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Identification of 4-benzylamino-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide derivatives as potent and orally bioavailable STAT6 inhibitors

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ABSTRACT

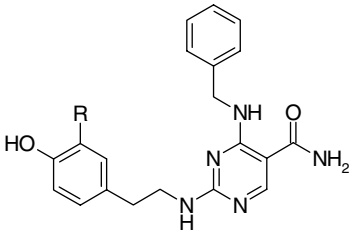
Signal transducers and activators of transcription 6 (STAT6) is a key regulator of the type 2 helper T (Th2) cell immune response and a potential therapeutic target for allergic diseases such as asthma and atopic diseases. To search for potent and orally bioavailable STAT6 inhibitors, we synthesized a series of 4-benzylaminopyrimidine-5-carboxamide derivatives and evaluated their STAT6 inhibitory activities. Among these compounds, 2-[(4-morpholin-4-ylphenyl)amino]-4-[(2,3,6-trifluorobenzyl)amino]pyrimidine-5-carboxamide (**25y**, YM-341619, AS1617612) showed potent STAT6 inhibition with an IC_{50} of 0.70 nM, and also inhibited Th2 differentiation in mouse spleen T cells induced by interleukin (IL)-4 with an IC_{50} of 0.28 nM without affecting type 1 helper T (Th1) cell differentiation induced by IL-12. In addition, compound **25y** showed an oral bioavailability of 25% in mouse.

1. Introduction

Naive $CD4^+$ T helper (Th) cells differentiate into two cell types, which are designated as type 1 helper T (Th1) and type 2 helper T (Th2) cells.[1] Th1 cells produce Th1 cytokines such as interferon-γ (IFN-γ), interleukin (IL)-2, and tumor necrosis factor-β for enhancement of cell-mediated 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. Th1 and Th2 responses are balanced under normal conditions, and an imbalance in these responses is thought to play an important role in the pathogenesis of allergic and autoimmune diseases.[2]

Differentiation of Th cells into Th2 cells is tightly regulated by several transcription factors.[3] Signal transducers and activators of transcription 6 (STAT6) is one of the most important transcription factors for differentiation of Th2 cells and regulation of signal transduction pathways induced by IL-4 and IL-13.[4] In STAT6-deficient mice, Th cells fail to differentiate into Th2 cells, and B cells are unable to produce immunoglobulin E.[5] In addition, antigen-induced airway hyperresponsiveness and eosinophil infiltration are significantly decreased.[6] These findings indicate that STAT6 might be an excellent therapeutic target for various allergic diseases, including asthma and atopic diseases.

We have previously reported a series of 2-{[2-(4-hydroxyphenyl)ethyl]amino}pyrimidine-5-carboxamide derivatives as novel STAT6 inhibitors (Fig. 1).[7] An investigation of their structure–activity relationships (SARs) for STAT6 inhibition showed that the



1a R=H
1b (AS1571499) R=Cl

Figure 1. Structures of 2-{[2-(4-hydroxyphenyl)ethyl]amino}pyrimidine-5-carboxamide derivatives.

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hydroxyl group at the 4-position of the benzene ring of the phenethyl moiety was important for activity and that the benzylamino group was a suitable substituent at C-4 of the pyrimidine ring. Selected 2-{[2-(4-hydroxyphenyl)ethyl]amino}pyrimidines showed potent STAT6 inhibition and, in particular, AS1517499 (**1b**) inhibited Th2 differentiation without influencing Th1 differentiation. However, preliminary pharmacokinetic studies revealed that these compounds were eliminated immediately in mouse after intravenous administration. It is well known that phenolic compounds commonly form conjugates at the hydroxyl group by sulfation and glucuronidation and, to a lesser extent, by acetylation and phosphorylation.[8] Therefore, we hypothesized that metabolic instability due to the phenolic moiety might be a reason for the poor pharmacokinetic profile.

Based on this hypothesis, we have attempted to find potent and orally bioavailable STAT6 inhibitors by two strategies. The first of these was introduction of substituents at positions *ortho* to the phenolic hydroxyl group for interference with the conjugation reaction, and this led to the design of the 2-(3,5-dichloro-4-hydroxyphenyl)ethyl derivative. The second strategy involved exploration of alternative structures without a phenolic hydroxyl group, instead of the 2-(4-hydroxyphenyl)ethylamino moiety of compound **1a**, including design of 2-phenylaminopyrimidine derivatives with alcoholic and heterocyclic moieties. In this report, we describe the synthesis and SARs of a series of 4-benzylaminopyrimidine-5-carboxamide derivatives, and also report the effects on Th1/Th2 differentiation and pharmacokinetic properties of selected compounds.

2. Chemistry

The synthesis of 2-{[2-(3,5-dichloro-4-hydroxyphenyl)ethyl]amino}pyrimidine derivative **6** is shown in Scheme 1. Chlorination of *N*-[2-(4-hydroxyphenyl)ethyl] acetamide (**2**) with *N*-chlorosuccinimide (NCS) gave dichloro derivative **3**, followed by hydrolysis to give 2-(3,5-dichloro-4-hydroxyphenyl)ethylamine (**4**). Substitution of the methylsulfonyl derivative **5**[7] by compound **4** provided the desired compound **6**.

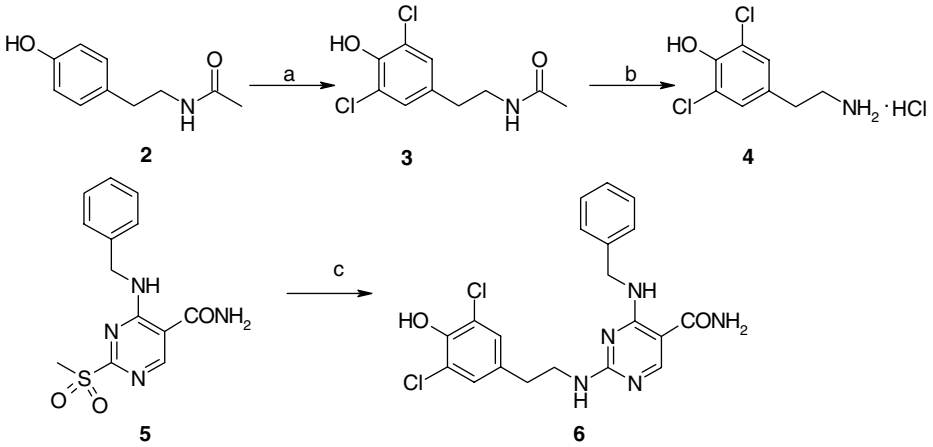
Scheme 2 shows the synthesis of 4-benzylaminopyrimidine derivatives **17**–**24**, **25a**–**e**, and **27**. Nucleophilic substitution reactions of ethyl 2,4-dichloropyrimidine-5-carboxylate (**7**) with various benzylamines were carried out at −70 °C in tetrahydrofuran (THF) to afford 4-benzylamino derivatives **8**–**10** in 67–72% yield. Hydrolysis of compounds **8**–**10** with 1 M NaOH in THF at 50 °C gave the corresponding carboxylic acids **11**–**13**, followed by treatment with oxalyl chloride and aqueous ammonia to give the carboxamides **14**–**16**, respectively. The chloro groups of **14**–**16** were substituted with the appropriate aniline derivatives to afford the desired 4-benzylaminopyrimidine-5-carboxamides **17**–**24**, **25a**–**c**, and **26**. Hydrogenation of the nitro group of **25c** furnished the amino derivative **25d**, and subsequent reaction of **25d** with acetyl chloride gave the acetamide derivative **25e**. Deprotection of the *tert*-butoxycarbonyl (Boc) group of **26** afforded the piperazine derivative **27**.

The synthesis of 2-[(4-morpholin-4-ylphenyl)amino]pyrimidine derivatives **25f**–**y** is outlined in Scheme 3. A substitution reaction of ethyl 2-chloro-4-methylsulfanylpyrimidine-5-carboxylate (**28**)[7] with 4-morpholinoaniline gave 2-amino-4-methylsulfanylpyrimidine derivative **29** as the sole product. Hydrolysis of **29** gave the carboxylic acid **30**, followed by condensation with aqueous ammonia in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole (HOBt) to afford the carboxamide **31**. The methylsulfinyl derivative**32** was obtained by oxidation of **31** with *m*-chloroperbenzoic acid (*m*-CPBA), and displacement of the methylsulfinyl group of **32** with the appropriate benzylamine and subsequent treatment with sodium bisulfite provided the desired molecules **25f**–**y**.

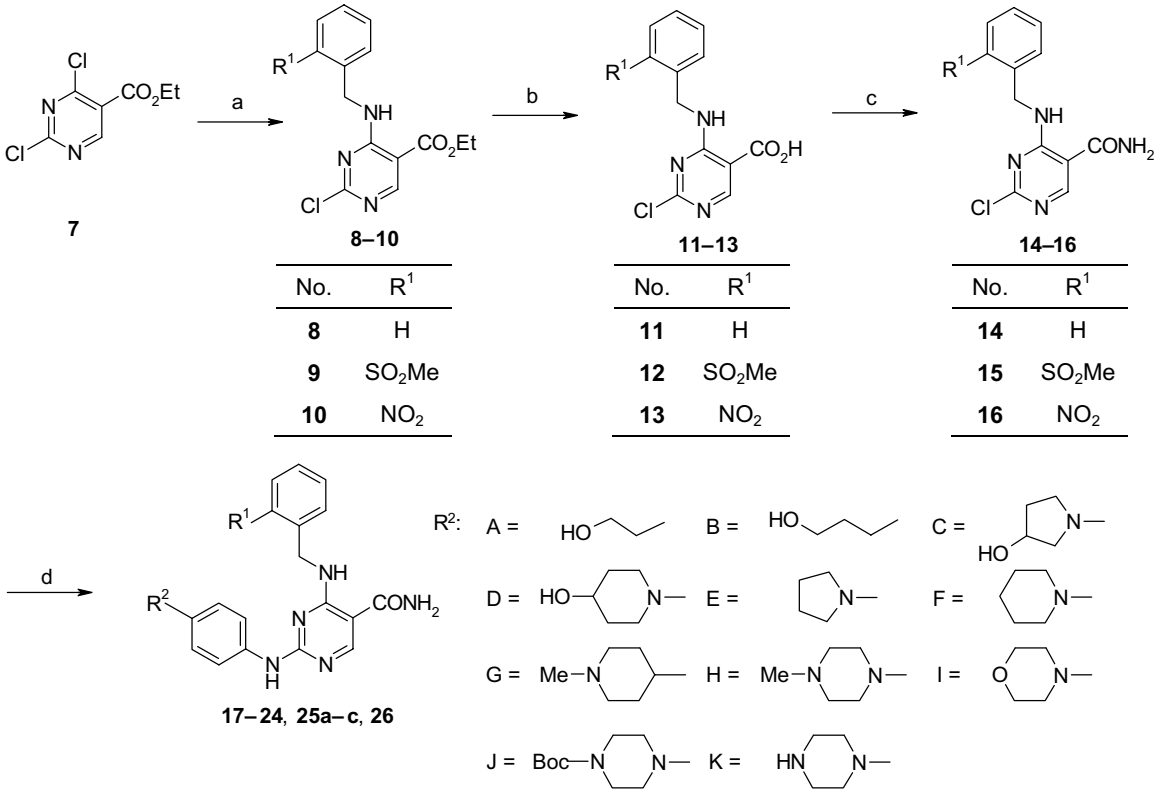
3. Results and discussion

The ability of the compounds to inhibit STAT6 activation was measured using a previously reported STAT6-dependent promoter reporter assay.[7] The activity of 2-{[2-(4-hydroxyphenyl)ethyl]amino}pyrimidine-5-carboxamide derivatives is shown in Table 1. As previously reported, the acidity of the phenolic group is an important factor in the activity; that is, compounds with more acidic hydroxyl groups tend to show more potent activity.[7] Li et al. have shown that the pK_a values of phenol, 2-chlorophenol, and 2,6-dichlorophenol are 10.09, 9.13, and 7.15, respectively,[9] and based on this report it is likely that the hydroxyl group of compound **6** is more acidic than those of compounds **1a** and **1b**, suggesting that **6** might show more potent activity than **1a** and **1b**. However, **6** was equipotent to **1a** and approximately 3-fold less potent than **1b**. These results indicate that the two chloro groups in compound **6** might interfere with an interaction between the hydroxyl group and a target protein in the STAT6 activation pathway.

