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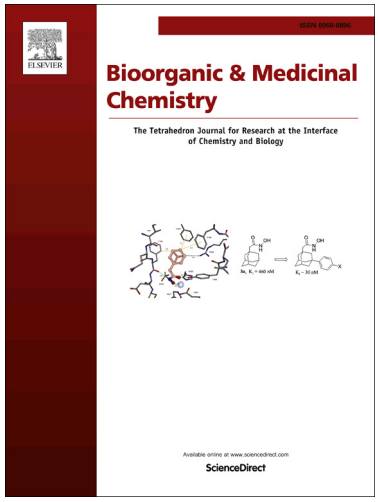

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Optimization of 2,4-diamino-5-fluoropyrimidine derivatives as protein kinase C theta inhibitors with mitigated time-dependent drug-drug interactions and P-gp liability

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Abstract

Protein kinase C theta (PKCθ) plays a critical role in T cell signaling and has therapeutic potential for T cell-mediated diseases such as transplant rejection and rheumatoid arthritis. Here, a series of 2,4-diamino-5-fluoropyrimidine derivatives were prepared and evaluated for their inhibition of PKCθ. Of these compounds, **14f** was found to exhibit potent PKCθ inhibitory activity and significantly weak CYP3A4 time-dependent inhibition (TDI) and P-glycoprotein (P-gp) liability.

1. Introduction

Immunological diseases such as transplant rejection and rheumatoid arthritis are characterized by inappropriate T cell activation that results in a pathogenic response.[1] In the field of transplantation, immunosuppressive agents are indispensable for the prevention of graft rejection. The calcineurin inhibitors (CNIs) such as FK506 and cyclosporine A, which reduce disease severity by preventing IL-2 production in T cells, are used as immunosuppressive agents. Treatment with CNIs after transplantation remarkably improves graft and patient survival. However, the clinical use of CNIs is reported to induce mechanism-based adverse effects such as nephrotoxicity and hypertension. Currently, combination therapy using CNIs and other immunosuppressive agents with an alternative mechanism is widely used to reduce adverse effects of CNIs by decreasing dose of CNIs. Regarding agents used in combination with CNIs, no concern of reversible and time-dependent inhibition (TDI) against CYP3A4 is necessary, as CNIs are mainly metabolized by CYP3A4 and the increased plasma concentration of CNIs due to CYP3A4 inhibition by a concomitant agent induces the occurrence of adverse effects. Mycophenolate mofetil (MMF), which exerts noncompetitive inhibition on the enzyme inosine monophosphate dehydrogenase (IMPDH) but does not inhibit CYP3A4, is often used in combination with CNIs to reduce the adverse effects. However, MMF also has side effects such as gastrointestinal tract disturbance.[2] Therefore, a new immunosuppressive drug with a distinct mechanism of action is urgently required.

The protein kinase C (PKC) family is one of the serine/threonine kinase families and has been classified into three subfamilies: classical (α, β, and γ), novel (δ, ε, η, and θ), and atypical (ζ and

λ).[3] Of these, PKCθ has recently been shown to play an important role in T cell signaling leading to the production of IL-2, which is of key importance in the immune response.[4,5] For example, the phenotype of PKCθ knockout mice is specific to T cell inactivation.[3] These results suggest the potential utility of a PKCθ inhibitor as a new immunosuppressive drug.

Although a large number of PKCθ inhibitors have been reported,[6] none have been launched as an immunosuppressive drug. Only Novartis Pharmaceuticals have reported development of Sotrastaurin (**1**) (Phase II, transplantation, Figure 1).[7] Clinical trials revealed that Sotrastaurin was effective in transplantation, but that it also increased area under the blood concentration-time curve (AUC) of FK506 as a combination drug by 2.0-fold, which may induce adverse effects.[8] This unfavorable outcome might be due to Sotrastaurin being a CYP3A4 inhibitor.[9] In this paper, to identify a novel PKCθ inhibitor with weak CYP3A4 inhibition, we focused on compound **2** described in the patent application from Rigel as our starting compound (Figure 1),[10] due to its good PKCθ inhibitory activity and synthetic accessibility. However, our investigation also revealed that compound **2** had CYP3A4 TDI properties that may cause adverse effects of combination drugs. In addition, compound **2** had a liability of being a substrate of P-glycoprotein (P-gp), which is an intestinal export pump and limits the oral bioavailability of substrate drugs. We therefore attempted to identify novel PKCθ inhibitors with weak CYP3A4 TDI and P-gp liability via modification of compound **2**. Here, we report the synthesis and biological evaluation of 2,4-diamino-5-fluoropyrimidine derivatives as PKCθ inhibitors.

2. Chemistry

As shown in Scheme 1, target compounds **5a** and **5b** were prepared from 2,4-dichloro-5-fluoropyrimidine (**3**). Treatment of compound **3** with various amines in methanol at room temperature in the presence of *N*,*N*-diisopropylethylamine (DIPEA) selectively gave **4a** and **4b**. *Ipso* substitution of these compounds with 3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)aniline under microwave irradiation with a catalytic amount of HCl gave target products **5a** and **5b**.

Compounds **9c** and its enantiomer **10c** were prepared via the alternative synthetic route depicted in Scheme 2. Treatment of compound **3** with sodium thiomethoxide selectively gave 2-chloro-5-fluoro-4-(methylsulfanyl)pyrimidine (**6**), which was then reacted with 3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)aniline, followed by oxidation with *m*-chloroperoxybenzoic acid (*m*-CPBA) to give a sulfoxide **8**. Displacement of the sulfoxide moiety by (3*R*) and (3*S*)-3-(aminomethyl)piperidine-1-carboxylic acid *tert*-butyl ester gave *tert*-butoxycarbonyl (Boc)-protected compounds **9a** and **10a**. Deprotection of the Boc group using trifluoroacetic acid (TFA) furnished compounds **9b** and **10b**, followed by reductive alkylation with formalin and sodium triacetoxyborohydride to give target compounds **9c** and **10c**, respectively.

The syntheses of various *N*-alkyl piperidine derivatives **11a**–**11d** are described in Scheme 3. Alkylation of compound **10b** with various alkyl bromides or triflates in the presence of DIPEA gave **11a**–**11d**.

Conversion of the tetrazole moiety on the phenyl ring to other heteroaryl rings is shown in Scheme 4. Treatment of compound **3** with (3*S*)-3-(aminomethyl)piperidine-1-carboxylic acid

tert-butyl ester and deprotection of Boc group following reductive alkylation with formalin and sodium triacetoxyborohydride gave the key intermediate **13** which was then reacted with various heteroaryl-substituted anilines to give **14a**–**14f** under acidic conditions.

The synthesis of aliphatic amine **17** and heteroaryl-substituted anilines **20** are described in Scheme 5 and 6, respectively. Ketone **15** was reacted with *p*-toluenesulfonylmethyl isocyanide (TosMIC) to give cyano compound **16**, which was then reduced with lithium alminium hydride (LAH) to give target product **17**. 3-Fluoronitrobenzene (**18**) was reacted with 4-chloro-1*H*-pyrazole, and nitro group was reduced with $SnCl_2 \cdot 2H_2O$ to give compound **20**.[11] Intermediates otherwise noted were commercially available.

3. Results and discussion

We evaluated the inhibitory activity of the compounds against human PKCθ *in vitro* by measuring fluorescence intensity after incubation with full-length human PKCθ and ATP. We also assessed the inhibitory activity of compounds against CYP3A4, the metabolic activity of human liver microsomes (HLMs) for midazolam was measured at 0 and 30 min after preincubation with test compounds. In the present study, the residual activity of CYP3A4 is shown as the percentage of metabolic activity remaining after 30 min preincubation, based on that at 0 min (i.e. without preincubation). In addition, we evaluated P-glycoprotein (P-gp) liability in LLC-PK1-MDR1 cells. Efflux ratio (ER) is the ratio of basolateral-to-apical permeability over apical-to-basolateral permeability of a compound. Net efflux ratio (NER) is the ratio of ER of MDR1 to wild type.

