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Synthesis and evaluation of 2-{[2-(4-hydroxyphenyl)-ethyl]amino}pyrimidine-5-carboxamide derivatives as novel STAT6 inhibitors

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Abstract—The STAT6 (signal transducers and activators of transcription 6) protein is activated by interleukin (IL)-4 and IL-13, and plays an important role in T-helper cell 2 (Th2) differentiation. STAT6 might therefore be an excellent therapeutic target for various allergic conditions, including asthma and atopic diseases. We synthesized a series of 2-{[2-(4-hydroxyphenyl)ethyl]amino}pyrimidine-5-carboxamide derivatives and evaluated their STAT6 inhibitory activities. Among these compounds, 4-(benzylamino)-2-{[2-(3-chloro-4-hydroxyphenyl)ethyl]amino}pyrimidine-5-carboxamide (**2t**, AS1517499) showed potent STAT6 inhibition with an IC_{50} value of 21 nM, and also inhibited IL-4-induced Th2 differentiation of mouse spleen T cells with an IC_{50} value of 2.3 nM and without influencing T-helper cell 1 (Th1) differentiation induced by IL-12.

1. Introduction

$CD4^+$ T helper (Th) cells are classified into two subsets that are referred to as Th1 and Th2.[1] Th1 cells produce interferon-γ (IFN-γ), interleukin (IL)-2, and tumor necrosis factor β, and enhance 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, and are involved in the development of humoral immunity for protection against extracellular pathogens. The important physiologic functions of Th1 and Th2 cells are mutually regulated and the immune system maintains a proper balance between Th1 and Th2 responses. Several diseases are thought to be caused by imbalance of these responses: a chronically ongoing Th1 response may result in inflammatory tissue damage, whereas an excessive Th2 response may be responsible for allergic diseases; therefore, the balance of the Th1/Th2 response is closely associated with human health and disease.[2] Recent studies have shown that Th2 cytokines contribute to allergic inflammatory responses by regulating immunoglobulin E (IgE) production and the functions of eosinophils and mast cells. In particular, IL-4 is thought to be a critical factor for Th2 differentiation and is known to regulate both IgE production by B cells and mast cell function.[3]

STAT6 (signal transducers and activators of transcription 6) is a member of the STAT family of transcription factors and is specifically activated by IL-4 and IL-13. The importance of STAT6 in Th2 differentiation has been established using STAT6-deficient mice, in which T cells fail to differentiate into Th2 cells in response to IL-4 and B cells are unable to produce IgE.[4] Since many studies indicate that Th2 cytokines and IgE are major players in allergic diseases,[5] STAT6 might be an excellent therapeutic target for the treatment of various allergic conditions, including asthma and atopic diseases. However, although a few STAT6 inhibitors such as TMC-264 have been reported,[6] no compounds have been investigated in clinical trials (Fig. 1). Therefore, to find novel STAT6 inhibitors, we performed high-throughput screening of our chemical libraries using a

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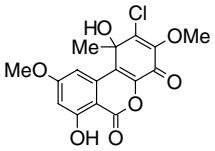
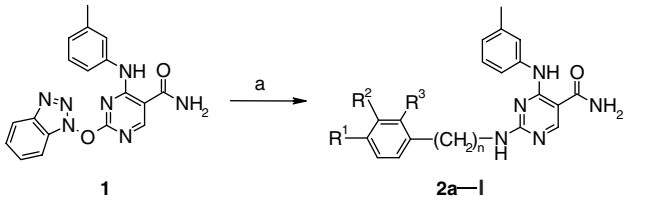


Figure 1. The Structure of TMC-264.

STAT6 reporter assay; this led to the identification of 2-{[2-(4-hydroxyphenyl)ethyl]amino}-4-[(3-methylphenyl)amino] pyrimidine-5-carboxamide (**2a**) as a STAT6 inhibitor. Herein, we describe the synthesis of a series of 2-{[2-(4-hydroxyphenyl)ethyl]amino}pyrimidine-5-carboxamide derivatives, their structure–activity relationships (SARs) for STAT6 inhibition, and the effect of one derivative on Th1/Th2 differentiation in mouse spleen T cells.

2. Chemistry

As shown in Scheme 1, 4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide derivatives **2a–l** were synthesized according to the procedure of Hisamichi et al.[7] The displacement reaction of 2-benzotriazo-l-yloxy-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide 1 with phenylalkylamines was carried out at 80 °C in *N*-methylpyrrolidinone (NMP) and afforded 4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide derivatives **2** with phenylalkylamino groups at C-2 of the pyrimidine ring.



1 **2a—l**

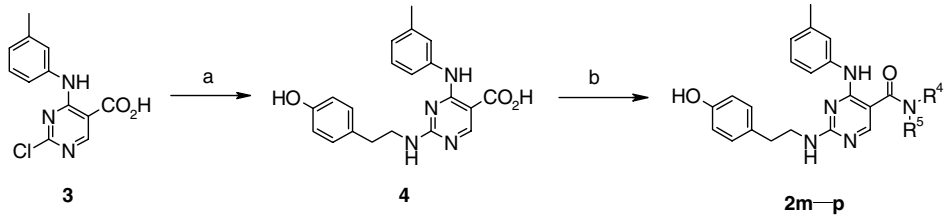
No.	R^1	R^2	R^3	n
2a	HO	H	H	2
2b	H	H	H	2
2c	MeO	H	H	2
2d	HO	H	H	1
2e	HO	H	H	3
2f	H	HO	H	2
2g	H	H	HO	2
2h	HO	Me	H	2
2i	HO	MeO	H	2
2j	HO	F	H	2
2k	HO	Cl	H	2
2l	HO	Br	H	2

Scheme 1. Reagents: (a) phenylalkylamine (see text and Section 5), NMP.

The synthesis of various *N*-alkylamide analogues of **2a** is described in Scheme 2. Treatment of 2-chloropyrimidine-5-carboxylic acid **3**[7] with tyramine, followed by condensation with the corresponding amines in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole (HOBt), resulted in formation of **2m–p**.

The synthetic route of analogues of **2a** with various substituents at C-4 of the pyrimidine ring is outlined in Scheme 3. Treatment of ethyl 2,4-dichloropyrimidine-5-carboxylate **5** with sodium thiomethoxide (NaSMe) in tetrahydrofuran (THF) at −10 °C in the presence of benzyltriethylammonium chloride ($BnEt_3NCl$) gave ethyl 2-chloro-4-methylsulfanylpyrimidine-5-carboxylate **6**. This displacement reaction proceeded very slowly below −20 °C, whereas ethyl 2,4-dimethylsulfanylpyrimidine-5-carboxylate was obtained as the major product above 0 °C. Reaction of **6** with tyramine proceeded selectively at C-2 of the pyrimidine ring to give 4-methylsulfanylpyrimidine derivative **7**. Hydrolysis of the ethoxycarbonyl group of **7** by 1 M NaOH and condensation using aqueous ammonia gave pyrimidine-5-carboxamide derivative **9**. The methylsulfanyl group of **9** was carefully oxidized with *m*-chloroperbenzoic acid (*m*-CPBA) to afford methylsulfinyl derivative **10**. In the case of using excess amounts of *m*-CPBA gave several unknown products and desirable methylsulfonyl derivative was not obtained in acceptable yield. Finally, displacement of the methylsulfinyl group of **10** with cyclohexylamine, benzylamine or aniline furnished compounds **2q**, **2r** or **2s**, respectively.

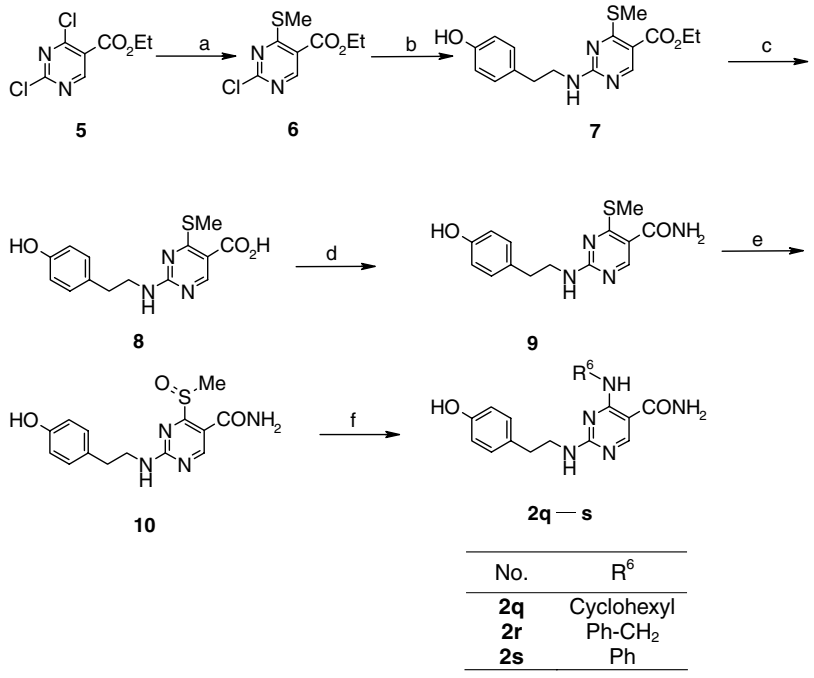
An alternative synthetic route for analogues of **2a** is described in Scheme 4. 4-Benzylamino-2-methylsulfanylpyrimidine derivatives **14** and **15**, which were readily prepared from commercially available 2-methylsulfanylpyrimidine derivative **11** according to the procedure in the literature,[7] were converted into the corresponding