The activity of 4-benzylaminopyrimidine derivatives without phenolic hydroxyl groups is shown in Table 2. The 4-[(2-hydroxyethyl)phenyl]amino derivative **17** showed equipotent activity to **1a**, and replacement of the ethylene linker of **17** to propylene (**18**) was tolerated. These results reveal that the phenolic hydroxyl



Scheme 1. Reagents and conditions: (a) NCS/DMF, 40 °C; (b) 6 M HCl/AcOH, reflux; (c) 4, $^{i}Pr_2NEt$/NMP, 80–90 °C.



No.	R^1
8	H
9	SO_2Me
10	NO_2

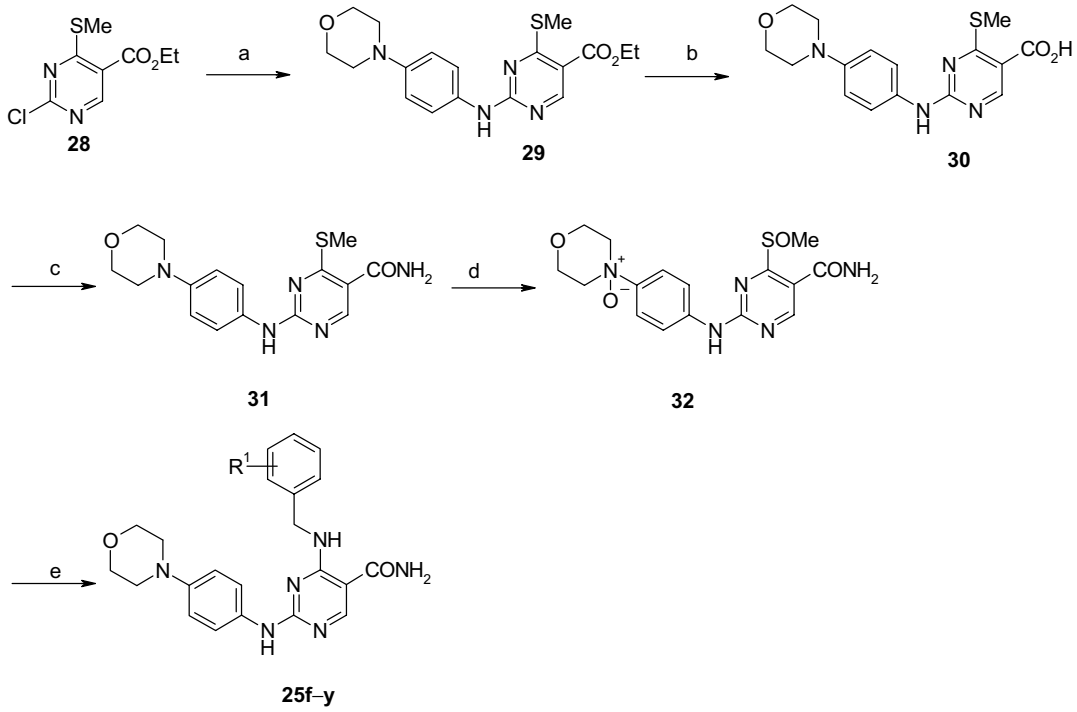
No.	R^1
11	H
12	SO_2Me
13	NO_2

No.	R^1
14	H
15	SO_2Me
16	NO_2

No.	R^1	R^2
17	H	A
18	H	B
19	H	C
20	H	D
21	H	E
22	H	F
23	H	G
24	H	H
25a	H	I
25b	SO_2Me	I
25c	NO_2	I
25d	NH_2	I
25e	NHCOMe	I
26	H	J
27	H	K

Scheme 2. Reagents and conditions: (a) benzylamine, 2-nitrobenzylamine, or 2-methylsulfonylbenzylamine, $^{i}Pr_2NEt$/THF, −70 °C; (b) 1 M NaOH/THF, 50 °C; (c) i—$(COCl)_2$, cat. DMF/CH_2Cl_2, ii—NH_4OH (28%), 0 °C; (d) R^2-C_6H_4-NH_2/NMP, 80–90 °C; (e) H_2, 10% Pd-C/DMF; (f) Ac_2O/pyridine; (g) 4 M HCl–dioxane/dioxane–H_2O.

group of **1a** may be interchangeable with alcoholic hydroxyl groups. Introduction of a 3-hydroxypyrrolidine group (**19**) instead of the hydroxyalkyl groups of **17** and **18** also retained the activity, and 4-hydroxypiperidine derivative **20** was about 6-fold more potent than **1a**. Removal of the hydroxyl groups (**21**, **22**) of **19** and **20** resulted in a 6- to 8-fold decrease of the activity. Interestingly, piperidine derivatives **20** and **22** tended to show more potent activity than the corresponding pyrrolidine derivatives **19** and **21**, respectively. Conversion of the piperidin-1-yl group of compound **22** to piperazines (**24**, **27**) and a morpholine (**25a**) caused a 17- to 19-fold increase in potency. These results suggest that terminal hetero atoms in R^2 may be required for potent STAT6 inhibitory activity, and this was supported by the 1-methylpiperidin-4-yl derivative **23** showing a potency 7 times higher than that of the



No.	R^1	No.	R^1
25f	2-Me	**25p**	3-OMe
25g	2-OMe	**25q**	3-OH
25h	2-OH	**25r**	4-F
25i	2-Cl	**25s**	2,3-F_2
25j	2-F	**25t**	2,4-F_2
25k	2-CF_3	**25u**	2,5-F_2
25l	2-SMe	**25v**	2,6-F_2
25m	3-F	**25w**	3,5-F_2
25n	3-Cl	**25x**	2,3,5-F_3
25o	3-CF_3	**25y**	2,3,6-F_3

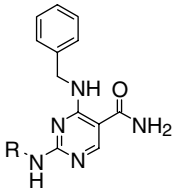
Scheme 3. Reagents and conditions: (a) 4-morpholinoaniline, 4 M HCl–dioxane/NMP, 90 °C; (b) 1 M NaOH/MeOH; (c) EDC·HCl, HOBt/DMF, then, NH_4OH (28%); (d) *m*-CPBA DMA, <5 °C; (e) benzylamines, $^iPr_2NEt$/DMA, 80–90 °C, then aqueous $NaHSO_3$.

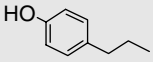
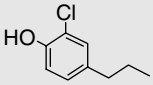
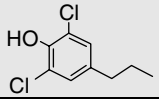
piperidin-1-yl derivative **22**. We hypothesized that the nitrogen and oxygen atoms of the piperazine and morpholine rings might act as hydrogen bond acceptors in interacting with a target protein.

The pharmacokinetic profiles of compounds **6** and **25a** in mouse are shown in Table 3. Compound **6** showed high total body clearance (CL_{tot}) and a short half-life ($t_{1/2}$) after intravenous administration, and low oral bioavailability. In contrast, compound **25a** had a decreased CL_{tot} and a somewhat longer $t_{1/2}$ relative to compound **6**. Furthermore, **25a** had an oral bioavailability of 30%. These results indicate that **25** might be metabolically more stable and preferable for oral administration compared to **6**.

The activity of 2-[(morpholine-4-yl)phenyl]aminopyrimidine derivatives with various substituted benzylamino groups at C-4 of the pyrimidine ring is shown in Table 4. Among the monofluorinated compounds, the 2-fluoro (**25j**) and 3-fluoro (**25m**) derivatives were about 4-fold more potent than the unsubstituted derivative **25a**; however, the 4-fluoro compound (**25r**) was 100-fold less potent. In the series of 2-substituted benzyl derivatives, the methyl (**25f**), methoxy (**25g**), and chloro (**25i**) derivatives showed comparable activity to **25a**. The hydroxyl (**25h**), trifluoromethyl (**25k**), nitro (**25c**), and methylsulfanyl (**25l**) derivatives were 3- to 5-fold less active than **25a**, and introduction of amino (**25d**), acetamide (**25e**), or methylsulfonyl (**25b**) groups decreased the activity dramatically. Among the 3-substituted benzyl derivatives, the hydroxyl (**25q**), methoxy (**25p**), and chloro (**25n**) derivatives were 3- to 6-fold less active than **25a**, and the trifluoromethyl derivative **25o** was 20-fold less active than **25a**. Among compounds with two fluoro groups, the 2,3-difluoro (**25s**), 2,5-difluoro (**25u**), and 2,6-difluoro (**25v**) derivatives were equipotent to the 2-fluoro (**25j**) and 3-fluoro (**25m**) compounds; however, the 3,5-difluoro derivative **25w** was unexpectedly 3-fold less active than the 3-fluoro derivative **25m**. As for the 4-fluoro derivative **25r**, the 2,4-difluoro compound **25t** displayed a drop in activity of greater than 2 orders of magnitude compared to

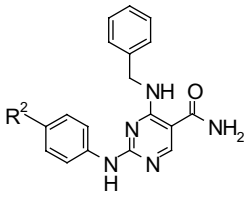
Table 1
STAT6 inhibitory activity of 2-{[2-(4-hydroxyphenyl)ethyl]amino}pyrimidine-5-carboxamide derivatives **1a**, **1b**, and **6**



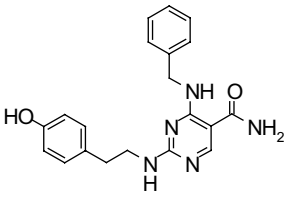
Compound	R	IC_{50}[a] (nM)
1a		88[b]
1b		21[b]
6		61

[a] IC_{50} values are averages of two or three experiments.
[b] See Ref. 7.

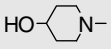
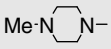
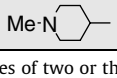
Table 2
STAT6 inhibitory activity of 2-benzylaminopyrimidine-5-carboxamide derivatives **17**–**24**, **25a**, and **27**



17—24, **25a**, **27**



1a

Compound	R^2	IC_{50}[a] (nM)
1a		88
17		95
18		81
19		98
20		16
21		650
22		120
24		6.2
27		7.0
25a		6.5
23		17

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

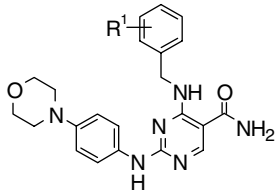
the 2,3-difluoro (**25s**), 2,5-difluoro (**25u**), and 2,6-difluoro (**25v**) derivatives. For the trifluorobenzyl compounds, the 2,3,5-trifluoro derivative **25x** maintained activity and the 2,3,6-trifluoro derivative **25y** showed the most potent activity with an IC_{50} of 0.70 nM. These results indicate that introduction of fluoro groups at the 2- or 3-position of the benzyl group was effective in enhancing activity.