Compound **2** showed potent PKCθ inhibitory activity, but the residual activity of CYP3A4 was only 43% (Table 1). This result shows that compound **2** exhibits TDI for CYP3A4. Further, the NER of **2** was 40 in an LLC-PK1-MDR1 permeability assay, implying that compound **2** might be a P-gp substrate that induces low *in vivo* exposure.[12]

To minimize TDI, we focused on the distance between the piperidine nitrogen atom and the pyrimidine core, as CYP3A4 substrates are reported to have a hydrogen bond acceptor atom 5.5-7.8 Å from the site of metabolism.[13] We therefore modified the distance between the piperidine nitrogen atom and pyrimidine core (hydrogen bond acceptor). Although insertion of a methylene linker between piperidine moiety and pyrimidine maintained PKCθ inhibitory activity, TDI of **5a** remained unchanged. We next investigated an alternative strategy for reducing lipophilicity, as

correlations between lipophilicity of compounds and CYP3A4 substrates have been reported.[14] Removal of four methyl groups of **5a** subsequently proved effective. Compound **5b** showed weak TDI and potent PKCθ inhibitory activity. Changing the position of the piperidine nitrogen atom to the 3-position also maintained good activity and weak TDI (**9c** and **10c**). Isomer preference was noted for these two compounds. The *S*-isomer **10c** had 10-fold more active than that of the *R*-isomer **9c**. Our investigation demonstrated that structural modifications reducing the lipophilicity of compounds successfully ameliorated TDI. In addition, removal of four methyl groups also improved P-gp liability by reducing the pKa of compound (P-gp NER of **10c** was 9.3). This improvement was consistent with previous reports that indicated good correlation between basicity of compounds and P-gp NER.[15] We therefore reduced the pKa of the nitrogen in the piperidine to further improve P-gp NER (Table 2). Although introduction of a methoxyethyl group into the piperidine moiety (**11b**) reduced the pKa, improvement of P-gp NER was not observed. In contrast, 2,2-difluoromethyl piperidine (**11c**) and trifluoromethyl peperidine (**11d**) showed reduced pKa values 6.29 and 4.81 respectively, and both improved the P-gp NER.[16] While this investigation demonstrated the importance of lowering pKa to decrease P-gp NER value, basicity was also deemed important for PKCθ inhibitory activity.

We next focused on the high topological polar surface area (TPSA) of compound **10c** with an associated cost to P-gp NER (TPSA of **10c**: 105.9 $Å^2$).[16,17] As an opening strategy to reduce the TPSA, we removed the methoxy substituent on the phenyl ring, but P-gp NER of desmethoxy derivative **14a** was not improved. We then replaced the N atom of tetrazole with a C or O atom to

further reduce TPSA, resulting in triazole **14b**, oxazole **14c** and pyrazole **14d**. Triazole **14b** did not substantially change the P-gp NER. In contrast, while oxazole **14c** and pyrazole **14d** decreased PKCθ inhibitory activity, they provided a sought after improvement in P-gp NER to 3.5 and 4.5. Our investigation demonstrated that structural modifications reducing the TPSA of compounds successfully improved the P-gp NER of compounds.

Of these compounds, we selected **14d** with good P-gp NER for further optimization to improve PKCθ inhibitory activity. Introduction of substituents to the pyrazole ring and their effect on PKCθ inhibitory activity are shown in Table 4. Introduction of a methyl group to **14d** slightly decreased PKCθ inhibitory activity (**14e**), while the chlorine derivative **14f** exhibited 9-fold more potent activity than **14d**, maintaining good P-gp NER and CYP3A4 TDI. Docking studies of other diaminopyrimidine compounds with PKCθ have suggested that the diaminopyrimidine binds to the hinge region at the ATP binding site via 2-point binding of the 2-amino substituent and the nitrogen of the pyrimidine at the 1 position with Leu461, i.e. first between the NH of the 2-amino substituent and carbonyl 'O' of Leu461 (NH···CO), second between the N of pyrimidine and NH of Leu461 (N···NH).[18,19] Electron withdrawal by the chlorine atom of **14f** is considered to decrease the electron density of the NH of 2-amino substituent, which might strengthen the hydrogen bond with Leu461 and improve *in vitro* activity.[20]

As a result of the above optimization, we identified novel PKC inhibitors such as compound **14f** with mitigated CYP3A4 TDI and P-gp liability. These results indicated that **14f** will be a promising immunosuppressive combination agent with CNIs such as FK506, because **14f** has little concern

about TDI compared to compound **2**. Further optimization and biological evaluation of this compound is being conducted at present and studies of diaminopyrimidine analogues will be disclosed in due course.

4. Conclusions

We investigated the SAR of PKCθ inhibitory activity, CYP3A4 TDI and P-gp liability to obtain a promising PKCθ inhibitor. We first reduced the lipophilicity of **2** to obtain **10c** with mitigated CYP3A4 TDI. We then investigated the optimization of P-gp liability by lowering pKa or reducing TPSA. Although we demonstrated the importance of lowering pKa to improve P-gp liability, basicity was also required for PKCθ inhibition (**11c** and **11d**). In contrast, the alternative strategy of reducing TPSA gave compound **14f** with improved P-gp liability and maintained PKCθ inhibitory activity. As a result of our optimization, we discovered the novel PKCθ inhibitor **14f**, which presented little concern of CYP3A4 TDI or P-gp liability.

5. Experimental section

5.1. Chemistry

^{1}H NMR spectra were recorded on a Varian VNS-400, JEOL JNM-LA400, or JEOL JNM-AL400 and chemical shifts were expressed in δ (ppm) values with tetramethylsilane as an internal reference (s=singlet, d=doublet, t=triplet, m=multiplet, dd=double doublet, tt=triple triplet, q=quartet, ddd=double double doublet, and br=broad peak). Mass spectra (MS) were recorded on a

Waters UPLC/SQD[413W]-LC/MS system. Elemental analyses were performed using a Yanaco MT-6 (C, H, N), Elementar Vario EL III (C, H, X), and Dionex ICS-3000 (S, halogene) and were within ± 0.4% of theoretical values. Electrospray ionization positive high-resolution mass spectrum (HRMS) was obtained using a Waters LCT Premier. Unless otherwise noted, all reagents and solvents obtained from commercial suppliers were used without further purification. The following abbreviations are used: *m*-CPBA, *m*-chloroperoxybenzoic acid; DIPEA, *N*,*N*-diisopropylethylamine; DME, 1,2-dimethoxy ethane; DMF, *N*,*N*-dimethylformamide; DMI, 1,3-dimethyl-2-imidazolidinone; IPA, isopropyl alcohol (2-propanol); LAH, lithium alminium hydride; NMP, *N*-methylpyrrolidone; TFA, trifluoroacetic acid; and THF, tetrahydrofuran. TosMIC, *p*-tolylsulfonylmethyl isocyanide.

5.1.1. 2-Chloro-5-fluoro-*N*-[(1,2,2,6,6-pentamethylpiperidin-4-yl)methyl]pyrimidin-4-amine (**4a**)

To a solution of 2,4-dichloro-5-fluoropyrimidine **3** (400 mg, 2.4 mmol) in MeOH (6 mL), 1-(1,2,2,6,6-pentamethylpiperidin-4-yl)methanamine **16** (480 mg, 2.6 mmol) and DIPEA (820 μL, 4.8 mmol) were added. The reaction mixture was stirred at room temperature for 16 h, concentrated in vacuo and triturated with (*n*-hexane-EtOAc) (50:50) to give **4a** (500 mg, 66%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 1.28 (6H, s), 1.47 (6H, s), 1.58–1.92 (5H, m), 2.64 (3H, s), 3.22–3.29 (2H, m), 8.09 (1H, d, J = 3.4 Hz), 8.13–8.36 (1H, m); MS (ESI) m/z 315, 317 $[M+H]^+$.

5.1.2. 2-Chloro-5-fluoro-*N*-[(1-methylpiperidin-4-yl)methyl]pyrimidin-4-amine (**4b**)

To a solution of **3** (500 mg, 3.0 mmol) in MeOH (5 mL), (1-methyl-4-piperidinyl)methanamine (380 mg, 3.0 mmol) was added. The reaction mixture was stirred at room temperature for 16 h and then diluted with $CHCl_3$ and basified with saturated aqueous $NaHCO_3$. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on silica gel with elution using ($CHCl_3$-MeOH) (97:3) to give **4b** (295 mg, 38%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 1.09–1.23 (2H, m), 1.48–1.66 (3H, m), 1.72–1.83 (2H, m), 2.12 (3H, s), 2.67–2.77 (2H, m), 3.17–3.25 (2H, m), 8.04 (1H, d, J = 3.6 Hz), 8.18 (1H, br s); MS (ESI) m/z 259, 261 $[M+H]^+$.

5.1.3.

5-Fluoro-N^2-[3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)phenyl]-N^4-[(1,2,2,6,6-pentamethylpiperidin-4-yl)methyl]pyrimidine-2,4-diamine (**5a**)

To a solution of **4a** (200 mg, 0.64 mmol) in IPA (2 mL), 3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)aniline (110 mg, 0.54 mmol) and 4 M HCl-EtOAc (310 μL, 1.3 mmol) were added in a 10 mL microwave vial. The reaction mixture was heated with stirring at 140 °C in a Biotage auto-sampling microwave reactor for 1 h. The reaction mixture was diluted with $CHCl_3$ and washed with H_2O. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on amino silica gel with elution using ($CHCl_3$-MeOH) (90:10) to give **5a** (100 mg, 33%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ

0.81–1.12 (2H, m), 0.86 (6H, s), 1.03 (6H, s), 1.40–1.52 (2H, m), 1.92–2.08 (1H, m), 2.14 (3H, s), 2.58 (3H, s), 3.14–3.23 (2H, m), 3.80 (3H, s), 6.77 (1H, t, *J* = 2.0 Hz), 7.56–7.74 (3H, m), 7.90 (1H, d, *J* = 3.8 Hz), 9.43 (1H, s); MS (ESI) *m*/*z* 484 $[M+H]^+$; HRMS (ESI) calcd for $C_{24}H_{34}FN_9O$ $[M+H]^+$: 484.2949, Found; 484.2946.