3 **4** **2m—p**

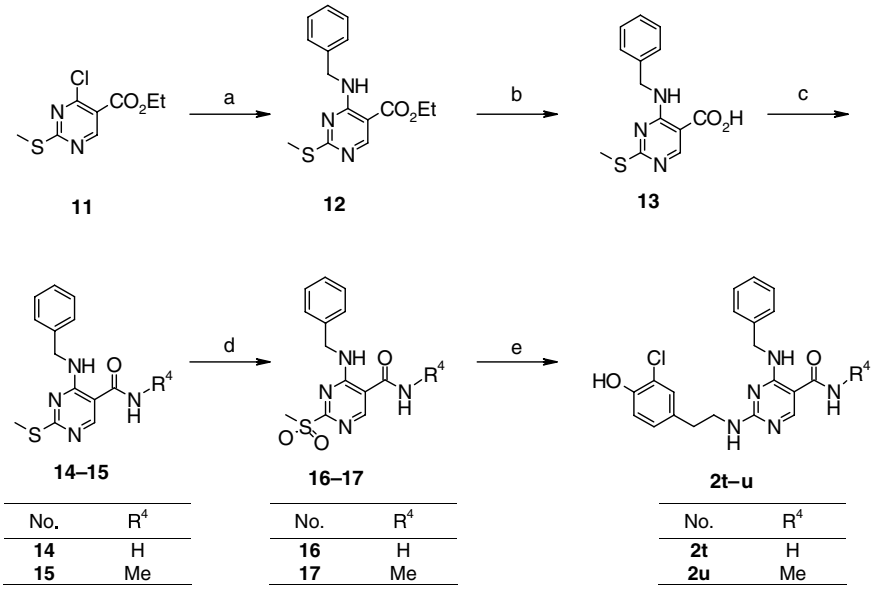
No.	NR^4R^5
2m	NHMe
2n	NHEt
2o	NH^{i}Pr
2p	NMe_2

Scheme 2. Reagents: (a) tyramine, NMP; (b) EDC·HCl, HOBt, R^4R^5NH, DMF, $^i Pr_2NEt$.



No.	R^6
2q	Cyclohexyl
2r	Ph-CH_2
2s	Ph

Scheme 3. Reagents: (a) NaSMe, $BnEt_3NCl$, THF; (b) tyramine, $^iPr_2NEt$, NMP; (c) 1 M NaOH, MeOH; (d) EDC·HCl, HOBt, NH_4OH, DMF; (e) *m*-CPBA, NMP; (f) R^6NH_2, $^iPr_2NEt$, NMP.



No.	R^4
14	H
15	Me

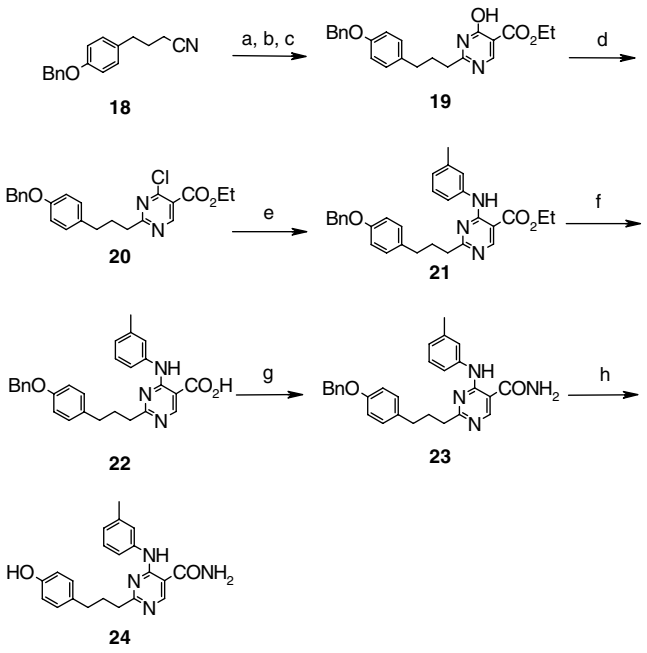
No.	R^4
16	H
17	Me

No.	R^4
2t	H
2u	Me

Scheme 4. Reagents: (a) benzylamine, $^iPr_2NEt$, MeCN; (b) 1 M NaOH, THF; (c) EDC·HCl, HOBt, NH_4OH or $MeNH_2$, DMF; (d) *m*-CPBA, NMP; (e) 2-(3-chloro-4-hydroxylphenyl)ethylamine, $^iPr_2NEt$, NMP.

methylsulfonyl derivatives **16** and **17**, respectively, by reaction with *m*-CPBA. Displacement of the methylsulfonyl group by 2-(3-chloro-4-hydroxy)phenethylamine proceeded smoothly at 80 °C and gave compounds **2t** and **2u**.

The preparation of 2-[3-(4-hydroxyphenyl)propyl]pyrimidine derivative **24** is shown in Scheme 5. Conversion of butyronitrile **18** into the corresponding amidine, followed by condensation with ethoxymethylenemalonate, gave the 2-{3-[4-(benzyloxy)phenyl]propyl}-4-hydroxypyrimidine derivative **19**. Chlorination of **19** and subsequent substitution with *m*-toluidine provided compound **21**. Hydrolysis of the ethoxycarbonyl group afforded carboxylic acid **22**, and condensation of the carboxylic acid using aqueous ammonia gave the pyrimidine-5-carboxamide derivative **23**. Finally, deprotection of the benzyl group using hydrogenation in the presence of palladium on carbon furnished compound **24**.



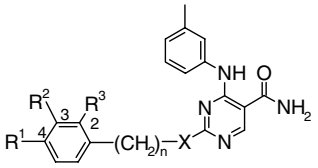
Scheme 5. Reagents: (a) HCl, EtOH–$CHCl_3$; (b) $AcONH_4$, EtOH; (c) diethyl ethoxymethylenemalonate, NaOMe, EtOH; (d) $POCl_3$, diethylaniline; (e) *m*-toluidine, $^{i}Pr_2NEt$, MeCN; (f) 1 M NaOH, EtOH–THF; (g) EDC·HCl, HOBt, NH_4OH, DMF; (h) H_2, 10% Pd–C, MeOH–THF.

3. Results and discussion

The ability of the compounds to inhibit STAT6 activation was measured in a STAT6-dependent promoter reporter assay utilizing FW4 reporter cells, which express both human IL-4 receptor α and human IL-2 receptor γ subunits and were stably transfected with an IL-4-responsive luciferase reporter plasmid.

The activities of derivatives with modifications of the 2-(4-hydroxyphenyl)ethylamino moiety are shown in Table 1. 2-[2-(4-Hydroxyphenyl)ethylamino]pyrimidine derivative **2a**, which was found in high-throughput screening, exhibited a moderate STAT6 inhibitory activity with an IC_{50} value of 190 nM. Removal or methylation of the hydroxyl group resulted in loss of activity (**2b** and **2c**) and a similar loss of activity was also observed in the case of conversion of the nitrogen into carbon at C-2 of the pyrimidine ring (**24**). These results indicate that the hydroxyl group and the NH moiety at C-2 of the pyrimidine ring are essential for STAT6 inhibition. Compounds **2d** and **2e**, in which the ethylene linker of **2a** was replaced by methylene and propylene, respectively, showed no activity. Transposition of the hydroxyl group of **2a** from the 4-position to the 3-position (**2f**) led to a 16-fold decrease in the activity, whereas the 2-hydroxyl derivative (**2g**) showed no activity. These results suggest that the position of the hydroxyl group has a significant influence on STAT6 inhibitory activity and that a 2-(4-hydroxyphenyl)ethyl moiety might place the hydroxyl group in a suitable position to increase potency. Introduction of a methyl group at the 3-position of **2a** was well tolerated and compounds with a halogen at the 3-position (**2j–l**) showed improved activity compared to compound **2a**. In particular, chloro derivative **2k** was 4-fold more potent than **2a**. In contrast, methoxy derivative **2i** was 3-fold less active than compound **2a**.