Table 3
Pharmacokinetic parameters of compounds **6** and **25a** in mouse[a]

	iv			po
	CL_{tot}[b] (mL/min/kg)	$t_{1/2}$[c] (h)	V_d[d] (mL/kg)	F[e] (%)
6[f]	168	0.06	931	1.0
25a[g]	59.6	0.76	3909	30

[a] Each value is an average of data from three animals.
[b] Total body clearance.
[c] Plasma half-life.
[d] Distribution volume.
[e] Bioavailability.
[f] Dosed at 1 mg/kg, iv and 10 mg/kg, po.
[g] Dosed at 1 mg/kg, iv and 3 mg/kg, po.

Table 4
STAT6 inhibitory activity of 4-benzylamino-2-(4-morpholin-4-ylphenyl)aminopyrimidine-5-carboxamide derivatives **25a**–**y**



Compound	R^1	IC_{50}[a] (nM)
25a	H	6.5
25j	2-F	1.4
25m	3-F	1.8
25r	4-F	650
25f	2-Me	11
25g	2-OMe	8.2
25h	2-OH	22
25i	2-Cl	7.9
25k	2-CF_3	25
25c	2-NO_2	38
25d	2-NH_2	310
25e	2-CH_3CONH	5200
25l	2-SMe	23
25b	2-SO_2Me	2000
25n	3-Cl	36
25o	3-CF3	130
25p	3-OMe	25
25q	3-OH	19
25s	2,3-F_2	2.1
25t	2,4-F_2	310
25u	2,5-F_2	1.6
25v	2,6-F_2	2.2
25w	3,5-F_2	5.5
25x	2,3,5-F_3	1.8
25y	2,3,6-F_3	0.70

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

To assess the benzyl moiety further, we performed a quantitative SAR (QSAR) analysis of the compounds shown in Table 4, using molecular mass, molecular volume, molar refractivity,[10] Verloop L, and B_1–B_5,[11] ellipsoidal volume, Wiener topological index,[12] bond dipole moment, and E-state indices[13] as descriptors. These parameters were calculated using TSAR.[14] Partition coefficient (*c*Log*P*) and molecular surface area (MSA) were calculated using CLOGP[15] and Molecular Operating Environment (MOE) software,[16] respectively. Selection of descriptors was performed with a genetic algorithm,[17] and the QuaSAR-Evolution algorithm provided by MOE was used for selection of variables.[14] The activities of the 2-(morpholin-4-yl)phenylaminopyrimidine derivatives were described

Table 5
pIC_{50} values and calculated physiochemical descriptors of compounds **20a–y**

Compound	R[1]	pIC_{50}	$cLogP$[a]	MSA[b]	μ_4[c]
25a	H	8.19	3.89	427.3	0.4696
25j	2-F	8.85	4.04	433.4	0.4696
25m	3-F	8.74	4.04	433.6	0.4827
25r	4-F	6.19	4.04	435.3	−0.9483
25f	2-Me	7.96	4.34	448.6	0.4696
25g	2-OMe	8.07	3.81	453.6	0.4696
25h	2-OH	7.66	3.18	438.4	0.4696
25i	2-Cl	8.10	4.61	444.9	0.4696
25k	2-CF_3	7.60	4.78	461.1	0.4696
25c	2-NO_2	7.42	3.56	453.6	0.4696
25d	2-NH_2	6.51	2.62	441.9	0.4696
25e	2-CH_3CONH	5.28	2.26	481.4	0.4696
25l	2-SMe	7.64	4.45	471.7	0.4696
25b	2-SO_2Me	5.70	2.25	483.8	0.4696
25n	3-Cl	7.44	4.61	447.1	0.4867
25o	3-CF3	6.89	4.78	461.1	0.4707
25p	3-OMe	7.60	3.81	458.9	0.4790
25q	3-OH	7.72	3.23	439.9	0.4790
25s	2,3-F_2	8.68	4.11	438.2	0.4827
25t	2,4-F_2	6.51	4.18	438.1	−0.9483
25u	2,5-F_2	8.26	4.18	437.9	0.4827
25v	2,6-F_2	8.66	4.18	431.0	0.4696
25w	3,5-F_2	8.25	4.18	438.9	0.4955
25x	2,3,5-F_3	8.74	4.25	442.7	0.4955
25y	2,3,6-F_3	9.15	4.25	439.0	0.4826

[a] $cLogP$ values were calculated by CLOGP.[15]
[b] MSA values were calculated by MOE.[16]
[c] μ_4 values were calculated by TSAR.[14]

by Eq. 1, which includes three physiochemical descriptors: $cLogP$, MSA, and bond dipole moment at the 4-position (μ_4) (Table 5).

$$pIC_{50} = 0.591cLogP - 0.0404MSA + 1.45\mu_4 + 22.9 \quad (1)$$

$$(n = 25, R = 0.905, R^2 = 0.820, \mathrm{RMSE} = 0.428, F = 31.8)$$

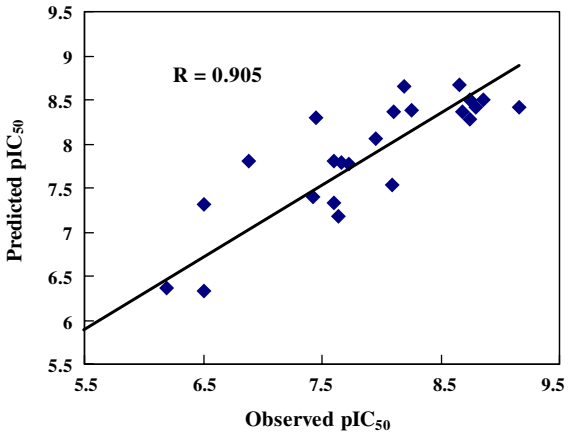


Figure 2. Correlation between calculated and observed pIC_{50} values of the 4-benzylaminopyrimidine derivatives listed in Table 4.

Eq. 1 has a good correlation coefficient ($R = 0.905$) and the correlation between the observed and predicted pIC_{50} values is shown in Figure 2. The positive coefficient for $cLogP$ indicates that lipophilic molecules have better STAT6 inhibitory activity, whereas the negative coefficient for MSA indicates that expansion of van der Waals surface decreases the activity. Furthermore, the positive coefficient of μ_4 suggests that these derivatives interact electrostatically with an electronegative region of a target protein at the 4-position of the phenyl ring of the benzylamino moiety. The decreased activities of the 4-fluoro derivatives **25r** and **25t** might be due to the strong electron-withdrawing group at this position.

Pharmacokinetic parameters for the most potent compound, **25y**, are shown in Table 6. This compound had a similar CL_{tot} and $t_{1/2}$ to **25a** and showed an oral bioavailability of 25% in mouse. These results indicate that introduction of fluoro groups at the 2-, 3-, and 6-positions of the benzene ring of the benzylamino moiety does not alter the pharmacokinetic profile.

The effect of compound **25y** on differentiation of T cells to Th subsets was examined using a previously reported method for determination of the degree of Th1 and Th2 differentiation based on production of IFN-γ and IL-4, respectively.[7] As shown in Figure 3, **25y** inhibited production of IL-4 with an IC_{50} of 0.28 nM, but showed no effect on production of IFN-γ. These results indicate that **25y** inhibits Th2 differentiation without influencing Th1 differentiation. In addition, **25y** inhibited antigen-induced eosinophil infiltration in the lung of mice by 71% compared to controls after

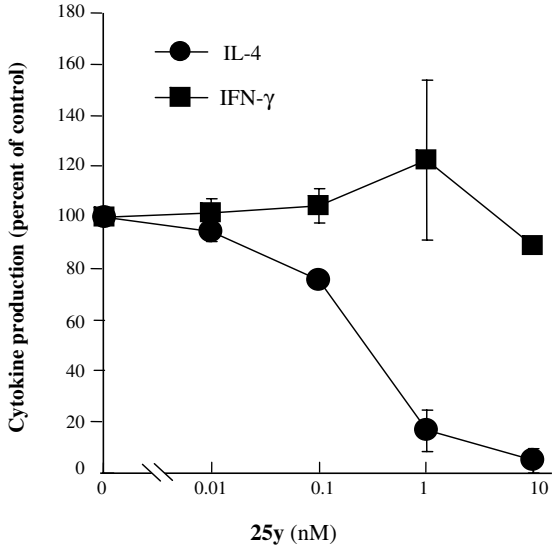


Figure 3. The effect of compound **25y** on cytokine production of spleen T cells from mice. Squares indicate the amount of IFN-γ produced in a culture in the presence of IL-12 to induce Th1 cells, and circles indicate the amount of IL-4 produced from Th2 cells differentiated in a culture with IL-4. The cytokine levels in the DMSO control were 1450 ± 150 ng/mL for IFN-γ and 8.8 ± 3.3 ng/mL for IL-4. Data are means ± SEM expressed as percentages relative to the DMSO control ($n = 3$).

Table 6
Pharmacokinetic parameters of compound **25y** in mouse[a]

	iv			po			
	CL_{tot}[c] (mL/min/kg)	$t_{1/2}$[d] (h)	V_d[e] (mL/kg)	C_{max}[f] (ng/mL)	T_{max}[g] (h)	AUC[h] (ng h/mL)	F[i] (%)
25y[b]	36.1	1.0	3117	80	0.5	114	25

[a] Each value is an average of data from three animals.
[b] Compound **25y** was administered as the hydrochloride salt. Dosed at 1 mg/kg, iv and 1 mg/kg, po.
[c–e,i] See footnote in Table 3.
[f] Peak height concentration.
[g] Time of peak concentration.
[h] Area under the blood concentration–time curve.

oral administration at a dose of 0.3 mg/kg.[18] Collectively, our findings indicate that compound **25y** (YM-341619, AS1617612) is the first potent and orally bioavailable STAT6 inhibitor, and we suggest that this compound could be useful for treatment of allergic diseases such as asthma and atopic diseases.

4. Conclusion

In an attempt to find potent and orally bioavailable STAT6 inhibitors, we introduced chloro groups at positions *ortho* to the phenolic hydroxyl group of the 2-(4-hydroxyphenyl)ethyl derivative **1a**, and explored alternative structures without phenolic hydroxyl groups in the 2-(4-hydroxyphenyl)ethyl moiety. The piperazine (**24**, **27**) and morpholine (**25a**) derivatives showed potent STAT6 inhibitory activity. Pharmacokinetic studies indicated that the morpholine derivative **25a** had an oral bioavailability of 30% in mouse. A QSAR study revealed that introduction of fluoro groups in the 2- or 3-position of the benzyl group of **25a** led to enhancement of activity. Among the fluorobenzyl derivatives, 2,3,6-trifluorobenzyl derivative **25y** was identified as the most potent STAT6 inhibitor with an IC_{50} of 0.70 nM. The QSAR study suggested that the activity was dependent on the lipophilicity and molecular surface area and on the bond dipole moment at the 4-position of the phenyl ring of the benzyl moiety. Compound **25y** had an oral bioavailability of 25% in mouse and a selective inhibitory effect on Th2 differentiation with an IC_{50} of 0.28 nM. These results show that **25y** (YM-341619, AS1617612) is the first potent and orally bioavailable STAT6 inhibitor, and this compound may 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). Compound **6** showed characteristic broad peaks in the ^{1}H NMR spectrum at room temperature, probably because of the presence of conformational isomers. Therefore, the ^{1}H NMR spectrum of compound **6** was measured at 80 °C to confirm the structure. 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.