5.1.4.

5-Fluoro-*N*^{2}-[3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)phenyl]-*N*^{4}-[(1-methylpiperidin-4-yl)methyl]pyrimidine-2,4-diamine dihydrochloride (**5b**)

To a solution of **4b** (120 mg, 0.48 mmol) in IPA (3 mL), 3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)aniline (98 mg, 0.48 mmol) and 4 M HCl-EtOAc (240 μL, 0.96 mmol) were added in a 10 mL microwave vial. The reaction mixture was heated with stirring at 140 °C in a Biotage auto-sampling microwave reactor for 1 h. The reaction mixture was washed with IPA to give an ivory solid. The solid was washed with EtOAc and MeOH to give **5b** (86 mg, 36%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 1.41–1.93 (2H, m), 1.74–1.77 (2H, m), 1.84–1.94 (1H, m), 2.60 (3H, s), 2.67–2.71 (3H, m), 2.78–2.87 (2H, m), 3.27–3.30 (2H, m), 3.34–3.37 (2H, m), 3.38 (3H, s), 6.96 (1H, br), 7.50–7.51 (1H, m), 7.58–7.59 (1H, m), 8.12–8.13 (1H, m), 8.66 (1H, br), 10.31 (1H, br); MS (ESI) *m*/*z* 428 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{26}FN_9O$ $[M+H]^+$:428.2322, Found: 428.2326.

5.1.5. 2-Chloro-5-fluoro-4-(methylsulfanyl)pyrimidine (**6**)

To a solution of **3** (3.0 g, 18.0 mmol) in THF (30 mL), sodium thiomethoxide (1.35 g, 19.3 mmol) was added under -30 °C in dry ice-acetone bath and stirred at -30 °C for 2 h. The reaction mixture was warmed to room temperature and poured into H_2O and extracted with EtOAc. The organic layer was then washed with H_2O, brine, dried over Na_2SO_4 and concentrated in vacuo to give **6** (24 g, 96%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.62 (3H, s), 8.09 (1H, d, J = 1.4 Hz); MS (ESI) m/z 179, 181 $[M+H]^+$.

5.1.6.

5-Fluoro-*N*-[3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)phenyl]-4-(methylsulfanyl)pyrimidin-2-amine (**7**)

To a solution of **6** (550 mg, 3.1 mmol) in IPA (5 mL), 3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)aniline (710 mg, 3.5 mmol) and 4 M HCl-EtOAc (850 μL, 3.4 mmol) were added in a 25 mL microwave vial. The reaction mixture was heated with stirring at 130 °C in a Biotage auto-sampling microwave reactor for 1 h. The reaction mixture was poured into H_2O and the precipitate was washed with H_2O to give **7** (890 mg, 83%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.58 (3H, s), 2.59 (3H, s), 3.82 (3H, s), 6.88 (1H, t, J = 2.0 Hz), 7.56 (1H, t, J = 2.0 Hz), 7.70 (1H, t, J = 1.8 Hz), 8.31 (1H, d, J = 2.0 Hz), 10.02 (1H, s); MS (ESI) m/z 348 $[M+H]^+$.

5.1.7.

5-Fluoro-*N*-[3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)phenyl]-4-(methylsulfinyl)pyrimidin-2-amine (**8**)

To a solution of **7** (800 mg, 2.3 mmol) in CH_2Cl_2 (8 mL), *m*-CPBA (650 mg, 2.9 mmol) was added at 0 °C and stirred for 3h at 0 °C. The reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo The residue was chromatographed on silica gel with elution using EtOAc to give **8** (450 mg, 54%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.62 (3H, s), 2.94 (3H, s), 3.84 (3H, s), 6.91 (1H, t, J = 1.9 Hz), 7.71–7.75 (2H, m), 8.77 (1H, d, J = 1.5 Hz), 10.52 (1H, s); MS (ESI) m/z 364 $[M+H]^+$.

5.1.8. ***tert*-Butyl (3*R*)-3-{[(5-fluoro-2-{[3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)phenyl]amino}pyrimidin-4-yl)amino]methyl}piperidine-1-carboxylate** (**9a**)

To a solution of **8** (300 mg, 0.83 mmol) in NMP (3 mL), (3*R*)-3-(aminomethyl)piperidine-1-carboxylic acid *tert*-butyl ester (210 mg, 0.99 mmol) and DIPEA (180 μL, 1.1 mmol) were added in a 10 mL microwave vial. The reaction mixture was heated with stirring at 110 °C in a Biotage auto-sampling microwave reactor for 0.5 h. The reaction mixture was poured into saturated aqueous NH_4Cl and collected by vacuum filtration to give **9a** (420 mg, 99%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 1.18–1.83 (5H, m), 1.32 (9H, s), 2.58

(3H, s), 2.63–2.84 (2H, m), 3.17–3.29 (2H, m), 3.62–3.93 (2H, m), 3.80 (3H, s), 6.78 (1H, t, *J* = 2.1 Hz), 7.54 (1H, t, *J* = 1.9 Hz), 7.59–7.64 (1H, m), 7.71–7.74 (1H, m), 7.92 (1H, d, *J* = 3.8 Hz), 9.44 (1H, s); MS (ESI) *m*/*z* 514 $[M+H]^+$.

5.1.9.

5-Fluoro-*N*^{2}-[3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)phenyl]-*N*^{4}-[(3*S*)-piperidin-3-ylmethyl] pyrimidine-2,4-diamine (**9b**)

To a solution of **9a** (400 mg, 0.78 mmol) in CH_2Cl_2 (4 mL), TFA (2.0 mL, 26 mmol) was added and stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo. The residue was added to saturated aqueous K_2CO_3 and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated in vacuo The residue was chromatographed on amino silica gel with elution using ($CHCl_3$-MeOH) (96:4) to give **9b** (260 mg, 81%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 0.80–1.71 (6H, m), 2.05–2.40 (2H, m), 2.57 (3H, s), 2.71–2.87 (2H, m), 3.14–3.24 (2H, m), 3.80 (3H, s), 6.77 (1H, t, *J* = 2.0 Hz), 7.52–7.75 (3H, m), 7.89 (1H, d, *J* = 3.8 Hz), 9.41 (1H, s); MS (ESI) *m*/*z* 414 $[M+H]^+$.

5.1.10.

5-Fluoro-*N*^{2}-[3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)phenyl]-*N*^{4}-{[(3*R*)-1-methylpiperidin-3-yl]methyl}pyrimidine-2,4-diamine (**9c**)

To a solution of **9b** (50 mg, 0.12 mmol) in CH_2Cl_2 (1 mL), 36% aqueous formaldehyde solution

(11 μL, 0.15 mmol) and sodium triacetoxyborohydride (33 mg, 0.16 mmol) were added and the mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with $CHCl_3$ and basified with saturated aqueous $NaHCO_3$. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on amino silica gel with elution using EtOAc to give **9c** (32 mg, 62%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 0.74–1.93 (7H, m), 2.09 (3H, s), 2.48–2.64 (2H, m), 2.57 (3H, s), 3.18–3.33 (2H, m), 3.79 (3H, s), 6.77 (1H, t, J = 2.0 Hz), 7.52–7.75 (3H, m), 7.89 (1H, d, J = 3.8 Hz), 9.42 (1H, s); MS (ESI) m/z 428 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{26}FN_9O$ $[M+H]^+$: 428.2322, Found: 428.2331. $[\alpha]_D^{21}$ (c = 0.1, MeOH) -5.5 °.

5.1.11. *tert*-Butyl (3*S*)-3-{[(5-fluoro-2-{[3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)phenyl]amino}pyrimidin-4-yl)amino]methyl}piperidine-1-carboxylate (**10a**)

Compound **10a** was prepared from compound **8** in 85% yield as a colorless solid, using a similar approach to that described for **9a**. ^{1}H NMR (DMSO-d_6) δ 1.16–1.85 (5H, m), 1.32 (9H, s), 2.31–2.78 (2H, m), 2.58 (3H, s), 3.15–3.29 (2H, m), 3.60–3.94 (2H, m), 3.80 (3H, s), 6.78 (1H, t, J = 2.1 Hz), 7.54 (1H, t, J = 2.1 Hz), 7.59–7.67 (1H, m), 7.71–7.76 (1H, m), 7.92 (1H, d, J = 3.7 Hz), 9.44 (1H, s); MS (ESI) m/z 514 $[M+H]^+$.

