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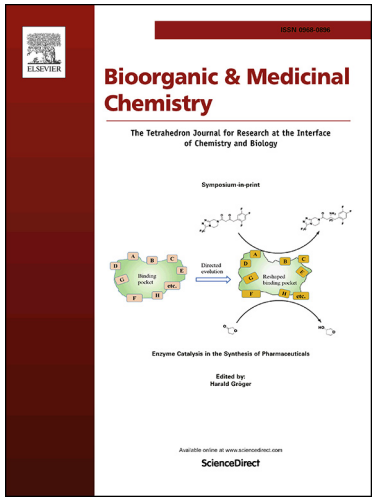

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Discovery of 2,4-diamino-5-cyanopyrimidine derivatives as protein kinase C theta inhibitors with mitigated time-dependent drug-drug interactions

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Key words: protein kinase C theta (PKCθ), CYP3A4 time-dependent inhibition (TDI), transplant rejection.

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. PKCθ inhibitors have emerged as effective immunomodulative agents for the prevention of transplant rejection. We previously reported that the 2,4-diamino-5-cyanopyrimidine derivative **2** was a potent PKCθ inhibitor; however, it exhibited CYP3A4 time-dependent inhibition (TDI). Here, we report the structural modification of compound **2** into **34** focusing on mitigating CYP3A4 TDI. Compound **34** exhibited potent *in vitro* activity with mitigated CYP3A4 TDI and efficacy in vivo transplant model.

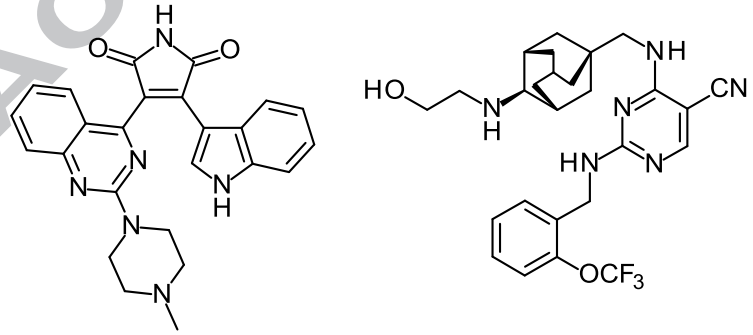
1. Introduction

Organ transplant rejection is commonly treated with immunosuppressive drugs such as calcineurin inhibitors.[1] Calcineurin inhibitors (CNIs) such as tacrolimus and cyclosporin A inhibit the phosphatase activity of calcineurin and the translocation of the nuclear factor of activated T cells (NF-AT) into the nucleus. While treatment with CNIs after transplantation remarkably improves graft and patient survival, the clinical use of CNIs reportedly induces mechanism-based adverse effects such as nephrotoxicity and hypertension.[2] Currently, CNI minimization protocols are widely used to reduce the adverse effects of CNIs by combining other immunosuppressive agents with different mechanisms of action. 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.

Protein kinase C (PKC) is a member of the family of serine/threonine kinases that consist of classical (α, β, and γ), novel (δ, ε, η, and θ), and atypical (ζ and λ) isoforms.[3] Of these isozymes, PKCθ exhibits a selective expression pattern in T cells, platelets, and skeletal muscle. A recent study showed that PKCθ plays an important role in T cell signaling, leading to the production of interleukin-2 (IL-2), which plays a key role in the immune response.[4] Moreover, PKCθ knockout mice exhibit a T cell inactivation-specific phenotype. The unique role of PKCθ in the immune system underlies its emergence as a potential target for the treatment of transplant rejection with reduced risk of systemic side effects. A large number of PKCθ inhibitors have been reported to

date.[5] For example, Novartis Pharmaceuticals developed Sotrastaurin (**1**) as an adjunctive drug in kidney and liver transplant rejection (Fig. 1).[6] Against this background, we previously reported the 2,4-diamino-5-cyanopyrimidine derivative **2** as an inhibitor of PKCθ (Fig. 1).[9] However, upon further investigation, we found that compound **2** had CYP3A4 TDI properties that may increase the plasma concentration of CNIs such as tacrolimus to cause adverse effects as a combination therapy in clinical treatment. In fact, clinical trials of Sotrastaurin (**1**) revealed that it was effective in transplantation, but that it also increased area under the blood concentration-time curve (AUC) of tacrolimus as a combination drug by 2.0-fold, which may induce adverse effects.[7] This unfavorable outcome might be due to Sotrastaurin being a CYP3A4 inhibitor.[8] To our knowledge, none of the reported PKCθ inhibitors have been launched.

Here, we attempted to identify a novel PKCθ inhibitor with mitigated CYP3A4 inhibition by modifying our lead compound **2**. We report the synthesis and biological evaluation of 2,4-diamino-5-cyanopyrimidine derivatives as PKCθ inhibitors.



Sotrastaurin（**1**） **2**

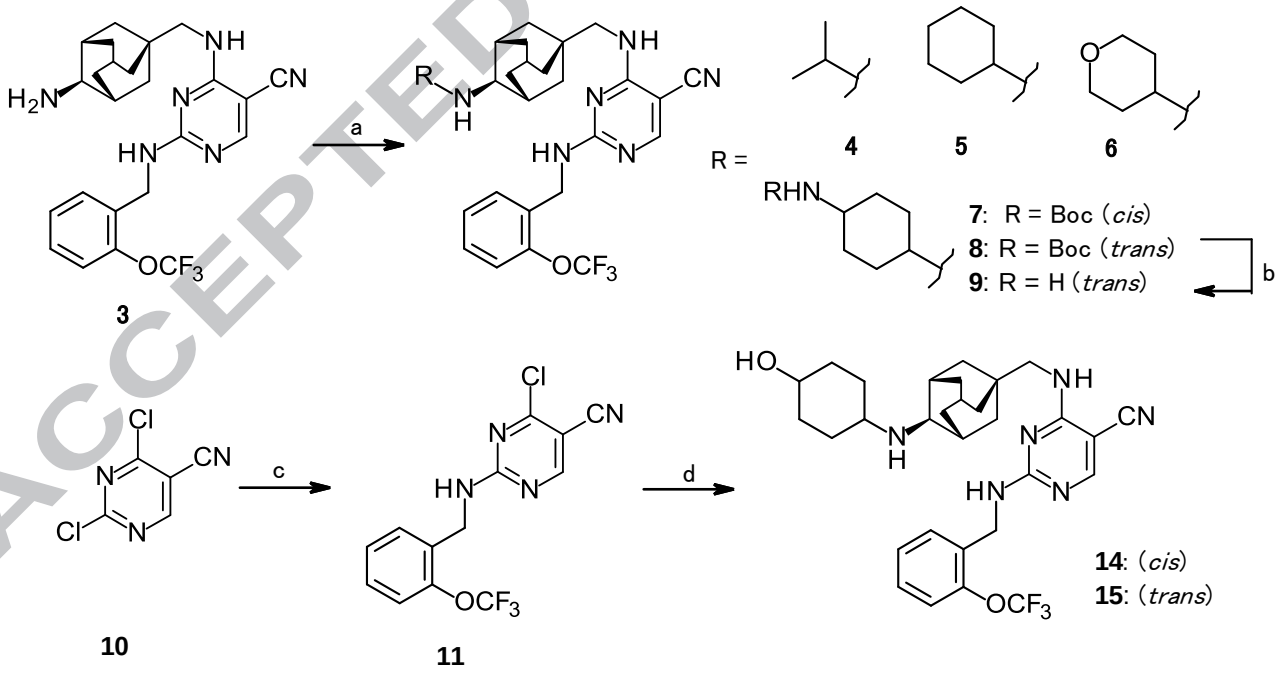
Figure 1. Structure of PKC inhibitors.

2. Chemistry

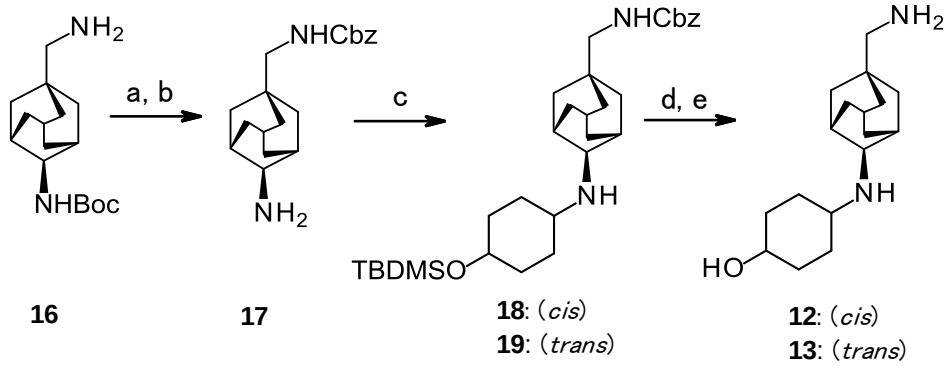
As shown in Scheme 1, target compounds **4**–**6** and **9** were prepared from compound **3**.[9] Reductive alkylation of compound **3**[9] with various ketones and sodium triacetoxyborohydride ($NaBH(OAc)_3$) gave compounds **4**–**8**. Subsequent chromatographic separation isolated the *cis* isomer **7** and *trans* isomer **8**. Deprotection of the *trans* isomer **8** using trifluoroacetic acid (TFA) furnished target compound **9**. Compounds **14** and **15** were prepared from 2,4-dichloro-5-cyanopyrimidine **10**. Treatment of **10** with 2-(trifluoromethoxy)benzylamine in the presence of DIPEA gave compound **11**. *Ipso* substitution with amine **12** or **13** gave *cis* isomer **14** or *trans* isomer **15**, respectively. The synthesis of intermediate aliphatic amines **12, 13** are described in Scheme 2. Protection of compound **16**[9] with benzyl chloroformate (Cbz), followed by deprotection of the Boc group using TFA gave **17**. Reductive alkylation with 4-(*tert*-butyldimethylsilyloxy)cyclohexanone and $NaBH(OAc)_3$ resulted in the formation of a mixture of *cis* and *trans* isomers. Chromatographic separation isolated the *cis* isomer **18** and *trans* isomer **19**. The two isomers were identified on the basis of the chemical shift of the proton at the α-position with respect to the oxygen atom. This proton resonates at higher ppm in the *cis* isomer (δ from 3.79 to 3.86 ppm) than the corresponding proton in the *trans* isomer (δ from 3.50 to 3.61 ppm).[9, 10] Deprotection of the TBDMS group using tetrabutylammonium fluoride (TBAF), followed by hydrogenation with Pd on carbon gave *cis* amine **12** and *trans* amine **13**. Intermediates otherwise noted were commercially available. Target compounds **30**–**39** were prepared from intermediate **20** (Scheme 3).[9] Displacement of the sulfoxide moiety by the corresponding amines, followed by deprotection of the Boc group gave **3**

