor for activity. Therefore, we designed fused bicyclic pyrimidine derivatives that may mimic the 4-aminopyrimidine-5-carboxamide system (Fig. 2).

In the discovery and development of new drugs, examination of the inhibitory activity against cytochrome P450 (CYP) is also an important issue, since CYP inactivation may produce unfavorable drug–drug interactions (DDIs) and toxicity. In particular, time-dependent inhibition (TDI) of CYP has recently become a concern in clinical practice with regard to the duration of the CYP inhibitory effect.[8] CYP3A4 is a major CYP isoform and is thought to be involved in the metabolism of approximately 50% of drugs used in humans. Since our previous STAT6 inhibitor **1b** showed TDI of CYP3A4, we assessed potential DDIs and evaluated CYP3A4 TDI in the novel series of STAT6 inhibitors. In this paper, we report the synthesis of novel fused bicyclic pyrimidine derivatives, together with their structure–activity relationships (SARs) and CYP3A4 profiles. We also describe the in vitro and in vivo pharmacological profiles of one derivative.

2. Chemistry

The syntheses of 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives and a 6,7-dihydro-5H-pyrrolo[2,3-*d*]pyrimidine derivative are shown in Scheme 1. A substitution reaction of methyl 1,6-dihydro-2-methylthio-6-oxo-5-pyrimidinecarboxylate (**2**)[9] with 4-morpholinoaniline gave the 2-phenylamino derivative **3**.[10] Chlorination of **3** with phosphorus trichloride in the presence of diethylaniline afforded the 4-chloro derivative **4**. Displacement of the chloro group of **4** with the appropriate benzylamine furnished 4-benzylamino derivatives **5a**–**d**. Hydrolysis of the compounds **5a**–**d** afforded carboxylic acids **6a**–**d**, followed by condensation with *N,O*-dimethylhydroxylamine to give *N*-methoxy-*N*-methylcarboxamide derivatives **7a**–**d**, respectively. Treatment of **7a**–**d** with lithium aluminum hydride afforded the corresponding aldehyde derivatives **8a**–**d** in 53–82% yields. A Wittig reaction of **8a**–**d** with (methoxymethyl)triphenylphosphonium chloride and subsequent treatment with concentrated HCl in methanol gave the desired 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives **9a**–**d**. Conversion of **9a** into 6,7-dihydoropyrrolopyrimidine derivative **10** was achieved by hydrogenation in the presence of palladium on carbon.[11]

Scheme 2 shows an alternative synthetic route for the 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives. Stille coupling of 4-amino-5-bromo-2-chloropyrimidine (**11**)[12] with (*Z*)-1-ethoxy-2-(tributhylstannyl)ethene using a catalytic amount of dichlorobis(triphenylphosphine) palladium in the presence of *n*-tetrabutylammonium chloride afforded alkenylpyrimidine **12** in 57% yield as a mixture of the *Z* and *E* isomers (*Z*/*E* > 20/1).[13] Cyclization of compound **12** with trifluoroacetic acid gave 2-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine **13** in good yield. Alkylation of the 7-position of compound **13** with 3-fluorobenzyl bromide and 3,5-difluorobenzyl bromide readily proceeded in the presence of potassium carbonate. A palladium catalyzed cross-coupling reaction of compounds **14a**–**b** with 4-morpholinoaniline and 4-(1-*tert*-butoxycarbonylpiperazin-4-yl)aniline provided compounds **9e** and **15**, respectively. Deprotection of the *tert*-butyldimethylsilyl (TBDMS) group of **18** and the Boc group of compound **20** gave the 2-hydroxyethyl derivative **19** and the 2-aminoethyl derivative **21**, respectively. Hydrolysis of **22** gave the acetic acid derivative **23**, followed by condensation with aqueous ammonia and methylamine in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole (HOBt) to afford the amide derivatives **24** and **25**, respectively.

3. Results and discussion

The ability of the compounds to inhibit STAT6 activation was measured in an IL-4-stimulated STAT6-dependent promoter reporter assay.[6] In this assay, the 7*H*-pyrrolo[2,3-*d*]pyrimidine derivative **9a** was identified as a new lead compound with moderate activity (Table 1). Reduction of the double bond in the pyrrole moiety (**10**) led to a decrease in activity. To understand the relationship between the molecular features and the activity, a molecular modeling study was performed. Superimposition of **9a** and **10** with the lowest-energy conformation of **1a** showed good overlap, except for the amino group of the carbamoyl moiety (Fig. 3). In contrast, the atomic charge distribution of the 4-aminopyrimidine-5-carboxamide moiety of **1a** and the pyrrolopyrimidine ring of **9a** differed from that of the dihydropyrrolopyrimidine ring of **10** (Fig. 4). For **1a** and **9a**, the negative charge (red) was distributed on the right side of the molecule, whereas **10** had a positive charge (blue) in the corresponding region. These results suggest that the 7*H*-pyrrolo[2,3-*d*]pyrimidine may mimic the 4-aminopyrimidine-5-carboxamide moiety electrostatically. The negative potential surface may interact with a positive region. Therefore, we hypothesized that the pyrrole moiety of **9a** and the 4-amino-5-carbamoyl moiety of **1a** might interact with a protonated site on the target protein.

The inhibitory activities of 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives with modification of the benzyl and morpholine moieties are shown in Table 2. In our previous work, we found that introducing a fluorine atom at the 2- or 3-position of the benzene ring in the benzyl moiety enhanced the activity, whereas the 4-fluoro derivative had diminished activity.[6] Similar SARs were observed in the 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives: the 2- and 3-fluoro derivatives (**9b**, **9d**) were about two- and threefold more potent than **9a**, respectively, and the 4-fluoro derivative **9c** was 10-fold less potent than **9a**. Interestingly, the 3,5-difluoro derivative **9e** was 10-fold more potent than **9a**.

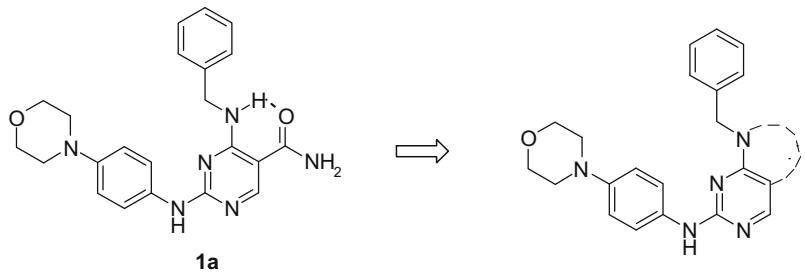
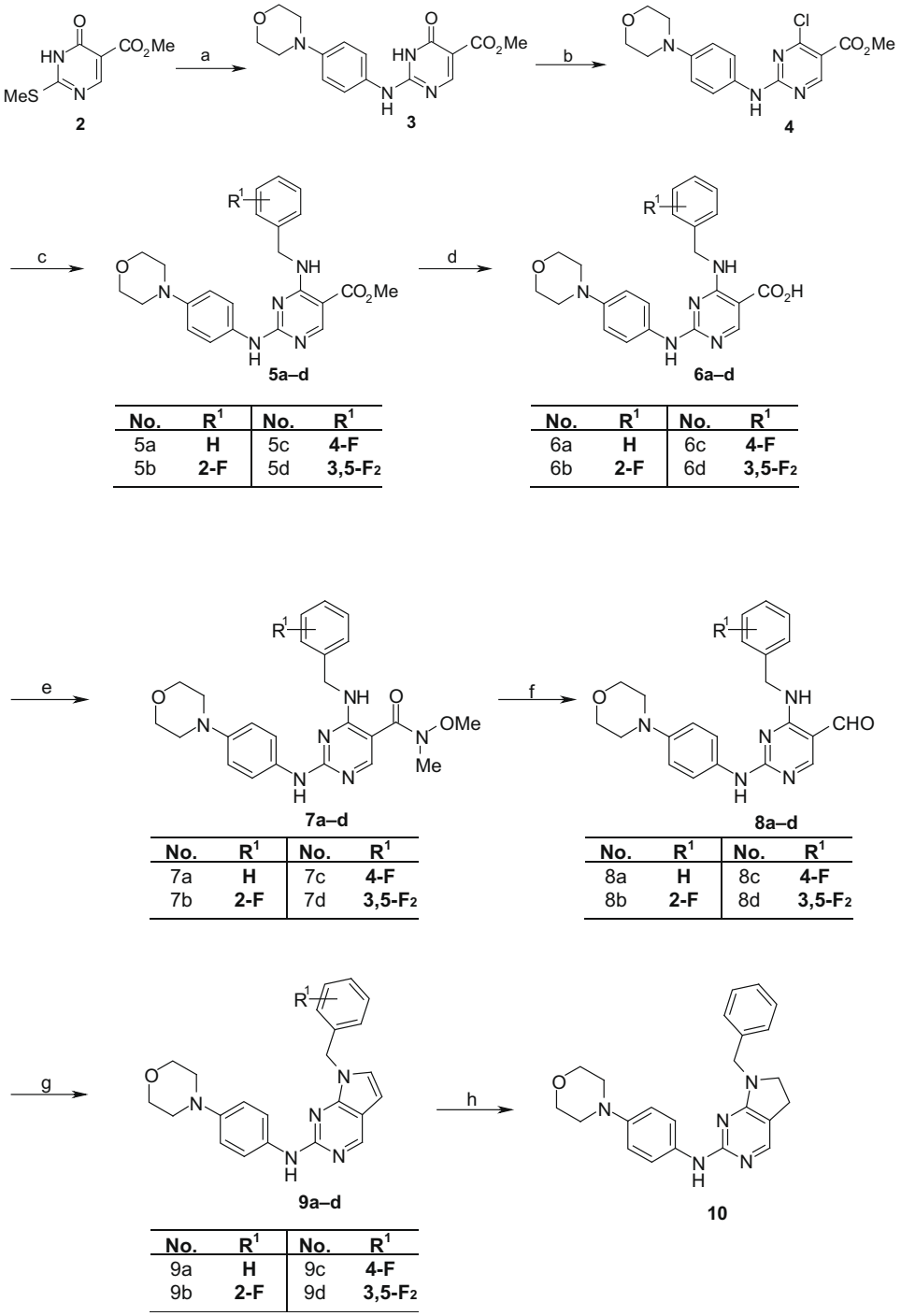


Figure 2. Design of novel fused pyrimidine derivatives.