5.1.1. *N*-[2-(3,5-dichloro-4-hydroxyphenyl)ethyl]acetamide (3)

NCS (8.61 g, 64.4 mmol) was added portionwise to a DMF (30 mL) solution of *N*-[2-(4-hydroxyphenyl)ethyl]acetamide **2** (5.50 g, 30.7 mmol) at 45 °C and the mixture was stirred for 1 h at 50 °C. The reaction mixture was concentrated and the resulting residue was diluted with H_2O and extracted with AcOEt. The organic layer was dried and concentrated in vacuo. The residue was chromatographed on silica gel with elution using AcOEt to give **3** (3.42 g, 45%) as a colorless solid, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 1.76 (3H, s), 2.60 (2H, t, J = 7.2 Hz), 3.19–3.24 (2H, m), 7.19 (2H, s), 7.85 (1H, br), 9.87 (1H, s); FAB MS m/e $[M+H]^+$ 248.

5.1.2. 2-(3,5-Dichloro-4-hydroxyphenyl)ethylamine hydrochloride (4)

A mixture of compound **3** (3.40 g, 13.7 mmol), acetic acid (60 mL), and 6 M HCl (20 mL) was refluxed for 12 h. The reaction mixture was concentrated in vacuo. EtOH (50 mL) was added to the residue and the mixture was concentrated in vacuo. The resulting solid was triturated with acetonitrile and collected by filtration to give **4** (2.98 g, 90%) as a colorless solid, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 2.81 (2H, t, J = 7.6 Hz), 2.99–3.04 (2H, m), 7.29 (2H, s), 8.085 (2H, br), 10.02 (1H, s); FAB MS m/e $[M+H]^+$ 206.

5.1.3. 4-(Benzylamino)-2-{[2-(3,5-dichloro-4-hydroxyphenyl) ethyl]amino}pyrimidine-5-carboxamide (6)

Compound **4** (555 mg, 1.81 mmol) was added to a solution of **5**[7] (660 mg, 2.72 mmol) and diisopropylamine (0.8 mL, 4.58 mmol) in NMP (6 mL) and the mixture was stirred for 1 h at 100 °C. The reaction 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–NH_4OH (28%) (300:10:1) to give a colorless solid, which was recrystallized from MeOH–THF to give **6** (162 mg, 21%) as a colorless solid. Mp 100–103 °C; ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.69 (2H, t, J = 7.0 Hz), 3.40–3.45 (2H, m), 4.62 (2H, d, J = 5.9 Hz), 6.92 (1H, br), 7.11 (4H, br), 7.20–7.23 (1H, m), 7.29–7.30 (4H, m), 8.38 (1H, s), 9.32 (1H, br s), 9.55 (1H, br s); FAB MS m/e $[M+H]^+$ 432. Anal. Calcd for $C_{20}H_{19}Cl_2N_5O_2 \cdot 0.7H_2O$: C, 53.99; H, 4.62; Cl, 15.94; N, 15.74. Found: C, 54.39; H, 4.65; Cl, 16.07; N, 15.32.

5.1.4. Ethyl 4-benzylamino-2-chloropyrimidine-5-carboxylate (8)

A THF (100 mL) solution of benzylamine (5.71 mL, 55 mmol) was added dropwise to a THF (200 mL) solution of ethyl 2,4-dichloropyrimidine-5-carboxylate **3** (10.05 g, 45.5 mmol) and diisopropylethylamine (7.9 mL, 45.5 mmol) at −70 °C and the mixture was stirred for 0.5 h at −70 °C. The reaction 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 hexane–AcOEt (20:1) to give **8** (9.51 g, 72%) as a colorless powder, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 1.31 (3H, t, J = 7.1 Hz), 4.32 (2H, q, J = 7.1 Hz), 4.68 (2H, d, J = 5.8 Hz), 7.24–7.35 (6H, m), 8.64 (1H, s), 8.93 (1H, br t, J = 5.8 Hz); FAB MS m/e $[M+H]^+$ 292.

5.1.5. Ethyl 2-chloro-4-{[2-(methylsulfonyl)benzyl]amino} pyrimidine-5-carboxylate (9)

Compound **9** was prepared from compound **7** and 2-(methylsulfonyl)benzylamine in 72% yield as a colorless powder, using a similar approach to that described for **8**. Compound **9** was used in the next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 1.26 (3H, t, J = 7.2 Hz), 3.25 (3H, s), 4.38 (2H, q, J = 7.2 Hz), 5.09 (2H, d, J = 6.6 Hz), 7.50 (1H, t, J = 7.3 Hz), 7.62 (1H, t, J = 7.3 Hz), 7.69 (1H, d, J = 7.8 Hz), 8.06 (1H, d, J = 7.8 Hz), 8.68 (1H, s), 9.13 (1H, br); FAB MS m/e $[M+H]^+$ 370.

5.1.6. Ethyl 2-chloro-4-[(2-nitrobenzyl)amino]pyrimidine-5-carboxylate (10)

Compound **10** was prepared from compound **7** and 2-nitrobenzylamine in 67% yield as a colorless powder, using a similar approach to that described for **8**. Compound **10** was used in the next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 1.38 (3H, t, J = 7.1 Hz), 4.37 (2H, q, J = 7.1 Hz), 5.04 (2H, d, J = 6.4 Hz), 7.45–7.51 (1H, m), 7.63 (1H, dt, J = 1.3, 7.5 Hz), 7.72 (1H, dd, J = 1.3, 8.3 Hz), 8.69 (1H, s), 9.10 (1H, br); FAB MS m/e $[M+H]^+$ 337.

5.1.7. 4-(Benzylamino)-2-chloropyrimidine-5-carboxylic acid (11)

1 M NaOH (34 mL) was added to a solution of **8** (9.44 g, 32.4 mmol) in THF (90 mL) and the mixture was stirred for 2 h at

50 °C. 1 M HCl (34 mL) was added and the resulting solid was collected by filtration to give **11** (8.45 g, 99%) as a colorless powder, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 4.67 (2H, d, J = 5.8 Hz), 7.24–7.38 (6H, m), 8.61 (1H, s), 9.04 (1H, br); FAB MS m/e $[M-H]^-$ 262.

5.1.8. 2-Chloro-4-{[2-(methylsulfonyl)benzyl]amino}pyrimidine-5-carboxylic acid (12)

Compound **12** was prepared from compound **9** in 88% yield as a colorless powder, using a similar approach to that described for **11**. Compound **12** was used in the next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 3.28 (3H, s), 5.08 (2H, d, J = 6.4 Hz), 7.49–7.54 (1H, m), 7.61–7.68 (2H, m), 8.02 (1H, d, J = 7.3 Hz), 8.67 (1H, s), 9.27 (1H, br t, J = 6.4 Hz); FAB MS m/e $[M+H]^+$ 342.

5.1.9. 2-Chloro-4-[(2-nitrobenzyl)amino]pyrimidine-5-carboxylic acid (13)

Compound **13** was prepared from compound **10** in 98% yield as a colorless powder, using a similar approach to that described for **11**. Compound **13** was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 4.96 (2H, d, J = 6.0 Hz), 7.57 (1H, d, J = 7.5 Hz), 7.73 (1H, dt, J = 1.3, 7.3 Hz), 8.08 (1H, dd, J = 1.3, 8.3 Hz), 8.63 (1H, s), 9.10 (1H, br t, J = 6.0 Hz), 13.77 (1H, br); FAB MS m/e $[M-H]^-$ 307.

5.1.10. 4-(Benzylamino)-2-chloropyrimidine-5-carboxamide (14)

Oxalyl dichloride (2.95 mL) and DMF (1 drop) were added to a suspension of **11** (8.63 g, 32.7 mmol) in CH_2Cl_2 (90 mL) and the mixture was stirred for 2 h at room temperature. The reaction mixture was concentrated and the resulting residue was poured into ice-NH_4OH (28%). The resulting solid was collected by filtration to give **14** (8.20 g, 95%) as a colorless powder, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 4.63 (2H, d, J = 5.8 Hz), 7.27–7.37 (6H, m), 7.69 (1H, br), 7.97 (0.5H, br), 8.21 (0.5H, br), 8.59 (1H, s), 9.59 (1H, br t, J = 5.8 Hz); FAB MS m/e $[M+H]^+$ 263.

5.1.11. 2-Chloro-4-{[2-(methylsulfonyl)benzyl]amino}pyrimidine-5-carboxamide (15)

Compound **15** was prepared from compound **12** in 75% yield as a colorless powder, using a similar approach to that described for **14**. Compound **15** was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 3.37 (3H, s), 4.99 (2H, d, J = 6.2 Hz), 7.52–7.58 (2H, m), 7.68–7.73 (2H, m), 7.94 (1H, d, J = 6.6 Hz), 8.22 (1H, br), 8.60 (1H, s), 9.71 (1H, br t, J = 6.2 Hz); FAB MS m/e $[M+H]^+$ 341.

5.1.12. 2-Chloro-4-[(2-nitrobenzyl)amino]pyrimidine-5-carboxamide (16)

Compound **16** was prepared from compound **13** in 81% yield as a colorless powder, using a similar approach to that described for **14**. Compound **16** was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 4.91 (2H, d, J = 6.0 Hz), 7.53–7.58 (2H, m), 7.71–7.76 (2H, m), 8.07 (1H, dd, J = 1.5, 8.6 Hz), 8.23 (1H, br), 8.61 (1H, s), 9.74 (1H, br t, J = 6.0 Hz), 13.77 (1H, br); FAB MS m/e $[M+H]^+$ 308.

5.1.13. 4-Benzylamino-2-{[4-(2-hydroxyethyl)phenyl]amino}pyrimidine-5-carboxamide hydrochloride (17)

4-(2-Hydroxyethyl)aniline (313 mg, 2.28 mmol) was added to a solution of **14** (400 mg, 1.52 mmol) in NMP (5 mL) and stirred for 0.5 h at 90 °C. The reaction mixture was cooled and diluted with AcOEt (50 mL). The resulting solid was collected by filtration and recrystallized from MeOH–THF to give **17** (327 mg, 54%) as an ivory solid. Mp 233–236 °C; ^{1}H NMR (DMSO-d_6) δ 2.70 (2H, t, J = 7.2 Hz), 3.59 (2H, t, J = 7.2 Hz), 4.70 (2H, d, J = 5.6 Hz), 7.18 (2H, d, J = 8.2 Hz), 7.25–7.38 (7H, m), 7.59 (1H, br s), 8.26 (1H, br s), 8.63 (1H, s), 10.37 (1H, s), 10.79 (1H, s); FAB MS m/e $[M+H]^+$ 364. Anal. Calcd for $C_{20}H_{21}N_5O_2 \cdot HCl \cdot 0.5H_2O$: C, 58.75; H, 5.67; N, 17.13; Cl, 8.87. Found: C, 58.84; H, 5.50; N, 17.07; Cl, 8.69.

5.1.14. *tert*-Butyl 4-(4-{[5-(aminocarbonyl)-4-(benzylamino)pyrimidine-2-yl]amino}phenyl)piperazine-1-carboxylate (26)

Compound **26** was prepared from compound **14** and *tert*-butyl 4-(4-aminophenyl)piperazine-1-carboxylate in 49% yield as a pale brown solid, using a similar approach to that described for **17**. ^{1}H NMR (DMSO-d_6) δ 1.42 (9H, s), 2.97–3.00 (4H,m), 3.42–3.45 (4H,m), 4.66 (2H, d, J = 5.9 Hz), 6.83 (2H, d, J = 8.8 Hz), 7.24–7.34 (7H, m), 7.51 (2H, d, J = 8.8 Hz), 8.51 (1H, s), 9.27 (1H, br), 9.50 (1H, br); FAB MS m/e $[M+H]^+$ 504.