and **21**–**29**. Reductive alkylation with

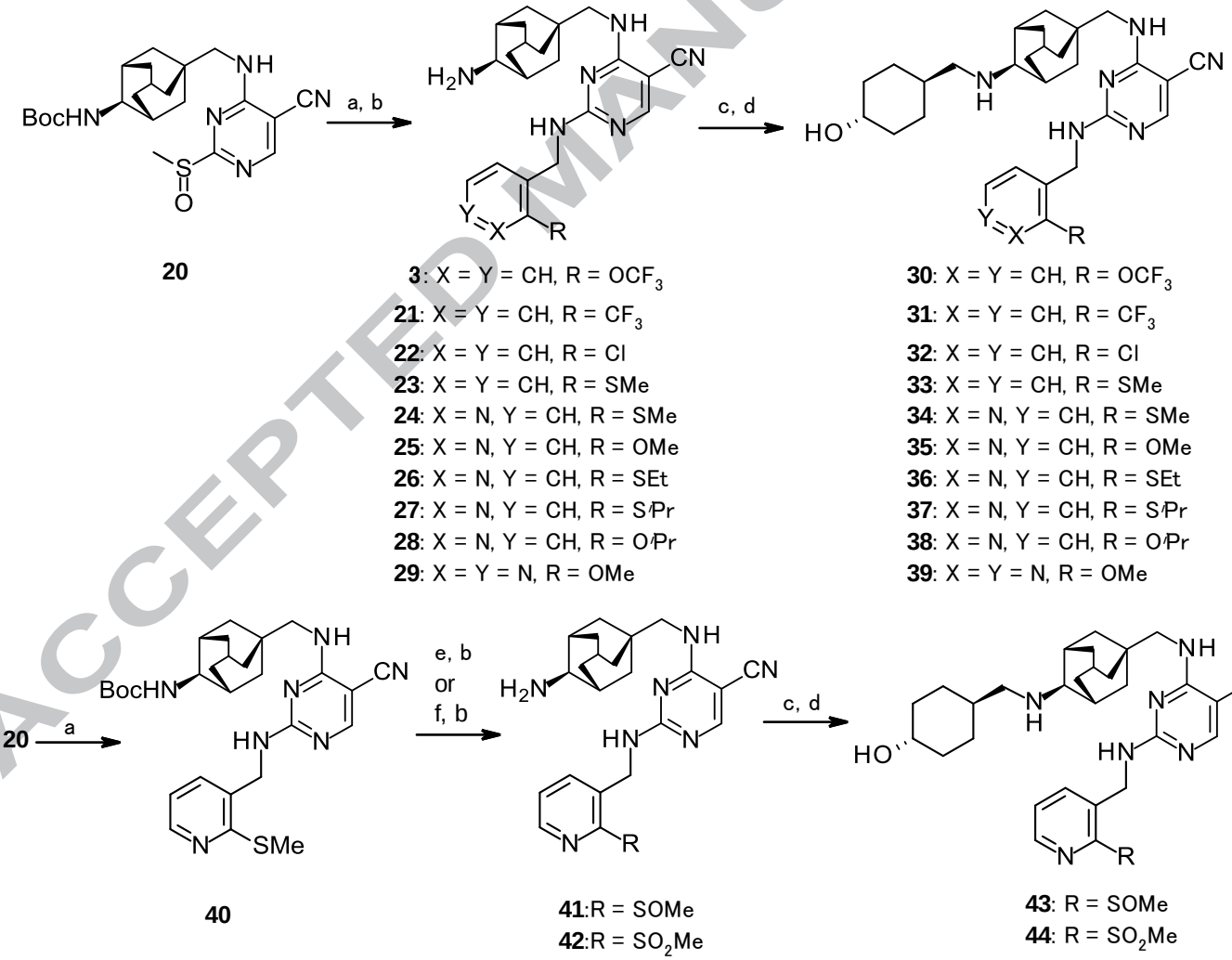
trans-4-((*tert*-butyldimethylsilyl)oxy)cyclohexanecarbaldehyde[11] and $NaBH(OAc)_3$, followed by deprotection of the *tert*-butyldimethylsilyl (TBDMS) group using HCl gave compounds **30**–**39**. Compounds **43** and **44** were also prepared from **20**. Displacement of the sulfoxide moiety by [2-(methylsulfanyl)pyridin-3-yl]methanamine gave Boc protected compound **40**, and subsequent oxidation of *m*-chloroperoxy benzoic acid (*m*-CPBA) gave sulfoxide **41** or sulfone **42**. Reductive alkylation with *trans*-4-((tert-butyldimethylsilyl)oxy)cyclohexanecarbaldehyde and subsequent deprotection gave **43** or **44**, respectively.



Scheme 1. Reagents and conditions: (a) various ketones, $NaBH(OAc)_3$, CH_2Cl_2 or THF, rt; (b) TFA, CH_2Cl_2, rt; (c) 2-(trifluoromethoxy)benzylamine, DIPEA, DMF, -50 °C; (d) amine **12** or **13**, DIPEA, DMI, rt.



Scheme 2. Reagents and conditions: (a) CbzCl, NEt_3, CH_2Cl_2, rt; (b) TFA, CH_2Cl_2, rt; (c) 4-(*tert*-butyldimethylsilyloxy)cyclohexanone, $NaBH(OAc)_3$, CH_2Cl_2, rt; (d) TBAF, THF, 70 °C; (e) H_2 (1atm), 10% Pd/C (wet), MeOH, 35 °C.



Scheme 3. Reagents and conditions: (a) various amines, DIPEA, DMF, rt; (b) TFA, CH_2Cl_2, rt; (c) *trans*-4-((*tert*-butyldimethylsilyl)oxy)cyclohexanecarbaldehyde, $NaBH(OAc)_3$, CH_2Cl_2, DMF, rt; (d) HCl, MeOH, rt; (e) *m*-CPBA (1.2 eq.), CH_2Cl_2, rt; (f) *m*-CPBA (3.8 eq.), CH_2Cl_2, rt.

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3. Results and Discussion

We evaluated the *in vitro* inhibitory activity of the compounds against human PKCθ by measuring the fluorescence intensity after incubation with the full-length human recombinant PKCθ and adenosine triphosphate (ATP). An IL-2 production inhibitory activity assay was used to assess the cellular potency of the compounds. We also assessed the inhibitory activity of the compounds against CYP3A4 by measuring the metabolic activity of human liver microsomes for midazolam at 0 and 30 min after preincubation with the test compounds. The residual activity of CYP3A4 is reported as the percentage of metabolic activity remaining after preincubation for 30 min compared to that at 0 min (i.e. without preincubation).

Compound **2** showed potent PKCθ inhibitory activity but the residual activity of CYP3A4 was only 36% (Table 1). This result suggests that compound **2** exhibits TDI for CYP3A4. To minimize TDI, we focused on the secondary amine, which reportedly undergoes CYP3A4-mediated *N*-hydroxylation to corresponding secondary hydroxylamines that inactivate CYP3A4 as metabolic intermediate complex formation with the nitroso metabolite forms through the secondary hydroxylamine and nitrone pathway.[12] We previously reported that basicity and the location of the amine at the 4-position of the pyrimidine derivative is essential for PKCθ inhibitor activity.[9] We therefore increased the steric hindrance around the adamantylamine moiety at the 4-position as an alternative strategy for disrupting the formation of a metabolite-intermediate complex to reduce TDI.[13] Table 1 shows the effect of replacement of hydroxy ethyl group to other steric substituents.