Table 1. STAT6 inhibitory activity of 4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide derivatives **2a–l** and **24**

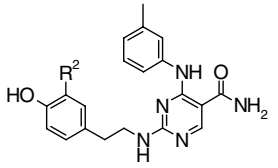


Compound	R^1	R^2	R^3	*n*	X	IC_{50}[a] (nM)
2a	HO	H	H	2	NH	190
2b	H	H	H	2	NH	16%[b]
2c	MeO	H	H	2	NH	49%[b]
2d	HO	H	H	1	NH	19%[b]
2e	HO	H	H	3	NH	23%[b]
2f	H	HO	H	2	NH	3000
2g	H	H	HO	2	NH	15%[b]
2h	HO	Me	H	2	NH	130
2i	HO	MeO	H	2	NH	660
2j	HO	F	H	2	NH	64
2k	HO	Cl	H	2	NH	45
2l	HO	Br	H	2	NH	83
24	HO	H	H	2	CH_2	32%[b]

[a] IC_{50} values were determined in triplicate in one experiment.
[b] % inhibition at 10 μm.

Vinogradova et al. have shown that chemical shifts of hydroxyl groups in substituted phenols correlate with acidity; compounds with higher chemical shifts are more acidic.[8] The ^1H NMR chemical shifts of the hydroxyl group of **2a** and **2h–l** are listed in Table 2. The hydroxyl groups of the halogen derivatives **2j–l** resonated at lower field compared to that of **2a**, and the chemical shift of the hydroxyl group in the methyl derivative **2h** was

Table 2. ^1H NMR chemical shifts of the proton of hydroxyl group and STAT6 inhibitory activity of **2a** and **2h–l**



Compound	R^2	δ[a] (ppm)	IC_{50}[b] (nM)
2a	H	8.90	190
2h	Me	8.73	130
2i	MeO	8.39	660
2j	F	9.29	64
2k	Cl	9.59	45
2l	Br	9.67	83

[a] ^1H NMR chemical shifts were measured in DMSO-d_6. See Section 5.
[b] IC_{50} values were determined in triplicate in one experiment.

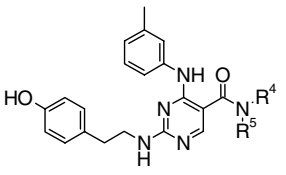
similar to that of **2a**. On the contrary, the hydroxyl group of the methoxy derivative **2i** resonated at higher field compared to that of **2a**. These data suggest that the hydroxyl group of halogen derivatives **2j–l**, which showed improved STAT6 inhibitory activity, might be more acidic than that in **2a**, and that the hydroxyl group in methoxy derivative **2i**, which had reduced activity, might be less acidic than that in **2a**. On the basis of these results, it appears likely that increased acidity of phenolic group is beneficial effect for the STAT6 inhibitory activity.

Next, the effect of the 5-carboxamide group on activity was assessed. As shown in Table 3, *N*-methyl amide **2m** exhibited activity comparable with that of **2a**, whereas replacement of the methyl group with larger substituents, such as ethyl (**2m**) or isopropyl (**2n**) groups, led to a decrease in activity. Furthermore, a greater decrease in activity was observed with the *N*,*N*-dimethyl amide derivative **2p**. These results suggest that the allowable steric bulk around the amide nitrogen might be small.

The effects of substituents at C-4 of the pyrimidine ring on STAT6 inhibitory activity are shown in Table 4. Whereas phenyl derivative **2s** was about 2-fold less active than **2a**, cyclohexyl analogue **2q** and benzyl analogue **2r** were about 2-fold more potent than compound **2a**. In the case of 4-benzylamino derivatives, introduction of a chloro group at the 3-position also improved the activity; compounds **2t** and **2u** showed potent STAT6 inhibitory activity with IC_{50} values of 21 and 30 nM, respectively.

The effect of the potent STAT6 inhibitor **2t** on differentiation of T cells to Th cell subsets was examined. Mouse spleen T cells stimulated by anti-CD3 antibody in the presence of IL-12 or IL-4 differentiate into Th1 or Th2 cells, respectively. As shown in Figure 2, in the presence of IL-12 compound **2t** showed no effect on the ability of spleen T cells to produce IFN-γ, which is a marker of Th1 differentiation. In contrast, in the presence of IL-4 compound **2t** inhibited production of IL-4, a marker of Th2 differentiation, with an IC_{50} value of 2.3 nM.[9] These results suggest that compound **2t** selectively inhibits Th2 differentiation without affecting Th1 differentiation; we note that a similar response to IL-4 was observed in spleen T cells from STAT6-deficient mice.[4] As far as we are aware, our results provide the first demonstration of a low molecular weight compound that selectively inhibits Th2 differentiation by regulation of the IL-4 signaling cascade.[10]

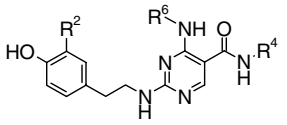
Table 3. STAT6 inhibitory activity of 2-{[2-(4-hydroxyphenyl)-ethyl]amino}-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide derivatives **2a** and **2m–p**



Compound	R^4	R^5	IC_{50}[a] (nM)
2a	H	H	190
2m	Me	H	170
2n	Et	H	380
2o	$CHMe_2$	H	1100
2p	Me	Me	8200

[a] IC_{50} values were determined by in triplicate in one experiment.

Table 4. STAT6 inhibitory activity of 2-{[2-(4-hydroxyphenyl)ethyl]amino}pyrimidine-5-carboxamide derivatives **2a** and **2q–u**



Compound	R^2	R^4	R^6	IC_{50}[a] (nM)
2a	H	H	3-Me–Ph	190
2s	H	H	Ph	410
2q	H	H	Cyclohexyl	100
2r	H	H	$PhCH_2$	88
2t	Cl	H	$PhCH_2$	21
2u	Cl	Me	$PhCH_2$	30

[a] IC_{50} values were determined by in triplicate in one experiment.

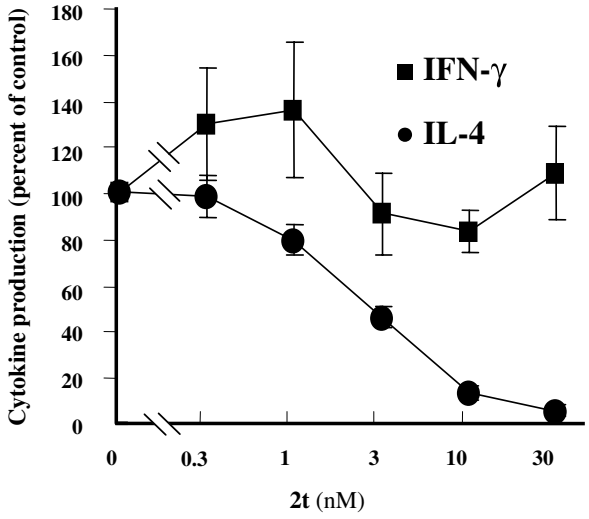


Figure 2. The effect of compound **2t** on cytokine production in T cells from spleen of mice. Squares indicate the amount of IFN-γ produced by cells cultured in the presence of IL-12, which induces differentiation to Th1 cells, and circles indicate the amount of IL-4 produced by cells cultured with IL-4, which induces Th2 cells. The cytokine levels in control cells (DMSO) were as follows (ng/ml): IFN-γ = 292 ± 84.0; IL-4 = 2.68 ± 0.27. Data are shown as means ± SEM expressed as percentages relative to values from the control cells ($n = 6$).

4. Conclusion

In conclusion, we have reported a series of 2-{[2-(4-hydroxyphenyl)ethyl]amino}pyrimidine-5-carboxamide derivatives as novel STAT6 inhibitors and investigated

the SARs of these derivatives. With regard to substituents at C-2 of the pyrimidine ring, a 2-(4-hydroxyphenyl)ethylamino moiety gives the best activity, and introduction of a halogen group at the 3-position of the phenol moiety led to further improvement of activity. Concerning the carboxamide moiety, introduction of a sterically less-hindered alkyl group, such as methyl, is tolerable. With respect to substituents at C-4 of the pyrimidine ring, benzylamine and cyclohexylamine were more favorable than aniline. On the basis of the SAR studies, we optimized the structure and identified **2t** as the most potent STAT6 inhibitor. Compound **2t** was subsequently shown to inhibit IL-4-induced Th2 differentiation of mouse spleen T cells, but had no influence on Th1 differentiation induced by IL-12. These results suggest that compound **2t** (AS1517499) might be useful for treatment of various allergic conditions caused by excess Th2 responses, such as asthma and atopic diseases. A further investigation of this novel class of STAT6 inhibitors will be reported in the near future.

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). Compounds with a phenylalkylamino group at C-2 of the pyrimidine ring showed characteristic broad peaks in the ^{1}H NMR spectra at room temperature, probably because of the presence of a conformational isomer. As an example of typical case, the methyl group of the 3-methylphenyl moiety at C-4 of the pyrimidine ring and both NH protons attached to the pyrimidine ring of compound **2a** were observed as broad signal. Therefore, the ^{1}H NMR spectra of selected compounds were measured at 80 °C to confirm the structure. Mass spectra were recorded with a Hitachi M-80 or a JEOL JMS-DX300 spectrometer. Drying of organic solutions during workup was performed over anhydrous $MgSO_4$. Column chromatography was carried out on silica gel (Kieselgel 60). Unless otherwise noted, all reagents and solvents obtained from commercial suppliers were used without further purification.