5.1.12.

5-Fluoro-*N*^{2}-[3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)phenyl]-*N*^{4}-[(3*R*)-piperidin-3-ylmethyl] pyrimidine-2,4-diamine (**10b**)

Compound **10b** was prepared from compound **10a** in 99% yield as a colorless solid, using a similar approach to that described for **9b**. ^{1}H NMR (DMSO-d_6) δ 0.80–1.77 (5H, m), 2.05–2.53 (3H, m), 2.58 (3H, s), 2.72–2.88 (2H, m), 3.14–3.24 (2H, m), 3.80 (3H, s), 6.78 (1H, t, J = 2.0 Hz), 7.52–7.76 (3H, m), 7.89 (1H, d, J = 3.8 Hz), 9.42 (1H, s); MS (ESI) m/z 414 $[M+H]^+$.

5.1.13.

5-Fluoro-*N*^{2}-[3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)phenyl]-*N*^{4}-{[(3*S*)-1-methylpiperidin-3-yl]methyl}pyrimidine-2,4-diamine (**10c**)

Compound **10c** was prepared from compound **10b** in 61% yield as a colorless solid, using a similar approach to that described for **9c**. ^{1}H NMR (DMSO-d_6) δ 0.73–1.93 (7H, m), 2.09 (3H, s), 2.44–2.70 (2H, m), 2.57 (3H, s), 3.12–3.36 (2H, m), 3.80 (3H, s), 6.78 (1H, t, J = 2.0 Hz), 7.52–7.76 (3H, m), 7.89 (1H, d, J = 3.8 Hz), 9.42 (1H, s); MS (ESI) m/z 428 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{26}FN_9O$ $[M+H]^+$: 428.2322, Found: 428.2332; Anal. Calcd for $C_{20}H_{26}FN_9O$: C, 56.19; H, 6.13; N, 29.49; F, 4.44. Found: C, 56.28; H, 6.15; N, 29.45; F, 4.47. $[\alpha]_D^{21}$ (c = 0.1, MeOH) 6.0 °.

5.1.14.

***N*^{4}-{[(3*S*)-1-Ethylpiperidin-3-yl]methyl}-5-fluoro-*N*^{2}-[3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)**

phenyl]pyrimidine-2,4-diamine (**11a**)

To a solution of **10b** (52 mg, 0.13 mmol) in NMP (500 μL), ethylbromide (12 μL, 0.16 mmol) and DIPEA (28 μL, 0.16 mmol) were added and the reaction mixture was stirred at 80 °C for 1h. To the reaction mixture $CHCl_3$ was added and chromatographed on amino silica gel with elution using (*n*-hexane-EtOAc) (1:6) to give **11a** (38 mg, 68%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 0.77–2.36 (9H, m), 0.94 (3H, t, J = 8.0 Hz), 2.40–2.78 (2H, m), 2.57 (3H, s), 3.17–3.37 (2H, m), 3.80 (3H, s), 6.78 (1H, t, J = 2.0 Hz), 7.52–7.76 (3H, m), 7.89 (1H, d, J = 3.8 Hz), 9.42 (1H, s); MS (ESI) *m*/*z* 442 $[M+H]^+$; HRMS (ESI) calcd for $C_{21}H_{28}FN_9O$ $[M+H]^+$: 442.2479, Found: 442.2479.

5.1.15.

5-Fluoro-*N*^{4}-{[(3*S*)-1-(2-methoxyethyl)piperidin-3-yl]methyl}-*N*^{2}-[3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)phenyl]pyrimidine-2,4-diamine (**11b**)

Compound **11b** was prepared from compound **10b** in 64% yield as a colorless solid, using a similar approach to that described for **11a**. ^{1}H NMR (DMSO-d_6) δ 0.79–1.93 (7H, m), 2.36–2.42 (2H, m), 2.57 (3H, s), 2.65–2.76 (2H, m), 3.18 (3H, s), 3.19–3.39 (4H, m), 3.80 (3H, s), 6.78 (1H, t, J = 2.0 Hz), 7.52–7.76 (3H, m), 7.89 (1H, d, J = 3.8 Hz), 9.42 (1H, s); MS (ESI) *m*/*z* 472 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{30}FN_9O_2$ $[M+H]^+$: 472.2585, Found: 472.2575.

5.1.16.

2-[(3*S*)-3-{[(5-Fluoro-2-{[3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)phenyl]amino}pyrimidin-4-

yl)amino]methyl}piperidin-1-yl]ethanol (**11c**)

To a solution of **10b** (59 mg, 0.14 mmol) in THF (1.2 ml), 2,2-difluoroethyl trifluoromethanesulfonate (37 mg, 0.17 mmol) and DIPEA (49 μL, 0.29 mmol) were added. The reaction mixture was refluxed for 14 h. After cooling to room temperature, the reaction mixture was diluted with $CHCl_3$ and basified with saturated aqueous $NaHCO_3$. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on silica gel with elution using ($CHCl_3$-MeOH) (97:3) to give **11c** (35mg, 51%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 0.80–0.88 (1H, m), 1.31–1.42 (1H, m), 1.53–1.59 (2H, m), 1.82–1.86 (2H, m), 2.05–2.11 (1H, m), 2.57 (3H, s), 2.61–2.70 (2H, m), 2.73–2.80 (2H, m), 3.16–3.31 (2H, m), 3.80 (3H, s), 6.07 (1H, tt, J = 55.9, 4.3 Hz), 6.78 (1H, t, J = 2.1 Hz), 7.54–7.59 (2H, m), 7.73 (1H, t, J = 1.8 Hz), 7.90 (1H, d, J = 3.7 Hz), 9.42 (1H, s). MS (ESI) m/z 478 $[M+H]^+$; HRMS (ESI) calcd for $C_{21}H_{26}F_3N_9O$ $[M+H]^+$: 478,2291, Found: 478.2288.

5.1.17.

5-Fluoro-*N*^{2}-[3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)phenyl]-*N*^{4}-{[(3*S*)-1-(2,2,2-trifluoroethyl)piperidin-3-yl]methyl}pyrimidine-2,4-diamine (**11d**)

Compound **11d** was prepared from compound **10b** in 64% yield as a colorless solid, using a similar approach to that described for **11c**. ^{1}H NMR (DMSO-d_6) δ 0.81–0.91 (1H, m), 1.33–1.43 (1H, m), 1.53–1.59 (2H, m), 1.84–1.91 (1H, m), 1.98–2.03 (1H, m), 2.21–2.27 (1H, m), 2.57 (3H, s), 2.77–2.80 (1H, m), 2.83–2.86 (1H, m), 3.10 (2H, q, J = 10.3 Hz), 3.21–3.25 (2H, m), 3.79 (3H,

s), 6.78 (1H, t, *J* = 2.1 Hz), 7.55–7.60 (2H, m), 7.72 (1H, t, *J* = 1.8 Hz), 7.90 (1H, d, *J* = 3.8 Hz), 9.43 (1H, s). MS (ESI) *m*/*z* 496 $[M+H]^+$; HRMS (ESI) calcd for $C_{21}H_{25}F_4N_9O_2$ $[M+H]^+$: 496.2196, Found: 496.2206.

5.1.18. 2-Chloro-5-fluoro-*N*-[(3*R*)-piperidin-3-ylmethyl]pyrimidin-4-amine (12)

To a solution of **3** (3.4 g, 20 mmol) in DMF (34 mL), (3*S*)-3-(aminomethyl)piperidine-1-carboxylic acid *tert*-butyl ester (5.2 g, 24 mmol) and DIPEA (4.5 mL, 26 mmol) were added. The reaction mixture was stirred at room temperature for 14 h. The reaction mixture was poured into saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with H_2O and brine and then dried over Na_2SO_4 and concentrated in vacuo to give a brown solid. To a solution of the residue in CH_2Cl_2 (60 mL), TFA (13 mL, 170 mmol) was added and stirred at room temperature for 4h. The reaction mixture was concentrated in vacuo. The residue was added to saturated aqueous K_2CO_3 and extracted with ($CHCl_3$-MeOH) (80/20). The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give **12** (4.2 g, 87%) as a pale solid. ^{1}H NMR (DMSO-d_6): 0.99–1.12 (1H, m), 1.24–1.39 (1H, m), 1.52–1.61 (1H, m), 1.66–1.79 (2H, m), 2.21 (1H, dd, *J* = 11.8, 9.4 Hz), 2.38–2.48 (1H, m), 2.77–2.85 (1H, m), 2.85–2.93 (1H, m), 3.15–3.24 (3H, m), 8.04 (1H, d, *J* = 3.2 Hz), 8.15-8.24 (1H, m); MS (ESI) *m*/*z* 245, 247 $[M+H]^+$.