No.	R^1	No.	R^1
5a	**H**	5c	**4-F**
5b	**2-F**	5d	**3,5-F$_2$**

No.	R^1	No.	R^1
6a	**H**	6c	**4-F**
6b	**2-F**	6d	**3,5-F$_2$**

No.	R^1	No.	R^1
7a	**H**	7c	**4-F**
7b	**2-F**	7d	**3,5-F$_2$**

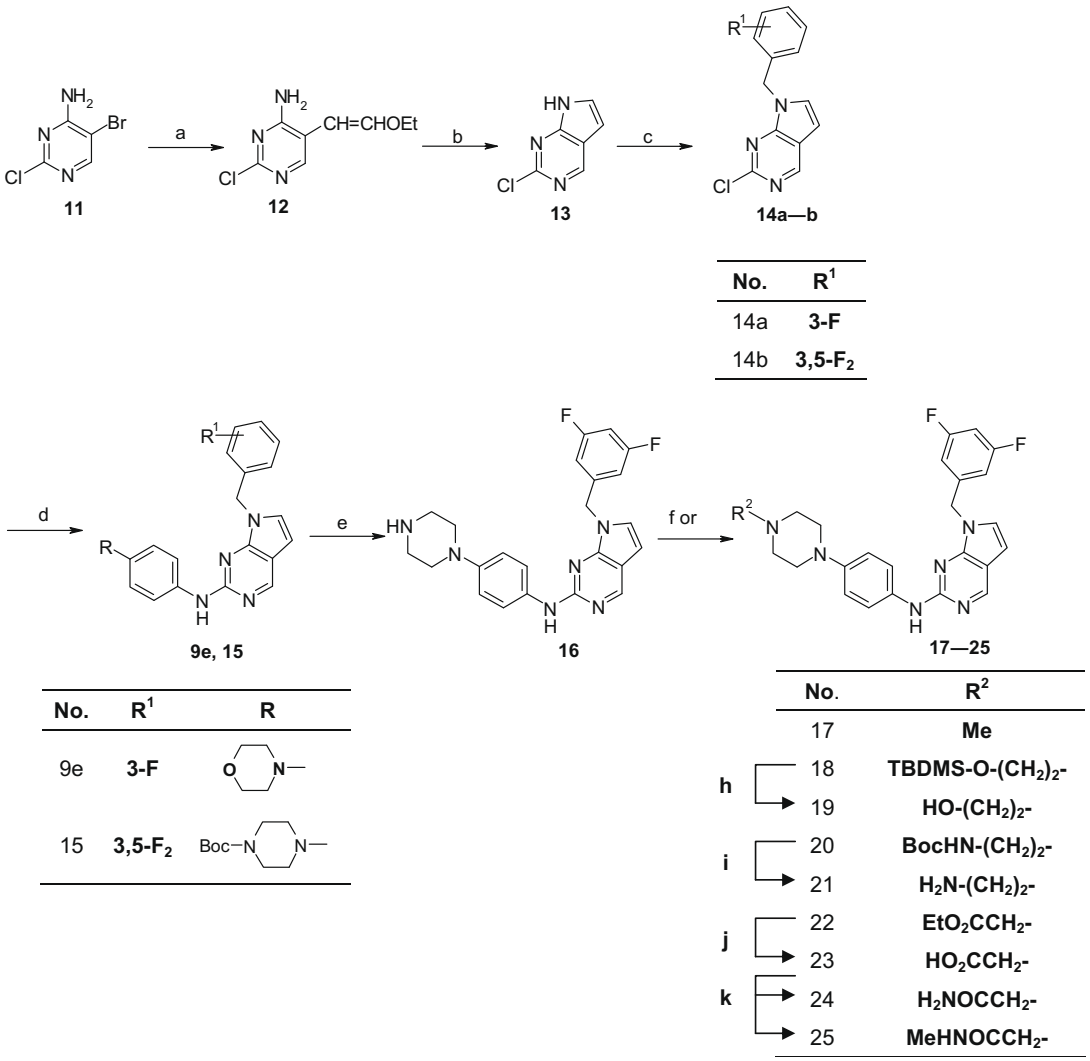
No.	R^1	No.	R^1
8a	**H**	8c	**4-F**
8b	**2-F**	8d	**3,5-F$_2$**

No.	R^1	No.	R^1
9a	**H**	9c	**4-F**
9b	**2-F**	9d	**3,5-F$_2$**

Scheme 1. Reagents and conditions: (a) 4-(morpholin-4-yl)aniline/EtOH, reflux; (b) $POCl_3$, diethylaniline/MeCN, reflux; (c) benzylamine derivatives, $^{i}Pr_2NEt$/dimethylacetamide, rt; (d) 1 M NaOH/MeOH–THF, reflux; (e) *N,O*-dimethylhydroxylamine hydrochloride, Et_3N, EDC·HCl, HOBt/DMF, rt; (f) $LiAlH_4$/THF, −78 to 0 °C; (g) (i) (methoxymethyl)triphenylphosphonium chloride, ^{n}BuLi/THF, (ii) concd HCl/MeOH, reflux; (h) H_2, 10% Pd–C/AcOEt–AcOH, rt.

Replacement of the morpholine group of **9e** with a piperazine group led to improved activity (**16**), and methylation of the terminal nitrogen of the piperazine gave further improvement in the activity (**17**). Replacing the methyl substituents with 2-hydroxyethyl (**19**), acetamide (**24**) and *N*-methylacetamide (**25**) retained the activity, whereas the 2-aminoethyl derivative (**21**) and acetic acid derivative (**23**) had decreased activity. These results indicate that neutral substituents at the terminal nitrogen of the piperazine moiety might give favorable activity over basic and acidic substituents.

To evaluate the inhibitory effects of the 7*H*-pyrrolo[2,3-*d*]-pyrimidine derivatives against CYP3A4, the metabolic activity of human liver microsomes (HLMs) for midazolam, a substrate for CYP3A4, was measured at 0 and 30 min after preincubation with the compounds. The residual activities of the HLMs in the presence of the pyrrolopyrimidine derivatives are shown in Table 2. The metabolic activity at 0 min after preincubation with compound **9a** was 88% compared to the activity without **9a**, but the activity at 30 min after preincubation was only 34% based on the activity at 0 min. These results show that **9a** exhibits TDI of CYP3A4. The



Scheme 2. Reagents and conditions: (*Z*)-1-ethoxy-2-(tributylstannyl)ethane, $PdCl_2(PPh_3)_2$, $^nBu_4NCl$/DMF, 140 °C; (b) trifluoroacetic acid/$Cl(CH_2)_2Cl$, 80 °C; (c) 3-fluorobenzyl bromide or 3,5-difluorobenzyl bromide, K_2CO_3/DMSO, 60 °C; (d) 4-(morphplin-4-yl)aniline and 4-(1-*tert*-butoxycarbonylpiperazin-4-yl)aniline, $Pd(OAc)_2$, *rac*-BINAP, Cs_2CO_3/dioxane, 80 °C; (e) 4 M HCl–dioxane/MeOH–THF, 50 °C; (f) CH_3I or $BrCH_2CO_2Et$, K_2CO_3/DMF, rt; (g) $TBDMSO(CH_2)_2Br$ or $BocHN(CH_2)_2Br$, NaI, Na_2CO_3/DMF, 100 °C; (h) $^nBu_4NF$/THF, rt; (i) 4 M HCl–AcOEt/MeOH, rt; (j) 1 M NaOH/MeOH–THF, 50 °C; (k) NH_4OH (28%) or aqueous methylamine (25%), EDC·HCl, HOBt/DMF.