While introduction of an isopropyl substituent did not improve TDI (**4**), more bulky substituents such as cyclohexane slightly improved TDI (**5**) compared to **4**. Conversion of the cyclohexane ring to a tetrahydropyranyl (THP) ring (**6**) improved PKCθ inhibitory activity with maintaining TDI, and *trans* cyclohexanol group improved both inhibitory activity and TDI (**15**). Isomer preference was noted for these compounds. The *trans* isomer **15** had greater improvement in TDI than the *cis* isomer **14**. Replacing the hydroxy group with an amino group slightly decreased the TDI (**9**). Insertion of a methylene linker between the cyclohexane moiety and pyrimidine proved slightly effective for improving TDI (**30**).

The above described optimizations ameliorated TDI compared with the lead compound **2**. However, the improvement was not sufficient and the solubility of compound **30** was low (< 1 μM in pH 6.8 buffer solution). They highlighted the need to improve the physicochemical properties.[14] We therefore focused on reducing lipophilicity because the lipophilicity of compounds is reportedly correlated with the solubility and CYP3A4 substrates.[15, 16] Given that our previous docking study suggested that the substituent of the phenyl ring at the 2-position was located at some distance from the hinge binding region of PKCθ,[9] modifications made here were expected to be acceptable. We therefore modified the substituent of the phenyl ring of compound **30** to reduce lipophilicity (Table 2). Replacement of the OCF_3 group with CF_3 or Cl group resulted in **31** or **32** did not improve TDI. On the other hand, we investigated SMe substituent (**33**) substantially improved TDI and water solubility. Replacement of the phenyl ring with a pyridine ring resulted in compound **34**, which had improved CYP3A4 TDI and water solubility, as well as PKCθ inhibitory activity (IC_{50} = 0.44 nM)

and IL-2 inhibitory activity (IC_{50} = 10 nM). Replacement of the S atom with an O atom maintained TDI and water solubility, but decreased PKCθ inhibitory activity and IL-2 inhibitory activity (**35**). Oxidation of **34** produced **43** and **44**, which exhibited decreased inhibitory activities. Increasing lipophilicity such as in **36**, **37** and **38** improved PKCθ inhibitory activity and IL-2 inhibitory activity, but didn't improve TDI compared with **34** and **35**. Further, introduction of a nitrogen atom to produce **39** resulted in a loss of PKCθ inhibitory activity.

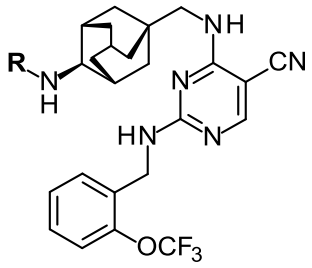
As a result of the above optimizations, we selected **34**, which had good *in vitro* potency and solubility, for further evaluation. Pharmacokinetic parameters for compound **34** were measured in rats (Table 3). Plasma concentration was measured after a single oral dose at 1 mg/kg. The dose resulted in a maximum plasma concentration (C_{max}) of 12.4 ng /mL, allowing bioavailability to be calculated as a ratio of the area under the plasma concentration-time curve of **34** after intravenous and oral administration; the bioavailability was 58.3%.

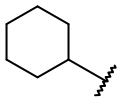
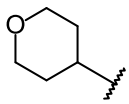
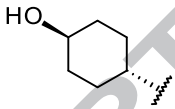
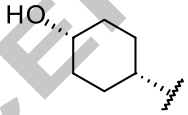
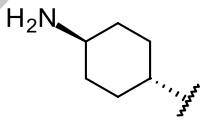
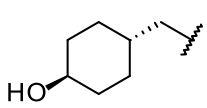
We also evaluated the inhibitory activity of **34** against other PKC isoforms and 27 serine/threonine and tyrosine kinases,[17] as some isozymes and kinases are known to cause side effects. For example, PKCδ-deficient mice exhibit hyperproliferation of B cells and overproduction of inflammatory cytokines.[18] A near-linear relationship was also noted between the dose of pan PKC inhibitor sotrastaurin and adverse effects such as gastrointestinal disorders and diarrhea.[19] As a result of evaluation, we found **34** was a potent and selective inhibitor of PKCθ.[17] The expression profile and selective inhibition exhibited by PKCθ may form a suitable balance between immunosuppression and minimization of systemic side effects.

We subsequently investigated the effect of **34** in combination on cardiac graft survival in rats (Table 4). In combination with tacrolimus (suboptimal dose: 0.02 mg/kg), **34** at 1 mg/kg b.i.d. drastically reduced graft rejection. We also found the *in vivo* efficacy of **34** to prevent allograft rejection in a monkey renal transplantation model.[20]

Table 1

Conversion of *N*-alkyl group on the adamantyl amine



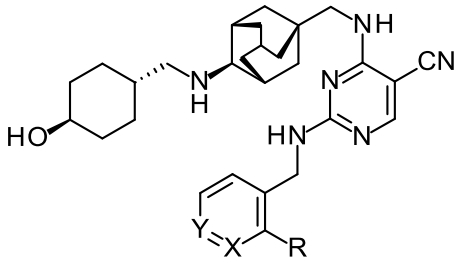
Compound	R	PKCθ IC_{50}[a] (nM)	CYP3A4 TDI[b] (residual activity, %)
2	-CH_2CH_2OH	0.70	36
4	-*i*Pr	< 1.0	29
5		1.9	43
6		<1.0	41
15		0.38	64
14		0.66	43
9		0.10	59
30		0.42	70

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

Table 2.

Conversion of the 2-trifluoromethoxy benzene ring



Compound	R	X	Y	PKCθ IC_{50}[a] (nM)	IL-2 IC_{50}[b] (nM)	CYP3A4 TDI[c] (residual activity, %)	Solubility[d] (μM)	ACDlogP[e]
30	OCF_3	CH	CH	0.42	11	70	<1	5.11
31	CF_3	CH	CH	0.57	17	63	NT[f]	5.10
32	Cl	CH	CH	1.4	22	77	1.1	5.10
33	SMe	CH	CH	0.94	16	92	45	4.98
34	SMe	N	CH	0.44	10	98	>50	4.07
35	OMe	N	CH	1.9	43	94	>50	3.49
43	SOMe	N	CH	32	170	NT[f]	>100	2.58
44	SO_2Me	N	CH	NT[f]	140	NT[f]	>100	2.60
36	SEt	N	CH	0.46	<3.0	80	16	4.46
37	S^{i}Pr	N	CH	0.23	3.0	66	<1	4.76
38	O^{i}Pr	N	CH	0.59	18	87	>100	4.13
39	OMe	N	N	>100	NT[f]	NT[f]	>100	2.72

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

[b] Inhibition of IL-2 production in Jurkat cells. IC_{50} values were determined in duplicate in one experiment.

[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).

[d] Solubility of the test compound in a buffer solution of pH 6.8.

[e] ACDlogP values were calculated by ACD/Percepta. (version 14.0.0)

[f] Not tested.

Table 3

Pharmacokinetic parameters of compound **34** in rats[a]

i.v. (1 mg/kg)				p.o. (1 mg/kg)			
AUC_{24h}[b] (ng·h/mL)	$t_{1/2}$[c] (h)	V_{ss}[d] (L/kg)	CL_{tot}[e] (mL/min/kg)	AUC_{24h}[b] (ng·h/mL)	C_{max}[f] (ng/mL)	t_{max}[g] (h)	F[h] (%)
248	3.97	12.9	66.7	145	12.4	3.33	58.3

[a] Each value is an average of data from three animals.

[b] Area under the plasma concentration versus curve from time zero to 24 hours after dosing.

[c] Elimination half-life from plasma.

[d] Volume of distribution at steady state.

[e] Total body clearance.

[f] Maximum plasma concentration.

[g] Time to reach maximum plasma concentration.

[h] Absolute oral bioavailability.

Table 4

Effect of compounds **34** concomitant treatment with tacrolimus on graft survival of rat cardiac transplantation model[a]

Compound	Combined drug	n	Graft survival time (days)	MST[b] (days)
Vehicle	none	-		6[c]
Vehicle	tacrolimus[d]	11	5, 5, 6, 6, 6, 7, 7, 7, 9, 11, 11	7[e]
1 mg/kg	tacrolimus[d]	9	9, 11, 13, 17, 20, 23, 24, 24, 26	20[e]

[a] Test compounds were orally administered.

[b] Median survival time.

[c] Standard data in our laboratory.

[d] 0.02 mg/kg, q.d., i.m.

[e] ref 19.

4. Conclusions

We investigated the structure activity relationship of novel compounds for PKCθ inhibitory activity and CYP3A4 TDI to obtain a promising PKCθ inhibitor. We introduced steric hindrance and reduced lipophilicity to mitigate CYP3A4 TDI. As a result of optimization, we discovered compound **34** with an IC_{50} value in the nano molar range, excellent aqueous solubility and mitigated CYP3A4 TDI. Compound **34** showed good selectivity for PKCθ over other PKC isoforms and kinases. Administration of **34** in a rat model of cardiac transplantation and a monkey model of renal transplantation demonstrated the potential of PKCθ as a new drug target for organ transplantation. This potent and selective PKCθ inhibitor might exhibit a suitable balance between immunosuppression and minimization of systemic side effects for improved treatment of transplant rejection.

5. Experimental section

5.1. Chemistry

^{1}H NMR spectra were recorded on a Bruker Avance 400, Bruker AV400M, Bruker Avance III HD, Varian VNS-400, or Varian 400 MR; and chemical shifts were expressed in δ (ppm) values with tetramethylsilane as an internal reference (s = singlet, d = doublet, t = triplet, m = multiplet, and br = broad peak). Mass spectra (MS) were recorded on Agilent 1100, Thermo Electron LCQ Advantage, or Waters UPLC/SQD. 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; DMF, *N*,*N*-dimethylformamide; DMI, 1,3-dimethyl-2-imidazolidinone; TBAF, tetrabutylammonium fluoride; TFA, trifluoroacetic acid; and THF, tetrahydrofuran.