5.1.19. 2-Chloro-5-fluoro-N-{[(3*S*)-1-methylpiperidin-3-yl]methyl}pyrimidin-4-amine (13)

To a solution of **12** (2.1 g, 8.6 mmol) in CH_2Cl_2 (21 mL) , 36% aqueous formaldehyde solution

(1.0 g, 13 mmol) and sodium triacetoxyborohydride (2.7 g, 13 mmol) were added and then stirred at room temperature for 1 h. The reaction mixture was diluted with $CHCl_3$ and basified with saturated aqueous $NaHCO_3$. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on amino silica gel with elution using $CHCl_3$ to give **13** (1.3 g, 59%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 0.82–0.97 (1H, m), 1.34–1.49 (1H, m), 1.54–1.70 (3H, m), 1.78–1.94 (2H, m), 2.12 (3H, s), 2.53–2.69 (2H, m), 3.12–3.29 (2H, m), 8.04 (1H, d, J = 3.5 Hz), 8.14–8.24 (1H, m); MS (ESI) m/z 259, 261 $[M+H]^+$.

5.1.20.

5-Fluoro-*N*^{4}-{[(3*S*)-1-methylpiperidin-3-yl]methyl}-*N*^{2}-[3-(5-methyl-1*H*-tetrazol-1-yl)phenyl]pyrimidine-2,4-diamine (**14a**)

To a solution of **13** (355 mg, 1.4 mmol) in IPA (14 mL), 3-(5-methyl-1*H*-tetrazol-1-yl)aniline (313 mg, 1.3 mmol) and 4 M HCl-EtOAc (1.0 mL, 4.1 mmol) were added and stirred at 120 °C for 9 h. The reaction mixture was diluted with $CHCl_3$ and washed with saturated aqueous $NaHCO_3$. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on amino silica gel with elution using ($CHCl_3$-MeOH) (90:10) to give **14a** (320 mg, 59%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 0.74–0.87 (1H, m), 1.28–1.41 (1H, m), 1.54–1.64 (3H, m), 1.74–1.92 (2H, m), 2.10 (3H, s), 2.52–2.70 (2H, m), 2.57 (3H, s), 3.15–3.28 (2H, m), 7.10–7.18 (1H, m), 7.47 (1H, t, J = 8.1 Hz), 7.55–7.63 (1H, m), 7.77–7.83 (1H, m), 7.90 (1H, d, J = 3.8 Hz), 8.24 (1H, t, J = 2.1 Hz), 9.49 (1H, s); MS (ESI) m/z 398 $[M+H]^+$;

HRMS (ESI) calcd for $C_{19}H_{24}FN_9$ $[M+H]^+$: 398.2217, Found: 398.2208.

5.1.21.

5-Fluoro-N^4-{[(3*S*)-1-methylpiperidin-3-yl]methyl}-N^2-[3-(1*H*-1,2,3-triazol-1-yl)phenyl]pyrimidine-2,4-diamine (**14b**)

Compound **14b** was prepared from compound **13** in 74% yield as a colorless solid, using a similar approach to that described for **14a**. ^{1}H NMR (DMSO-d_6) δ 0.81–0.95 (1H, m), 1.27–1.42 (1H, m), 1.50–1.64 (3H, m), 1.71–1.82 (1H, m), 1.83–1.94 (1H, m), 2.07 (3H, s), 2.47–2.66 (2H, m), 3.23–3.37 (2H, m), 7.27–7.33 (1H, m), 7.41 (1H, t, *J* = 8.1 Hz), 7.52–7.61 (1H, m), 7.65–7.72 (1H, m), 7.90 (1H, d, *J* = 3.8 Hz), 7.95 (1H, d, *J* = 3.8 Hz), 8.57 (1H, d, *J* = 2.1 Hz), 8.71 (1H, d, *J* = 1.2 Hz), 9.4 (1H, s); MS (ESI) *m*/*z* 383 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{23}FN_8$ $[M+H]^+$: 383.2108, Found: 383.2106.

5.1.22.

5-Fluoro-N^4-{[(3*S*)-1-methylpiperidin-3-yl]methyl}-N^2-[3-(1,3-oxazol-5-yl)phenyl]pyrimidine-2,4-diamine (**14c**)

Compound **14c** was prepared from compound **13** in 72% yield as a colorless solid, using a similar approach to that described for **14a**. ^{1}H-NMR (DMSO-d_6) δ 0.80–0.95 (1H, m), 1.30–1.46 (1H, m), 1.52–1.71 (3H, m), 1.74–1.84 (1H, m), 1.84–1.98 (1H, m), 2.08 (3H, s), 2.42–2.70 (2H, m), 3.23–3.39 (2H, m), 7.21–7.27 (1H, m), 7.31 (1H, t, *J* = 7.9 Hz), 7.50–7.56 (1H, m), 7.57 (1H, s),

7.63–7.69 (1H, m), 7.88 (1H, d, *J* = 3.8 Hz), 8.20–8.24 (1H, m), 8.42 (1H, s), 9.19 (1H, s); MS (ESI) *m*/*z* 383 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{23}FN_6O$ $[M+H]^+$: 383.1996, Found: 383.1994.

5.1.23.

5-Fluoro-*N*^{4}-{[(3*S*)-1-methylpiperidin-3-yl]methyl}-*N*^{2}-[3-(1*H*-pyrazol-1-yl)phenyl]pyrimidine-2,4-diamine (**14d**)

Compound **14d** was prepared from compound **13** in 86% yield as a colorless solid, using a similar approach to that described for **14a**. ^{1}H NMR (DMSO-d_6) δ 0.82–0.97 (1H, m), 1.31–1.45 (1H, m), 1.53–1.68 (3H, m), 1.74–1.97 (2H, m), 2.08 (3H, s), 2.50–2.71 (2H, m), 3.21–3.41 (2H, m), 6.52 (1H, dd, *J* = 2.4, 1.6 Hz), 7.24–7.34 (2H, m), 7.48–7.59 (2H, m), 7.71 (1H, d, *J* = 1.6 Hz), 7.88 (1H, d, *J* = 4.0 Hz), 8.34 (1H, d, *J* = 2.4 Hz), 8.44 (1H, dd, *J* = 1.6, 1.6 Hz), 9.24 (1H, s); MS (ESI) *m*/*z* 382 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{24}FN_7$ $[M+H]^+$: 382.2155, Found: 382.2148.

5.1.24.

5-Fluoro-*N*^{4}-{[(3*S*)-1-methylpiperidin-3-yl]methyl}-*N*^{2}-[3-(4-methyl-1*H*-pyrazol-1-yl)phenyl]pyrimidine-2,4-diamine (**14e**)

Compound **14e** was prepared from compound **13** in 93% yield as a white solid, using a similar approach to that described for **14a**. ^{1}H NMR (DMSO-d_6) δ 0.83–0.97 (1H, m), 1.32–1.45 (1H, m), 1.53–1.69 (3H, m), 1.74–1.84 (1H, m), 1.84–1.96 (1H, m), 2.08 (3H, s), 2.10 (3H, s), 2.52–2.62 (1H, m), 2.62–2.70 (1H, m), 3.26–3.38 (2H, m), 7.17–7.23 (1H, m), 7.27 (1H, dd, *J* = 8.0, 8.0 Hz),

7.46–7.56 (3H, m), 7.88 (1H, d, *J* = 3.6 Hz), 8.10 (1H, s), 8.38 (1H, dd, *J* = 2.0, 2.0 Hz), 9.21 (1H, s); MS (ESI) *m*/*z* 396 $[M+H]^+$; HRMS (ESI) calcd for $C_{21}H_{26}FN_7$ $[M+H]^+$: 396.2312, Found: 396.2308.

5.1.25.

***N*^{2}-[3-(4-Chloro-1*H*-pyrazol-1-yl)phenyl]-5-fluoro-*N*^{4}-{[(3*S*)-1-methylpiperidin-3-yl]methyl}pyrimidine-2,4-diamine** (**14f**)

Compound **14j** was prepared from compound **13** in 92% yield as a white solid, using a similar approach to that described for **14a**. ^{1}H NMR (DMSO-d_6) δ 0.80–0.95 (1H, m), 1.30–1.44 (1H, m), 1.52–1.68 (3H, m), 1.73–1.96 (2H, m), 2.08 (3H, s), 2.49–2.70 (2H, m), 3.24–3.39 (2H, m), 7.21–7.27 (1H, m), 7.32 (1H, dd, *J* = 8.0, 8.0 Hz), 7.49–7.58 (2H, m), 7.83 (1H, d, *J* = 0.4 Hz), 7.89 (1H, d, *J* = 3.6 Hz), 8.45 (1H, dd, *J* = 2.2, 2.2 Hz), 8.65 (1H, d, *J* = 0.4 Hz), 9.28 (1H, s); MS (ESI) *m*/*z* 416, 418 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{23}ClFN_7$ $[M+H]^+$: 416.1766, Found: 416.1770; Anal. Calcd for $C_{20}H_{23}ClFN_7$: C, 57.76; H, 5.57; N, 23.57; Cl, 8.52; F, 4.57. Found: C, 57.79; H, 5.46; N, 23.64; Cl, 8.49; F, 4.59. $[\alpha]_D^{21}$ (*c* = 0.1, MeOH) -4.5 °.