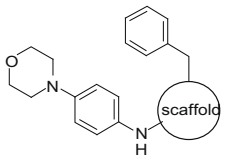
morpholine derivatives **9b**–**e** also produced time-dependent decreases of CYP3A4 activity. In the piperazine series, compound **16** did not show TDI of CYP3A4, but did produce moderate inhibition of CYP3A4 at 0 min after preincubation. Introduction of a methyl group at the terminal nitrogen of the piperazine moiety in **16** led to decreased CYP3A4 inhibition at 0 min (compound **17**). The *N*-(2-hydroxy)ethyl and *N*-methylacetamide derivatives (**19**, **25**) showed similar CYP3A4 profiles to **17**. The 2-aminoethyl derivative (**21**) showed no TDI, but the residual activity at 0 min after preincubation was only 65%. These findings suggest that primary and secondary amino groups (**16**, **21**) are important in CYP3A4 inhibition. Further improvement of the CYP profile was found for the acetic acid and acetamide derivatives (**23**, **24**), which showed no CYP inhibition and weak TDI. These data suggest that TDI of CYP by the 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives might be related to the lipophilicity of the compounds, since the calculated log *D* values of the piperazine derivatives at pH 7.4 ($c\,\mathrm{Log}\,D_{7.4}$) are <4, whereas those for the morpholine derivatives are >4 (Table 2). TDI is often caused by generation of reactive metabolites[8] and we speculate that the less lipophilic piperazine derivatives might be metabolically more stable than the morpholine derivatives.

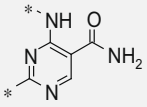
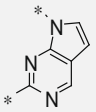
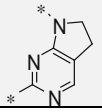
The results in Table 2 suggest that **24** is the most promising compound from the perspectives of STAT6 inhibition and the CYP3A4 profile. Therefore, the effect of **24** on differentiation of T cells to Th subsets was examined using a previously reported method.[15] The extent of Th1 and Th2 differentiation was determined based on production of IFN-γ and IL-4, respectively. As shown in Figure 5, **24** inhibited production of IL-4 with an IC_{50} of 2.4 nM, but showed no effect on production of IFN-γ. These observations indicate that **24** inhibits Th2 differentiation without influencing Th1 differentiation. To evaluate its potency as an anti-asthmatic drug, **24** was tested in an antigen-induced mouse asthmatic model, and was found to suppress eosinophil infiltration in the lung in a dose-dependent manner after oral administration (0.03–0.3 mg/kg, po, Fig. 6). Collectively, our findings indicate that compound **24** (AS1810722) is a potent and orally active STAT6 inhibitor, and we suggest that this compound may be useful for treatment of allergic diseases such as asthma and atopic diseases.

4. Conclusion

In an attempt to find novel STAT6 inhibitors, we synthesized fused bicyclic pyrimidine derivatives and evaluated their activities.

Table 1
STAT6 inhibitory activity of 4-benzylaminopyrimidine-5-carboxamide derivative **1a** and fused bicyclic pyrimidine derivatives **9a** and **10**



Compound	Scaffold	IC_{50}[a] (nM)
1a		6.5
9a		400
10		8300

[a] IC_{50} values are averages from two or three experiments.

Among these compounds, the 7*H*-pyrrolo[2,3-*d*]pyrimidine derivative **9a** showed moderate activity. The atomic charge distribution of **9a** suggested that the 7*H*-pyrrolo[2,3-*d*]pyrimidine moiety could mimic a 4-aminopyrimidine-5-carboxamide moiety. Optimization of the benzyl group and morpholine moiety of **9a** led to identification of a piperazinylacetamide derivative (**24**, AS1810722) that showed potent STAT6 inhibitory activity and a good CYP3A4 profile. Compound **24** (AS1810722) inhibited in vitro Th2 differentiation with an IC_{50} of 2.4 nM without affecting Th1 cell differentiation, and also suppressed eosinophil infiltration in an antigen-induced mouse asthmatic model after oral administration. These results suggest that **24** is a novel potent and orally active STAT6 inhibitor that could be useful for treatment of allergic diseases such as asthma and atopic diseases.

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). Mass spectra were recorded with a Hitachi M-80 or a JEOL JMS-DX300 spectrometer. Organic solutions were dried over anhydrous $MgSO_4$ during work-up. Column chromatography was carried out on silica gel (Kieselgel 60). Unless otherwise noted, all commercial reagents and solvents were used without further purification.

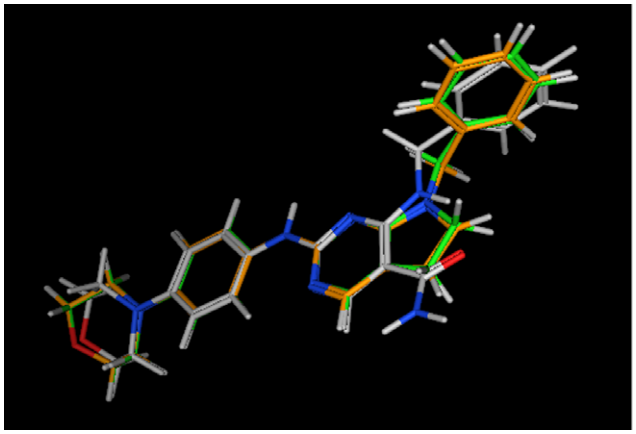


Figure 3. Superposition of **1a** (gray), **9a** (orange) and **10** (green).

5.1.1. Methyl 2-[(4-morpholin-4-ylphenyl)amino]-6-oxo-1,6-dihydropyrimidine-5-carboxylate (3)

4-Morpholinoaniline (32.7 g, 187 mmol) was added to a mixture of **2**[9] (35.0 g, 174.8 mmol) and EtOH (350 mL) and the mixture was refluxed for 10 h. The mixture was cooled to room temperature and resulting precipitate was collected by filtration and washed with EtOH (100 mL), MeOH (100 mL) and hexane (100 mL). A mixture of the crude solid and DMF (250 mL) was stirred for 1 h at 130 °C. The reaction mixture was cooled to room temperature, and MeOH (250 mL) was added. The resulting solid was collected by filtration and washed with MeOH (100 mL) to give **3** (48.6 g, 84%) as a pale green solid. ^{1}H NMR (DMSO-d_6) δ 3.06–3.09 (4H, m), 3.69 (3H, s), 3.72–3.75 (4H, m), 6.93 (2H, d, J = 9.2 Hz), 7.37 (2H, d, J = 9.2 Hz), 8.41 (1H, s), 9.14 (1H, br), 10.95 (1H, br); FAB MS m/e $[M+H]^+$ 331.

5.1.2. Methyl 4-chloro-2-[(4-morpholin-4-ylphenyl)amino]-pyrimidine-5-carboxylate (4)

Diethylaniline (5.8 mL, 36.3 mmol) was added to a mixture of **3** (10.0 g, 30.3 mmol) and phosphorus trichloride (5.6 mL, 60.5 mmol) and the mixture was refluxed for 3.5 h at 90 °C. The reaction mixture was cooled to room temperature and concentrated in vacuo. AcOEt (200 mL) was added to the residue and resulting solid was collected by filtration. The solid was dissolved in AcOEt–THF and the organic layer was washed successively with H_2O and saturated aqueous NaCl. The organic layer was dried and concentrated in vacuo. The resulting solid was triturated with MeOH and collected by filtration to give 4 (5.18 g, 49%) as a pale green solid, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 3.14–3.16 (4H, m), 3.86–3.89 (4H, m), 3.91 (3H, s), 6.93 (2H, d, J = 8.8 Hz), 7.41 (1H, br), 7.47 (2H, d, J = 8.8 Hz), 8.89 (1H, s); FAB MS m/e $[M+H]^+$ 349, 351.

5.1.3. Methyl 4-(benzylamino)-2-[(4-morpholin-4-ylphenyl)-amino]pyrimidine-5-carboxylate (5a)

Benzylamine (344 mg, 3.21 mmol) was added to a solution of **4** (800 mg, 2.29 mmol) and diisopropylethylamine (0.56 mL, 3.21 mmol) in dimethylacetamide (8 mL) and the mixture was stirred for 3 h at room temperature. The reaction mixture was then diluted with H_2O and the resulting solid was collected by filtration and washed successively with MeOH and Et_2O to give **5a** (920 mg, 96%) as a colorless solid, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 3.01–3.08 (4H, m), 3.71–3.74 (4H, m), 3.78 (3H, s), 4.70 (2H, d, J = 5.9 Hz), 6.81 (2H, d, J = 8.8 Hz), 7.23–7.27 (1H, m), 7.33–7.35 (4H, m), 7.47 (2H, d, J = 8.8 Hz), 8.55 (1H, s), 8.65 (1H, br), 9.60 (1H, s); FAB MS m/e $[M+H]^+$ 420.

5.1.4. Methyl 4-[(2-fluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxylate (5b)

Compound **5b** was prepared from compound **4** and 2-fluorobenzylamine in 93% yield as a colorless solid, using a similar approach to that described for **5a**, and used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 3.00–3.03 (4H, m), 3.72–3.74 (4H, m), 3.79 (3H, s), 4.75 (2H, d, J = 5.8 Hz), 6.78 (2H, d, J = 8.8 Hz), 7.14–7.16 (1H, m), 7.25–7.32 (3H, m), 7.42 (2H, d, J = 8.8 Hz), 8.55 (1H, s), 8.65 (1H, br), 9.58 (1H, s); FAB MS m/e $[M+H]^+$ 438.