5.1.15. 4-Benzylamino-2-{[4-(3-hydroxypropyl)phenyl]amino}pyrimidine-5-carboxamide (18)

4-(3-Hydroxypropyl)aniline hydrochloride (342 mg, 1.82 mmol) was added to a solution of **14** (400 mg, 1.52 mmol) in NMP (8 mL) and stirred for 2 h at 90 °C. The reaction mixture was cooled and poured into H_2O (50 mL), and neutralized with saturated aqueous $NaHCO_3$. The mixture was extracted with AcOEt and the organic layer was washed successively with H_2O and saturated aqueous NaCl, dried and concentrated in vacuo. The resulting ivory solid was recrystallized from MeOH to give **18** (76 mg, 13%) as a colorless solid. Mp 205–206 °C; ^{1}H NMR (DMSO-d_6,) δ 1.64–1.71 (2H, m), 2.52–2.55 (2H, m), 3.38–3.42 (2H, m), 4.43 (2H, t, J = 5.4 Hz), 4.68 (2H, d, J = 6.4 Hz), 7.02 (2H, d, J = 8.3 Hz), 7.14 (1H, br s), 7.22–7.26 (1H, m), 7.33–7.36 (4H, m), 7.54 (2H, d, J = 8.3 Hz), 7.79 (1H, br s), 8.32 (1H, s), 9.39 (1H, s), 9.52 (1H, br s); FAB MS m/e $[M+H]^+$ 378. Anal. Calcd for $C_{21}H_{23}N_5O_2$: C, 66.83; H, 6.14; N, 18.55. Found: C, 66.43; H, 6.11; N, 18.54.

5.1.16. 4-Benzylamino-2-{[4-(4-hydroxypiperidin-1-yl)phenyl]amino}pyrimidine-5-carboxamide (20)

Compound **20** was prepared from compound **14** and 4-(4-hydroxylpiperidine-1-yl)aniline in 28% yield as a colorless solid, using a similar approach to that described for **18**: mp 188–189 °C (dec) (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 1.43–1.52 (2H, m), 1.79–1.82 (2H, m), 2.73 (2H, dt, J = 2.9, 11.3 Hz), 3.39–3.42 (2H, m), 3.54-3.61 (1H, m), 4.64 (1H, d, J = 4.4 Hz), 4.66 (2H, d, J = 5.9 Hz), 6.80 (2H, d, J = 8.8 Hz), 7.12 (1H, br s), 7.22–7.28 (1H, m), 7.33–7.36 (4H, m), 7.47 (2H, d, J = 8.3 Hz), 7.75 (1H, br s), 8.51 (1H, s), 9.22 (1H, s), 9.49 (1H, br s); FAB MS m/e $[M+H]^+$ 419. Anal. Calcd for $C_{23}H_{26}N_6O_2 \cdot 0.3H_2O$: C, 65.17; H, 6.32; N, 19.83. Found: C, 65.14; H, 6.46; N, 19.88.

5.1.17. 4-Benzylamino-2-[(4-piperidin-1-ylphenyl)amino]pyrimidine-5-carboxamide (22)

Compound **22** was prepared from compound **14** and 4-(piperidine-1-yl)aniline dihydrochloride in 34% yield as a colorless needle, using a similar approach to that described for **18**: mp 203–204 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 1.47–1.52 (2H, m), 1.58–1.64 (4H, m), 3.00–3.03 (4H, m), 4.66 (2H, d, J = 5.9 Hz), 6.79 (2H, d, J = 8.8 Hz), 7.12 (1H, br s), 7.22–7.28 (1H, m), 7.32–7.36 (4H, m), 7.47 (2H, d, J = 8.3 Hz), 7.75 (1H, br s), 8.51 (1H, s), 9.23 (1H, s), 9.49 (1H, br s); FAB MS m/e $[M+H]^+$ 403. Anal. Calcd for $C_{23}H_{26}N_6O$: C, 68.63; H, 6.51; N, 20.88. Found: C, 68.92; H, 6.59; N, 20.94.

5.1.18. 4-Benzylamino-2-{[4-(4-methylpiperazin-1-yl)phenyl]amino}pyrimidine-5-carboxamide (24)

Compound **24** was prepared from compound **14** and 4-(4-methylpiperazin-1-yl)aniline trihydrochloride in 23% yield as an ivory solid, using a similar approach to that described for **18**: mp 202–

204 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 2.24 (3H, s), 2.47–2.49 (4H, m), 3.04–3.06 (4H, m), 4.66 (2H, d, J = 5.9 Hz), 6.89 (2H, d, J = 9.3 Hz), 7.12 (1H, br s), 7.23–7.28 (1H, m), 7.33–7.34 (4H, m), 7.50 (2H, d, J = 9.3 Hz), 7.74 (1H, br s), 8.51 (1H, s), 9.25 (1H, s), 9.49 (1H, s); FAB MS m/e $[M+H]^+$ 418. Anal. Calcd for $C_{23}H_{27}N_7O{\cdot}1.2H_2O$: C, 62.91; H, 6.75; N, 22.33. Found: C, 62.72; H, 6.55; N, 22.37.

5.1.19. 4-(Benzylamino)-2-{[4-(3-hydroxypyrrolidine-1-yl)phenyl]amino}pyrimidine-5-carboxamide (19)

4-(3-Hydroxypyrrolidine-1-yl)aniline (540 mg, 3.03 mmol) and 4 M HCl–dioxane (0.75 mL) were added to a solution of **14** (400 mg, 1.52 mmol) in NMP (5 mL) and stirred for 2 h at 90 °C. The reaction mixture was cooled and poured into H_2O (50 mL), and neutralized with saturated aqueous $NaHCO_3$. The mixture was extracted with AcOEt–THF (2:1), and the organic layer was washed successively with H_2O and saturated aqueous NaCl, dried and concentrated in vacuo. The residue was chromatographed on silica gel with elution using $CHCl_3$–MeOH (30:1 to 10:1), to give crude solid (168 mg), which was recrystallized from MeOH–THF to give **19** (103 mg, 17%) as a colorless solid. Mp 217–221 °C; ^{1}H NMR (DMSO-d_6) δ1.84–1.88 (1H, m), 2.01–2.07(1H, m), 3.01 (1H, d, J = 8.3 Hz), 3.16–3.28 (2H, m), 3.36–3.39 (1H, m), 4.38 (1H, br s), 4.65 (2H, d, J = 5.9 Hz), 4.89 (1H, d, J = 3.9 Hz), 6.39 (2H, d, J = 8.8 Hz), 7.05 (1H, br s), 7.22–7.28 (1H, m), 7.33–7.36 (4H, m), 7.42 (2H, d, J = 8.8 Hz), 7.68 (1H, br s), 8.49 (1H, s), 9.10 (1H, s), 9.47 (1H, s); FAB MS m/e $[M+H]^+$ 405. Anal. Calcd for $C_{22}H_{24}N_6O_2{\cdot}0.1H_2O$: C, 65.04; H, 6.00; N, 20.69. Found: C, 64.94; H, 5.83; N, 20.42.

5.1.20. 4-Benzylamino-2-{[4-(1-methylpiperidin-4-yl)phenyl]-amino}pyrimidine-5-carboxamide (23)

Compound **23** was prepared from compound **14** and 4-(1-methylpiperidin-4-yl)aniline in 45% yield as a colorless solid, using a similar approach to that described for **19**: mp 203–204 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 1.55–1.70 (4H, m), 1.93 (2H, dt, J = 2.4, 11.7 Hz), 2.17 (3H, s), 4.68 (2H, d, J = 5.9 Hz), 7.06 (2H, d, J = 8.8 Hz), 7.15 (1H, br s), 7.23–7.28 (1H, m), 7.33–7.37 (4H, m), 7.55 (2H, d, J = 8.8 Hz), 7.77 (1H, br s), 8.54 (1H, s), 9.37 (1H, s), 9.52 (1H, br t, J = 5.9 Hz); FAB MS m/e $[M+H]^+$ 417. Anal. Calcd for $C_{24}H_{28}N_6O{\cdot}H_2O$: C, 66.34; H, 6.96; N, 19.34. Found: C, 66.61; H, 7.04; N, 19.14.

5.1.21. 4-Benzylamino-2-[(4-morpholin-4-ylphenyl)amino]-pyrimidine-5-carboxamide (25a)

Compound **25a** was prepared from compound **14** and 4-morpholinoaniline in 43% yield as a colorless needle, using a similar approach to that described for **19**: mp 248–250 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 2.99–3.02 (4H, m), 3.71–3.74 (4H, m), 4.66 (2H, d, J = 6.3 Hz), 6.81 (2H, d, J = 9.3 Hz), 7.14 (1H, br s), 7.23–7.28 (1H, m), 7.32–7.35 (4H, m), 7.51 (2H, d, J = 9.3 Hz), 7.75 (1H, br s), 8.51 (1H, s), 9.27 (1H, s), 9.50 (1H, s); FAB MS m/e $[M+H]^+$ 405. Anal. Calcd for $C_{22}H_{24}N_6O_2$: C, 65.33; H, 5.98; N, 20.78. Found: C, 65.26; H, 6.03; N, 20.70.

5.1.22. 4-{[2-(Methylsulfonyl)benzyl]amino}-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25b)

Compound **25b** was prepared from compound **15** and 4-morpholinoaniline in 39% yield as a colorless solid, using a similar approach to that described for **19**: mp 264–265 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 2.98–3.00 (4H, m), 3.20 (3H, s), 3.71–3.73 (4H, m), 5.06 (2H, d, J = 6.4 Hz), 6.73 (2H, d, J = 8.8 Hz), 7.14 (1H, br s), 7.36 (2H, br), 7.49–7.56 (2H, m), 7.64–7.70 (1H, m), 7.51 (2H, d, J = 9.3 Hz), 7.75 (1H, br s), 7.97 (1H, dd, J = 1.4, 8.3 Hz), 8.54 (1H, s), 9.22 (1H, s), 9.55 (1H, br s); FAB MS m/e $[M+H]^+$ 483. Anal. Calcd for $C_{23}H_{26}N_6O_4S$: C, 57.25; H, 5.43; N, 17.42; S, 6.65. Found: C, 57.06; H, 5.57; N, 17.16; S, 6.51.

5.1.23. 2-[(4-Morpholin-4-ylphenyl)amino]-4-[(2-nitrobenzyl) amino]pyrimidine-5-carboxamide (25c)

Compound **25c** was prepared from compound **16** and 4-morpholinoaniline in 50% yield as a yellow solid, using a similar approach to that described for **19**: mp 264–265 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 2.99–3.01 (4 H, m), 3.72–3.75 (4H, m), 4.95 (2H, d, J = 5.9 Hz), 6.71 (2H, br d, J = 7.8 Hz), 7.28 (3H, br), 7.51–7.53 (2H, m), 7.69–7.72 (2H, m), 8.14 (1H, d, J = 7.8 Hz), 8.54 (1H, s), 9.25 (1H, br s), 9.62 (1H, br s); FAB MS m/e $[M+H]^+$ 450. Anal. Calcd for $C_{22}H_{23}N_7O_4$: C, 58.79; H, 5.16; N, 21.81. Found: C, 58.83; H, 5.09; N, 21.60.