5.1.1.

4-[({(1*s*,3*R*,4*s*,5*S*,7*s*)-4-[(Propan-2-yl)amino]adamantan-1-yl}methyl)amino]-2-({[2-(trifluoromethoxy)phenyl]methyl}amino)pyrimidine-5-carbonitrile (4)

To a solution of **3** (50 mg, 0.11 mmol) in CH_2Cl_2 (2.5 mL), acetone (23 μL, 0.32 mmol) and sodium triacetoxyborohydride (67 mg, 0.32 mmol) were added and then stirred at room temperature for 5 h. The reaction mixture was basified with saturated aqueous $NaHCO_3$ and extracted with EtOAc. 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) to give **4** (53 mg, 98%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ ppm 0.94 (6H, d, J = 6.2 Hz), 0.97–1.97 (14H, m), 2.46 and 2.65 (total 1H, each brs), 2.70–2.75 (1H, m), 2.94 and 3.15 (total 2H, each d, J = 6.3 Hz), 4.54 (2H, d, J = 6.0 Hz), 7.16–7.35 (5H, m), 7.90 and 8.14 (total 1H, each t, J = 6.3 Hz), 8.18 (1H, s); MS (ESI) *m*/*z* 515 $[M+H]^+$; HRMS (ESI) calcd for $C_{27}H_{34}F_3N_6O$ $[M+H]^+$: 515.2741, Found: 515.2740.

5.1.2.

4-[({(1*s*,3*R*,4*s*,5*S*,7*s*)-4-(Cyclohexylamino)adamantan-1-yl}methyl)amino]-2-({[2-(trifluoromethoxy)phenyl]methyl}amino)pyrimidine-5-carbonitrile (5)

Compound **5** was prepared from compound **3** in 91% yield as a colorless solid, using similar approach to that described for **4**. ^{1}H NMR (DMSO-d_6) δ ppm 0.91–1.22 (14H, m), 1.45–1.99 (11H, m), 2.32 (1H, m), 2.93 and 3.15 (total 2H, each d, J = 6.3Hz), 4.54 (2H, d, J = 6.0 Hz), 7.16–7.35 (5H, m), 7.89 and 8.14 (total 1H, each t, J = 6.3 Hz), 8.18 (1H, s); MS (ESI) m/z 555 $[M+H]^+$; HRMS (ESI) calcd for $C_{30}H_{38}F_3N_6O$ $[M+H]^+$: 555.3054, Found: 555.3047.

5.1.3.

4-[({(1*s*,3*R*,4*s*,5*S*,7*s*)-4-[(Oxan-4-yl)amino]adamantan-1-yl}methyl)amino]-2-({[2-(trifluoromethoxy)phenyl]methyl}amino)pyrimidine-5-carbonitrile (6)

Compound **6** was prepared from compound **3** in 67% yield as a colorless solid, using similar approach to that described for **4**. ^{1}H NMR (DMSO-d_6) δ ppm 1.02–1.89 (18H, m), 2.54–2.74 (2H, m), 2.94 and 3.17 (total 2H, each d, J = 6.3Hz), 3.27 (2H, t, J = 11.6 Hz), 3.81 (2H, d, J = 11.6 Hz), 4.54 (2H, d, J = 6.0 Hz), 7.16–7.34 (5H, m), 7.89 and 8.14 (total 1H, each t, J = 6.3Hz), 8.18 (1H, s); MS (ESI) m/z 557 $[M+H]^+$; HRMS (ESI) calcd for $C_{29}H_{36}F_3N_6O_2$ $[M+H]^+$: 557.2846, Found: 557.2839.

5.1.4.

4-[({(1*s*,3*R*,4*s*,5*S*,7*s*)-4-[(*cis*-4-*tert*-Butoxycarbonylaminocyclohexyl)amino]adamantan-1-yl}methyl)amino]-2-({[2-(trifluoromethoxy)phenyl]methyl}amino)pyrimidine-5-carbonitrile (7) and 4-[({(1*s*,3*R*,4*s*,5*S*,7*s*)-4-[(*trans*-4-*tert*-Butoxycarbonylaminocyclohexyl)amino]adamantan-1-yl}methyl)amino]-2-({[2-(trifluoromethoxy)phenyl]methyl}amino)pyrimidine-5-carbonitrile (8)

To a solution of **3** (75 mg, 0.16 mmol) in CH_2Cl_2 (5 mL) and THF (2 mL), 4-(*tert*-butoxycarbonylamino)cyclohexanone (76 mg, 0.36 mmol) and sodium triacetoxyborohydride (150 mg, 0.71 mmol) were added and then stirred at room temperature. The reaction mixture was basified with 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 amino silica gel with elution using (*n*-hexane-EtOAc) (1:1) to give **7** (30 mg, 29%, less polar) and **8** (27 mg, 25%, high polar). **7** ^{1}H NMR (DMSO-d_6) δ ppm 0.89–1.89 (24H, m), 1.37 (9H, m), 2.39–2.44 (1H, m), 2.90–2.97 (2H, m), 4.51–.4.58 (2H, m), 6.54–6.66 (1H, m), 7.22–7.40 (5H, m), 8.10–8.16 (1H, m), 8.18 (1H, s); MS (ESI) *m*/*z* 670 $[M+H]^+$; **8** ^{1}H NMR (DMSO-d_6) δ ppm 0.89–1.90 (24H, m), 1.37 (9H, m), 2.18–2.29 (1H, m), 2.88–2.98 (2H, m), 4.46–.4.59 (2H, m), 6.61–6.73 (1H, m), 7.22–7.42 (5H, m), 8.01–8.16 (1H, m), 8.18 (1H, s); MS (ESI) *m*/*z* 670 $[M+H]^+$;

5.1.5.

4-[({(1*s*,3*R*,4*s*,5*S*,7*s*)-4-[(*trans*-4-Aminocyclohexyl)amino]adamantan-1-yl}methyl)amino]-2-({[

2-(trifluoromethoxy)phenyl]methyl}amino)pyrimidine-5-carbonitrile (9)

To a solution of **8** (21 mg, 0.03 mmol) in CH_2Cl_2 (2 mL), TFA (0.12 mL, 1.6 mmol) was added and stirred at room temperature. The reaction mixture was concentrated in vacuo. The residue was chromatographed on amino silica gel with elution using ($CHCl_3$-MeOH) to give **9** (13 mg, 72%). ^{1}H NMR (DMSO-d_6) δ ppm 0.94–2.67 (27H, m), 2.93–3.15 (2H, m), 4.53–4.55 (2H, m), 7.15–7.35 (5H, m), 7.89–8.15 (1H, m), 8.18 (1H, s); MS (ESI) *m*/*z* 570 $[M+H]^+$; HRMS (ESI) calcd for $C_{30}H_{39}F_3N_7O$ $[M+H]^+$: 570.3163, Found: 570.3162.

5.1.6. 4-Chloro-2-({[2-(trifluoromethoxy)phenyl]methyl}amino)pyrimidine-5-carbonitrile (11)

To a solution of 2,4-dichloropyrimidine-5-carbonitrile **10** (5.1 g, 29 mmol) in DMF (50 mL), 2-(trifluoromethoxy)benzylamine (5.9 g, 31 mmol) in DMF (13 mL) and DIPEA (6.1 mL, 35 mmol) were added under -50 °C in dry ice-acetone bath and stirred at -50 °C. The reaction mixture was diluted with EtOAc and washed with H_2O, dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel with elution using $CHCl_3$ to give **11** (4.0 g, 42%). ^{1}H NMR (DMSO-d_6) δ ppm 4.58–4.69 (2H, m), 7.31–7.50 (4H, m), 8.75 and 8.77 (1H, each s), 9.23–9.33 (1H, br); MS (ESI) *m*/*z* 351, 353 $[M+Na]^+$.

5.1.7.

4-[({(1s,3R,4s,5S,7s)-4-[(*cis*-4-Hydroxycyclohexyl)amino]adamantan-1-yl}methyl)amino]-2-({[

2-(trifluoromethoxy)phenyl]methyl}amino)pyrimidine-5-carbonitrile (14)

To a solution of **11** (43 mg, 0.13 mmol) in DMI (0.6 mL), **12** (30 mg, 0.11 mmol) and DIPEA (75 μL, 0.43 mmol) were added and stirred at room temperature over night. The reaction mixture was diluted with $CHCl_3$ and chromatographed on amino silica gel with elution using ($CHCl_3$-MeOH) to give **14** (42 mg, 68%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ ppm 0.71–2.70 (24H, m), 2.90–3.19 (2H, m), 3.56–3.64 (1H, m), 4.23–4.59 (3H, m), 7.14–7.41 (5H, m), 7.90 and 8.14 (total 1H, each t, J = 6.3 Hz), 8.18 (1H, s); MS (ESI) m/z 571 $[M+H]^+$; HRMS (ESI) calcd for $C_{30}H_{38}F_3N_6O_2$ $[M+H]^+$: 571.3003, Found: 571.3001.

5.1.8.