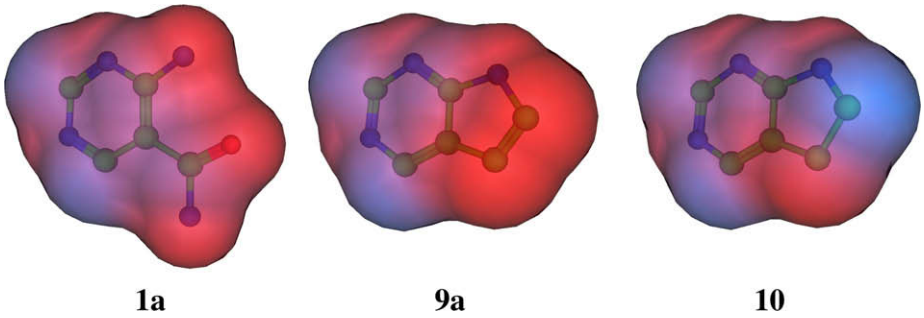


Figure 4. Atomic charge distributions of the 4-aminopyrimidine-5-carboxamide moiety, pyrrolopyrimidine ring, and dihydropyrrolopyrimidine ring of **1a**, **9a**, and **10**, respectively. Compounds are colored blue for nitrogen, red for oxygen and green for carbon. Connolly surfaces around the core scaffolds are colored red for negative charge and blue for positive charge. See Section 5.

5.1.5. Methyl 4-[(4-fluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxylate (5c)

Compound **5c** was prepared from compound **4** and 4-fluorobenzylamine in 96% yield as a pale brown solid, using a similar approach to that described for **5a**, and used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 3.02–3.04 (4H, m), 3.72–3.74 (4H, m), 3.78 (3H, s), 4.67 (2H, d, J = 5.6 Hz), 6.82 (2H, d, J = 8.8 Hz), 7.13–7.17 (2H, m), 7.35–7.37 (2H, m), 7.47 (2H, d, J = 8.8 Hz), 8.54 (1H, s), 8.65 (1H, br), 9.57 (1H, s); FAB MS m/e [M+H]$^+$ 438.

5.1.6. Methyl 4-[(3,5-difluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxylate (5d)

Compound **5d** was prepared from compound **4** and 3,5-difluorobenzylamine in 92% yield as a pale yellow solid, using a similar approach to that described for **5a**, and used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 3.01–3.03 (4H, m), 3.72–3.74 (4H, m), 3.80 (3H, s), 4.68 (2H, d, J = 6.0 Hz), 6.79 (2H, d, J = 8.3 Hz), 7.02–7.10 (3H, m), 7.39 (2H, br), 8.55 (1H, s), 8.73 (1H, s), 9.58 (1H, br); FAB MS m/e [M+H]$^+$ 455.

5.1.7. 4-(Benzylamino)-2-[(4-morpholin-4-ylphenyl)amino]-pyrimidine-5-carboxylic acid (6a)

1 M NaOH (12.9 mL) was added to a solution of **5a** (2.00 g, 4.61 mmol) in MeOH (20 mL) and THF (20 mL) and the mixture was stirred for 10 h at 60 °C. 1 M HCl (14 mL) was added and the resulting solid was collected by filtration and washed with H_2O to give **6a** (1.83 g, 98%) as a colorless solid, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 3.01–3.03 (4H, m), 3.71–3.74 (4H, m), 4.70 (2H, d, J = 5.9 Hz), 6.81 (2H, d, J = 8.8 Hz), 7.25–7.27 (1H, m), 7.33–7.35 (4H, m), 7.49 (2H, d, J = 8.8 Hz), 8.51 (1H, s), 8.75 (1H, br), 9.48 (1H, s), 12.56 (1H, br); FAB MS m/e [M+H]$^+$ 406.

5.1.8. 4-[(2-Fluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)-amino]pyrimidine-5-carboxylic acid (6b)

Compound **6b** was prepared from compound **5b** in 96% yield as a colorless solid, using a similar approach to that described for **6a**, and used in the next reaction without further purification. colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.01–3.03 (4H, m), 3.71–3.75 (4H, m), 4.74 (2H, d, J = 5.3 Hz), 6.78 (2H, d, J = 8.8 Hz), 7.12–7.42 (4H, m), 7.43 (2H, d, J = 8.8 Hz), 8.52 (1H, s), 8.74 (1H, br), 9.50 (1H, br), 12.61 (1H, br); FAB MS m/e [M+H]$^+$ 424.

5.1.9. 4-[(4-Fluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxylic acid (6c)

Compound **6c** was prepared from compound **5c** in 96% yield as a pale brown solid, using a similar approach to that described for **6a**, and used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 3.01–3.04 (4H, m), 3.72–3.77 (4H, m), 4.67 (2H, d, J = 5.6 Hz), 6.82 (2H, d, J = 8.8 Hz), 7.13–7.18 (2H, m), 7.35–7.39 (2H, m), 7.48 (2H, d, J = 8.8 Hz), 8.51 (1H, s), 8.77 (1H, br), 9.47 (1H, s), 12.55 (1H, br); FAB MS m/e [M+H]$^+$ 424.

5.1.10. 4-[(3,5-Difluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxylic acid (6d)

Compound **6d** was prepared from compound **5d** in 95% yield as a pale yellow solid, using a similar approach to that described for **6a**, and used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 3.01–3.03 (4H, m), 3.72–3.74 (4H, m), 4.68 (2H, d, J = 5.2 Hz), 6.79 (2H, d, J = 8.8 Hz), 7.02–7.10 (3H, m), 7.39 (2H, d, J = 8.8 Hz), 8.52 (1H, s), 8.85 (1H, br), 9.50 (1H, br), 12.62 (1H, br); FAB MS m/e [M−H]$^-$ 440.

5.1.11. 4-(Benzylamino)-*N*-methoxy-*N*-methyl-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (7a)

EDC·HCl (1.02 g, 5.30 mmol) and HOBt (718 mg, 5.30 mmol) were added to a mixture of **6a** (1.43 g, 3.53 mmol) in DMF (20 mL). After stirring for 30 min at room temperature, *N,O*-dimethylhydroxylamine hydrochloride (516 mg, 5.30 mmol) and triethylamine (1.72 mL, 12.4 mmol) were added and the mixture was stirred for 24 h at room temperature. The reaction mixture was then diluted with H_2O and extracted with AcOEt–THF. The organic layer was washed successively with H_2O and saturated aqueous NaCl. The organic layer was dried and concentrated in vacuo. The resulting pale yellow solid was recrystallized from THF–MeOH to give **7a** (1.15 g, 73%) as a pale pink solid.^{1}H NMR (DMSO-d_6) δ 3.00–3.03 (4H, m), 3.23 (3H, s), 3.61 (3H, s), 3.71–3.74 (4H, m), 4.64 (2H, d, J = 5.9 Hz), 6.81 (2H, d, J = 8.8 Hz), 7.22–7.27 (1H, m), 7.33–7.40 (4H, m), 7.49 (2H, d, J = 8.8 Hz), 8.38 (1H, s), 8.45 (1H, br), 9.28 (1H, br); FAB MS m/e [M+H]$^+$ 449.

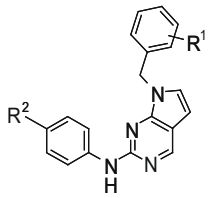
5.1.12. 4-[(2-Fluorobenzyl)amino]-*N*-methoxy-*N*-methyl-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (7b)

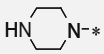
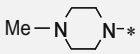
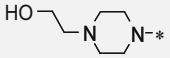
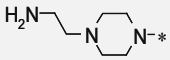
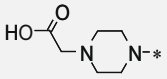
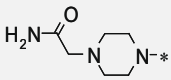
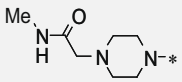
Compound **7d** was prepared from compound **6b** in 51% yield as a dark red solid, using a similar approach to that described for **7a**, and used in the next reaction without recrystallization. ^{1}H NMR (DMSO-d_6) δ 2.99–3.02 (4H, m), 3.24 (3H, s), 3.63 (3H, s), 3.71–3.75 (4H, m), 4.68 (2H, d, J = 5.8 Hz), 6.77 (2H, d, J = 8.8 Hz), 7.12–7.17 (1H, m), 7.21–7.32 (3H, m), 7.43 (2H, d, J = 8.8 Hz), 8.32 (1H, br), 8.39 (1H, br), 9.30 (1H, s); FAB MS m/e [M+H]$^+$ 467.

5.1.13. 4-[(4-Fluorobenzyl)amino]-*N*-methoxy-*N*-methyl-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (7c)

Compound **7c** was prepared from compound **6c** in 71% yield as a pale brown solid, using a similar approach to that described for **7a**, and used in the next reaction without recrystallization. ^{1}H NMR (DMSO-d_6) δ 3.00–3.03 (4H, m), 3.23 (3H, s), 3.61 (3H, s),

Table 2
STAT6 inhibitory activity, residual CYP3A4 activities and *c* Log $D_{7.4}$ of 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives **9a**–**e**, **16**, **17**, **19**, **21** and **23**–**25**



Compound	R^1	R^2	IC_{50} [a](nM)	CYP3A4[b] Preincubation time		*c* Log $D_{7.4}$[c]
				0 min	30 min	
1b			0.7	100	31	3.44
9a	H		400	88	34	4.03
9b	2-F		220	78	32	4.19
9d	3-F		120	80	37	4.29
9c	4-F		4100	82	43	4.19
9e	3,5-F_2		37	97	56	4.53
16	3,5-F_2		12	74	95	1.36
17	3,5-F_2		3.2	90	88	3.85
19	3,5-F_2		5.6	86	86	3.21
21	3,5-F_2		34	65	105	1.19
23	3,5-F_2		26	100	98	0.73
24	3,5-F_2		1.9	104	85	2.92
25	3,5-F_2		4.7	85	77	3.12

[a] IC_{50} values are averages from two or three experiments.
[b] Residual activities of HLM for metabolism of midazolam in the presence of 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives (5 μM) were determined following preincubation for 0 and 30 min. See Section 5.
[c] *c* Log $D_{7.4}$ values were calculated using Pallas PrologD (ver.3.0, CompuDrug).[14]

3.72–3.74 (4H, m), 4.61 (2H, d, *J* = 6.0 Hz), 6.81 (2H, d, *J* = 8.8 Hz), 7.13–7.17 (2H, m), 7.35–7.38 (2H, m), 7.47 (2H, d, *J* = 8.8 Hz), 8.30 (1H, br), 8.37 (1H, br), 9.27 (1H, br); FAB MS *m*/*e* $[M+H]^+$ 467.

