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Discovery of a potent and selective Axl inhibitor in preclinical model

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ABSTRACT

Axl and Mer are a members of the TAM (Tyro3-Axl-Mer) family of receptor tyrosine kinases, which, when activated, can promote tumor cell survival, proliferation, migration, invasion, angiogenesis, and tumor-host interactions. Chronic inhibition of Mer leads to retinal toxicity in mice. Therefore, successful development of an Axl targeting agent requires ensuring that it is safe for prolonged treatment. Here, to clarify whether enzyme inhibition of Mer by a small molecule leads to retinal toxicity in mice, we designed and synthesized Axl/Mer inhibitors and Axl-selective inhibitors. We identified an Axl/Mer dual inhibitor **28a**, which showed retinal toxicity at a dose of 100 mg/kg in mice. Subsequent derivatization of a pyridine derivative led to the discovery of a pyrimidine derivative, **33g**, which selectively inhibited the activity of Axl over Mer without retinal toxicity at a dose of 100 mg/kg in mice. Additionally, the compound displayed in vivo anti-tumor effects without influencing body weight in a Ba/F3-Axl isogenic subcutaneous model.

1. Introduction

Drug resistance to conventional, targeted therapy and immune checkpoint inhibitors is a major cause of failure of anticancer treatment. Understanding of resistance mechanisms and identification of the molecules driving resistance will allow development of targeted agents that can destroy the drug resistant cancer. Axl is a member of the TAM (Tyro3-Axl-Mer) family of receptor tyrosine kinases, which, when activated, can promote tumor cell survival, proliferation, migration and invasion, angiogenesis, and tumor host interactions.[1] Axl is often highly expressed in resistant tumor cells that show mesenchymal phenotypes.[2–3] Furthermore, Axl is involved in acquired drug resistance to multiple chemotherapies and molecular targeted-, radio- and immuno therapies.[4–9] Inhibition of Axl signaling suppresses such resistance in a variety of cancer types.[10] Therefore, we consider Axl to be a crucial drug target to eradicate therapy-resistant cancer cells to improve treatment outcomes.

Several small molecular inhibitors showing Axl inhbitory activity[11] have already been developed, e.g. gilteritinib (ASP2215),[12] TP-0903,[13] foretinib (XL-880),[14] and S49076.[15] However, these agents are multi-kinase inhibitors with high potency against not only Axl but also other relevant kinases, including FLT3, c-Met (also known as MET), ALK, and VEGFR2 kinase (also known as KDR). Recently, some selective inhibitors have been developed: BGB324[16] and DS-1205b[17] were reported as selective Axl inhibitors; ONO-7475[18] and RXDX-106[19] were reported as a selective Axl/Mer inhibitor (Fig. 1).

Abbreviations: ALK, anaplastic lymphorma kinase; Boc, *tert*-butoxycarbonyl; Cbz, benzyloxycarbonyl; DCM, dichloromethane; DDR1, discoidin domain receptor 1; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EtOH, ethanol; Et_3N, triethylamine; EtOAc, ethyl acetate; EtONa, sodium ethoxide; FLT3, fms-like tyrosine kinase 3; FLT4, fms-related tyrosine kinase 4; HATU, 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-Oxide Hexafluorophosphate; *i*Pr_2NEt, *N*,*N*-Diisopropylethylamine; K_2CO_3, potassium carbonate; KDR, kinase insert domain receptor; MeOH, methanol; $MgSO_4$, magnesium sulfate; *i*-PrOH, 2-propanol; $NaHCO_3$, sodium hydrogen carbonate; NH_4Cl, ammonium chloride; NMP, *N*-methylpyrrolidone; RT, room temperature; TAM, Tyro3-Axl-Mer; TBME, *tert*-butyl methyl ether; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLR, toll-like receptor; VEGFR2, vascular endothelial growth factor receptor 2.