4-[({(1*s*,3*R*,4*s*,5*S*,7*s*)-4-[(*trans*-4-Hydroxycyclohexyl)amino]adamantan-1-yl}methyl)amino]-2-({[2-(trifluoromethoxy)phenyl]methyl}amino)pyrimidine-5-carbonitrile (15)

Compound **15** was prepared from compound **11** in 53% yield as a colorless solid, using similar approach to that described for **14**. ^{1}H NMR (DMSO-d_6) δ ppm 0.69–2.69 (24H, m), 2.88–3.17 (2H, m), 3.22–3.41 (1H, m), 4.41–4.59 (3H, m), 7.13–7.43 (5H, m), 7.90 and 8.14 (total 1H, each t, J = 6.3 Hz), 8.18 (1H, s); MS (ESI) m/z 571 $[M+H]^+$; HRMS (ESI) calcd for $C_{30}H_{38}F_3N_6O_2$ $[M+H]^+$: 571.3003, Found: 571.3001.

5.1.9. Benzyl {[(1s,3R,4s,5S,7s)-4-aminoadamantan-1-yl]methyl}carbamate (17)

To solution of **16** (500 mg, 1.8 mmol) and NEt_3 (0.3 mL, 2.1 mmol) in CH_2Cl_2 (10 mL), Carbobenzoxy Chloride (Cbz-Cl) 0.28 mL, 2.0 mmol) was added at 0^{o}C and stirred at room

temperature for 4 h. The reaction mixture was diluted with EtOAc, washed with 0.1N aqueous HCl, H_2O, saturated aqueous $NaHCO_3$ and brine. The separated organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on amino silica gel with elution using (*n*-hexane-EtOAc) (3:1) to give a white solid (739 mg). To a solution of the residue in CH_2Cl_2 (5.9 mL), TFA (5.9 mL, 76 mmol) was added at 0 ^oC and stirred at room temperature for 2 h. The reaction mixture was concentrated, then diluted with CH_2Cl_2 and basified with aqueous K_2CO_3 and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give **17** (560 mg, 100%) as a colorless oil. ^1H NMR (DMSO-d_6) δ ppm 1.19–1.29 (2H, m), 1.33–1.51 (6H, m), 1.60–1.68 (2H, m), 1.74–1.82 (1H, m), 1.93–2.04 (2H, m), 2.28 (2H, br), 2.70 (2H, d, *J* = 6.4Hz), 2.77–2.83 (1H, m), 5.01 (2H, s), 7.18 (1H, t, *J* = 6.4Hz), 7.27–7.41 (5H, m); MS (ESI) *m*/*z* 315 $[M+H]^+$.

5.1.10. Benzyl ({(1s,3R,4s,5S,7s)-4-[(*cis*-4-{[*tert*-butyl(dimethyl)silyl]oxy}cyclohexyl)amino]adamantan-1-yl} methyl)carbamate (18) and Benzyl ({(1s,3R,4s,5S,7s)-4-[(*trans*-4-{[*tert*-butyl(dimethyl)silyl]oxy}cyclohexyl)amino]adamantan-1-yl }methyl)carbamate (19)

To a solution of **17** (760 mg, 2.4 mmol) and 4-(*tert*-butyldimethylsilyloxy)cyclohexanone (1.1 g, 4.8 mmol) in CH_2Cl_2 (23 mL), sodium triacetoxyborohydride (1.0 g, 4.8 mmol) was added and then stirred at room temperature for 4 h. The reaction mixture was extracted with EtOAc and saturated

aqueous $NaHCO_3$. The organic layer was washed with H_2O, brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on amino silica gel with elution using (*n*-hexane-EtOAc) (10:1 to 1:1) to give **18** (670 mg, 53%, less polar) as a colorless oil and **19** (440 mg, 34%, high polar) as a colorless oil. **18** ^{1}H NMR (DMSO-d_6) δ ppm 0.02 (6H, s), 0.87 (9H, s), 1.08–1.29 (3H, m), 1.33–1.64 (14H, m), 1.70–1.82 (3H, m), 1.91–2.00 (2H, m), 2.42–2.54 (1H, m), 2.62–2.69 (1H, m), 2.70 (2H, d, *J* = 6.3 Hz), 3.79–3.86 (1H, m, α-position of the oxygen atom), 5.00 (2H, s), 7.18 (1H, t, *J* = 6.3 Hz), 7.27–7.41 (5H, m); MS (ESI) *m*/*z* 527 $[M+H]^+$. **19** ^{1}H NMR (DMSO-d_6) δ ppm 0.02 (6H, s), 0.85 (9H, s), 0.92–1.29 (7H, m), 1.32–1.48 (6H, m), 1.68–1.85 (7H, m), 1.89–1.98 (2H, m), 2.31–2.44 (1H, m), 2.61–2.67 (1H, m), 2.70 (2H, d, *J* = 6.3 Hz), 3.50–3.61 (1H, m, α-position of the oxygen atom)), 5.00 (2H, s), 7.18 (1H, t, *J* = 6.3 Hz), 7.27–7.41 (5H, m); MS (ESI) *m*/*z* 527 $[M+H]^+$.

5.1.11. *cis*-4-{[(1*R*,2*s*,3*S*,5*s*,7*s*)-5-(Aminomethyl)adamantan-2-yl]amino}cyclohexan-1-ol (12)

To a solution of **18** (640 mg, 1.2 mmol) in THF (13 mL), TBAF (1 M THF solution) (3.7 mL, 3.7 mmol) was added and stirred at 70 °C for 13 h. The reaction mixture was concentrated in vacuo. The residue was extracted with $CHCl_3$/H_2O/brine. The organic layer was washed with brine 4 times, dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel with elution using ($CHCl_3$-MeOH-28% aqueous NH_3) (20:1:0 to 8:1:0.1) to give a colorless solid (450 mg). To the obtained product (430 mg) in MeOH (13 ml), 10% Pd on carbon (85 mg, 50% wet) was added and stirred at 35 °C under H_2 for 2.5 h. The reaction mixture was diluted with MeOH and

filtered through a Celite® pad. The filtrate was concentrated in vacuo to give **12** (310 mg, 95% from **18**) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ ppm 1.08–3.28 (28H, m), 4.07–4.15 (1H, m), 4.46–4.72 (1H, m); MS (ESI) *m*/*z* 279 $[M+H]^+$.

5.1.12. *trans*-4-{[(1*R*,2*s*,3*S*,5*s*,7*s*)-5-(Aminomethyl)adamantan-2-yl]amino}cyclohexan-1-ol (13)

Compound **13** was prepared from compound **19** in quantitative yield as a colorless solid, using similar approach to that described for **12**. ^{1}H NMR (DMSO-d_6) δ ppm 1.25–3.39 (28H, m), 3.75–3.83 (1H, m), 4.47 (1H, br s); MS (ESI) *m*/*z* 279 $[M+H]^+$

5.1.13.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-Aminoadamantan-1-yl]methyl}amino)-2-{[2-(trifluoromethoxy)benzyl] amino}pyrimidine-5-carbonitrile (3)

To a solution of **16** (2.1 g, 4.7 mmol) in DMI (20 mL), 2-(trifluoromethoxy)benzylamine (1.8 g, 9.4 mmol) was added at 0 °C and stirred at room temperature for 1 h. The reaction mixture was poured into H_2O, collected by vaccum filtration and chromatographed on silica gel with elution using (*n*-hexane-EtOAc) (8:2 to 7:3) to give a colorless solid. To a suspension of obtained product in CH_2Cl_2 (30 mL), TFA (14 mL) was added at 0 °C and stirred at room temperature for 1 h. The reaction mixture was concentrated in vascuo. To the residue, saturated aqueous $NaHCO_3$ was added and the precipitate was washed with H_2O and *n*-hexane to give **3** (1.7 g, 76%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ ppm 1.01–2.02 (15H, m), 2.63 and 2.79 (total 1H, each s), 2.94 and 3.14

(total 2H, each d, *J* = 6.4Hz), 4.54 (2H, d, *J* = 6.1Hz), 7.14–7.34 (5H, m), 8.90 and 8.14 (total 1H, each t, *J* = 6.4Hz), 8.18 (1H, s); MS (ESI) *m*/*z* 473 $[M+H]^+$; HRMS (ESI) calcd for $C_{24}H_{28}F_3N_6O$ $[M+H]^+$: 473.2271, Found: 473.2276. Anal. Calcd for $C_{24}H_{27}F_3N_6O$: C, 61.01; H, 5.76; N, 17.79; F, 12.06. Found: C, 61.08; H, 5.84; N, 17.47; F, 11.84.

5.1.14.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-Aminoadamantan-1-yl]methyl}amino)-2-{[2-(trifluoromethyl)benzyl]amino}pyrimidine-5-carbonitrile (21)

Compound **21** was prepared from compound **20** in 80% yield as a colorless solid, using similar approach to that described for **3**. ^{1}H NMR (DMSO-d_6) δ ppm 0.95–2.09 (15H, m), 2.50–2.58 and 2.77–2.83 (1H, each m), 2.90 and 3.17 (2H, each d, *J* = 6.4Hz), 4.61–4.72 (2H, m), 7.14–7.76 (5H, m), 7.92–8.01 and 8.16–8.27 (total 2H, each m); MS (ESI) *m*/*z* 457 $[M+H]^+$; HRMS (ESI) calcd for $C_{24}H_{28}F_3N_6$ $[M+H]^+$: 457.2322, Found: 457.2321.