5.1.14. 4-[(3,5-Difluorobenzyl)amino]-*N*-methoxy-*N*-methyl-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (7d)

Compound **7d** was prepared from compound **6d** in 66% yield as a pale brown solid, using a similar approach to that described for **7a**, and used in the next reaction without recrystallization. ^{1}H NMR (DMSO-d_6) δ 3.00–3.02 (4H, m), 3.25 (3H, s), 3.62 (3H, s), 3.72–3.74 (4H, m), 4.62 (2H, d, *J* = 6.0 Hz), 6.79 (2H, d, *J* = 8.8 Hz), 7.02–7.09 (3H, m), 7.40 (2H, d, *J* = 8.8 Hz), 8.32 (1H, br), 8.35 (1H, s), 9.27 (1H, br); FAB MS *m*/*e* $[M+H]^+$ 485.

5.1.15. 4-(Benzylamino)-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carbaldehyde (8a)

A solution of **7a** (1.87 g, 4.17 mmol) in THF (20 mL) was added dropwise to a suspension of lithium aluminum hydride (174 mg, 4.49 mmol) and THF (20 mL) at −78 °C. The mixture was stirred for 2.5 h below 0 °C. H_2O (0.17 g), 15% aqueous NaOH solution (0.17 g) and H_2O (0.51 g) were successively added to the mixture at 0 °C. The resulting precipitate was filtered and the filtrate was concentrated in vacuo, and the residue was chromatographed on silica gel with elution using $CHCl_3$–MeOH (50:1–25:1) to give **8a** (1.50 g, 81%) as a yellow solid, which was used in the next reaction without further purification.^{1}H NMR (DMSO-d_6) δ 3.02–3.05 (4H, m), 3.71–3.74 (4H, m), 4.71 (2H, d, *J* = 5.7 Hz), 6.83 (2H, d, *J* = 8.6 Hz), 7.25–7.28 (1H, m), 7.33–7.35 (4H, m), 7.50 (2H, d,

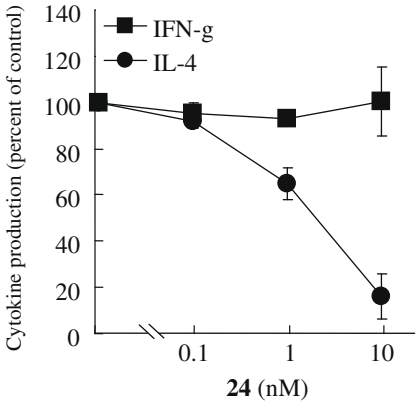
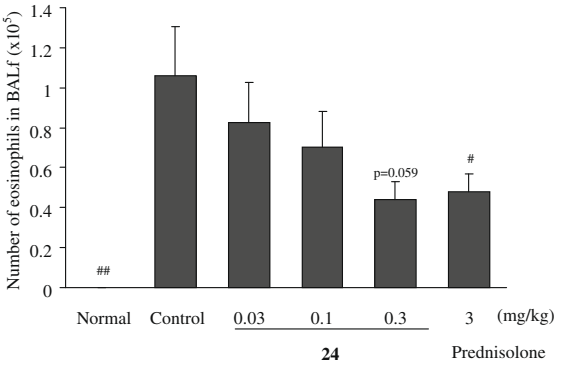


Figure 5. The effect of compound **24** on cytokine production of spleen T cells from mice. Squares indicate the amount of IFN-γ produced in a culture in the presence of IL-12 to induce Th1 cells. Circles indicate the amount of IL-4 produced from Th2 cells differentiated in a culture with IL-4. The cytokine levels in the DMSO control were 1280 ± 60 ng/mL for IFN-γ and 3.9 ± 0.5 ng/mL for IL-4. Data are means ± SEM expressed as percentages relative to the DMSO control ($n = 3$).



n=10

#<0.05: significant difference from control as determined by Student's *t*-test

Figure 6. Effects of compound **24** and prednisolone on accumulation of eosinophils in bronchoalveolar lavage fluid (BALF) in ovalbumin (OVA)-sensitized mice.

J = 8.6 Hz), 8.46 (1H, s), 9.05 (1H, br), 9.54 (1H, s), 9.84 (1H, br); FAB MS m/e $[M+H]^+$ 390.

5.1.16. 4-[(2-Fluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carbaldehyde (8b)

Compound **8b** was prepared from compound **7b** in 53% yield as a yellow solid, using a similar approach to that described for **8a**, and used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 3.02–3.05 (4H, m), 3.72–3.75 (4H, m), 4.75 (2H, d, J = 5.7 Hz), 6.80 (2H, d, J = 8.8 Hz), 7.13–7.17 (1H, m), 7.23–7.34 (3H, m), 7.45 (2H, d, J = 8.8 Hz), 8.47 (1H, s), 9.08 (1H, br), 9.56 (1H, s), 9.86 (1H, br); FAB MS m/e $[M+H]^+$ 408.

5.1.17. 4-[(4-Fluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carbaldehyde (8c)

Compound **8c** was prepared from compound **7c** in 82% yield as a yellow solid, using a similar approach to that described for **8a**, and used in the next reaction without further purification.^{1}H NMR (DMSO-d_6) δ 3.03–3.06 (4H, m), 3.72–3.75 (4H, m), 3.78 (3H, s), 4.68 (2H, d, J = 5.6 Hz), 6.84 (2H, d, J = 8.8 Hz), 7.13–7.18 (2H, m), 7.35–7.38 (2H, m), 7.50 (2H, d, J = 8.8 Hz), 8.45 (1H, s), 9.10 (1H, br), 9.53 (1H, s), 9.83 (1H, br); FAB MS m/e $[M+H]^+$ 408.

5.1.18. 4-[(3,5-Difluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carbaldehyde (8d)

Compound **8d** was prepared from compound **7d** in 75% yield as a yellow solid, using a similar approach to that described for **8a**, and used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 3.02–3.04 (4H, m), 3.72–3.75 (4H, m), 4.68 (2H, d, J = 5.6 Hz), 6.81 (2H, d, J = 8.3 Hz), 7.02–7.11 (3H, m), 7.41 (2H, d, J = 8.3 Hz), 8.47 (1H, s), 9.12 (1H, br), 9.56 (1H, s), 9.83 (1H, br); FAB MS m/e $[M+H]^+$ 426.

5.1.19. 7-Benzyl-*N*-(4-morpholin-4-ylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (9a)

n-BuLi (1.58 M) solution in hexane (4.7 mL, 7.45 mmol) was added dropwise to a mixture of (methoxymethyl)triphenylphosphonium chloride (2.55 g, 7.45 mmol) at 0–5 °C and the mixture was stirred for 10 min. To the resulting red solution, a solution of **8a** (1.45 g, 3.72 mmol) in THF (50 mL) was added dropwise at 0 °C and the mixture was stirred for 2 h at room temperature. The mixture was then diluted with AcOEt and washed successively with H_2O and saturated aqueous NaCl. The organic layer was dried and concentrated in vacuo, and the residue was chromatographed on silica gel with elution using $CHCl_3$–MeOH (50:1–30:1) to give the crude methoxyvinyl derivative (1.0 g) as a yellow solid. A mixture of the crude methoxyvinyl derivative, concentrated HCl (5 mL) and MeOH (50 mL) was refluxed for 16 h. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was diluted with $CHCl_3$ and basicified with saturated aqueous $NaHCO_3$. The organic layer was separated from the aqueous phase, washed with saturated aqueous NaCl and then dried and concentrated in vacuo. The resulting solid was recrystallized from THF–MeOH to give **9a** (725 mg, 51% for 2 steps) as a colorless solid. Mp 153–155 °C; ^{1}H NMR (DMSO-d_6) δ 3.01–3.03 (4H, m), 3.72–3.75 (4H, m), 5.34 (2H, s), 6.43 (1H, d, J = 3.4 Hz), 6.87 (2H, d, J = 8.8 Hz), 7.24–7.36 (6H, m), 7.69 (2H, d, J = 8.8 Hz), 8.65 (1H, s), 9.16 (1H, s); FAB MS m/e $[M+H]^+$ 386. Anal. Calcd for $C_{23}H_{23}N_5O$: C, 71.67; H, 6.01; N, 18.17. Found: C, 71.63; H, 6.04; N, 18.26.