5.1.1. 2-{[2-(4-Hydroxyphenyl)ethyl]amino}-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide (2a). Tyramine (5.31 g, 38.8 mmol) was added to a solution of **1**[7] (7.00 g, 19.4 mmol) in NMP (140 mL) and the mixture was stirred for 3 h at 80 °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 $CHCl_3$–MeOH–NH_4OH (28%) (500:10:1) to give a colorless solid, which was recrystallized from AcOEt–hexane to give **2a** (5.30 g, 75%) as a colorless solid. Mp 178–180 °C; ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.26 (3H, s), 2.75 (2H, t, J = 7.2 Hz), 3.46–3.52 (2H, m), 6.67 (2H, d, J = 8.0 Hz), 6.84 (1H, d, J = 7.6 Hz), 6.99 (2H, d, J = 8.0 Hz), 7.14–7.19 (2H, m), 7.33 (1H, br s), 7.50 (1H, s), 7.53 (1H, s), 8.55 (1H, s), 8.90 (1H, s), 11.42 (1H, s); FAB MS *m/e* $(M+H)^+$ 364. Anal. Calcd for $C_{20}H_{21}N_5O_2$: C, 66.10; H, 5.82; N, 19.27. Found: C, 66.09; H, 5.87; N, 19.30.

5.1.2. 4-[(3-Methylphenyl)amino]-2-[(2-phenylethyl)amino]pyrimidine-5-carboxamide (2b). Compound **2b** was prepared from compound **1** and 2-phenylethylamine in 38% yield as a colorless solid, using a similar approach to that described for **2a**: mp 162–165 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.25 (3H, s), 2.85 (2H, t, J = 7.2 Hz), 3.52–3.54 (2H, m), 6.85 (1H, d, J = 7.2 Hz), 7.15–7.30 (7H, m), 7.46–7.52 (3H, m), 7.83 (1H, br s), 7.51 (2H, br), 8.54 (1H, s), 11.42 (1H, s); FAB MS *m/e* $(M+H)^+$ 348. Anal. Calcd for $C_{20}H_{21}N_5O$: C, 69.14; H, 6.09; N, 20.16. Found: C, 68.94; H, 5.78; N, 19.99.

5.1.3. 2-{[2-(4-Methoxyphenyl)ethyl]amino}-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide (2c). Compound **2c** was prepared from compound **1** and 2-(4-methoxyphenyl)ethylamine in 40% yield as a colorless solid, using a similar approach to that described for **2a**: mp 171–172 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.26 (3H, s), 2.80 (2H, t, J = 7.2 Hz), 3.49–3.54 (2H, m), 3.72 (3H, s), 6.82–6.84 (3H, m), 7.16–7.21 (5H, m), 7.33 (1H, br s), 7.49 (2H, br), 8.54 (1H, s), 11.41 (1H, 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, 66.70; H, 5.98; N, 18.50.

5.1.4. 2-[(4-Hydroxybenzyl)amino]-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide (2d). Compound **2d** was prepared from compound **1** and 4-hydroxybenzylamine in 44% yield as a colorless solid, using a similar approach to that described for **2a**: mp 280–281 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.26 (3H, s), 4.42 (2H, s), 6.68 (2H, d, J = 7.8 Hz), 6.81 (1H, d, J = 6.8 Hz), 7.10–7.19 (4H, m), 7.24–7.95 (4H, m), 8.56 (1H, s), 9.22 (1H, s), 11.43 (1H, s); FAB MS *m/e* $(M+H)^+$ 350. Anal. Calcd for $C_{19}H_{19}N_5O_2$: C, 65.32; H, 5.48; N, 20.04. Found: C, 65.29; H, 5.49; N, 20.33.

5.1.5. 2-{[3-(4-Hydroxyphenyl)propyl]amino}-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide (2e). Compound **2e** was prepared from compound **1** and 3-(4-hydroxyphenyl)propylamine in 45% yield as a pale-yellow solid, using a similar approach to that described for **2a**: mp 203–204 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6, 80 °C) δ 1.76–1.83 (2H, m), 2.29 (3H, s), 2.52–2.56 (2H, m), 3.28–3.30 (2H, m), 6.65 (2H, d, J = 7.8 Hz), 6.84 (1H, d, J = 7.4 Hz), 6.98 (2H, d, J = 7.8 Hz), 7.18 (1H, t, J = 7.8 Hz), 7.21–7.83 (5H, m), 8.55 (1H, s), 9.08 (1H, s), 11.43 (1H, 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, 66.92; H, 6.29; N, 18.49.

5.1.6. 2-{[2-(3-Hydroxyphenyl)ethyl]amino}-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide (2f). Compound **2f** was prepared from compound **1** and 2-(3-hydroxyphenyl)ethylamine hydrochloride with diisopropylethylamine in 26% yield as a colorless solid, using a similar approach to that described for **2a**: mp 192–193 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.26 (3H, s), 2.78 (2H, t, J = 6.8 Hz), 3.50–3.55 (2H, m), 6.59–6.64 (3H, m), 6.83 (1H, d, J = 8.0 Hz), 7.06 (1H, t, J = 7.6 Hz), 7.17 (1H, t, J = 7.6 Hz), 7.21 (1H, br s), 7.34 (1H, br s), 7.49–7.53 (2H, m), 8.55 (1H, s), 9.01 (1H, s), 11.42 (1H, s); FAB MS *m/e* $(M+H)^+$ 364. Anal. Calcd for $C_{20}H_{21}N_5O_2$: C, 66.10; H, 5.82; N, 19.27. Found: C, 66.18; H, 5.92; N, 19.53.

5.1.7. 2-{[2-(2-Hydroxyphenyl)ethyl]amino}-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide (2g). Compound **2g** was prepared from compound **1** and 2-(2-hydroxyphenyl)ethylamine in 46% yield as a pale-yellow solid, using a similar approach to that described for **2a**: mp 255–257 °C (EtOH–THF); ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.25 (3H, s), 2.83 (2H, t, J = 7.2 Hz), 3.51–3.56 (2H, m), 6.70 (1H, t, J = 7.6 Hz), 6.78–6.83 (2H, m), 6.99–7.06 (2H, m), 7.13–7.19 (2H, m), 7.33 (1H, br s), 7.51 (2H, br), 8.54 (1H, s), 9.12 (1H, s), 11.42 (1H, s); FAB MS *m/e* $(M+H)^+$ 364. Anal. Calcd for $C_{20}H_{21}N_5O_2$: C, 66.10; H, 5.82; N, 19.27. Found: C, 65.95; H, 5.96; N, 19.27.

5.1.8. 2-{[2-(4-Hydroxy-3-methylphenyl)ethyl]amino}-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide (2h). Compound **2h** was prepared from compound **1** and 2-(4-hydroxy-3-methylphenyl)ethylamine in 16% yield as a colorless solid, using a similar approach to that described for **2a**: mp 209–211 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.08 (3H, s), 2.26 (3H, s), 2.71 (2H, t, J = 7.2 Hz), 3.46–3.59 (2H, m), 6.66 (1H, d, J = 8.0 Hz), 6.80–6.85 (3H, m), 7.14–7.18 (2H, m), 7.33 (1H, br s), 7.50 (2H, br), 8.54 (1H, s), 8.73 (1H, s), 11.42 (1H, 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, 66.60; H, 6.16; N, 18.57.

5.1.9. 2-{[2-(4-Hydroxy-3-methoxyphenyl)ethyl]amino}-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide (2i). Compound **2i** was prepared from compound **1** and 2-(4-hydroxy-3-methoxylphenyl)ethylamine in 25% yield as a colorless solid, using a similar approach to that described for **2a**: mp 182–183 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.25 (3H, s), 2.76 (2H, t, J = 7.2 Hz), 3.50–3.55 (2H, m), 3.71 (3H, s), 6.60 (1H, d, J = 7.6 Hz), 6.67 (1H, d, J = 8.0 Hz), 6.75 (1H, s), 6.83 (1H, d, J = 7.2 Hz), 7.15 (1H, t, J = 8.0 Hz), 7.20 (1H, br s), 7.33 (1H, br s), 7.50 (2H, br), 8.39 (1H, s), 8.54 (1H, s), 11.42 (1H, s); FAB MS *m/e* $(M+H)^+$ 384. Anal. Calcd for $C_{21}H_{23}N_5O_3$: C, 64.11; H, 5.89; N, 17.80. Found: C, 64.18; H, 5.87; N, 17.77.

5.1.10. 2-{[2-(3-Fluoro-4-hydroxyphenyl)ethyl]amino}-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide (2j). Compound **2j** was prepared from compound **1** and 2-(3-fluoro-4-hydroxyphenyl)ethylamine in 16% yield as a pale-yellow solid, using a similar approach to that described for **2a**: mp 208–209 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.26 (3H, s), 2.76 (2H, t, J = 7.2 Hz), 3.48–3.53 (2H, m), 6.79–6.85 (3H, m), 6.93 (1H, d, J = 8.4 Hz), 7.15 (1H, d, J = 8.0 Hz), 7.21 (1H, br s), 7.33 (1H, br s), 7.49 (2H, br), 8.54 (1H, s), 9.29 (1H, s), 11.41 (1H, s); FAB MS *m/e* $(M+H)^+$ 382. Anal. Calcd for $C_{20}H_{20}FN_5O_2$: C, 62.98; H, 5.29; F, 4.98; N, 18.36. Found: C, 62.91; H, 5.30; F, 5.07; N, 18.36.