5.1.15.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-Aminoadamantan-1-yl]methyl}amino)-2-{[2-(chlorophenyl)methyl]amino}pyrimidine-5-carbonitrile (22)

Compound **22** was prepared from compound **16** in 69% yield as a colorless solid, using similar approach to that described for **3**. ^{1}H NMR (DMSO-d_6) δ ppm 1.07–2.03 (15H, m), 2.62 and 2.80 (total 1H, each br s), 2.94 and 3.16 (total 2H, each d, *J* = 6.3Hz), 4.51-4.54 (2H, m), 7.15–7.31 (4H,

m), 7.42 (1H, d, *J* = 7.4Hz), 7.92 and 8.19 (total 1H, each t, *J* = 6.3Hz), 8.18 (1H, s); MS (ESI) *m*/*z* 423 $[M+H]^+$; HRMS (ESI) calcd for $C_{23}H_{28}ClN_6$ $[M+H]^+$: 423.2058, Found: 423.2059.

5.1.16.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-Aminoadamantan-1-yl]methyl}amino)-2-{[2-(methylsulfanyl)benzyl]amino}pyrimidine-5-carbonitrile (23)

Compound **23** was prepared from compound **20** in 79% yield as a colorless solid, using similar approach to that described for **3**. ^{1}H NMR (DMSO-d_6) δ ppm 1.07–2.03 (15H, m), 2.49 (3H, s), 2.63 and 2.81 (total 1H, each brs), 2.95 and 3.15 (total 2H, each d, *J* = 6.3Hz), 4.44 (2H, d, *J* = 5.8Hz), 7.06–7.27 (5H, m), 7.84 and 8.11 (total 1H, each t, *J* = 6.0Hz), 8.16 (1H, s); MS (ESI) *m*/*z* 435 $[M+H]^+$; HRMS (ESI) calcd for $C_{24}H_{31}N_6S$ $[M+H]^+$: 435.2325, Found: 435.2324.

5.1.17.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-Aminoadamantan-1-yl]methyl}amino)-2-({[2-(methylsulfanyl)pyridin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (24)

Compound **24** was prepared from compound **20** in 69% yield as a colorless solid, using similar approach to that described for **3**. ^{1}H NMR (DMSO-d_6) δ ppm 1.01–2.07 (15H, m), 2.54 and 2.55 (total 3H, each s), 2.58–2.82 (1H, m), 2.90–3.18 (2H, m), 4.29–4.41 (2H, m), 7.02–7.11 (1H, m), 7.14–7.41 (2H, m), 7.86–8.23 (2H, m), 8.29–8.37 (1H, m); MS (ESI) *m*/*z* 436 $[M+H]^+$; HRMS (ESI) calcd for $C_{23}H_{30}N_7S$ $[M+H]^+$: 436.2278, Found: 436.2278.

5.1.18.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-Aminoadamantan-1-yl]methyl}amino)-2-({[2-(methoxy)pyridin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (25)

Compound **25** was prepared from compound **20** in 80% yield as a colorless solid, using similar approach to that described for **3**. ^{1}H NMR (DMSO-d_6) δ ppm 1.02–2.05 (15H, m), 2.61–2.82 (1H, m), 2.90–3.17 (2H, m), 3.89 and 3.92 (total 3H, each s), 4.34–4.41 (2H, m), 6.87–6.95 (1H, m), 7.10–7.31 (1H, m), 7.36–7.43 (1H, m), 7.78–8.09 (2H, m), 8.16 (1H, s); MS (ESI) *m*/*z* 420 $[M+H]^+$; HRMS (ESI) calcd for $C_{23}H_{30}N_7O$ $[M+H]^+$: 420.2506, Found: 420.2507.

5.1.19.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-Aminoadamantan-1-yl]methyl}amino)-2-({[2-(ethylsulfanyl)pyridin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (26)

Compound **26** was prepared from compound **20** in 70% yield as a colorless solid, using similar approach to that described for **3**. ^{1}H NMR (DMSO-d_6) δ ppm 1.06–2.02 (15H, m), 1.32 (3H, t, *J* = 8.0 Hz), 2.61–2.79 (1H, m), 2.93–3.17 (2H, m), 3.22 (2H, q, *J* = 8.0 Hz), 4.31–4.34 (2H, m), 7.04–7.35 (3H, m), 7.87–8.17 (2H, m), 8.29–8.33 (1H, m); MS (ESI) *m*/*z* 450 $[M+H]^+$; HRMS (ESI) calcd for $C_{24}H_{32}N_7S$ $[M+H]^+$: 450.2434, Found: 450.2434.

5.1.20.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-Aminoadamantan-1-yl]methyl}amino)-2-({[2-(isopropylsulfanyl)pyridin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (27)

Compound **27** was prepared from compound **20** in 71% yield, using similar approach to that described for **3**. ^{1}H NMR (DMSO-d_6) δ ppm 1.07–2.02 (21H, m), 2.62–2.80 (1H, m), 2.94–3.16 (2H, m), 4.07–4.14 (1H, m), 4.29–4.33 (2H, m), 7.03–7.39 (3H, m), 7.82–8.11 (1H, m), 8.17 (1H, s), 8.30–8.33 (1H, m); MS (ESI) *m*/*z* 464 $[M+H]^+$.

5.1.21.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-Aminoadamantan-1-yl]methyl}amino)-2-({[2-(isopropyloxy)pyridin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (28)

Compound **28** was prepared from compound **20** in 80% yield, using similar approach to that described for **3**. ^{1}H NMR (DMSO-d_6) δ ppm 1.06–2.03 (21H, m), 2.61–2.79 (1H, m), 2.94–3.15 (2H, m), 4.34–4.38 (2H, m), 5.26–5.34 (1H, m), 6.83–8.01 (5H, m), 8.17 (1H, s); MS (ESI) *m*/*z* 448 $[M+H]^+$.

5.1.22.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-Aminoadamantan-1-yl]methyl}amino)-2-({[2-(methoxy)pyridazin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (29)

Compound **29** was prepared from compound **20** in 72% yield as a colorless solid, using similar approach to that described for **3**. ^{1}H NMR (DMSO-d_6) δ ppm 0.95–2.08 (13H, m), 2.58–3.19 (3H,

m), 3.22–3.40 (2H, m), 4.03–4.16 (3H, m), 4.32–4.48 (2H, m), 7.16–7.27 (1H, m), 7.28–8.23 (3H, m), 8.71–8.84 (1H, m); MS (ESI) *m*/*z* 421 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{29}N_8O$ $[M+H]^+$: 421.2459, Found: 421.2460.

5.1.23.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-{[(*trans*-4-Hydroxycyclohexyl)methyl]amino}adamantan-1-yl]methyl}amino)-2-({[2-(trifluoromethoxy)phenyl]methyl}amino)pyrimidine-5-carbonitrile (30)

Under ice-cooling, to a solution of (1*S*,4*S*)-4-((*tert*-butyldimethylsilyl)oxy)cyclohaxanecarbaldehyde (28 mg, 0.12 mmol) in CH_2Cl_2 (1.5 mL), **3** (50 mg, 0.11 mmol) and sodium triacetoxyborohydride (67 mg, 0.32 mmol) were added and then stirred at room temperature for 2 h. To the reaction mixture, (1*S*,4*S*)-4-((*tert*-butyldimethylsilyl)oxy)cyclohaxanecarbaldehyde (10 mg, 0.04 mmol) was added and stirred at room temperature for 1 h. The reaction mixture was basified with saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was washed with H_2O, brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on amino silica gel with elution using (*n*-hexane- $CHCl_3$) (2:8) to give a white solid (41 mg). To the obtained product (38 mg, 0.05 mmol) in MeOH (0.7 mL), 1N aqueous HCl (10 mL) was added under ice-cooling and stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo and diluted with $CHCl_3$, basified with saturated aqueous $NaHCO_3$. The mixture was extracted with 10% MeOH/$CHCl_3$, the organic layer was washed with H_2O, brine, dried over $MgSO_4$ and concentrated

in vacuo. The residue was chromatographed on amino silica gel with elution using ($CHCl_3$-MeOH) (97:3 to 90:10) to give **30** (29 mg, 49% from **3**) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ ppm 0 .78–2.62 (26H, m), 2.89–3.18 (2H, m), 3.25–3.37 (1H, m), 4.41–4.45 (1H, m), 4.50–4.58 (2H, m), 7.10–7.40 (5H, m), 7.85–8.21 (2H, m); MS (ESI) *m*/*z* 585 $[M+H]^+$; HRMS (ESI) calcd for $C_{31}H_{40}F_3N_6O_2$ $[M+H]^+$: 585.3159, Found: 585.3153.

5.1.24.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-{[(*trans*-4-Hydroxycyclohexyl)methyl]amino}adamantan-1-yl]methyl}amino)-2-({[2-(trifluoromethyl)phenyl]methyl}amino)pyrimidine-5-carbonitrile (31)

Compound **31** was prepared from compound **21** in 70% yield as a colorless solid, using similar approach to that described for **30**. ^{1}H NMR (DMSO-d_6) δ ppm 0.79–2.58 (26H, m), 2.86–3.19 (2H, m), 3.26–3.38 (1H, m), 4.43–4.48 (1H, m), 4.62–4.71 (2H, m), 7.18–7.73 (5H, m), 7.93–8.01 and 8.16–8.27 (total 2H, each m); MS (ESI) *m*/*z* 569 $[M+H]^+$; HRMS (ESI) calcd for $C_{31}H_{40}F_3N_6O$ $[M+H]^+$: 569.3210, Found: 569.3200.