5.1.20. 7-(2-Fluorobenzyl)-*N*-(4-morpholin-4-ylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (9b)

Compound **9b** was prepared from compound **8b** in 28% yield as a pale brown solid, using a similar approach to that described for **9a**: mp 162–164 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 3.01–3.03 (4H, m), 3.73–3.75 (4H, m), 5.40 (2H, s), 6.45 (1H, d, J = 3.9 Hz), 6.87 (2H, d, J = 8.8 Hz), 7.12–7.18 (2H, m), 7.23–7.27 (2H, m), 7.31–7.37 (1H, m), 7.67 (2H, d, J = 8.8 Hz), 8.65 (1H, s), 9.16 (1H, s); FAB MS m/e $[M+H]^+$ 404. Anal. Calcd for $C_{23}H_{22}N_5OF$: C, 68.47; H, 5.50; N, 17.36; F, 4.71. Found: C, 68.23; H, 5.57; N, 17.52; F, 4.63.

5.1.21. 7-(4-Fluorobenzyl)-*N*-(4-morpholin-4-ylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (9c)

Compound **9c** was prepared from compound **8c** in 29% yield as a pale yellow solid, using a similar approach to that described for **9a**: mp 170–172 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 3.02–3.04 (4H, m), 3.73–3.75 (4H, m), 5.32 (2H, s), 6.43 (1H, d, J = 3.4 Hz), 6.88 (2H, d, J = 8.8 Hz), 7.15–7.20 (2H, m), 7.28 (1H, d, J = 3.4 Hz), 7.33–7.36 (2H, m), 7.68 (2H, d, J = 8.8 Hz), 8.65 (1H, s), 9.16 (1H, s); FAB MS m/e $[M+H]^+$ 404. Anal. Calcd for $C_{23}H_{22}N_5OF$: C, 68.47; H, 5.50; N, 17.36; F, 4.71. Found: C, 68.32; H, 5.65; N, 17.36; F, 4.67.

5.1.22. 7-(3,5-Difluorobenzyl)-*N*-(4-morpholin-4-ylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (9d)

Compound **9d** was prepared from compound **8d** in 57% yield as a colorless solid, using a similar approach to that described for **9a**: mp 203–206 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 3.01–3.04 (4H, m), 3.73–3.75 (4H, m), 5.33 (2H, s), 6.45 (1H, d, J = 3.4 Hz), 6.88 (2H, d, J = 9.3 Hz), 7.09–7.13 (1H, m), 7.31 (1H, d, J = 3.4 Hz), 7.38–7.45 (2H, m), 7.67 (2H, d, J = 9.3 Hz), 8.66 (1H, s), 9.16 (1H,

s); FAB MS *m*/*e* $[M+H]^+$ 422. Anal. Calcd for $C_{23}H_{21}N_5OF_2$: C, 65.55; H, 5.02; N, 16.62; F, 9.02. Found: C, 65.54; H, 5.08; N, 16.83; F, 9.08.

5.1.23. 7-Benzyl-*N*-(4-morpholin-4-ylphenyl)-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (10)

Palladium (10%) on carbon (150 mg) was added to a solution of **9a** (500 mg, 130 mmol) in AcOEt (7 mL) and acetic acid (0.7 mL). The mixture was stirred for 6 h at room temperature under a hydrogen atmosphere. The mixture was then filtered with Celite and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel with elution using $CHCl_3$–MeOH–NH_4OH(28%) (200:10:1) to give a colorless solid, which was recrystallized from AcOEt to give **10** (149 mg, 36%) as a colorless solid. Mp 175–177 °C; ^{1}H NMR (DMSO-d_6) δ 2.28 (2H, t, J = 8.2 Hz), 2.86–2.90 (4H, m), 3.43 (2H, t, J = 8.2 Hz), 3.70–3.73 (4H, m), 4.56 (2H, s), 6.82 (2H, d, J = 9.1 Hz), 7.26–7.38 (5H, m), 7.61 (2H, d, J = 9.1 Hz), 7.65 (1H, s), 8.74 (1H, s); FAB MS *m*/*e* $[M+H]^+$ 388. Anal. Calcd for $C_{23}H_{25}N_5O$: C, 71.29; H, 6.50; N, 18.07. Found: C, 71.07; H, 6.45; N, 18.15.

5.1.24. 2-Chloro-5-(2-ethoxyvinyl)pyrimidin-4-amine (12)

Dichlorobis(triphenylphosphine)palladium (2.74 g, 3.91 mmol) and *n*-tetrabutylammonium chloride (21.7 g, 78.1 mmol) were added to a mixture of **11**[12] (16.3 g, 78.1 mmol), (*Z*)-1-ethoxy-2-(tributylstannyl)ethane (42.3 g, 117 mmol) and DMF (150 mL), and the mixture was heated at 150 °C for 1 h under an argon atomosphere. The reaction mixture was cooled to room temperature and diluted with AcOEt (300 mL) and H_2O (300 mL), and then filtered with Celite. The organic layer was separated from the aqueous layer and the aqueous layer was extracted with AcOEt. The combined organic layers were washed with saturated aqueous NaCl, dried and concentrated in vacuo. The residue was chromatographed on silica gel with elution using hexane–AcOEt (3:1–1:1) to give **12** (8.84 g, 57%) as a colorless solid, which was used in the next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 1.34 (3H, t, J = 7.1 Hz), 4.02 (2H, q, J = 7.1 Hz), 4.95 (1H, d, J = 7.1 Hz), 5.64 (2H, br), 6.31 (1H, d, J = 7.1 Hz), 8.25 (1H, s); FAB MS *m*/*e* $[M+H]^+$ 200.

5.1.25. 2-Chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (13)

Trifluoroacetic acid (10 mL) was added to a solution of **12** (9.34 g, 46.8 mmol) in dichloroethane (100 mL) and the mixture was stirred at 100 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was diluted with AcOEt–THF and basicified with saturated aqueous $NaHCO_3$. The organic layer was separated from the aqueous phase and the aqueous layer was extracted with AcOEt–THF. The combined organic layers were washed with saturated aqueous NaCl, dried and concentrated in vacuo. The residue was chromatographed on silica gel with elution using hexane–AcOEt (2:1), followed by hexane–AcOEt–THF (7:7:6) to give **13** (6.61 g, 92%) as an ivory solid, which was used in the next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 6.64–6.66 (1H, m), 7.41–7.43 (1H, m), 8.89 (1H, s), 10.74 (1H, br); FAB MS *m*/*e* $[M+H]^+$ 154.

5.1.26. 2-Chloro-7-(3-fluorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (14a)

3-Fluorobenzyl bromide (257 mg, 1.36 mmol) was added to a mixture of **13** (173 mg, 1.13 mmol) and potassium carbonate (187 mg, 1.35 mmol) in DMSO (4 mL) and the mixture was stirred at 60 °C for 2 h. The mixture was then diluted with AcOEt and extracted with H_2O. The organic layer was washed with saturated aqueous NaCl, dried and concentrated in vacuo. The residue was chromatographed on silica gel with elution using hexane–AcOEt (3:1–2:1) to give **14a** (334 mg, 94%) as an ivory solid, which was used in the next reaction without further purification.^{1}H NMR ($CDCl_3$) δ 5.43 (2H, s), 6.60 (1H, d, J = 3.7 Hz), 6.86–6.91 (1H, m), 6.97–7.03 (2H, m), 7.16 (1H, d, J = 3.7 Hz), 7.28–7.34 (1H, m), 8.32 (1H, s); FAB MS *m*/*e* $[M+H]^+$ 262.

5.1.27. 2-Chloro-7-(3,5-difluorobenzyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (14b)

Compound **14b** was prepared from compound **13** and 3,5-difluorobenzyl bromide in 99% yield as a pale brown solid, using a similar approach to that described for **14a**: ^{1}H NMR ($CDCl_3$) δ 5.41 (2H, s), 6.63 (1H, d, J = 3.6 Hz), 6.68–6.78 (3H, m), 7.16 (1H, d, J = 3.6 Hz), 8.84 (1H, s); FAB MS *m*/*e* $[M+H]^+$ 280.

5.1.28. 7-(3-Fluorobenzyl)-*N*-(4-morpholin-4-ylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (9e)

Palladium diacetate (14 mg, 0.061 mmol), 2'-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP, 57 mg, 0.092 mmol) and cesium carbonate (239 mg, 0.73 mmol) were added to a mixture of **14a** (160 mg, 0.61 mmol), 4-morpholinoaniline (120 mg, 0.67 mmol) and 1,4-dioxane (3.2 mL), and the mixture was heated at 80 °C for 3 h under an argon atmosphere. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was chromatographed on silica gel with elution using hexane–AcOEt–THF (2:1:1) to give a crude solid (147 mg, 60%), which was recrystallized from EtOH to give **9e** (56 mg, 23%) as an ivory solid. Mp 183–184 °C; ^{1}H NMR (DMSO-d_6) δ 3.01–3.03 (4H, m), 3.72–3.75 (4H, m), 5.35 (2H, s), 6.45 (1H, d, J = 3.4 Hz), 6.87 (2H, d, J = 9.3 Hz), 7.08–7.13 (1H, m), 7.32 (1H, d, J = 3.4 Hz), 7.36–7.41 (1H, m), 7.65 (2H, d, J = 9.2 Hz), 8.66 (1H, s), 9.16 (1H, s); FAB MS *m*/*e* $[M+H]^+$ 404. Anal. Calcd for $C_{23}H_{22}N_5OF$: C, 68.47; H, 5.50; N, 17.36; F, 4.71. Found: C, 68.43; H, 5.64; N, 17.33; F, 4.69.