5.1.11. 2-{[2-(3-Chloro-4-hydroxyphenyl)ethyl]amino}-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide (2k). Compound **2k** was prepared from compound **1** and 2-(3-chloro-4-hydroxyphenyl)ethylamine in 55% yield as a colorless solid, using a similar approach to that described for **2a**: mp 176–177 °C (MeOH–AcOEt); ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.26 (3H, s), 2.76 (2H, t, J = 7.2 Hz), 3.47–3.52 (2H, m), 6.84–6.87 (2H, m), 6.95 (1H, d, J = 8.4 Hz), 7.14–7.19 (2H, m), 7.26 (1H, br s), 7.35 (1H, br s), 7.49 (2H, br), 8.54 (1H, s), 9.59 (1H, s), 11.43 (1H, s); FAB MS *m/e* $(M+H)^+$ 398. Anal. Calcd for $C_{20}H_{20}ClN_5O_2$: C, 60.38; H, 5.07; Cl, 8.91; N, 17.60. Found: C, 66.00; H, 5.07; Cl, 8.76; N, 17.67.

5.1.12. 2-{[2-(3-Bromo-4-hydroxyphenyl)ethyl]amino}-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide (2l). Compound **2l** was prepared from compound **1** and 2-(3-bromo-4-hydroxyphenyl)ethylamine in 27% yield as a colorless solid, using a similar approach to that described for **2a**: mp 179–180 °C (EtOH–THF); ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.27 (3H, s), 2.76 (2H, t, J = 7.2 Hz), 3.47–3.52 (2H, m), 6.84 (1H, d, J = 8.4 Hz), 6.99 (1H, d, J = 8.0 Hz), 7.17–7.30 (4H, m), 7.49 (2H, br), 8.54 (1H, s), 9.67 (1H, s), 11.40 (1H, s); FAB MS *m/e* $(M)^+$ 442. Anal. Calcd for $C_{20}H_{20}BrN_5O_2$: C, 54.31; H, 4.56; Br, 18.07; N, 15.83. Found: C, 54.33; H, 4.45; Br, 17.80; N, 15.82.

5.1.13. 2-{[2-(4-Hydroxyphenyl)ethyl]amino}-4-[(3-methylphenyl)amino]pyrimidine-5-carboxylic acid (4). Tyramine hydrochloride (2.39 g, 13.7 mmol) and diisopropylethylamine (5.26 mL, 30.2 mmol) were added to a solution of **3**[7] (1.81 g, 6.86 mmol) in NMP (15 mL) and the mixture was stirred for 14 h at 80 °C. The reaction mixture was then diluted with H_2O (150 mL) and the resulting solid was collected by filtration, triturated with THF–MeOH, and collected by filtration to give **4** (1.23 g, 49%), which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 2.25–2.32 (3 H, br), 2.70–2.75 (2H, m), 3.43–3.48 (2H, m), 6.67 (2H, d, J = 8.2 Hz), 6.86–7.04 (3H, m), 7.20 (1H, t, J = 7.6 Hz), 7.49–7.57 (2H, m), 7.65–7.85 (1H, m), 8.53–8.62 (1H, br), 9.18 (1H, s), 10.49–10.56 (1H, br), 12.18 (1H, br); FAB MS *m/e* $(M+H)^+$ 365.

5.1.14. 2-{[2-(4-Hydroxyphenyl)ethyl]amino}-*N*-methyl-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide (2m). EDC·HCl (223 mg, 1.16 mmol) and HOBt (157 mg, 1.16 mmol) were added to a suspension of **4** (352 mg, 0.97 mmol) in DMF (4 mL). After stirring for 10 min at room temperature, methylamine hydrochloride (79 mg, 1.16 mmol) and diisopropylethylamine (0.38 mL, 2.13 mmol) were added and the mixture was

stirred for 16 h at room temperature. The reaction mixture was 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 **2m** (154 mg, 42%) as a brown amorphous solid. ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.26 (3H, s), 2.73–2.77 (5H, m), 3.46–3.52 (2H, m), 6.67 (2H, d, J = 8.4 Hz), 6.84 (1H, d, J = 7.2 Hz), 6.99 (2H, d, J = 8.4 Hz), 7.14–7.18 (2H, m), 7.51 (2H, br), 8.13 (1H, br s), 8.48 (1H, s), 8.90 (1H, s), 11.30 (1H, s); FAB MS *m/e* $(M+H)^+$ 378. Anal. Calcd for $C_{21}H_{23}N_5O_2 \cdot 0.2H_2O$: C, 66.19; H, 6.19; N, 18.38. Found: C, 66.28; H, 6.17; N, 18.28.

5.1.15. N-Ethyl-2-{[2-(4-hydroxyphenyl)ethyl]amino}-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide (2n). Compound **2n** was prepared from compound **4** and 70% aqueous ethylamine by a procedure similar to that described for **2m**, excluding diisopropylethylamine. **2n** was obtained in 56% yield as a colorless solid: mp 179–180 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6, 80 °C) δ 1.13 (3 H, t, J = 7.2 Hz), 2.26 (3 H, s), 2.74 (2H, t, J = 7.2 Hz), 3.24–3.31 (2H, m), 3.46–3.51 (2H, m), 6.66 (2H, d, J = 8.4 Hz), 6.83 (1H, d, J = 7.2 Hz), 6.98 (2H, d, J = 8.4 Hz), 7.14–7.18 (2H, m), 7.50 (2H, br), 8.16 (1H, br s), 8.50 (1H, s), 8.91 (1H, s), 11.31 (1H, s); FAB MS *m/e* $(M+H)^+$ 392. Anal. Calcd for $C_{22}H_{25}N_5O_2$: C, 67.50; H, 6.44; N, 17.89. Found: C, 67.39; H, 6.54; N, 18.09.

5.1.16. 2-{[2-(4-Hydroxyphenyl)ethyl]amino}-4-[(3-methylphenyl)amino]-*N*-(*iso*-propyl)pyrimidine-5-carboxamide (2o). Compound **2o** was prepared from compound **4** and *iso*-propylamine by a procedure that of described for **2m**, excluding diisopropylethylamine. Compound **2o** was obtained in 53% yield as a colorless solid: mp 196–197 °C (AcOEt –hexane); ^{1}H NMR (DMSO-d_6, 80 °C) δ 1.16 (6H, d, J = 6.8 Hz), 2.26 (3H, s), 2.75 (2H, t, J = 7.2 Hz), 3.44–3.51 (2H, m), 4.07–4.14 (2H, m), 6.67 (2H, d, J = 8.4 Hz), 6.84 (1H, d, J = 7.2 Hz), 6.99 (2H, d, J = 8.4 Hz), 7.14–7.18 (2H, m), 7.52 (2H, br), 8.16 (1H, br s), 8.50 (1H, s), 8.91 (1H, s), 11.33 (1H, s); FAB MS *m/e* $(M+H)^+$ 406. Anal. Calcd for $C_{23}H_{27}N_5O_2$: C, 68.13; H, 6.71; N, 17.27. Found: C, 68.25; H, 6.94; N, 17.37.

5.1.17. 2-{[2-(4-hydroxyphenyl)ethyl]amino}-*N*,*N*-dimethyl-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide (2p). Compound **2p** was prepared from compound **4** and 50% aqueous dimethylamine by a procedure that of described for **2m**, excluding diisopropylethylamine. Compound **2p** was obtained in 42% yield as a colorless solid: mp 172–173 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.25 (3H, s), 2.73 (2H, t, J = 7.2 Hz), 3.01 (6H, s), 3.44–3.50 (2H, m), 6.66 (2H, d, J = 8.4 Hz), 6.83 (1H, d, J = 7.2 Hz), 6.98 (2H, d, J = 8.4 Hz), 7.04 (1H, br), 7.15 (1H, t, J = 8.0 Hz), 7.45–7.49 (2H, m), 8.05 (1H, s), 8.50 (1H, s), 8.90 (1H, s), 9.25 (1H, br); FAB MS *m/e* $(M+H)^+$ 392. Anal. Calcd for $C_{22}H_{25}N_5O_2$: C, 67.50; H, 6.44; N, 17.89. Found: C, 67.52; H, 6.51; N, 17.83.

5.1.18. Ethyl 2-chloro-4-(methylsulfanyl)pyrimidine-5-carboxylate (6). NaSMe (4.12 g, 58.9 mmol) and benzyltriethylammonium chloride (128 mg, 0.56 mmol) were added to a solution of ethyl 2,4-dichloropyrimidine-5-carboxylate (**5**, 12.4 g, 56.1 mmol) in THF (180 mL) and the mixture was stirred at −10 °C for 3 h. The reaction mixture was then diluted with Et_2O and ice water, and the resulting solid was collected by filtration to give **6** (7.0 g, 54%), which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 1.33 (3H, t, J = 7.1 Hz), 2.50 (3H, s), 4.35 (2H, q, J = 7.1 Hz), 8.90 (1H, s). FAB MS *m/e* $(M+H)^+$ 233.