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The main function of TAM family members in normal tissues is clearance of apoptotic cells and dampening of Toll-like receptor (TLR)-dependent inflammatory responses and natural killer cell activity.[20–21] Chronic inhibition of Mer leads to retinal toxicity in a mouse model.[22] In Mer knock-out mice, retinal pigment epithelial cells cannot clear apoptotic-like outersegment debris, resulting in retinal degeneration. The retinal toxicity of the multi kinase inhibitors as mentioned above and the four selective Axl inhibitors shown in Fig. 1 has not been published, except in patents where the retinal toxicity of DS-1205b has been mentioned. To clarify whether enzyme inhibition of Mer by small molecules leads to retinal toxicity in mice, we focused on the selective Axl/Mer inhibitors RXDX-106 and ONO-7475. We speculated that the Axl selectivity of these compounds might be increased if the right end part were convert to the uracil or hexahydroquinoline-2,5-dione moiety, because some multi kinase inhibitors consist of a smaller moiety like pyridone or cyclopropane dicarboxamide. In the present study, we replaced a pyridine and a pyrimidine in the quinoline moiety of RXDX-106 and ONO-7475 in an attempt to produce an original Axl inhibitor with drug-like properties.

We report the synthesis of a series of pyridine and pyrimidine compounds and the selection of the optimal compound for Axl inhibition by analysis of structure–activity relationships (SARs), optimization of drug-likeness parameters, and pathological evaluation for in vivo retinal toxicity.

2. Chemistry

The primary purpose of the chemistry research was to develop a novel Axl inhibitor. RXDX-106 and ONO-7475 each contain a quinoline moiety. To investigate the effect of uracil substituents on the pyridine framework, we synthesized pyridine derivatives of RXDX-106 as shown in Schemes 1 and 2. To produce uracil-5-carboxylic acid derivatives **7a-c**, **3** was synthesized from **1** by amination using ammonia, and **4** was produced from **3** by ureidation with 4-fluorophenyl isocyanate. Cyclization of **4** in the presence of sodium ethoxide generated **5**. Alkylation of **5** with iodomethane or 2-iodopropane or cyclopentyl bromide, followed by hydroxylation, generated the derivatives **7a-c**, respectively. A different synthetic route was used to produce uracil-5-carboxylic acid derivative **7d**. Compound **1** was aminated with cyclopropylamine to produce **2**, and then cyclization with 4-fluorophenyl isocyanate in the presence of K_2CO_3 generated **6d**. Hydroxylation of **6d** under acidic condition generated **7d**.

Various aniline derivatives, **19a-e**, were created to investigate the substituent effect of fluorine. The synthetic route differed depending on the position of the fluorine. To produce aniline derivatives **19a-c**, **17a-b** were synthesized from 2-amino-4-chloropyridine (**15**) by nucleophilic aromatic substitution (S_NAr) in the presence of nitrophenol derivatives **16a-b**. Amidation of **17a** with N,*N*-dimethylglycine, and amidation of **17a-b** with 1-Boc-piperidine-4-ylacetic acid generated **18a-c**. Deprotection of the Boc group of **18b-c**, followed by reductive amination with formaldehyde, afforded **18d-e**, respectively. Reduction of the nitro group of **18a** and **18d-e** with Pd/C afforded **19a-c**, respectively. Condensation of **19a-b** and **19d** with **7b** generated **20a-c**, respectively. Condensation of **19c** with **7d** and **7c** generated **20d-e**, respectively. To synthesize aniline derivatives **19d-e, 14a-b** were synthesized from 4-chloropyridine-2-carboxamide (**8**) by S_NAr in the presence of aminophenol derivatives **9a-b**. Protection of the aniline moiety of **10a-b** by benzyloxycarbonyl (Cbz), followed by Hofmann rearrangement, afforded **12a-b**, respectively. Condensation of **12a-b** with 1-Boc-piperidine-4-ylacetic acid, followed by deprotection of the Boc group and reductive amination with formaldehyde generated **19d-e**, respectively. Condensation of **19d** with **7b** produced **20c**.

Pyridine derivatives containing hexahydroquinoline-2,5-dione were synthesized as shown in Scheme 3. Carboxylic acid (**24**) was synthesized by cyclization of ethyl (ethoxymethylene)cyanoacetate (**21**), followed by amidation and hydroxylation. Compounds **25a-d** were synthesized by condensation of **19b-e** with **24**.

With the aim of improving the potency and the microsomal stability of **20a-e** and **25a-d**, ureidopyridine derivatives **28a-b** were synthesized from **10b** by condensation with **7b** and **24**, followed by Hofmann rearrangement and ureidation via phenyl carbamate as shown in Scheme 4.

To investigate Axl selectivity over Mer in the cell based assay using Ba/F3 driven with activity of each kinase, pyrimidine derivatives **33a-j** were synthesized as shown in Scheme 5. Compound **31** was synthesized by S_NAr from aminochloropyrimidine (**29**) in the presence of aminophenol (**30**). Condensation of **31** with **7a-d** afforded **32a-d**, respectively. Amidation of **32a** with cyclopropanecarbonyl chloride generated **33a**. Ureidation of **32a-d** with the corresponding cyclic amine via phenyl carbamate produced **33b-j**, respectively.