5.1.25.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-{[(*trans*-4-Hydroxycyclohexyl)methyl]amino}adamantan-1-yl]methyl}amino)-2-{[2-(chlorophenyl)methyl]amino}pyrimidine-5-carbonitrile (32)

Compound **32** was prepared from compound **22** in 74% yield as a colorless solid, using similar approach to that described for **30**. ^{1}H NMR (DMSO-d_6) δ ppm 0.79–2.03 (23H, m), 2.20–2.61 (3H,

m), 2.89–3.19 (2H, m), 3.26–3.37 (1H, m), 4.40–4.45 (1H, m), 4.48–4.56 (2H, m), 7.12–7.45 (5H, m), 7.87–8.22 (2H, m); MS (ESI) *m*/*z* 535, 537 $[M+H]^+$; HRMS (ESI) calcd for $C_{30}H_{40}ClN_6O$ $[M+H]^+$: 535.2947, Found: 535.2946.

5.1.26.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-{[(*trans*-4-Hydroxycyclohexyl)methyl]amino}adamantan-1-yl]methyl}amino)-2-({[2-(methylsulfanyl)phenyl]methyl}amino)pyrimidine-5-carbonitrile (33)

Compound **33** was prepared from compound **23** in 58% yield as a colorless solid, using similar approach to that described for **30**. ^{1}H NMR (DMSO-d_6) δ ppm 0.79–2.03 (23H, m), 2.21–2.62 (6H, m), 2.90–3.19 (2H, m), 3.24–3.39 (1H, m), 4.38–4.50 (3H, m), 7.04–7.31 (5H, m), 7.78–8.22 (2H, m); MS (ESI) *m*/*z* 547 $[M+H]^+$; HRMS (ESI) calcd for $C_{31}H_{43}N_6OS$ $[M+H]^+$: 547.3214, Found: 547.3217.

5.1.27.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-{[(*trans*-4-Hydroxycyclohexyl)methyl]amino}adamantan-1-yl]methyl}amino)-2-({[2-(methylsulfanyl)pyridin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (34)

Compound **34** was prepared from compound **24** in 82% yield as a colorless solid, using similar approach to that described for **30**. ^{1}H NMR (DMSO-d_6) δ ppm 0.79–2.04 (23H, m), 2.21–2.62 (6H, m), 2.88–3.18 (2H, m), 3.24–3.38 (1H, m), 4.30–4.40 (2H, m), 4.41–4.45 (1H, m), 7.03–7.12 (1H, m), 7.13–7.41 (2H, m), 7.84–8.21 (2H, m), 8.26–8.37 (1H, m); MS (ESI) *m*/*z* 548 $[M+H]^+$; HRMS

(ESI) calcd for $C_{30}H_{42}N_7OS$ $[M+H]^+$: 548.3166 , Found: 548.3166; Anal. Calcd for $C_{30}H_{41}N_7OS$: C, 65.78; H, 7.54; N, 17.90; S, 5.85. Found: C, 65.74; H, 7.57; N, 17.89; S, 5.94.

5.1.28.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-{[(*trans*-4-Hydroxycyclohexyl)methyl]amino}adamantan-1-yl]methyl}amino)-2-({[2-(methoxy)pyridin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (35)

Compound **35** was prepared from compound **25** in 90% yield as a colorless solid, using similar approach to that described for **30**. ^{1}H NMR (DMSO-d_6) δ ppm 0.79–2.03 (23H, m), 2.22–2.61 (3H, m), 2.88–3.19 (2H, m), 3.25–3.38 (1H, m), 3.89 and 3.92 (total 3H, each s), 4.33–4.46 (3H, m), 6.86–6.96 (1H, m), 7.08–7.34 (1H, m), 7.34–7.44 (1H, m), 7.75–8.09 (2H, m), 8.16 (1H, s); MS (ESI) *m*/*z* 532 $[M+H]^+$; HRMS (ESI) calcd for $C_{30}H_{42}N_7O_2$ $[M+H]^+$: 532.3395, Found: 532.3394.

5.1.29.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-{[(*trans*-4-Hydroxycyclohexyl)methyl]amino}adamantan-1-yl]methyl}amino)-2-({[2-(ethylsulfanyl)pyridin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (36)

Compound **36** was prepared from compound **26** in 37% yield as a colorless solid, using similar approach to that described for **30**. ^{1}H NMR (DMSO-d_6) δ ppm 0.81–2.31 (27H, m), 1.32 (3H, t, *J* = 8.0 Hz), 2.93–3.17 (2H, m), 3.22 (2H, q, *J* = 8.0Hz), 4.31–4.36 (2H, m), 4.43 (1H, d, *J* = 4.0 Hz), 7.03–7.38 (3H, m), 7.85–8.17 (2H, m), 8.26–8.33 (1H, m); MS (ESI) *m*/*z* 562 $[M+H]^+$; HRMS

(ESI) calcd for $C_{31}H_{44}N_7OS$ $[M+H]^+$: 562.3323, Found: 562.3318.

5.1.30.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-{[(*trans*-4-Hydroxycyclohexyl)methyl]amino}adamantan-1-yl]methyl}amino)-2-({[2-(*iso*-propylsulfanyl)pyridin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (37)

Compound **37** was prepared from compound **27** in 89% yield as a colorless solid, using similar approach to that described for **30**. ^{1}H NMR (DMSO-d_6) δ ppm 0.82–2.32 (33H, m), 2.94–3.17 (2H, m), 4.08–4.15 (1H, m), 4.29–4.33 (2H, m), 4.42–4.43 (1H, m), 7.03–7.08 (1H, m), 7.16–7.39 (2H, m), 7.84–8.17 (1H, m), 8.17 (1H, s), 8.27–8.33 (1H, m); MS (ESI) *m*/*z* 576 $[M+H]^+$; HRMS (ESI) calcd for $C_{32}H_{46}N_7OS$ $[M+H]^+$: 576.3479, Found: 576.3477.

5.1.31.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-{[(*trans*-4-Hydroxycyclohexyl)methyl]amino}adamantan-1-yl]methyl}amino)-2-({[2-(*iso*-propyloxy)pyridin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (38)

Compound **38** was prepared from compound **28** in 100% yield as a colorless solid, using similar approach to that described for **30**. ^{1}H NMR (DMSO-d_6) δ ppm 0.82–2.67 (33H, m), 2.95–3.18 (2H, m), 4.32–4.42 (3H, m), 5.28–5.34 (1H, m), 6.83–7.40 (3H, m), 7.65–8.00 (2H, m), 8.16 (1H, s); MS (ESI) *m*/*z* 560 $[M+H]^+$; HRMS (ESI) calcd for $C_{32}H_{46}N_7O_2$ $[M+H]^+$: 560.3708, Found: 560.3707.

5.1.32.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-{[(*trans*-4-Hydroxycyclohexyl)methyl]amino}adamantan-1-yl]methyl}amino)-2-({[2-(methoxy)pyridazin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (39)

Compound **39** was prepared from compound **29** in 59% yield as a colorless solid, using similar approach to that described for **30**. ^{1}H NMR (DMSO-d_6) δ ppm 0.76–1.66 (16H, m), 1.76–1.91 (6H, m), 1.94–2.36 (3H, m), 2.80–3.20 (2H, m), 3.25–3.30 (2H, m), 4.05–4.13 (3H, m), 4.34–4.46 (3H, m), 7.16–7.26 (1H, m), 7.29–8.32 (3H, m), 8.71–8.80 (1H, m); MS (ESI) *m*/*z* 533 $[M+H]^+$; HRMS (ESI) calcd for $C_{29}H_{41}N_8O_2$ $[M+H]^+$: 533.3347, Found: 533.3341.

5.1.33.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-*tert*-Butoxycarbonylaminoadamantan-1-yl]methyl}amino)-2-({[2-(methylsulfanyl)pyridin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (40)

To a solution of **20** (700 mg, 1.6 mmol) in DMF (7 mL), [2-(methylsulfanyl)pyridin-3-yl]methanamine (360 mg, 2.4 mmol) and DIPEA (410 μL, 2.4 mmol) were added at 0 ^{o}C and stirred at room temperature for 1 h. The reaction mixture was diluted with EtOAc/H_2O and extracted with EtOAc. The organic layer was washed with H_2O, brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel with elution using ($CHCl_3$-MeOH) (98:2 to 90:10) to give **40** (730 mg, 86%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ ppm 1.02–1.98 (22H, m), 2.54 and 2.55 (total 3H, each s), 2.90–3.19 (2H, m), 3.28–3.51 (1H, m), 4.31–4.41 (2H, m), 6.63–6.84 (1H, m), 7.03–7.13 (1H, m), 7.20–7.41 (2H, m), 7.87–7.93 and 8.14–8.24 (total 2H, each m), 8.29–8.38 (1H, m); MS (ESI) *m*/*z* 558 $[M+Na]^+$.