5.1.19. Ethyl 2-{[2-(4-hydroxyphenyl)ethyl]amino}-4-(methylsulfanyl)pyrimidine-5-carboxylate (7). Tyramine hydrochloride (5.34 g, 7.55 mmol) and diisopropylethylamine (11.2 mL, 64.2 mmol) were added to a solution of **6** (6.49 g, 30.7 mmol) in NMP (60 mL) and the mixture was stirred for 16 h at 80 °C. The reaction mixture was then diluted with AcOEt (600 mL) and washed successively with H_2O and saturated aqueous NaCl. The organic layer was dried and concentrated, and the resulting solid was triturated with MeCN to give **7** (6.42 g, 69%), which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 1.28 (3H, t, J = 7.1 Hz), 2.37 (1H, s, SCH_3), 2.43 (2H, s, SCH_3), 2.72–2.77 (2H, m), 3.48–3.55 (2H, m), 4.22 (2H, q, J = 7.1 Hz), 6.67 (2H, d, J = 8.2 Hz), 7.01 (2H, d, J = 8.2 Hz), 8.09 (0.33H, br t, J = 5.8 Hz, NH), 8.13 (0.67 H, br t, J = 5.8 Hz, NH), 8.54 (0.67 H, s, CH at C-6 of pyrimidine), 8.63 (0.33 H, s, CH at C-6 of pyrimidine), 9.17 (1H, s). FAB MS *m/e* $(M+H)^+$ 334.

5.1.20. 2-{[2-(4-Hydroxyphenyl)ethyl]amino}-4-(methylsulfanyl)pyrimidine-5-carboxylic acid (8). One molar of NaOH (90 mL) was added to a solution of **7** (12.0 g, 36.0 mmol) in MeOH (180 mL) and the mixture was refluxed for an hour. One molar of HCl (90 mL) was added and the resulting solid was collected by filtration and washed successively with H_2O and MeCN–Et_2O (1:1) to give **8** (10.7 g, 97%), which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 2.35 (1H, s, SCH_3), 2.41 (2H, s, SCH_3), 2.70–2.77 (2H, m), 3.48–3.54 (2H, m), 6.67 (2H, d, J = 8.0 Hz), 7.02 (2H, d, J = 8.0 Hz), 7.90 (0.33 H, br t, J = 5.8 Hz, NH), 8.02 (0.67 H, br t, J = 5.8 Hz, NH), 8.50 (0.67 H, s, CH at C-6 of pyrimidine), 8.59 (0.33 H, s, CH at C-6 of pyrimidine), 9.18 (1H, br), 12.58 (1H, br). FAB MS *m/e* $(M+H)^+$ 306.

5.1.21. 2-{[2-(4-Hydroxyphenyl)ethyl]amino}-4-(methylsulfanyl)pyrimidine-5-carboxamide (9). EDC·HCl (8.06 g, 42.0 mmol) and HOBt (5.68 g, 42.0 mmol) were added to a suspension of **8** (10.7 g, 35.0 mmol) in NMP (100 mL). After stirring for 2 h at room temperature, 28% NH_4OH (10.3 mL) was added and the mixture was stirred for 1 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 H_2O and MeCN–Et_2O (1:1) to give 9 (10.5 g, 98%) as a colorless powder, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6)

δ 2.3–2.37 (3 H, br), 2.71–2.76 (2H, m), 3.47 (2H, br), 6.67 (2H, d, J = 8.4 Hz), 7.01 (2H, d, J = 8.4 Hz), 7.06 (1H, br), 7.61 (1H, br), 7.73 (1H, br), 8.40–8.45 (1H, br), 9.17 (1H, s). FAB MS *m/e* $(M+H)^+$ 305.

5.1.22. 2-{[2-(4-Hydroxyphenyl)ethyl]amino}-4-(methylsulfinyl)pyrimidine-5-carboxamide (10). *m*-CPBA (7.09 g) was added portionwise to a solution of **9** (10.0 g, 32.9 mmol) in NMP (100 mL) at below 5 °C and the mixture was stirred for 1 h. The reaction mixture was then diluted with H_2O, and the resulting solid was collected by filtration and washed successively with H_2O, Et_2O, and MeCN–Et_2O (1:1) to give **10** (9.68 g, 92%) as a pale-yellow powder, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 2.75 (3H, s), 2.78 (2H, br), 3.49–3.58 (2H, m), 6.65–6.69 (2H, m), 7.03–7.09 (2H, m), 7.43 (1H, br), 8.00 (1H, br), 8.23 (0.4H, br, NH), 8.45 (0.6H, br, NH), 8.70 (0.4 H, s, CH at C-6 of pyrimidine), 8.78 (0.6H, s, CH at C-6 of pyrimidine), 9.15 (0.4H, s, OH), 9.18 (0.6H, s, OH). FAB MS *m/e* $(M+H)^+$ 321.

5.1.23. 4-(Cyclohexylamino)-2-{[2-(4-hydroxyphenyl)ethyl]amino}pyrimidine-5-carboxamide (2q). Cyclohexylamine (372 mg, 3.75 mmol) was added to a solution of **10** (800 mg, 2.50 mmol) and diisopropylethylamine (0.87 mL, 5.0 mmol) in NMP (8 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 (39:1) to give a colorless solid, which was recrystallized from MeOH–AcOEt to give **2q** (547 mg, 62%) as a colorless solid. Mp 185–186 °C; ^{1}H NMR (DMSO-d_6, 80 °C) δ 1.23–1.40 (6H, m), 1.55 (1H, br), 1.66–1.70 (2H, m), 1.89–1.91 (2H, m), 2.71 (2H, t, J = 7.2 Hz), 3.39–3.40 (2H, m), 3.96 (1H, br), 6.67 (2H, d, J = 8.4 Hz), 6.80 (1H, br), 6.99–7.01 (3H,m), 8.34 (1H, s), 8.89 (1H, s), 8.96 (1H, br); FAB MS *m/e* $(M+H)^+$ 356. Anal. Calcd for $C_{19}H_{25}N_5O_2$: C, 64.20; H, 7.09; N, 19.70. Found: C, 64.17; H, 7.42; N, 19.60.

5.1.24. 4-(Benzylamino)-2-{[2-(4-hydroxyphenyl)ethyl]amino}pyrimidine-5-carboxamide (2r). Compound **2r** was prepared from compound **10** and benzylamine in 48% yield as a colorless solid, using a similar approach to that described for **2r**: mp 208–209 °C (MeOH–THF); ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.67 (2H, t, J = 7.6 Hz), 3.38–3.41 (2H, m), 4.63 (2H, d, J = 5.6 Hz), 6.64 (2H, d, J = 8.4 Hz), 6.84 (1H, br), 6.93 (2H, d, J = 8.4 Hz), 7.08 (1H, br), 7.22–7.24 (1H, m), 7.28–7.31 (5H, m), 8.39 (1H, s), 8.80 (1H, s), 9.31 (1H, br); FAB MS *m/e* $(M+H)^+$ 364. Anal. Calcd for $C_{20}H_{21}N_5O_2$: C, 66.10; H, 5.82; N, 19.27. Found: C, 66.13; H, 5.78; N, 19.32.

5.1.25. 4-Anilino-2-{[2-(4-hydroxyphenyl)ethyl]amino}-pyrimidine-5-carboxamide (2s). Compound **2s** was prepared from compound **10** and aniline in 30% yield as a pale-yellow solid, using a similar approach to that described for **2r**: mp 248–249 °C (MeOH); ^{1}H NMR (DMSO-d_6, 80 °C) δ 2.74 (2H, t, J = 8.4 Hz), 3.45–3.51 (2H, m), 6.68 (2H, d, J = 8.4 Hz), 6.99–7.04 (3H, m), 7.11–7.34 (5H, m), 7.67 (2H, d, J = 8.4 Hz), 8.55 (1H, s), 8.91 (1H, s), 11.47 (1H, br); FAB MS *m/e* $(M+H)^+$ 350. Anal. Calcd for $C_{19}H_{19}N_5O_2 \cdot 0.2H_2O$: C, 64.65; H, 5.54; N, 19.84. Found: C, 64.82; H, 5.53; N, 19.75.

5.1.26. Ethyl 4-(benzylamino)-2-(methylsulfanyl)pyrimidine-5-carboxylate (12). Benzylamine (3.22 g, 30.1 mmol) was added to a solution of ethyl 4-chloro-2-(methylsulfanyl)pyrimidine-5-carboxylate (**11**, 7.00 g, 30.1 mmol) and diisopropylethylamine (5.24 mL, 30.1 mmol) in MeCN (70 mL) at 0 °C and the mixture was stirred for 5 h at room temperature. 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 to give **12** (8.75 g, 96%) as a pale-yellow solid, which was used in the next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 1.36 (3H, t, J = 7.1 Hz), 2.48 (3H, s), 4.31 (2H, q, J = 7.1 Hz), 4.76 (2H, d, J = 5.9 Hz), 7.24–7.34 (5H, m), 8.60 (1H, br), 8.65 (1H, s); FAB MS *m/e* $(M+H)^+$ 304.