5.1.26. 1,2,2,6,6-Pentamethylpiperidine-4-carbonitrile (**16**)

Under argon atmosphere, to a solution of **15** (3.0 g, 18 mmol) and TosMIC (3.8 g, 19 mmol) in DME (63 mL) and *tert*-BuOH (21 mL), *tert*-BuOK (4.0 g, 35 mmol) was added at 0 °C. The reaction mixture was allowed to room temperature and stirred for 2 h. The reaction mixture was

poured into H_2O and extracted with EtOAc. The organic layer was washed with brine and dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was triturated with (*n*-hexane-EtOAc) (50:50) to give **16** (1.8 g, 56%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.00 (6H, s), 1.16 (6H, s), 1.63–1.74 (2H, m), 1.78–1.88 (2H, m), 2.22 (3H, s), 2.73–2.84 (1H, m); MS (ESI) *m*/*z* 181 $[M+H]^+$.

5.1.27. 1-(1,2,2,6,6-Pentamethylpiperidin-4-yl)methanamine (**17**)

Under argon atmosphere, to a suspension of LAH (210 mg, 5.5 mmol) in THF (20 mL), **16** (1.0 g, 5.5 mmol) was added at 0 °C. The reaction mixture was stirred at 0 °C for 3 h then cautiously quenched with saturated aqueous Rochelle's salt and stirred at room temperature for 15 min. The precipitate was filtrated and concentrated under reduced pressure. The residue was chromatographed on amino silica gel with elution using ($CHCl_3$-MeOH) (98:2) to give **17** (1.0 g, 98%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 0.97–1.17 (4H, m), 1.01 (6H, s), 1.14 (6H, s), 1.48–1.58 (2H, m), 1.61–1.75 (1H, m), 2.25 (3H, s), 2.53 (2H, d, J = 6.4 Hz); MS (ESI) *m*/*z* 185 $[M+H]^+$.

5.1.28. 4-Chloro-1-(3-nitrophenyl)-1*H*-pyrazole (**19**)

To a solution of **20** (400 mg, 2.8 mmol) in DMI (2.4 mL), 4-chloro-1*H*-pyrazole (440 mg, 4.3 mmol) and K_2CO_3 (0.78 g, 5.7 mmol) were added and stirred at 130 °C for 16 h. The mixture was partitioned with EtOAc and H_2O. The organic layer was washed with brine, dried over anhydrous

Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed on amino silica gel with elution using (*n*-hexane-EtOAc) (90:10) to give **21** (583 mg, 92%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 7.82 (1H, dd, *J* = 8.0, 8.0 Hz), 8.00 (1H, d, *J* = 0.4 Hz), 8.16–8.21 (1H, m), 8.27–8.32 (1H, m), 8.64 (1H, dd, *J* = 2.4, 2.4 Hz), 9.04 (1H, d, *J* = 0.4 Hz); MS (ESI) *m*/*z* 224, 226 $[M+H]^+$.

5.1.29. 3-(4-Chloro-1*H*-pyrazol-1-yl)aniline (**20**)

To a solution of **19** (580 mg, 2.6 mmol) in EtOH (14 mL), $SnCl_2 \cdot 2H_2O$ (2.3 g, 10 mmol) was added and refluxed for 6 h. The mixture was partitioned with EtOAc and saturated aqueous $NaHCO_3$ and filtered with celite. The residue was extracted, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed on amino silica gel with elution using (*n*-hexane-EtOAc) (45:55) to give **22** (467 mg, 94%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 5.38 (2H, s), 6.49–6.54 (1H, m), 6.84–6.88 (1H, m), 7.01 (1H, dd, *J* = 2.0, 2.0 Hz), 7.10 (1H, dd, *J* = 8.0, 8.0 Hz), 7.79 (1H, d, *J* = 0.8 Hz), 8.59 (1H, d, *J* = 0.8 Hz); MS (ESI) *m*/*z* 194, 196 $[M+H]^+$.

5.2. Biology

5.2.1. PKCθ inhibitory activity (enzyme assay)

The reaction mixture contained STK Substrate 1-biotin, full-length human PKCθ and ATP. Our

compound was dissolved in dimethyl sulfoxide and added to the reaction mixture. The reaction mixture was incubated at room temperature for 60 min, followed by incubation with Sa-XL665 and STK Antibody Cryptate for 60 min. The enzyme reaction rate was measured in fluorescence intensity at 620 nm (Cryptate) and 665 nm (XL665). The activity is expressed as "PKCθ IC_{50} (nM)" in Tables.

5.2.2. Assessment of inhibition potency of CYP3A4 using human liver microsomes

The test compounds (5 μM) were added to human liver microsomes (HLMs) (0.1 mg/mL) in potassium sodium phosphate buffer (100 mM, pH 7.4) containing NADPH (1 mM) and ethylenediaminetetraacetic acid (EDTA, 0.1 mM). For reversible inhibition study, midazolam was added to the reaction mixture without pre-incubation. For time-dependent inhibition study, midazolam was added to the mixture after pre-incubation for 30 min at 37 °C. The reaction was stopped by addition of acetonitrile after incubation for 20 min at 37 °C. The metabolite of midazolam (1'-hydroxymidazolam) was analyzed by LC/MS/MS and the metabolic activity was determined. The residual metabolic activity of HLMs for midazolam in the time-dependent inhibition study was expressed as the percentage of that in the reversible inhibition study.

5.2.3. Transcellular transport study in LLC-PK1-MDR1 cells

Wild type or MDR1-expressing LLC-PK1 cells (LLC-PK1-WT or LLC-PK1-MDR1, respectively) cultured for 5 days on a BD Falcon 96-Multiwell 1.0 μm Insert System were

pre-incubated with transport buffer (HBSS, pH7.4, for the apical and basolateral sides) for 1h. After aspiration of the transport buffer, the donor solution (transport buffer (0.5% DMSO) containing the test compound (1 μM) and Texas Red (1 μM)) was added to the apical or basolateral side for the influx or efflux transport study, respectively, and the receiver solution (transport buffer (0.5% DMSO)) was added to the opposite side. After incubation for 3h, the test compound in both sides was analyzed by LC/MS/MS and the apparent permeability was determined. Efflux ratio (ER) was calculated by dividing the apparent permeability in the direction from the basolateral to the apical side by that in the opposite direction. Net efflux ratio (NER) was the ratio of ER of LLC-PK1-MDR1 to LLC-PK1-WT. Texas Red was used for the estimation of the apparent permeability via paracellular transport.

Acknowledgments

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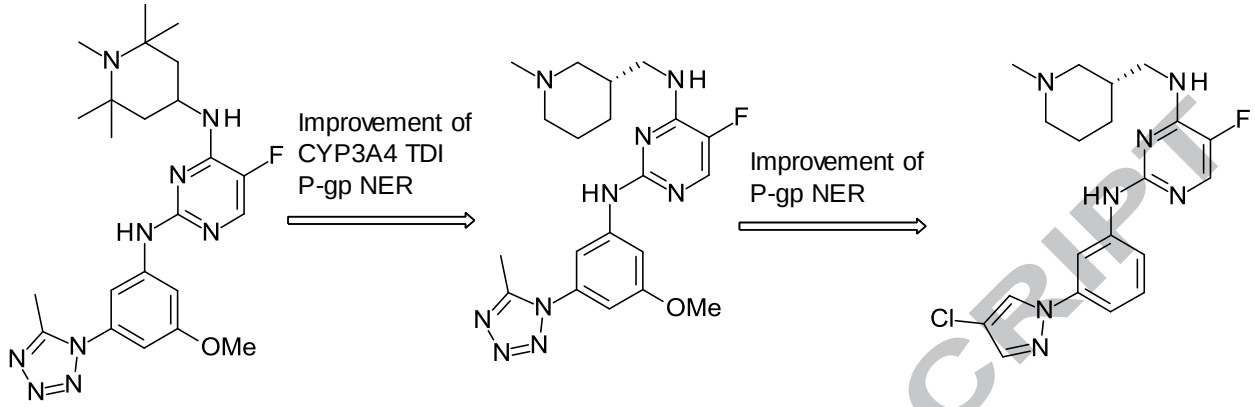
Graphical abstract