5.1.29. *tert*-Butyl 4-(4-{[7-(3,5-difluorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]amino}phenyl)piperazine-1-carboxylate (15)

Compound **15** was prepared from compound **14b** and *tert*-butyl 4-(4-aminophenyl)piperazine-1-carboxylate in 84% yield as a pale brown solid, using a similar approach to that described for **9e**: ^{1}H NMR (DMSO-d_6) δ 1.42 (9H, s), 2.98–3.00 (4H, m), 3.45–3.47 (4H, m), 5.36 (2H, s), 6.47 (1H, d, J = 3.6 Hz), 6.88 (2H, d, J = 9.2 Hz), 6.97–6.98 (2H, m), 7.14–7.18 (2H, m), 7.33 (1H, d, J = 3.6 Hz), 7.64 (2H, d, J = 9.2 Hz), 8.67 (1H, br), 9.18 (1H, s); FAB MS *m*/*e* $[M+H]^+$ 521.

5.1.30. 7-(3,5-Difluorobenzyl)-*N*-(4-piperazin-1-ylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine dihydrochloride (16)

4 M HCl–AcOEt (47 mL) was added to a mixture of **15** (24.4 g, 46.9 mmol) and MeOH–THF (1:1, 400 mL), and the mixture was stirred at 80 °C for 13 h. The reaction mixture was cooled to room temperature and concentrated in vacuo to give **16** (23.0 g, 99%) as a yellow solid recrystallized from aqueous EtOH. Mp 243–249 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 3.23 (4H, br), 3.33–3.35 (4H, m), 5.37 (2H, s), 6.67 (1H, d, J = 3.7 Hz), 6.99–7.04 (4H, m), 7.17–7.22 (1H, m), 7.55 (2H, d, J = 9.0 Hz), 7.60 (1H, d, J = 3.7 Hz), 8.85 (1H, s), 9.23 (1H, br), 10.18 (1H, s); FAB MS *m*/*e* $[M+H]^+$ 421. Anal. Calcd for $C_{23}H_{22}N_6OF_2{\cdot}2HCl{\cdot}4H_2O$: C, 48.86; H, 5.70; N, 14.86; F, 6.72; Cl, 12.54. Found: C, 48.92; H, 5.49; N, 14.70; F, 6.79; Cl, 12.79.

5.1.31. 7-(3,5-Difluorobenzyl)-*N*-[4-(4-methylpiperazin-1-yl)phenyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (17)

Dipotassium carbonate (840 mg, 6.08 mmol) and iodomethane (0.13 mL, 2.09 mmol) were added to a DMF (9.3 mL) solution of **16** (926 mg, 1.88 mmol) and the mixture was stirred for 2 h at room temperature. The mixture was then diluted with AcOEt–THF and washed successively with H_2O and saturated aqueous NaCl. The organic layer was dried and concentrated in vacuo. The resultant solid was recrystallized from AcOEt–MeOH to give **17** (150, 15%) as a pale yellow solid. Mp 181–184 °C; ^{1}H NMR

(DMSO-d_6) δ 2.42–2.48 (4H, m), 2.50 (3H, s), 3.03–3.06 (4H, m), 5.35 (2H, s), 6.46 (1H, d, J = 3.6 Hz), 6.85 (2H, d, J = 9.1 Hz), 6.97–7.01 (2H, m), 7.13–7.19 (1H, m), 7.33 (1H, d, J = 3.6 Hz), 7.61 (2H, d, J = 9.1 Hz), 8.66 (1H, s), 9.16 (1H, s); FAB MS m/e $[M+H]^+$ 435. Anal. Calcd for $C_{24}H_{24}N_6F_2{\cdot}0.2H_2O$: C, 65.80; H, 5.61; N, 19.18; F, 8.67. Found: C, 65.66; H, 5.56; N, 19.25; F, 8.53.

5.1.32. *N*-{4-[4-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)piperazin-1-yl]phenyl}-7-(3,5-difluorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (18)

(2-Bromoethoxy)-*tert*-butyldimethylsilane (630 mg, 2.64 mmol) and sodium iodide (394 mg, 2.64 mmol) were added to a mixture of **16** (926 mg, 1.88 mmol), disodium carbonate (751 mg, 7.10 mmol) and DMF (9 mL). The mixture was stirred for 4 h at 100 °C. The mixture was then diluted with AcOEt–THF and washed successively with H_2O and saturated aqueous NaCl. The organic layer was dried and concentrated in vacuo to give **18** (676 mg, 62%) as an ivory solid, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 0.06 (6H, s), 0.89 (9H, s), 2.47 (2H, t, J = 6.0 Hz), 2.57–2.59 (4H, m), 3.01–3.03 (4H, m), 3.72 (2H, t, J = 6.0 Hz), 5.35 (2H, s), 6.46 (1H, d, J = 3.6 Hz), 6.85 (2H, d, J = 9.2 Hz), 6.97–6.99 (2H, m), 7.14–7.19 (1H, m), 7.31 (1H, d, J = 3.6 Hz), 7.61 (2H, d, J = 9.2 Hz), 8.66 (1H, s), 9.15 (1H, s); FAB MS m/e $[M+H]^+$ 579.

5.1.33. 2-[4-(4-{[7-(3,5-Difluorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]amino}phenyl)piperazin-1-yl]ethanol (19)

A 1 M solution of *n*-tetrabuthylammonium fluoride in THF (5.7 mL) was added to a solution of **18** (655 mg, 1.13 mmol) and THF (10 mL), and the mixture was stirred for 16 h at room temperature. The mixture was then diluted with AcOEt and washed successively with H_2O and saturated aqueous NaCl. The organic layer was dried and concentrated in vacuo. The resultant solid was recrystallized from AcOEt to give **19** (305 mg, 58%) as an ivory solid. Mp 160–163 °C; ^{1}H NMR (DMSO-d_6) δ 2.43–2.46 (2H, m), 2.56–2.58 (4H, m), 3.02–3.05 (4H, m), 3.54 (2H, dt, J = 6.0, 6.8 Hz), 4.43 (1H, br), 5.35 (2H, s), 6.46 (1H, d, J = 3.6 Hz), 6.85 (2H, d, J = 9.2 Hz), 6.97–6.99 (2H, m), 7.13–7.19 (1H, m), 7.33 (1H, d, J = 3.6 Hz), 7.61 (2H, d, J = 9.2 Hz), 8.66 (1H, s), 9.15 (1H, s); FAB MS m/e $[M+H]^+$ 465. Anal. Calcd for $C_{25}H_{26}N_6F_2O{\cdot}0.3H_2O$: C, 63.90; H, 5.71; N, 17.88; F, 8.09. Found: C, 63.70; H, 5.62; N, 17.83; F, 8.18.

5.1.34. *tert*-Butyl {2-[4-(4-{[7-(3,5-difluorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]amino}phenyl)piperazin-1-yl]-ethyl}carbamate (20)

Compound **20** was prepared from compound **16** and 2-(*tert*-butoxycarbonylamino)ethyl bromide in 59% yield as a pale yellow solid, using a similar approach to that described for **18**, and used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 1.38 (9H, s), 2.37 (2H, t, J = 6.4 Hz), 2.53 (4H, br), 3.02–3.08 (6H, m), 5.35 (2H, s), 6.45 (1H, d, J = 3.6 Hz), 6.67 (1H, br), 6.85 (2H, d, J = 9.2 Hz), 6.97–6.99 (2H, m), 7.14–7.18 (1H, m), 7.34 (1H, d, J = 3.6 Hz), 7.61 (2H, d, J = 9.2 Hz), 8.31 (1H, s), 9.15 (1H, s); FAB MS m/e $[M+H]^+$ 564.

5.1.35. *N*-{4-[4-(2-Aminoethyl)piperazin-1-yl]phenyl}-7-(3,5-difluorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine hydrochloride (21)

4 M HCl–AcOEt (7.2 mL) was added to a solution of **20** (651 mg, 1.16 mmol) in MeOH (20 mL), and the mixture was stirred for 16 h at room temperature. The resulting solid was collected by filtration and washed with AcOEt. The solid was recrystallized from AcOEt–MeOH to give **21** (240 mg, 36%) as a yellow solid. Mp 169–171 °C; ^{1}H NMR (DMSO-d_6) δ 3.17–3.26 (4H, m), 3.36–3.43 (4H, m), 3.68–3.76 (4H, m), 5.37 (2H, s), 6.54 (1H, d, J = 3.6 Hz), 7.01–7.04 (3H, m), 7.17–7.23 (1H, m), 7.56–7.59 (3H, m), 8.42 (3H, br), 8.84 (1H, s), 10.09 (1H, br), 11.38 (1H, br); FAB MS m/e $[M+H]^+$ 464. Anal. Calcd for $C_{25}H_{27}N_7F_2{\cdot}3.1HCl{\cdot}3H_2O$: C, 47.62; H, 5.77; N, 15.55; F, 6.03; Cl, 17.43. Found: C, 47.33; H, 5.71; N, 15.35; F, 6.39; Cl, 17.32.