5.1.27. 4-(Benzylamino)-2-(methylsulfanyl)pyrimidine-5-carboxylic acid (13). One molar of NaOH (32 mL) was added to a solution of **12** (8.06 g, 27.3 mmol) in THF (80 mL) and MeOH (10 mL) and the mixture was stirred for 5 h at 50 °C. One molar of HCl (32 mL) was added and the resulting solid was collected by filtration to give **13** (7.35 g, quant.) as a colorless powder, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 2.40 (3H, s), 4.71 (2H, d, J = 5.9 Hz), 7.22–7.34 (5H, m), 8.54 (1H, s), 8.88 (1H, br t, J = 5.9 Hz), 13.26 (1H, br s); FAB MS *m/e* $(M+H)^+$ 276.

5.1.28. 4-(Benzylamino)-2-(methylsulfanyl)pyrimidine-5-carboxamide (14). EDC·HCl (4.18 g, 21.8 mmol) and HOBt (2.94 g, 21.8 mmol) were added to a solution of **13** (5.00 g, 18.2 mmol) in DMF (50 mL). After stirring for 30 min at room temperature, 28% aqueous ammonia (5.4 mL) was added and the mixture was stirred for 16 h at room temperature. The reaction mixture was diluted with AcOEt and washed successively with water and saturated aqueous NaCl. The organic layer was dried and concentrated in vacuo to give **14** (4.89 g, 98%) as a colorless powder, which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 2.40 (3H, s), 4.67 (2H, d, J = 5.9 Hz), 7.21–7.33 (5H, m), 7.46 (1H, br s), 8.04 (1H, br s), 8.53 (1H, s), 9.48 (1H, br t, J = 5.9 Hz); FAB MS *m/e* $(M+H)^+$ 275.

5.1.29. 4-(Benzylamino)-*N*-methyl-2-(methylsulfanyl)pyrimidine-5-carboxamide (15). Compound **15** was prepared from compound **13** and methylamine hydrochloride with equimolar of diisopropylamine in 95% yield as a colorless solid, using a similar approach to that described for **14**. Compound **15** was used for next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 2.40 (3H, s), 2.74 (3H, d, J = 4.5 Hz), 4.65 (2H, d, J = 5.9 Hz), 7.24–7.36 (5H, m), 8.45 (1H,

s), 8.52–8.53 (1H, m), 9.34 (1H, br t, J = 5.9 Hz); FAB MS m/e $(M+H)^+$ 289.

5.1.30. 4-(Benzylamino)-2-(methylsulfonyl)pyrimidine-5-carboxamide (16). *m*-CPBA (1.62 g) was added portionwise to a solution of **14** (1.00 g, 3.75 mmol) in NMP (10 mL) below 5 °C and the mixture was stirred for 3 h. The reaction mixture was then diluted with $CHCl_3$ and washed successively with saturated aqueous $NaHCO_3$, 10% aqueous Na_2SO_3, H_2O, and saturated aqueous NaCl. The organic layer was dried and concentrated in vacuo. H_2O was added to the residue, and the resulting solid was collected by filtration to give **16** (1.02 g, 91%) as a colorless powder, which was used in the next reaction without further purification. ^1H NMR (DMSO-d_6) δ 3.25 (3H, s), 4.70 (2H, d, J = 5.9 Hz), 7.24–7.39 (5H, m), 7.84 (1H, br s), 8.34 (1H, br s), 8.79 (1H, s), 9.67 (1H, br t, J = 5.9 Hz); FAB MS m/e $(M+H)^+$ 307.

5.1.31. 4-(Benzylamino)-*N*-methyl-2-(methylsulfonyl)pyrimidine-5-carboxamide (17). Compound **17** was prepared from compound **15** in 85% yield as a colorless powder, using a similar approach to that described for **16**. Compound **17** was used for next reaction without further purification. ^1H NMR (DMSO-d_6) δ 2.77 (3H, d, J = 4.5 Hz), 3.23 (3H, s), 4.68 (2H, d, J = 5.9 Hz), 7.25–7.38 (5H, m), 8.67 (1H, s), 8.77–8.78 (1H, m), 9.43 (1H, br t, J = 5.9 Hz); FAB MS m/e $(M+H)^+$ 321.

5.1.32. 4-(Benzylamino)-2-{[2-(3-chloro-4-hydroxyphenyl)ethyl]amino}pyrimidine-5-carboxamide (2t). 2-(3-Chloro-4-hydroxyphenyl)ethylamine hydrochloride (765 mg, 3.68 mmol) was added to a solution of **16** (750 mg, 2.45 mmol) and diisopropylamine (1.10 mL, 6.13 mmol) in NMP (7.5 mL) and the mixture was stirred for 1 h at 100 °C. The reaction mixture was then diluted with AcOEt and THF, 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 **2t** (280 mg, 29%) as a colorless solid. Mp 151–153 °C; ^1H NMR (DMSO-d_6, 80 °C) δ 2.68 (2H, t, J = 7.2 Hz), 3.40–3.42 (2H, m), 4.63 (2H, d, J = 6.0 Hz), 6.83–6.91 (3H, m), 7.10 (2H, br), 7.22–7.24 (1H, m), 7.28–7.31 (5H, m), 8.39 (1H, s), 9.33 (1H, br s), 9.57 (1H, s); FAB MS m/e $(M+H)^+$ 398. Anal. Calcd for $C_{20}H_{20}ClN_5O_2$: C, 60.38; H, 5.07; Cl, 8.91; N, 17.60. Found: C, 60.25; H, 4.97; Cl, 9.04; N, 17.75.

5.1.33. 4-(Benzylamino)-2-{[2-(3-chloro-4-hydroxyphenyl)ethyl]amino}-*N*-methylpyrimidine-5-carboxamide (2u). Compound **2u** was prepared from compound **2u** in 33% yield as a pale brown amorphous solid, using a similar approach to that described for **2t**: ^1H NMR(DMSO-d_6, 80 °C) δ 2.68–2.74 (5H, m), 3.40–3.45 (2H, m), 4.63 (2H, d, J = 5.2 Hz), 6.84–6.91 (3H, m), 6.99 (1H, br), 7.10 (1H, s), 7.21–7.25 (1H, m), 7.28–7.31 (4H, m), 7.99 (1H, br), 8.33 (1H, s), 9.30 (1H, br s), 9.59 (1H, s); FAB MS m/e $(M+H)^+$ 412. Anal. Calcd for $C_{21}H_{22}ClN_5O_2{\cdot}0.5H_2O$: C, 59.93; H, 5.51; Cl, 8.42; N, 16.64. Found: C, 59.64; H, 5.42; Cl, 8.15; N, 16.28.

5.1.34. Ethyl 2-{3-[4-(benzyloxy)phenyl]propyl}-4-oxo-1,4-dihydropyrimidine-5-carboxylate (19). Hydrogen chloride was bubbled through a solution of **18** (1.50 g, 5.97 mmol) in EtOH (30 mL) and $CHCl_3$ (15 mL) for 30 min at below 5 °C. After stirring for 2 h below 5 °C, the reaction mixture was concentrated in vacuo. The residue was combined with Et_2O (50 mL) and concentrated in vacuo, and then combined with EtOH (30 mL) and ammonia acetate (920 mg, 11.9 mmol) before being refluxed for 2 h. The mixture was again concentrated in vacuo, and a suspension of the resulting residue in EtOH (50 mL) was combined with sodium methoxide (1.61 mg, 29.9 mmol) and diethyl ethoxymethylenemalonate (2.58 g, 11.9 mmol) and stirred for 3 h at room temperature. The reaction mixture was concentrated in vacuo, and the residue was acidified with 1 M HCl and extracted with AcOEt. The organic layer was washed with water and saturated aqueous NaCl, dried, and concentrated in vacuo. The resulting residue was chromatographed on silica gel with elution using $CHCl_3$–MeOH (40:1) to give **19** (820 mg, 35%) as an amorphous solid, which was used in the next step without further purification: ^1H NMR ($CDCl_3$) δ 1.37 (3H, t, J = 7.1 Hz), 2.07–2.17 (2H, m), 2.66 (2H, t, J = 7.7 Hz), 2.79 (2H, t, J = 7.7 Hz), 4.36 (2H, q, J = 7.1 Hz), 5.02 (2H, s), 6.88 (2H, d, J = 8.6 Hz), 7.10 (2H, d, J = 8.6 Hz), 7.32–7.44 (5H, m), 8.72 (1H, s), 12.70 (1H, br s); FAB MS m/e $(M+H)^+$ 393.