2

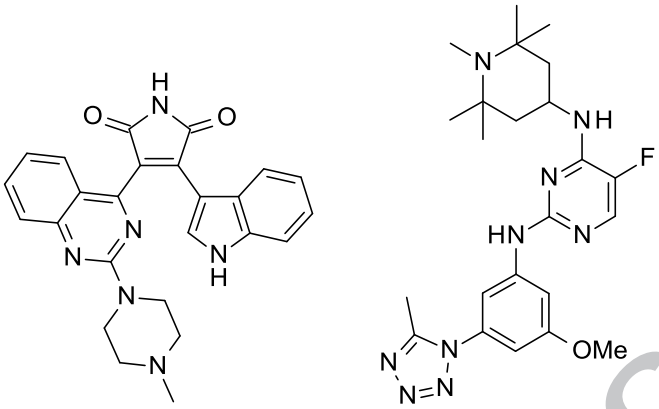
PKCθ IC_{50} = 1.5 nM
CYP3A4 TDI = 43%
P-gp NER = 40

10c

PKCθ IC_{50} = 0.32 nM
CYP3A4 TDI = 85%
P-gp NER = 9.3

14f

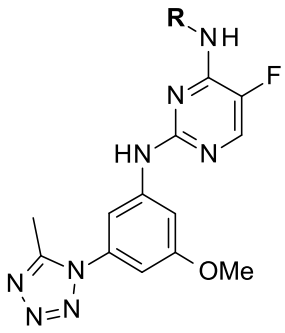
PKCθ IC_{50} = 3.0 nM
CYP3A4 TDI = 98%
P-gp NER = 2.3



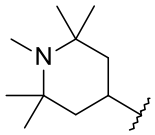
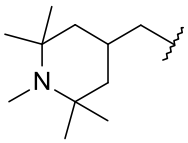
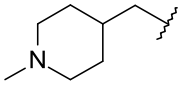
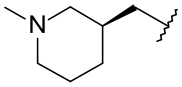
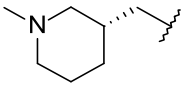
Sotrastaurin (**1**) **2**

Figure 1. Structure of PKC inhibitors.

Table 1. Conversion of pentamethylpiperidyl group of compound **2**.



Compound	R	PKCθ IC_{50}[a]	CYP3A4 TDI (residual	ACDlogP[c]	P-gp NER[d]	pKa[c]

		(nM)	activity, %)[b]			
2		1.5	43	4.00	40	10.21
5a		4.0	48	3.76	24	10.85
5b		0.68	89	1.93	8.0	9.19
9c		5.2	86	2.54	8.2	9.40
10c		0.32	85	2.54	9.3	9.40

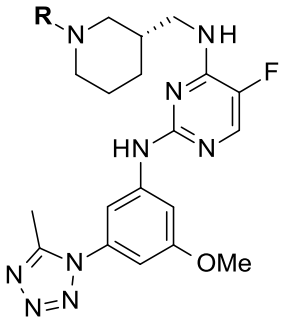
[a] IC_{50} values were determined in duplicate in one experiment.

[b] Activities of HLMs for metabolism of midazolam were measured and residual activities are shown as percentage of remained metabolic activity following preincubation for 30 min in presence of test compounds (5 μM).

[c] ACDlogP and pKa values were calculated with ACD/PhysChem Batch (version 12.01).

[d] Net efflux ratio was LLC-PK1-MDR1 efflux ratio to LLC-PK1-wild type efflux ratio.

Table 2. Conversion of *N*-alkyl group on the piperidine side chain



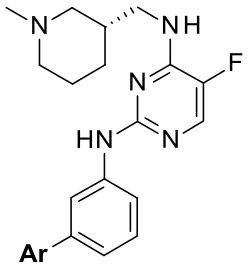
Compound	R	PKCθ IC_{50}[a] (nM)	P-gp NER[b]	pKa[c]
10c	Me	0.32	9.3	9.40
11a	Et	0.14	19	9.25
11b	$MeOCH_2CH_2$	0.46	9.6	8.43
11c	CHF_2CH_2	30	4.0	6.29
11d	CF_3CH_2	>100	2.4	4.81

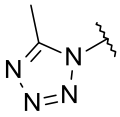
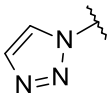
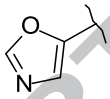
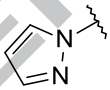
[a] IC_{50} values were determined in duplicate in one experiment.

[b] Net efflux ratio was ratio of LLC-PK1-MDR1 efflux ratio to LLC-PK1-wild type efflux ratio.

[c] pKa values were calculated with ACD/PhysChem Batch (version 12.01).

Table 3. Conversion of the tetrazole moiety on the phenyl ring



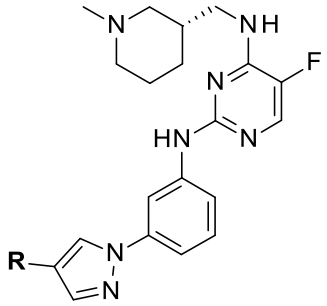
Compound	Ar	PKCθ IC_{50}[a] (nM)	P-gp NER[b]	TPSA[c]
10c	-	0.32	9.3	105.9
14a		2.1	11.7	96.7
14b		10	10.7	83.8
14c		27	4.5	79.1
14d		28	3.5	70.9

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

[b] Net efflux ratio was ratio of LLC-PK1-MDR1 efflux ratio to LLC-PK1-wild type efflux ratio.

[c] TPSA values were calculated with ACD/PhysChem Batch (version 12.01).

Table 4. PKCθ inhibitory activity and P-gp liability of pyrazole derivatives

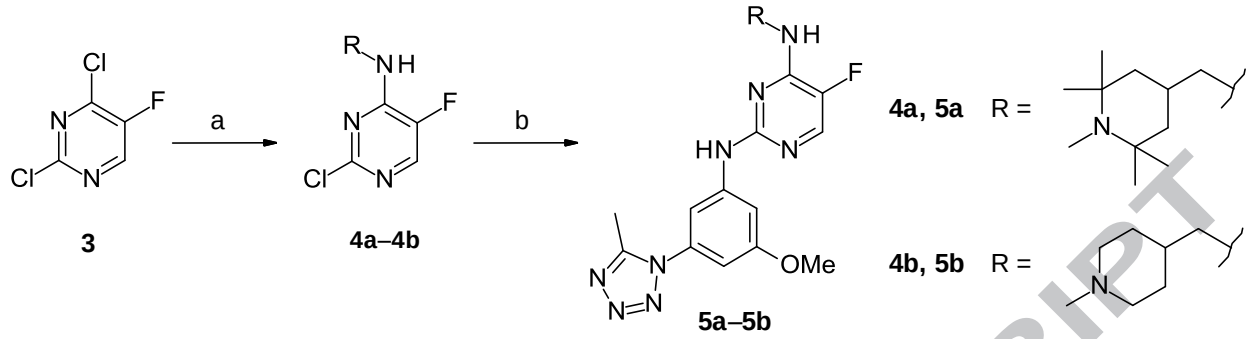


Compound	R	PKCθ IC_{50}[a] (nM)	P-gp NER[b]	CYP3A4 TDI (residual activity, %)[c]
14d	H	28	3.5	85
14e	Me	32	-	-
14f	Cl	3.0	2.3	98

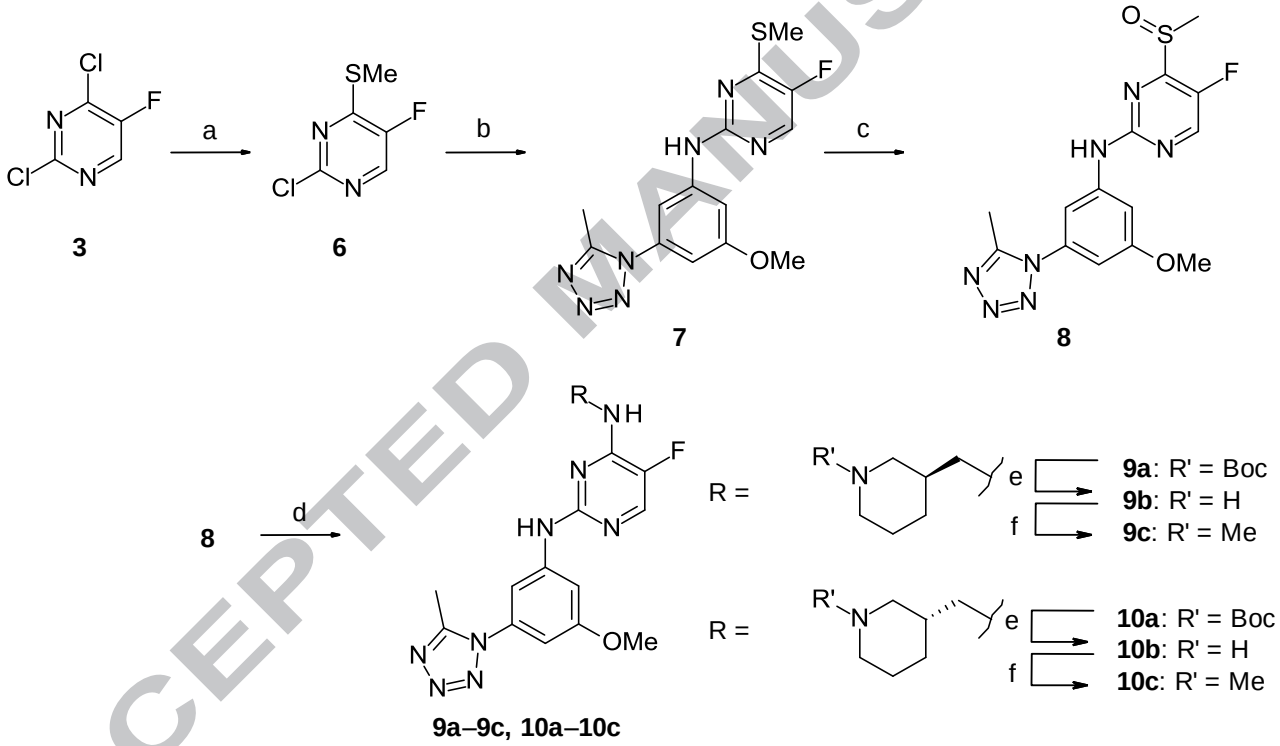
[a] IC_{50} values were determined in duplicate in one experiment.