5.1.24. 4-(Benzylamino)-2-[(4-pyrrolidine-1-ylphenyl)-amino]pyrimidine-5-carboxamide hydrochloride (21)

4-Pyrrolidine-1-ylaniline (500 mg, 3.08 mmol) and 4 M HCl–dioxane (0.75 mL) were added to a solution of **14** (400 mg, 1.52 mmol) in NMP (5 mL) and stirred for 2 h at 90 °C. The reaction mixture was cooled and poured into H_2O (50 mL), and neutralized with saturated aqueous $NaHCO_3$. The mixture was extracted with AcOEt–THF(2:1), and the organic layer was washed successively with H_2O and saturated aqueous NaCl, dried and concentrated in vacuo. The residue was chromatographed on silica gel with elution using $CHCl_3$–MeOH (100:1 to 20:1), to give crude product (227 mg). 4 M HCl–dioxane (0.5 mL) was added to a solution of the crude product in MeOH–THF (1:1, 10 mL) and the mixture was concentrated in vacuo. The resulting solid was recrystallized from MeOH–THF–H_2O to give **21** (206 mg, 31%) as a colorless solid. Mp 234–238 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.94–1.98 (4H, m), 3.26 (4H, br), 4.66 (2H, br s), 6.56 (2H, br s), 7.24–7.36 (7H, m), 7.65 (1H, br s), 8.23 (1H, br s), 8.55 (1H, br s), 10.28 (1H, s), 10.36 (1H, s); FAB MS m/e $[M+H]^+$ 389. Anal. Calcd for $C_{22}H_{24}N_6O_2{\cdot}HCl{\cdot}H_2O$: C, 59.65; H, 6.14; N, 18.97; Cl, 8.00. Found: C, 59.53; H, 6.18; N, 18.86; Cl, 8.15.

5.1.25. 4-[(2-Aminobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25d)

Palladium (10%) on carbon (100 mg) was added to a solution of **25c** (860 mg, 1.91 mmol) in DMF (10 mL) and the mixture was stirred for 16 h at room temperature under a hydrogen atmosphere. The mixture was filtered with Celite and the filtrate was concentrated in vacuo. The resulting solid was recrystallized from MeOH–THF to give **25d** (508 mg, 63%) as a colorless solid: mp 213–214 °C; ^{1}H NMR (DMSO-d_6) δ 3.00–3.03 (4H, m), 3.71–3.73 (4H, m), 4.50 (2H, d, J = 5.9 Hz), 5.02 (2H, br), 6.48 (1H, t, J = 7.3 Hz), 6.64 (1H, d, J = 7.3 Hz), 6.84 (2H, d, J = 8.8 Hz), 6.96 (2H, dt, J = 1.5, 8.1 Hz), 7.00–7.03 (2H, m), 7.55 (2H, d, J = 8.8 Hz), 7.72 (1H, br), 8.49 (1H, s), 9.27 (1H, br s); FAB MS m/e $[M+H]^+$ 420. Anal. Calcd for $C_{22}H_{25}N_7O_2$: C, 62.72; H, 6.03; N, 23.27. Found: C, 62.50; H, 5.87; N, 23.11.

5.1.26. 4-{[2-(Acetylamino)benzyl]amino}-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25e)

Acetic anhydride (0.1 mL) was added to a solution of **25d** (304 mg, 0.92 mmol) in pyridine (5 mL) at 0 °C and the mixture was stirred for 0.5 h at room temperature. The reaction mixture was diluted with water and the resulting solid was collected by filtration and washed with hot MeOH–THF to give **25e** (141 mg, 42%) as a colorless needle: mp 265–266 °C; ^{1}H NMR (DMSO-d_6) δ 3.00–3.02 (4H, m), 3.71–3.74 (4H, m), 4.67 (2H, d, J = 5.9 Hz), 6.82 (2H, d J = 9.3 Hz), 7.09–7.28 (4H, m), 7.48–7.52 (3H, m), 7.77 (1H, br), 8.51 (1H, s), 9.26 (1H, br), 9.43 (1H, s), 9.46 (1H, br); FAB MS m/e $[M-H]^-$ 460. Anal. Calcd for $C_{24}H_{27}N_7O_3$: C, 62.46; H, 5.90; N, 21.24. Found: C, 62.36; H, 6.05; N, 21.21.

5.1.27. 4-Benzylamino-2-[(4-piperazin-1-ylphenyl)amino]-pyrimidine-5-carboxamide (27)

4 M HCl–dioxane (1.5 mL) was added to a solution of **26** (738 mg, 1.47 mmol) in dioxane (10 mL) and the mixture was stirred for 0.5 h at room temperature. H_2O (3 mL) was added and the reaction mixture was stirred for 2 h at 90 °C. The mixture was then diluted with AcOEt (30 mL) and THF (20 mL) and basicified with saturated aqueous $NaHCO_3$. 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, and the resulting solid was collected by filtration and washed with EtOH. The collected solid was recrystallized from EtOH–THF to give **27** (413 mg, 70%) as an ivory solid: mp 223–226 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.81–2.83 (4H, m), 2.94–2.96 (4H, m), 4.66 (2H, d, J = 5.8 Hz), 6.78 (2H, d, J = 9.3 Hz), 7.14 (1H, br), 7.23–7.28 (1H, m), 7.32–7.34 (4H, m), 7.48 (2H, d, J = 9.3 Hz), 7.74 (1H, br), 8.51 (1H, s), 9.24 (1H, s), 9.49 (1H, s); FAB MS m/e $[M+H]^+$ 404. Anal. Calcd for $C_{22}H_{25}N_7O{\cdot}0.2H_2O$: C, 64.91; H, 6.29; N, 24.09. Found: C, 64.94; H, 6.20; N, 23.85.

5.1.28. Ethyl 4-(methylsulfanyl)-2-[(4-morpholin-4-ylphenyl)-amino]pyrimidine-5-carboxylate (29)

4-Morpholinoaniline (15.45 g, 86.7 mmol) and 4 M HCl–dioxane (39.4 mL) were added to a solution of **28**[7] (18.3 g, 78.8 mmol) in NMP (210 mL) and the mixture was stirred for 5 h at 90 °C. The reaction mixture was then diluted with AcOEt (600 mL) and THF (200 mL) and basicified with saturated aqueous $NaHCO_3$. The resulting precipitate was collected and washed with MeOH to **29** (16.4 g, 56%) as an ivory solid, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 1.32 (3H, t, J = 7.2 Hz), 2.45 (3H, s), 3.05–3.07 (4H, m), 3.72–3.74 (4H, m), 4.25 (2H, q, J = 7.1 Hz), 6.92 (2H, d, J = 8.8 Hz), 7.58 (2H, d, J = 8.8 Hz), 8.67 (1H, s), 10.01 (1H, br); FAB MS m/e $[M-H]^-$ 373.

5.1.29. 4-(Methylsulfanyl)-2-[(4-morpholin-4-ylphenyl)amino]-pyrimidine-5-carboxylic acid (30)

1 M NaOH (82 mL) was added to a solution of **29** (20.6 g, 55.0 mmol) in MeOH (100 mL) and THF (100 mL) and the mixture was stirred for 14 h at 50 °C. 1 M HCl (82 mL) was added and the resulting solid was collected by filtration and washed with H_2O to give **30** (18.2 g, 96%) as a pale brown solid, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 2.43 (3H, s), 3.05–3.08 (4H, m), 3.73–3.75 (4H, m), 6.93 (2H, d, J = 8.8 Hz), 7.58 (2H, d, J = 8.8 Hz), 8.64 (1H, s), 9.91 (1H, br); FAB MS m/e $[M-H]^-$ 345.

5.1.30. 4-(Methylsulfanyl)-2-[(4-morpholin-4-ylphenyl)amino]-pyrimidine-5-carboxamide (31)

EDC·HCl (12.6 g, 65.8 mmol) and HOBt (8.9 g, 65.8 mmol) were added to a solution of **30** (19.0 g, 54.9 mmol) in DMF (200 mL). After stirring for 20 min at room temperature, 28% NH_4OH (10.3 mL) was added 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 with H_2O to give **31** (14.4 g, 98%) as a colorless solid, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 2.38 (3H, s), 3.04–3.06 (4H, m), 3.72–3.74 (4H, m), 6.91 (2H, d, J = 9.2 Hz), 7.18 (1H, br), 7.58 (2H, d, J = 9.2 Hz), 7.74 (1H, br), 8.52 (1H, s), 9.66 (1H, br); FAB MS m/e $[M-H]^-$ 346.

5.1.31. 4-(Methylsulfinyl)-2-{[4-(4-oxidomorpholin-4-yl)-phenyl]amino}pyrimidine-5-carboxamide (32)

m-CPBA (2.56 g) was added portionwise to a solution of **31** (1.00 g, 2.89 mmol) in DMA (10 mL) at below 5 °C and the mixture was stirred for 3 h. The reaction mixture was then diluted with H_2O, and the resulting solid was collected by filtration and concentrated in vacuo. The residue was chromatographed on Daisogel® SP-120-40/60-ODS-B (Daiso Co. Ltd, Osaka, Japan) with elution using H_2O–MeOH (5:1 to 2:1) to give **32** (650 mg, 62%) as a yellow solid: ^{1}H NMR (DMSO-d_6) δ 2.82 (3H, s), 2.86–2.91 (2H, m), 3.76–3.79 (2H, m), 3.87–4.03 (2H, m), 4.36–4.42 (2H, m), 7.65 (0.5H, br), 7.85 (0.5H, br), 8.06 (4.5H, br), 8.20 (0.5H, br), 8.95 (1H, s), 10.76 (1H, br); FAB MS m/e $[M]^+$ 361.

5.1.32. 4-[(2-Methylbenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25f)

2-Methylbenzylamine (289 mg, 2.39 mmol) was added to a solution of **32** (300 mg, 0.795 mmol) and diisopropylethylamine (0.16 mL, 0.954 mmol) in NMP (5 mL) and the mixture was stirred for 3 h at 80 °C and cooled to room temperature. 0.42 M aqueous sodium bisulfite (4 mL) and 28% NH_4OH (0.5 mL) were added and the mixture was extracted with $CHCl_3$. The organic layer was washed with saturated aqueous NaCl, dried and concentrated in vacuo, and the residue was chromatographed on silica gel with elution using $CHCl_3$–MeOH (100:1 to 25: 1) to give a crude solid, which was recrystallized from MeOH–THF to give **25f** (79 mg, 24%) as a pale yellow solid. Mp 245–248 °C; ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.31 (3H, s), 2.99–3.02 (4H, m), 3.71–3.74 (4H, m), 4.63 (2H, d, J = 5.9 Hz), 6.78 (2H, d J = 8.8 Hz), 7.13–7.23 (5H, m), 7.47 (2H, d, J = 8.8 Hz), 7.75 (1H, br), 8.52 (1H, s), 9.27 (1H, br), 9.40 (1H, s); FAB MS m/e $[M+H]^+$ 419. Anal. Calcd for $C_{23}H_{26}N_6O_2{\cdot}0.3H_2O$: C, 65.17; H, 6.32; N, 19.83. Found: C, 65.11; H, 6.19; N, 19.78.