5.1.35. Ethyl 2-{3-[4-(benzyloxy)phenyl]propyl}-4-chloropyrimidine-5-carboxylate (20). Diethylaniline (0.55 mL, 3.46 mmol) was added to a mixture of **19** (755 mg, 1.92 mmol) and phosphorus trichloride (2 mL) and the mixture was stirred for 1 h at 90 °C. The reaction mixture was then poured into ice water and the resulting solid was collected by filtration and washed with AcOEt to give **20** (767 mg, 97%) as a pale brown solid, which was used in the next step without further purification: ^1H NMR ($CDCl_3$) δ 1.42 (3H, t, J = 7.3 Hz), 2.10–2.17 (2H, m), 2.66 (2H, t, J = 7.8 Hz), 3.00 (2H, t, J = 7.8 Hz), 4.43 (2H, q, J = 7.3 Hz), 5.04 (2H, s), 6.89 (2H, d, J = 8.3 Hz), 6.94 (2H, d, J = 8.3 Hz), 7.30–7.44 (5H, m), 9.04 (1H, s).

5.1.36. Ethyl 2-{3-[4-(Benzyloxy)phenyl]propyl}-4-[(3-methylphenyl)amino]pyrimidine-5-carboxylate (21). *m*-Toluidine (206 mg, 1.92 mmol) was added to a solution of **20** (750 mg, 1.83 mmol) and diisopropylethylamine (0.33 mL, 1.92 mmol) in MeCN (70 mL) and the mixture was stirred for 2 h at 60 °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 hexane–AcOEt (5:1) to give **21** (616 mg, 70%) as an amorphous solid: ^1H NMR ($CDCl_3$) δ 1.42 (3H, t, J = 7.7 Hz), 1.58 (3H, s), 2.10–2.20 (2H, m), 2.65 (2H, t, J = 7.1 Hz), 2.88 (2H, t, J = 7.5 Hz), 4.40

(2H, q, $J = 7.1$ Hz), 5.03 (2H, s), 6.88 (2H, d, $J = 8.8$ Hz), 6.93–6.95 (1H, m), 7.11 (2H, d, $J = 8.6$ Hz), 7.22 (1H, d, $J = 7.9$ Hz), 7.31–7.44 (5H, m), 7.55–7.60 (3H, m), 8.91 (1H, s), 10.25 (1H, s); FAB MS *m/e* $(M+H)^+$ 482.

5.1.37. 2-{3-[4-(Benzyloxy)phenyl]propyl}-4-[(3-methylphenyl)amino]pyrimidine-5-carboxylic acid (22). One molar of NaOH (2.7 mL) was added to a solution of **21** (640 mg, 1.33 mmol) in EtOH (5 mL) and THF (5 mL), and the mixture was stirred for 16 h at room temperature. One molar of HCl (2.7 mL) was added and the resulting solid was collected by filtration and washed successively with MeOH–H_2O (1:1) to give **22** (595 mg, 99%) as a colorless powder, which was used in the next reaction without further purification: ^{1}H NMR (DMSO-d_6) δ 1.99–2.09 (2H, m), 2.27 (3H, s), 2.58 (2H, t, $J = 7.2$ Hz), 2.77 (2H, t, $J = 7.5$ Hz), 5.06 (2H, s), 6.89–6.95 (3H, m), 7.10 (2H, d, $J = 8.6$ Hz), 7.21–7.27 (1H, m), 7.32–7.45 (5H, m), 7.55–7.57 (2H, m), 8.81 (1H, s), 10.60 (1H, br s); FAB MS *m/e* $(M+H)^+$ 454.

5.1.38. 2-{3-[4-(Benzyloxy)phenyl]propyl}-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide (23). EDC·HCl (292 mg, 1.52 mmol) and HOBt (206 mg, 1.52 mmol) were added to a solution of **22** (578 mg, 1.27 mmol) in DMF (15 mL). After stirring for 30 min at room temperature, 28% NH_4OH (0.74 mL) was added and the mixture was stirred for 16 h at room temperature. 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 (60:1) to give **23** (452 mg, 79%) as an ivory-colored powder: ^{1}H NMR (DMSO-d_6) δ 2.04 (2H, quint., $J = 7.5$ Hz), 2.27 (3H, s), 2.58 (2H, t, $J = 7.5$ Hz), 2.75 (2H, t, $J = 7.5$ Hz), 5.06 (2H, s), 6.88–6.92 (3H, m), 7.12 (2H, d, $J = 8.4$ Hz), 7.19–7.24 (1H, m), 7.31–7.45 (5H, m), 7.53–7.55 (2H, m), 7.76 (1H, br s), 8.31 (1H, br s), 8.81 (1H, s), 11.30 (1H, s); FAB MS *m/e* $(M+H)^+$ 453.

5.1.39. 2-[3-(4-hydroxyphenyl)propyl]-4-[(3-methylphenyl)amino]pyrimidine-5-carboxamide (24). Palladium (10%) on carbon (100 mg) was added to a solution of **23** (417 mg, 0.92 mmol) in MeOH (4 mL) and THF (6 mL), and the mixture was stirred for 14 h at room temperature under a hydrogen atmosphere. The mixture was filtered with Celite and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel with elution using $CHCl_3$–MeOH (20:1) to give a crude solid, which was recrystallized from MeOH–THF to give **24** (253 mg, 76%) as a pale-yellow solid: mp 240–241 °C; ^{1}H NMR (DMSO-d_6) δ 1.98–2.06 (2H, m), 2.28 (3H, s), 2.53 (2H, t, $J = 7.3$ Hz), 2.74 (2H, t, $J = 7.8$ Hz), 6.66 (2H, d, $J = 8.6$ Hz), 6.89 (1H, d, $J = 7.3$ Hz), 6.94 (2H, d, $J = 8.3$ Hz), 7.22 (1H, t, $J = 7.8$ Hz), 7.54–7.56 (2H, m), 7.76 (1H, br s), 8.32 (1H, br s), 8.81 (1H, s), 9.12 (1H, s), 11.31 (1H, s); FAB MS *m/e* $(M+H)^+$ 363. Anal. Calcd for $C_{21}H_{22}N_4O_2$: C, 69.59; H, 6.12; N, 15.46. Found: C, 69.51; H, 6.16; N, 15.41.

5.2. Biology

5.2.1. Plasmid construction. An IL-4-inducible luciferase reporter plasmid carrying copies of the CCAAT-enhancer binding protein (C/EBP) binding site and the STAT6 binding site was constructed as previously described.[11]The oligonucleotide containing the C/EBP and STAT6 binding sites used in the construction of the plasmid had the sequence 5′-TCGAGCGCTGTTGCT CAATCGACTTCCCAAGAACAGAGCTGTTGCTC AATCGACTTCCCAAGAACAGAA-3′. The synthesized oligonucleotides were annealed and then ligated into the *Xho*I–*Bgl*II site of the pGL2-TATA plasmid.[12]

5.2.2. Cells and cell culture. FW4 is a BAF-B03-derived cell line expressing human IL-2Rβ, IL-2Rγ, and IL-4R.[13] FW4 cells were obtained by co-transfection of pGL2-CS with a blasticidin resistance gene by electroporation, as previously described.[12] The cells were cultured in RPMI1640 medium (Gibco) supplemented with 10% fetal bovine serum (FBS) and 10% (v/v) WEHI-3B conditioned medium as a source of IL-3.[14]

5.2.3. Luciferase assay. FW4 reporter cells (1×10^4 cells/0.1 mL RPMI1640 supplemented with 10% (v/v) FBS and 10% (v/v) WEHI-3B conditioned medium) cultured in 96-well plates were stimulated with human IL-4 (1 ng/mL) (Genzyme) for 16 h. The test compounds were added to each well 30 min before stimulation. The final DMSO concentrations were 0.1%. The reaction was stopped by addition of 50 μL of solubilization buffer (10 mM Tris–HCl, pH 7.8, 0.5 mM $MgCl_2$, 10 mM dithiothreitol, and 0.1% (v/v) Triton X-100). Luciferase activities were measured with a ML3000 luminometer (Dynatech Laboratories, Inc.) after addition of 50 μL of substrate solution (5 mM luciferin, 2 mM coenzyme A, 2 mM ATP, 0.5 mM $MgCl_2$, and 2 mM $Mg(OH)_2$ in 10 mM Tris–HCl, pH 7.8). Relative activities were calculated as follows: Relative activity (%) = 100 × [relative light units (RLU) of sample upon stimulation − RLU for unstimulated sample]/(RLU upon stimulation − RLU unstimulated).

5.2.4. 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 medium (Gibco) supplemented with 10% fetal bovine serum (FBS) and 5×10^{-5} M 2-mercaptoethanol, stimulated with 10 μg/mL plate-bound anti-CD3 antibody (Cedarlane), and incubated with 10 ng/mL of mouse IL-2 (PeproTech) plus 10 ng/mL of mouse IL-12 (PeproTech) (Th1) or 10 ng/mL of 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. The test compound was incubated through 5 days. The concentrations of DMSO derived from stock solution were 0.1%. The cultured cells were washed and re-stimulated with plate-bound anti-CD3 antibody in the absence of the test compound, and supernatants

were collected 16 h later. Cytokine ELISAs were performed using antibodies recommended by Pharmingen.

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