5.1.36. Ethyl [4-(4-{[7-(3,5-difluorobenzyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidin-2-yl]amino}phenyl)piperazin-1-yl]acetate (22)

Ethyl bromoacetate (0.2 mL) was added to a mixture of **16** (780 mg, 1.58 mmol), dipotassium carbonate (813 mg, 5.88 mmol) and DMF (20 mL) and the mixture was stirred for 4 h at 100 °C. The mixture was then diluted with AcOEt and washed successively with H_2O and saturated aqueous NaCl. The organic layer was dried and concentrated in vacuo. The residue was chromatographed on silica gel with elution using $CHCl_3$–MeOH (100:1 to 50:1) to give a crude solid, which was recrystallized from EtOH to give **22** (561 mg, 75%) as a pale brown solid. ^{1}H NMR (DMSO-d_6) δ 1.21 (3H, t, J = 7.3 Hz), 2.65–2.62 (4H, m), 3.04–3.06 (4H, m), 3.27 (2H, s), 4.11 (2H, q, J = 7.3 Hz), 5.35 (2H, s), 6.46 (1H, d, J = 3.9 Hz), 6.86 (2H, d, J = 9.3 Hz), 6.96–7.00 (2H, m), 7.13–7.18 (1H, m), 7.33 (1H, d, J = 3.9 Hz), 7.62 (2H, d, J = 9.3 Hz), 8.66 (1H, s), 9.15 (1H, s); FAB MS m/e $[M+H]^+$ 507.

5.1.37. [4-(4-{[7-(3,5-Difluorobenzyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidin-2-yl]amino}phenyl)piperazin-1-yl]acetic acid (23)

1 M NaOH (1.9 mL) was added to a solution of **22** (490 mg, 0.97 mmol) and MeOH–THF (1:1, 20 mL), and the mixture was stirred at 50 °C for 15 h.1 M HCl (2.0 mL) was added to the mixture and resulting solid was collected by filtration and washed with H_2O. The crude solid was recrystallized from EtOH–THF to give **23** (284 mg, 61%) as a colorless solid. Mp 107–110 °C; ^{1}H NMR (DMSO-d_6) δ 2.73–2.76 (4H, m), 3.08–3.10 (4H, m), 3.22 (2H, s), 5.35 (2H, s), 6.46 (1H, d, J = 3.4 Hz), 6.86 (2H, d, J = 9.3 Hz), 6.96–7.01 (2H, m), 7.13–7.19 (1H, m), 7.33 (1H, d, J = 3.4 Hz), 7.62 (2H, d, J = 9.3 Hz), 8.66 (1H, s), 9.16 (1H, s); FAB MS m/e $[M-H]^-$ 477. Anal. Calcd for $C_{25}H_{24}N_6F_2O_2{\cdot}0.6H_2O$: C, 61.37; H, 5.19; N, 17.18; F, 7.77. Found: C, 61.20; H, 5.23; N, 17.23; F, 7.90.

5.1.38. 2-[4-(4-{[7-(3,5-Difluorobenzyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidin-2-yl]amino}phenyl)piperazin-1-yl]acetamide (24)

EDC·HCl (75 mg, 0.39 mmol) and HOBt (53 mg, 0.39 mmol) were added to a mixture of **23** (157 mg, 0.33 mmol) in DMF (5 mL). After stirring for 30 min at room temperature, 28% aqueous ammonia (1.0 mL) was added, and the mixture was stirred for 16 h at room temperature. The reaction mixture was then diluted with H_2O and extracted with AcOEt–THF. The organic layer was washed successively with H_2O and saturated aqueous NaCl and then dried and concentrated in vacuo. The residue was chromatographed on silica gel with elution using $CHCl_3$–MeOH (30:1 to 20:1) to give crude solid, which was recrystallized from EtOH to give **24** (95 mg, 60%) as a colorless solid. Mp 201–203 °C; ^{1}H NMR (DMSO-d_6) δ 2.58–2.60 (4H, m), 2.92 (2H, s), 3.08–3.10 (4H, m), 5.35 (2H, s), 6.46 (1H, d, J = 3.2 Hz), 6.83 (2H, d, J = 9.2 Hz), 6.96–7.01 (2H, m), 7.13–7.22 (1H, m), 7.33 (1H, d, J = 3.2 Hz), 7.62 (2H, d, J = 9.2 Hz), 8.66 (1H, s), 9.16 (1H, s); FAB MS m/e $[M+H]^+$ 478. Anal. Calcd for $C_{25}H_{25}N_7F_2O$: C, 62.88; H, 5.28; N, 20.53; F, 7.96. Found: C, 62.72; H, 5.32; N, 20.50; F, 8.10.

5.1.39. 2-[4-(4-{[7-(3,5-Difluorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]amino}phenyl)piperazin-1-yl]-*N*-methylacetamide hydrochloride (25)

EDC·HCl (78 mg, 0.41 mmol) and HOBt (56 mg, 0.41 mmol) were added to a mixture of **23** (150 mg, 0.31 mmol) in DMF (5 mL). After stirring for 30 min at room temperature, 40% methylamine in MeOH solution (0.1 mL) was added and the mixture was stirred for 16 h at room temperature. The reaction mixture was then diluted with H_2O and extracted with AcOEt. The

organic layer was washed successively with H_2O and saturated aqueous NaCl, and was then dried and concentrated in vacuo. The residue was chromatographed on silica gel with elution using $CHCl_3$–MeOH (50:1 to 30:1). The collected fraction was concentrated to give an amorphous solid. 4 M HCl–AcOEt (1 mL) was added to the amorphous solid, and the mixture was concentrated. The residue was triturated with EtOH–AcOEt to give **25** (100 mg, 29%) as a pale yellow solid. Mp 154–156 °C; ^{1}H NMR (DMSO-d_6) δ 2.68 (3H, d, J = 4.4 Hz), 3.16 (2H, br), 3.33 (2H, br), 3.56 (2H, br), 3.74 (2H, br), 4.02 (2H, s), 5.38 (2H, s), 6.72 (1H, d, J = 3.4 Hz), 7.02 (2H, d, J = 9.3 Hz), 7.03–7.07 (2H, m), 7.18–7.24 (1H, m), 7.53 (2H, d, J = 9.3 Hz), 7.67 (1H, d, J = 3.4 Hz), 8.69 (1H, br q, J = 4.4 Hz), 8.93 (1H, s), 10.55 (1H, s); FAB MS m/e $[M+H]^+$ 492. Anal. Calcd for $C_{26}H_{27}N_7F_2O\cdot2.8HCl\cdot0.7H_2O$: C, 51.51; H, 5.19; N, 16.17; F, 6.27; Cl, 16.37. Found: C, 51.51; H, 5.21; N, 16.15; F, 6.34; Cl, 16.07.

5.2. Molecular modeling

The geometry of **1a** was optimized using the Conformation Import function in the Molecular Operating Environment (MOE) program.[16] Structure alignment of **1a**, **9a** and **10** was performed with the Flexible Alignment tool in MOE, using the geometry of **1a** as a template and the MMFF94x force field.

5.3. Calculation of atomic charge distribution

MOE (revision 2007.0902) was used to build the ligand structures of **1a**, **9a**, and **10**. The molecular conformations were optimized at the semiempirical AM1 level and electrostatic potential-fitted atomic partial charges were calculated using the MOPAC 7.1 module in MOE. Connolly surfaces[17] that surround the van der Waals surfaces of the core scaffolds were generated and colored with red for negative charge and blue for positive charge, using the MOE program.

5.4. Measurement of residual activities of HLMs for metabolism of midazolam

The test compounds (5 μM) were pre-incubated with HLM (0.1 mg/mL) in the presence of NADPH (1 mM) and ethylenediaminetetraacetic acid (EDTA, 0.1 mM) in potassium sodium phosphate buffer (100 mM, pH 7.4). Midazolam was added to the reaction mixture following pre-incubation for 0 and 30 min at 37 °C. The reaction was stopped by addition of acetonitrile after 20 min incubation. The metabolite of midazolam (1′-hydroxymidazolam) was analyzed by LC–MS, comprising an HPLC system (Waters 2795) to separate the peaks and atmospheric pressure electrospray ionization MS (Waters ZQ). The residual midazolam 1′-hydroxylase activity in the presence of 5 μM of the test compound was expressed as the percentage activity of that in a control incubation performed without compound. Verapamil was used as a positive control CYP inactivator.

5.5. In vitro T cell differentiation

The effect of compound **24** on Th1 and Th2 differentiation from naive Th cells in mice were determined as described previously.[15]

5.6. OVA-induced pulmonary eosinophilia in actively sensitized rats

Female Balb/c mice were actively sensitized by intraperitoneal injection of OVA-containing aluminum hydroxide gel. This was repeated twice at four-day intervals. At 12 days after the first sensitization, the mice were exposed to an aerosolized solution of OVA for 1 h. At 72 h after OVA exposure, mice were sacrificed and BAL was performed. Compound **24** was administered orally 30 min before, and 24 and 48 h after OVA exposure. Prednisolne was administered orally 2 h before, and 24 and 48 h after OVA exposure. The number of eosinophils was determined as described previously.[7b]

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