5.1.33. 4-[(2-Methoxybenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25g)

Compound **25g** was prepared from compound **32** and 2-methoxybenzylamine in 18% yield as a colorless solid, using a similar approach to that described for **25f**: mp 210–213 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 3.00–3.03 (4H, m), 3.72–3.74 (4H, m), 3.85 (3H, s), 4.61 (2H, d, J = 5.8 Hz), 6.79 (2H, d, J = 8.8 Hz), 6.89 (1H, t, J = 7.3 Hz), 7.04 (1H, t, J = 8.3 Hz), 7.10 (1H, br), 7.18 (1H, t, J = 7.3 Hz), 7.25 (2H, dt, J = 1.5, 7.8 Hz), 7.51 (2H, d, J = 8.8 Hz), 7.71 (1H, br), 8.50 (1H, s), 9.24 (1H, br), 9.40 (1H, s); FAB MS m/e $[M+H]^+$ 435. Anal. Calcd for $C_{23}H_{26}N_6O_3{\cdot}0.2H_2O$: C, 63.06; H, 6.07; N, 19.18. Found: C, 63.09; H, 6.01; N, 19.23.

5.1.34. 4-[(2-Hydroxybenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25h)

Compound **25h** was prepared from compound **32** and 2-hydroxybenzylamine in 22% yield as a pale yellow solid, using a similar approach to that described for **25f**: mp 257–261 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 3.01–3.04 (4H, m), 3.72–3.74 (4H, m), 4.58 (2H, d, J = 5.4 Hz), 6.73 (1H, t, J = 7.3 Hz), 6.83–6.86 (3H, m), 7.05–7.10 (3H, m), 7.56 (2H, d, J = 9.3 Hz), 7.70 (1H, br), 8.49 (1H, s), 9.25 (1H, br), 9.39 (1H, br), 9.64 (1H, s); FAB MS m/e $[M+H]^+$ 421. Anal. Calcd for $C_{23}H_{26}N_6O_2{\cdot}0.2H_2O$: C, 62.32; H, 5.80; N, 19.82. Found: C, 62.08; H, 5.81; N, 19.71.

5.1.35. 4-[(2-Chlorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25i)

Compound **25i** was prepared from compound **32** and 2-chlorobenzylamine in 15% yield as a colorless solid, using a similar approach to that described for **25f**: mp 263–267 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 2.99–3.01 (4H, m), 3.72–3.74 (4H, m), 4.72 (2H, d, J = 5.9 Hz), 6.76 (2H, d, J = 8.8 Hz), 7.14 (1H, br), 7.28–7.29 (3H, m), 7.38–7.40 (1H, m), 7.50–7.52 (2H, m), 7.77 (1H, br), 8.53 (1H, s), 9.27 (1H, br), 9.54 (1H, s); FAB MS m/e $[M+H]^+$ 439. Anal. Calcd for $C_{22}H_{23}N_6O_2Cl{\cdot}0.5H_2O$: C, 58.99; H, 5.40; N, 18.76; Cl, 7.91. Found: C, 58.86; H, 5.20; N, 18.59; Cl, 8.02.

5.1.36. 4-[(2-Fluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25j)

Compound **25j** was prepared from compound **32** and 2-fluorobenzylamine in 35% yield as a colorless needle, using a similar approach to that described for **25f**: mp >260 °C (dec) (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 3.01–3.03 (4H, m), 3.72–3.74 (4H, m), 4.71 (2H, d, J = 5.9 Hz), 6.80 (2H, d, J = 8.8 Hz), 7.13–7.34 (5H, m), 7.45 (2H, d, J = 8.8 Hz), 7.79 (1H, br), 8.52 (1H, s), 9.34 (1H, s), 9.54 (1H, s); FAB MS m/e [M+H]$^+$ 423. Anal. Calcd for $C_{22}H_{23}N_6O_2F$: C, 62.55; H, 5.49; N, 19.89; F, 4.50. Found: C, 62.20; H, 5.45; N, 19.82; F, 4.51.

5.1.37. 2-[(4-Morpholin-4-ylphenyl)amino]-4-[(2-trifluoromethylbenzyl)amino]pyrimidine-5-carboxamide (25k)

Compound **25k** was prepared from compound **32** and 2-trifluoromethylbenzylamine in 15% yield as a yellow solid, using a similar approach to that described for **25f**: mp 263–267 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 2.96–2.98 (4H, m), 3.71–3.73 (4H, m), 4.87 (2H, d, J = 5.4 Hz), 6.70 (2H, br d, J = 7.3 Hz), 7.15 (1H, br), 7.37 (2H, br d, J = 7.3 Hz), 7.48–7.50 (2H, m), 7.63 (1H, t, J = 7.4 Hz), 7.77–7.91 (2H, m), 8.55 (1H, s), 9.28 (1H, br), 9.56 (1H, s); FAB MS m/e [M+H]$^+$ 473. Anal. Calcd for $C_{23}H_{23}N_6O_2F_3$: C, 58.47; H, 4.91; N, 17.79; F, 12.06. Found: C, 58.25; H, 4.98; N, 17.77; F, 12.06.

5.1.38. 4-{[2-(Methylsulfanyl)benzyl]amino}-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide hydrochloride (25l)

Compound **25l** was prepared from compound **32** and 2-methylsulfanylbenzylamine in 11% yield and isolated as a yellow solid, using a similar approach to that described for **25f**: mp 212–213 °C (dec) (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 3.10 (4H, m), 3.74–3.77 (4H, m), 4.66 (2H, d, J = 5.3 Hz), 6.92 (2H, br), 7.13–7.15 (2H, m), 7.30–7.37 (4H, m), 7.60 (1H, br), 8.18 (1H, br), 8.52 (1H, br), 10.19 (1H, br), 10.27 (1H, br); FAB MS m/e [M+H]$^+$ 451. Anal. Calcd for $C_{23}H_{26}N_6O_2S$·HCl: C, 56.72; H, 5.59; N, 17.26; Cl, 7.28; S, 6.58. Found: C, 56.44; H, 5.49; N, 17.19; Cl, 7.20; S, 6.52.

5.1.39. 4-[(3-Fluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25m)

Compound **25m** was prepared from compound **32** and 3-fluorobenzylamine in 30% yield as a pale yellow solid, using a similar approach to that described for **25f**: mp 246–249 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 2.97–3.04 (4H, m), 3.71–3.74 (4H, m), 4.67 (2H, d, J = 5.8 Hz), 6.80 (2H, d, J = 8.8 Hz), 7.04–7.18 (4H, m), 7.35–7.41 (1H, m), 7.46 (2H, d, J = 8.8 Hz), 7.75 (1H, br), 8.52 (1H, s), 9.27 (1H, s), 9.53 (1H, s); FAB MS m/e [M+H]$^+$ 423. Anal. Calcd for $C_{22}H_{23}N_6O_2F$·0.2H_2O: C, 62.02; H, 5.54; N, 19.73; F, 4.46. Found: C, 61.82; H, 5.43; N, 19.75; F, 4.76.

5.1.40. 4-[(3-Chlorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25n)

Compound **25n** was prepared from compound **32** and 3-chlorobenzylamine in 25% yield as a colorless solid, using a similar approach to that described for **25f**: mp 251–254 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 3.00–3.03 (4H, m), 3.72–3.74 (4H, m), 4.65 (2H, d, J = 5.8 Hz), 6.79 (2H, d, J = 8.8 Hz), 7.18 (1H, br), 7.27–7.30 (2H, m), 7.34–7.38 (2H, m), 7.44–7.42 (2H, m), 7.75 (1H, br), 8.52 (1H, s), 9.27 (1H, br), 9.54 (1H, s); FAB MS m/e [M+H]$^+$ 439. Anal. Calcd for $C_{22}H_{23}N_6O_2Cl$·0.2H_2O: C, 59.71; H, 5.33; N, 18.99; Cl, 8.01. Found: C, 59.82; H, 5.11; N, 18.98; Cl, 7.71.

5.1.41. 2-[(4-Morpholin-4-ylphenyl)amino]-4-[(3-trifluoromethylbenzyl)amino]pyrimidine-5-carboxamide (25o)

Compound **25o** was prepared from compound **32** and 3-trifluoromethylbenzylamine in 17% yield as a pale yellow solid, using a similar approach to that described for **25f**: mp 232–234 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 2.98–3.00 (4H, m), 3.71–3.74 (4H, m), 4.74 (2H, d, J = 5.8 Hz), 6.76 (2H, d, J = 8.8 Hz), 7.15 (1H, br), 7.43 (2H, br d, J = 8.8 Hz), 7.54–7.62 (3H, m), 7.69 (1H, s), 7.73 (1H, br), 8.52 (1H, s), 9.28 (1H, br), 9.58 (1H, s); FAB MS m/e [M+H]$^+$ 473. Anal. Calcd for $C_{23}H_{23}N_6O_2F_3$: C, 58.47; H, 4.91; N, 17.79; F, 12.06. Found: C, 58.70; H, 5.04; N, 17.69; F, 12.00.

5.1.42. 4-[(3-Methoxybenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25p)

Compound **25p** was prepared from compound **32** and 3-methoxybenzylamine in 29% yield as a colorless solid, using a similar approach to that described for **25f**: mp 220–222 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 3.00–3.02 (4H, m), 3.70 (3H, s), 3.71–3.74 (4H, m), 4.63 (2H, d, J = 5.4 Hz), 6.71–6.80 (3H, m), 6.82–6.89 (2H, m), 7.11 (1H, br), 7.23–7.27 (1H, m), 7.51 (2H, d, J = 8.8 Hz), 7.75 (1H, br), 8.51 (1H, s), 9.28 (1H, br), 9.49 (1H, s); FAB MS m/e [M+H]$^+$ 435. Anal. Calcd for $C_{23}H_{26}N_6O_3$·0.2H_2O: C, 63.06; H, 6.07; N, 19.18. Found: C, 62.93; H, 5.92; N, 19.16.

5.1.43. 4-[(3-Hydroxybenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25q)

Compound **25q** was prepared from compound **32** and 3-hydroxybenzylamine in 31% yield as a colorless solid, using a similar approach to that described for **25f**: mp 241–244 °C (dec) (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 3.00–3.02 (4H, m), 3.71–3.74 (4H, m), 4.58 (2H, d, J = 5.9 Hz), 6.62–6.41 (1H, m), 6.72–6.75 (2H, m), 6.81 (2H, d, J = 8.8 Hz), 7.11–7.14 (2H, m), 7.50 (2H, d, J = 8.8 Hz), 7.75 (1H, br), 8.51 (1H, s), 9.26 (1H, br), 9.35 (1H, br), 9.47 (1H, s); FAB MS m/e [M+H]$^+$ 421. Anal. Calcd for $C_{23}H_{26}N_6O_2$·0.2H_2O: C, 62.32; H, 5.80; N, 19.82. Found: C, 62.26; H, 5.94; N, 19.79.

5.1.44. 4-[(4-Fluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25r)

Compound **25r** was prepared from compound **32** and 4-fluorobenzylamine in 25% yield as a colorless solid, using a similar approach to that described for **25f**: mp 253–257 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 3.01–3.03 (4H, m), 3.72–3.74 (4H, m), 4.64 (2H, d, J = 6.1 Hz), 6.82 (2H, d, J = 9.1 Hz), 7.10 (1H, br), 7.14–7.17 (2H, m), 7.35–7.38 (2H, m), 7.50 (2H, d, J = 9.2 Hz), 7.75 (1H, br), 8.50 (1H, s), 9.25 (1H, s), 9.48 (1H, s); FAB MS m/e [M+H]$^+$ 423. Anal. Calcd for $C_{22}H_{23}N_6O_2F$·0.2H_2O: C, 62.02; H, 5.54; N, 19.73; F, 4.46. Found: C, 61.81; H, 5.48; N, 19.69; F, 4.73.