5.1.34.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-Aminoadamantan-1-yl]methyl}amino)-2-({[2-(methanesulfinyl)pyridin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (41)

To a solution of **40** (100mg, 0.19 mmol) in CH_2Cl_2 (3 mL), 75% *m*-CPBA (47 mg, 0.21 mmol) was added at 0 ^oC and stirred at 0 ^oC for 2 h. To the reaction mixture, 75% *m*-CPBA (4.3 mg, 0.019 mmol) was added at 0 ^oC and stirred at room temperature for 2 h. To the reaction mixture, aqueous $NaHCO_3$ was added and extracted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$, H_2O, brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel with elution using ($CHCl_3$-MeOH) (98:2 to 89:11) to give a colorless solid (120 mg). To a suspension of the obtained product (110 mg, 0.2 mmol) in CH_2Cl_2 (1.7 mL), TFA (1.5 mL, 20 mmol) was added at 0 ^oC and stirred at room temperature for 0.5 h. The reaction mixture was concentrated in vascuo. The residue was chromatographed on amino silica gel with elution using ($CHCl_3$-MeOH) (10:1) to give **41** (73 mg, 95% from **40**) as a colorless solid. ^1H NMR (DMSO-d_6) δ ppm 1.03–2.06 (15H, m), 2.59–2.81 (1H, m), 2.84 and 2.85 (total 3H, each s), 2.89–3.19 (2H, m), 4.69–4.92 (2H, m), 7.16–7.36 (1H, m), 7.49–7.56 (1H, m), 7.72–7.85 (1H, m), 7.90–8.22 (2H, m), 8.55–8.64 (1H, m); MS (ESI) *m*/*z* 452 $[M+H]^+$; HRMS (ESI) calcd for $C_{23}H_{30}N_7OS$ $[M+H]^+$: 452.2227, Found: 452.2232.

5.1.35.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-Aminoadamantan-1-yl]methyl}amino)-2-({[2-(methanesulfonyl)pyridin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (42)

Compound **42** was prepared from compound **40** in 73% yield as a pale yellow solid, using similar approach to that described for **41**. ^{1}H NMR (DMSO-d_6) δ ppm 0.96–2.07 (15H, m), 2.52–2.83 (1H, m), 2.88–3.20 (2H, m), 3.43 (3H, s), 4.87–4.96 (2H, m), 7.19–7.37 (1H, m), 7.62–7.70 (1H, m), 7.76–7.85 (1H, m), 7.88–8.24 (2H, m), 8.50–8.59 (1H, m); MS (ESI) *m*/*z* 468 $[M+H]^+$; HRMS (ESI) calcd for $C_{23}H_{30}N_7O_2S$ $[M+H]^+$: 468.2176, Found: 468.2179.

5.1.36.

4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-{[(*trans*-4-Hydroxycyclohexyl)methyl]amino}adamantan-1-yl]methyl}amino)-2-({[2-(methanesulfinyl)pyridin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (43)

Compound **43** was prepared from compound **41** in 79% yield as a colorless solid, using similar approach to that described for **30**. ^{1}H NMR (DMSO-d_6) δ ppm 0.80–2.58 (26H, m), 2.84 and 2.86 (total 3H, each s), 2.88–3.17 (2H, m), 3.25–3.38 (1H, m), 4.42–4.45 (1H, m), 4.69–4.93 (2H, m), 7.16–7.37 (1H, m), 7.48–7.57 (1H, m), 7.71–7.86 (1H, m), 7.89–8.23 (2H, m), 8.52–8.64 (1H, m); MS (ESI) *m*/*z* 564 $[M+H]^+$; HRMS (ESI) calcd for $C_{30}H_{42}N_7O_2S$ $[M+H]^+$: 564.3115, Found: 564.3116.

5.1.37.

**4-({[(1*s*,3*R*,4*s*,5*S*,7*s*)-4-{[(*trans*-4-Hydroxycyclohexyl)methyl]amino}adamantan-1-yl]methyl}a

mino)-2-({[2-(methanesulfonyl)pyridin-3-yl]methyl}amino)pyrimidine-5-carbonitrile (44)

Compound **44** was prepared from compound **42** in 63% yield as a colorless solid, using similar approach to that described for **30**. ^{1}H NMR (DMSO-d_6) δ ppm 0.78–2.55 (26H, m), 2.88–3.20 (2H, m), 3.25–3.37 (1H, m), 3.43 (3H, s), 4.41–4.47 (1H, m), 4.86–4.97 (2H, m), 7.19–7.39 (1H, m), 7.61–7.71 (1H, m), 7.75–7.86 (1H, m), 7.86–8.18 (1H, m), 8.18 and 8.21 (total 1H, each s), 8.45–8.60 (1H, m); MS (ESI) *m*/*z* 580 $[M+H]^+$; HRMS (ESI) calcd for $C_{29}H_{41}N_8O_2$ $[M+H]^+$: 580.3064, Found: 580.3061.

5.2. Biology

5.2.1. PKCθ inhibitory activity (enzyme assay)

The reaction mixture contained STK Substrate 1-biotin, recombinant 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 Eu^{3+} Cryptate for 60 min. The enzyme reaction rate was measured in fluorescence intensity at 620 nm (Eu^{3+} Cryptate) and 665 nm (XL665). The activity is expressed as "PKCθ IC_{50} (nM)" in Tables.

5.2.2. IL-2 inhibitory activity (cellular assay)

Jurkat cells transiently transfected with the pGL3-IL2 pro 43 plasmid were incubated for 14 h with test compounds in medium containing anti-CD3 and anti-CD28 antibodies, followed by the

addition of substrate solution to measure the firefly luciferase activity.

5.2.3. Rat cardiac transplantation model

ACI rats were used as cardiac donors and Lewis rats as cardiac recipients. All procedures were conducted under aseptic conditions. Rats were intraperitoneally anesthetized with pentobarbital (40 mg/kg). Abdominal vascularized heterotopic cardiac transplantation was conducted in accordance with a previously reported method. Compound **34** was dissolved in propylene glycol. Compound **34** was orally administered in combination with tacrolimus. Beginning on the operation day, all test compounds were administered for 14 consecutive days. Cardiac allograft function was assessed by daily palpation for 28 days, and graft rejection was defined as the cessation of palpable cardiac graft beats. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc. Further, the Astellas Pharma Inc. Tsukuba Research Center was awarded Accreditation Status by the AAALAC International. All efforts were made to minimize the number of animals used and to avoid suffering and distress.

5.2.4. 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.

5.2.5. Aqueous solubility

Small volumes of DMSO solutions of the test compounds were diluted to 130 μM by adding the aqueous buffer solution of pH 6.8. After incubation at 25 °C for 20 h, precipitates were separated by filtration. The solubility was determined by HPLC analysis of each filtrates.

5.2.6. Pharmacokinetic study

The pharmacokinetic characterization of compound **34** was conducted in female SD rats. Compound **34** was intravenously administered at 1 mg/kg in a mixture of DMF/propylene glycol/1N HCl/ saline (25/25/0.2/49.8) solution, and orally administered at 1 mg/kg in a mixture of DMF/propylene glycol/1N HCl/ saline (25/25/0.2/49.8) solution. Blood samples were taken at multiple time points up to 24 h after a single administration of the compound. Concentrations of unchanged compound in plasma were determined using LC-MS/MS. Pharmacokinetic parameters after i.v. and p.o. administration were calculated by noncompartmental analysis using Phoenix WinNonlin version 6.3 software (Certara USA, Inc., Princeton, NJ, USA).

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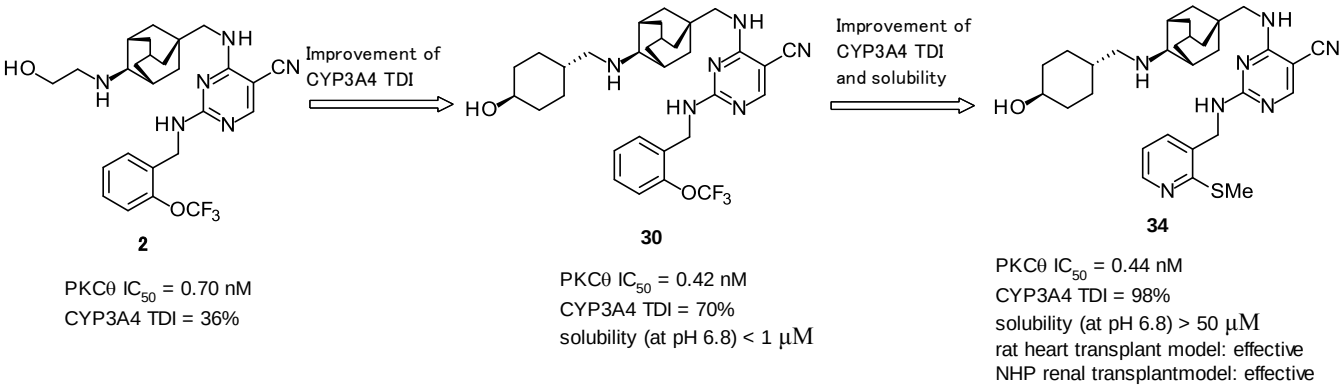
Graphical abstract



2

PKCθ IC_{50} = 0.70 nM
CYP3A4 TDI = 36%

30

PKCθ IC_{50} = 0.42 nM
CYP3A4 TDI = 70%
solubility (at pH 6.8) < 1 μM

34

PKCθ IC_{50} = 0.44 nM
CYP3A4 TDI = 98%
solubility (at pH 6.8) > 50 μM
rat heart transplant model: effective
NHP renal transplantmodel: effective