[b] Net efflux ratio was ratio of LLC-PK1-MDR1 efflux ratio to LLC-PK1-wild type efflux ratio.

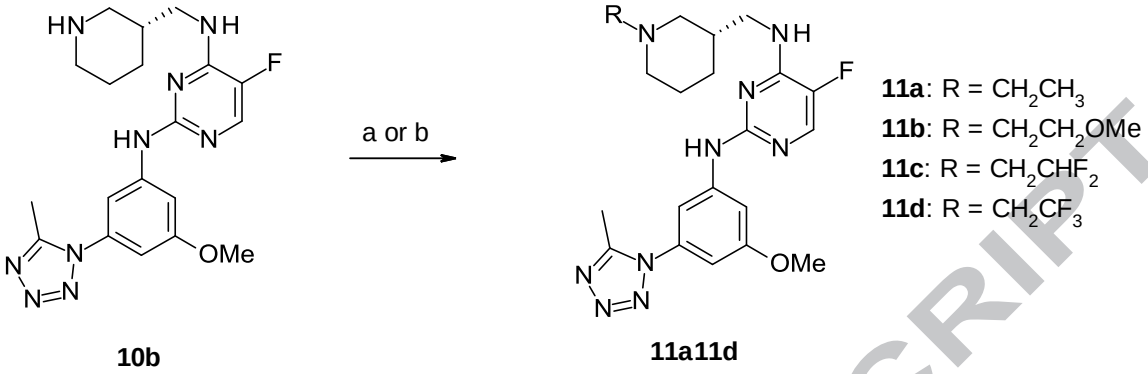
[c] Activities of HLMs for metabolism of midazolam were measured and residual activities are shown as percentage of remained metabolic activity following preincubation for 30 min in presence of test compounds (5 μM).



Scheme 1. Reagents and conditions: (a) RNH_2, DIPEA, MeOH, rt, 16 h; (b) 3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)aniline, HCl, IPA, microwave, 140 °C, 1 h.

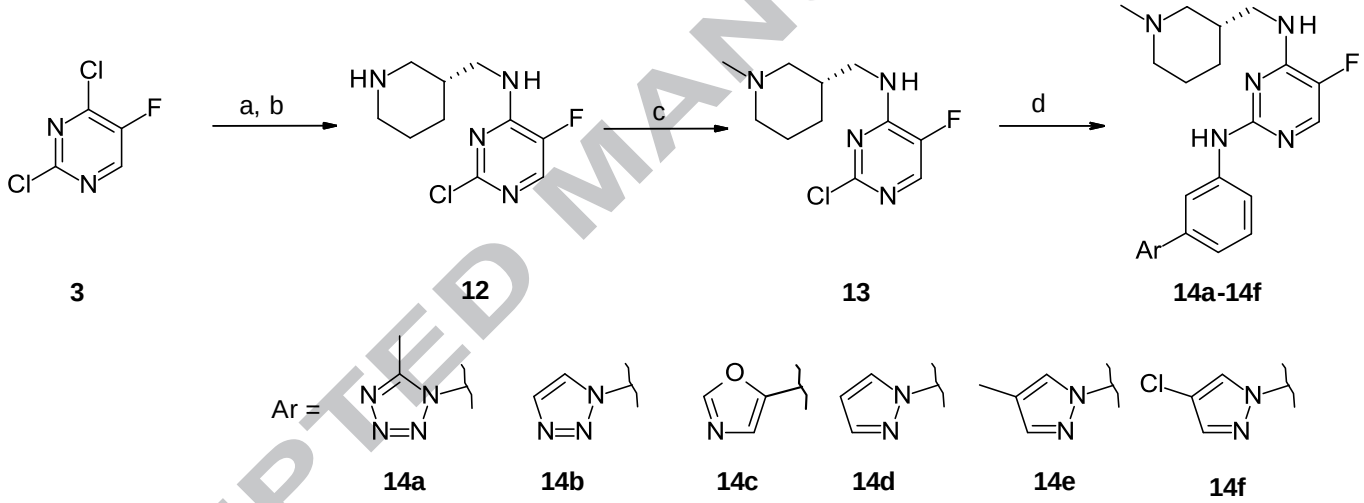


Scheme 2. Reagents and conditions: (a) NaSMe, THF, -30 °C, 2 h; (b) 3-methoxy-5-(5-methyl-1*H*-tetrazol-1-yl)aniline, HCl, IPA, microwave, 130 °C , 1 h; (c) *m*-CPBA, CH_2Cl_2, 0 °C, 3 h; (d) RNH_2, DIPEA, NMP, microwave, 120 °C, 1 h; (e) TFA, CH_2Cl_2, rt, 1 h; (f) 36% HCHO aq., $NaBH(OAc)_3$, CH_2Cl_2, rt, 3 h.



10b **11a11d**

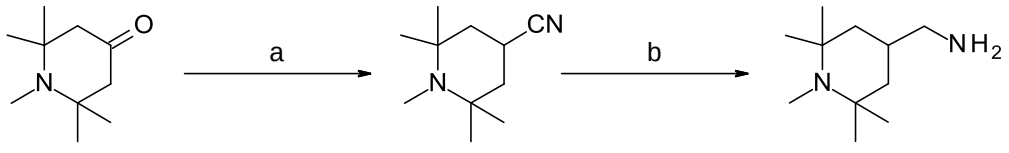
Scheme 3. Reagents and conditions: (a) RBr, DIPEA, NMP, microwave, 80 °C, 1 h (for **11a** and **11b**); (b) $ROSO_2CF_3$, DIPEA, THF, reflux, 14 h (for **11c** and **11d**).



3 **12** **13** **14a-14f**

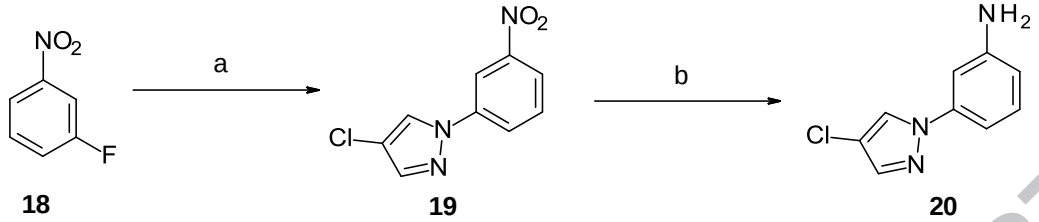
14a **14b** **14c** **14d** **14e** **14f**

Scheme 4. Reagents and conditions: (a) *tert*-butyl (3*S*)-3-(aminomethyl)piperidine-1-carboxylate, DIPEA, DMF, rt, 14 h; (b) TFA, CH_2Cl_2, rt, 4h; (c) 36% HCHO aq., $NaBH(OAc)_3$, CH_2Cl_2, rt, 1 h; (d) $ArNH_2$, HCl, IPA, 120 °C, 9 h.



15 **16** **17**

Scheme 5. Reagents and conditions: (a) TosMIC, ^tBuOK, DME, rt, 2 h; (b) LAH, THF, 0 °C, 3 h.



18 **19** **20**

Scheme 6. Reagents and conditions: (a) 4-Cl-1*H*-pyrazole, K_2CO_3, DMI, 130 °C, 16 h; (b) $SnCl_2 \cdot 2H_2O$, EtOH, reflux, 6 h.