5.1.45. 4-[(2,3-Difluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25s)

Compound **25s** was prepared from compound **32** and 4-fluorobenzylamine in 32% yield as a yellow solid, using a similar approach to that described for **25f**: mp 242–245 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 2.97–3.03 (4H, m), 3.72–3.74 (4H, m), 4.74 (2H, d, J = 5.9 Hz), 6.77 (2H, d, J = 8.8 Hz), 7.10–7.17 (3H, m), 7.28–7.35 (1H, m), 7.41 (2H, d, J = 8.8 Hz), 7.78 (1H, br), 8.53 (1H, s), 9.29 (1H, s), 9.53 (1H, s); FAB MS m/e [M+H]$^+$ 441. Anal. Calcd for $C_{22}H_{22}N_6O_2F_2$·0.2H_2O: C, 59.51; H, 5.08; N, 18.93; F, 8.56. Found: C, 59.39; H, 4.94; N, 18.86; F, 8.46.

5.1.46. 4-[(2,4-Difluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25t)

Compound **25t** was prepared from compound **32** and 2,4-difluorobenzylamine in 27% yield as a colorless solid, using a similar approach to that described for **25f**: mp 232–234 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 3.00–3.03 (4H, m), 3.72–3.74 (4H, m), 4.67 (2H, d, J = 5.9 Hz), 6.80 (2H, d, J = 8.8 Hz), 7.00–7.05 (1H, m), 7.12 (1H, br), 7.26–7.34 (2H, m), 7.46 (2H, d, J = 8.8 Hz), 7.76 (1H, br), 8.52 (1H, s), 9.28 (1H, s), 9.47 (1H, s); FAB MS m/e [M+H]$^+$ 441. Anal. Calcd for $C_{22}H_{22}N_6O_2F_2$·0.4H_2O: C, 59.03; H, 5.13; N, 18.77; F, 8.49. Found: C, 59.08; H, 5.00; N, 18.77; F, 8.69.

5.1.47. 4-[(2,5-Difluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25u)

Compound **25u** was prepared from compound **32** and 2,5-difluorobenzylamine in 26% yield as a pale brown solid, using a similar approach to that described for **25f**: mp 243–245 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 2.97–3.05 (4H, m), 3.72–3.74 (4H, m), 4.68 (2H, d, J = 5.9 Hz), 6.78 (2H, d, J = 8.8 Hz), 7.05 (1H, br), 7.11–7.17 (2H, m), 7.28–7.33 (1H, m), 7.43 (2H, d, J = 8.8 Hz), 7.79 (1H, br), 8.53 (1H, s), 9.30 (1H, s), 9.50 (1H, s); FAB MS m/e [M+H]$^+$ 441. Anal. Calcd for $C_{22}H_{22}N_6O_2F_2$: C, 59.99; H, 5.03; N, 19.08; F, 8.63. Found: C, 59.95; H, 4.98; N, 19.02; F, 8.51.

5.1.48. 4-[(2,6-Difluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25v)

Compound **25v** was prepared from compound **32** and 2,6-difluorobenzylamine in 32% yield as a colorless solid, using a similar approach to that described for **25f**: mp 245–248 °C (dec) (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 3.03–3.05 (4H, m), 3.72–3.75 (4H, m), 4.79 (2H, d, J = 5.9 Hz), 6.89 (2H, d, J = 8.8 Hz), 7.10 (1H, br), 7.12–7.18 (2H, m), 7.40–7.47 (1H, m), 7.64 (2H, d, J = 8.8 Hz), 7.75 (1H, br), 8.52 (1H, s), 9.35 (1H, s), 9.47 (1H, s); FAB MS m/e [M+H]$^+$ 441. Anal. Calcd for $C_{22}H_{22}N_6O_2F_2$: C, 59.99; H, 5.03; N, 19.08; F, 8.63. Found: C, 59.98; H, 4.93; N, 19.04; F, 8.55.

5.1.49. 4-[(3,5-Difluorobenzyl)amino]-2-[(4-morpholin-4-ylphenyl)amino]pyrimidine-5-carboxamide (25w)

Compound **25w** was prepared from compound **32** and 3,5-difluorobenzylamine in 44% yield as a colorless solid, using a similar approach to that described for **25f**: mp 227–230 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 2.98–3.03 (4H, m), 3.72–3.74 (4H, m), 4.61 (2H, d, J = 5.8 Hz), 6.79 (2H, d, J = 8.8 Hz), 7.01–7.11 (4H, m), 7.42 (2H, d, J = 8.8 Hz), 7.70 (1H, br), 8.52 (1H, s), 9.29 (1H, s), 9.54 (1H, s); FAB MS m/e [M+H]$^+$ 441. Anal. Calcd for $C_{22}H_{22}N_6O_2F_2{\cdot}0.2H_2O$: C, 59.51; H, 5.08; N, 18.93; F, 8.56. Found: C, 59.13; H, 5.00; N, 18.90; F, 8.92.

5.1.50. 2-[(4-Morpholin-4-ylphenyl)amino]-4-[(2,3,5-trifluorobenzyl)amino]pyrimidine-5-carboxamide (25x)

Compound **25x** was prepared from compound **32** and 2,3,5-trifluorobenzylamine in 23% yield as a colorless solid, using a similar approach to that described for **25f**: mp 229–231 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 2.99–3.02 (4H, m), 3.72–3.75 (4H, m), 4.73 (2H, d, J = 5.9 Hz), 6.77 (2H, d, J = 8.8 Hz), 6.90 (1H, br), 7.16 (1H, br), 7.39–7.46 (3H, m), 7.79 (1H, br), 8.54 (1H, s), 9.31 (1H, s), 9.53 (1H, s); FAB MS m/e [M+H]$^+$ 441. Anal. Calcd for $C_{22}H_{21}N_6O_2F_3{\cdot}0.7H_2O$: C, 56.10; H, 4.79; N, 17.84; F, 12.10. Found: C, 55.74; H, 4.54; N, 17.49; F, 12.39.

5.1.51. 2-[(4-Morpholin-4-ylphenyl)amino]-4-[(2,3,6-trifluorobenzyl)amino]pyrimidine-5-carboxamide (25y)

Compound **25y** was prepared from compound **32** and 2,3,6-trifluorobenzylamine in 20% yield as a brown solid, using a similar approach to that described for **25f**: mp 226–229 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6) δ 3.00–3.08 (4H, m), 3.72–3.75 (4H, m), 4.83 (2H, d, J = 5.9 Hz), 6.87 (2H, d, J = 8.8 Hz), 7.13 (1H, br), 7.16–7.22 (1H, m), 7.44–7.53 (1H, m), 7.57 (2H, d, J = 8.8 Hz), 7.76 (1H, br), 8.52 (1H, s), 9.34 (1H, s), 9.54 (1H, s); FAB MS m/e [M+H]$^+$ 441. Anal. Calcd for $C_{22}H_{21}N_6O_2F_3{\cdot}0.7H_2O$: C, 56.10; H, 4.79; N, 17.84; F, 12.10. Found: C, 56.49; H, 4.63; N, 17.48; F, 12.03.

5.1.52. 2-[(4-Morpholin-4-ylphenyl)amino]-4-[(2,3,6-trifluorobenzyl)amino]pyrimidine-5-carboxamide (25y) hydrochloride

Compound **25y** hydrochloride was prepared from compound **25y** using a similar manner to that described for **21**: mp 275–276 °C (EtOH–H_2O); ^{1}H NMR (DMSO-d_6) δ 3.13–3.15 (4H, m), 3.78–3.80 (4H, m), 4.86 (2H, d, J = 5.9 Hz), 7.05 (2H, br), 7.17–7.23 (1H, m), 7.45–7.54 (3H, m), 7.63 (1H, br), 8.23 (1H, br), 8.60 (1H, s), 10.34 (1H, s), 10.48 (1H, s); FAB MS m/e [M+H]$^+$ 441. Anal. Calcd for $C_{22}H_{21}N_6O_2F_3{\cdot}HCl$: C, 53.39; H, 4.48; N, 16.98; Cl, 7.16, F, 11.52. Found: C, 53.44; H, 4.30; N, 16.87; Cl, 11.39; F, 11.39.

5.2. QSAR analysis

Calculations of molecular mass, molecular volume, molar refractivity, Verloop L and B_1–B_5, ellipsoidal volume, Wiener topological index, bond dipole moment, and E-state indices were performed with TSAR.[14] $c\log P$ and MSA were calculated using CLOGP[15] and MOE,[16] respectively, and selection of descriptors was performed with the QuaSAR-Evolution algorithm.[15] IC_{50} values were converted to free energies on a negative log scale (pIC_{50}). Multiple linear regression analysis was performed with Excel 2003 (Microsoft Corp.). Combinations of descriptors with correlation coefficients >0.4 were discarded to avoid the colinearity problem. The following statistical parameters were determined for each regression equation: the number of points, n; the goodness of fit, R^2; the significance of the regression model, F; and the root mean square error, RMSE.

5.3. Biology

5.3.1. STAT6 reporter assay

The IL-4-inducible luciferase reporter plasmid and STAT6 reporter cells (FW4 reporter cells) were constructed as described previously.[7]

5.3.2. In vitro T cell differentiation

C57BL/6 mice were purchased from Charles River Laboratories. Spleen T cells were purified from total spleen cells using a nylon wool column. For in vitro differentiation assays, 1×10^6 T cells/mL were cultured in RPMI1640 (GIBCO) medium supplemented with 10% fetal bovine serum (FBS) and 5×10^{-5}M 2-mercaptoethanol and stimulated with 10 μg/mL plate-bound anti-CD3 (Cedarlane). The cells were then incubated with 10 ng/mL mouse IL-2 (PeproTech) plus 10 ng/mL mouse IL-12 (PeproTech) (Th1) or 10 ng/mL mouse IL-4 (PeproTech) plus 1 μg/mL of anti-CD28 (Pharmingen) (Th2) for 2 days. The cells were then cultured in the medium with the same concentrations of IL-2, IL-12, and IL-4 for another 3 days. Test compounds were added to each well for 5 days. The final DMSO concentration was 0.1%. The cultured cells were washed and restimulated with plate-bound anti-CD3 in the absence of test compound, and supernatants were collected 16 h later. Cytokine ELISAs were performed using antibodies recommended by Pharmingen.

5.4. Pharmacokinetic study

Pharmacokinetic studies were performed in 8-week-old female Balb/c mice purchased from Charles River Laboratories. Food and water were provided ad libitum. Pharmacokinetic experiments were performed after overnight fasting. For intravenous administration, compound **6** (1 mg/kg) was dissolved in 1% EtOH, 0.5% Cremophor EL, and 98.5% saline solution, compound **25a** (1 mg/kg) was dissolved in 0.5% DMSO, 1% Cremophor EL, and 98.5% saline solution, and compound **25y** (1 mg/kg) was dissolved in 1% DMSO, 1% Cremophor EL, and 98% saline solution. The compounds were administered by tail vein injection at a volume of 10 mL/kg. For oral administration, **6** (10 mg/kg), **25a** (3 mg/kg), and **25y** (1 mg/kg) were dissolved in 0.5% methylcellulose solution and administered at a volume of 10 mL/kg. Whole blood samples were taken from the abdominal vena cava of mice at 0.1, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h after intravenous administration and 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h after oral administration. Extraction of compounds from mice plasma was performed on 96-well Oasis® HLB

solid phase extraction plates.[19] The extracts were analyzed by high performance liquid chromatography-tandem mass spectroscopy (HPLC-MS-MS) using Waters 2790 Separations® and Micromass Quattro-Ultima® instruments and an XTerra MSC_{18} column (50×4.6 mm; 5 μm).

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