3. Results and discussion

To investigate intrinsic inhibitory activity against target kinases, the pyridine and pyrimidine derivatives were firstly evaluated with two assays, a cell-free Axl kinase assay and a cell-free Mer kinase assay. Additionally, some compounds were evaluated with a cell proliferation assay against Axl-dependent Ba/F3 cells (Ba/F3-Axl) and Mer-dependent Ba/F3 cells (Ba/F3-Mer). A solubility assay and microsomal stability assay in mouse liver microsomes were also conducted for drug-like properties.

Table 1 lists the results of the inhibitory activity, solubility, and microsomal stability assays for the pyridine derivatives containing a uracil moiety. Regarding the substituents on R^3, **20b** showed more potent inhibitory activity than **20a** in the cell-free Axl and Mer assays. Regarding the substituents on R^4, **20e** showed more potent inhibitory activity than **20d** in the cell-free Axl and Mer assays. No clear effect of fluorine substituents on R^1 and R^2 was seen. The Axl inhibitory activity of **20b** and **20e** was more than 10-fold higher that the Mer inhibitory activity in the cell-free assays, indicating Axl selectivity; however only a small difference was observed in the cell-based assay.

Table 2 lists the result for the equivalent analyses of pyridine derivatives containing hexahydroquinoline-2,5-dione, **25a-d**. In general, it was found that the hexahydroquinoline-2,5-dione derivatives showed moderate potency, but weaker potency than the uracil derivatives in the cell-free assay. Regarding the effect of fluorine substituents on R^1 and R^2, **25d** which had fluorine substituents on both R^1 and R^2, showed the highest Axl selectivity in the cell-free assay (Table 2), but this selectivity

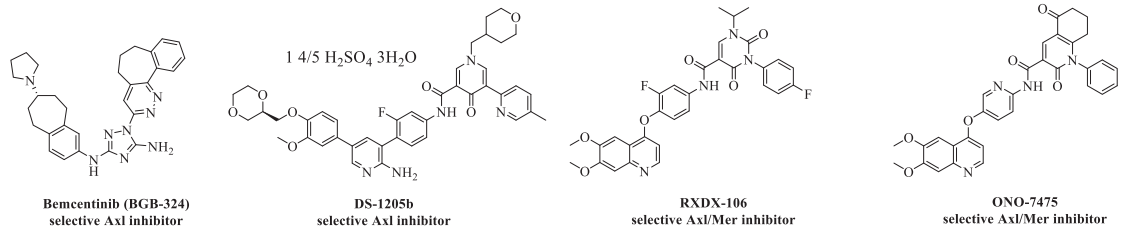
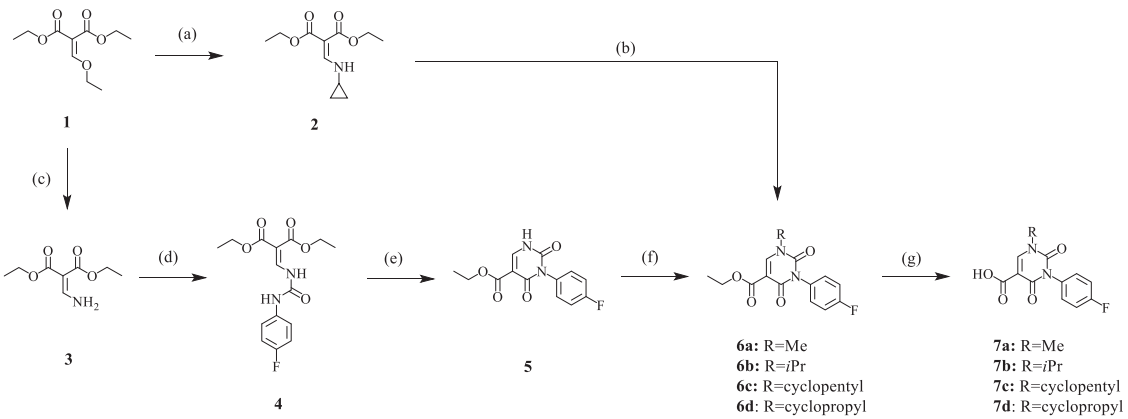
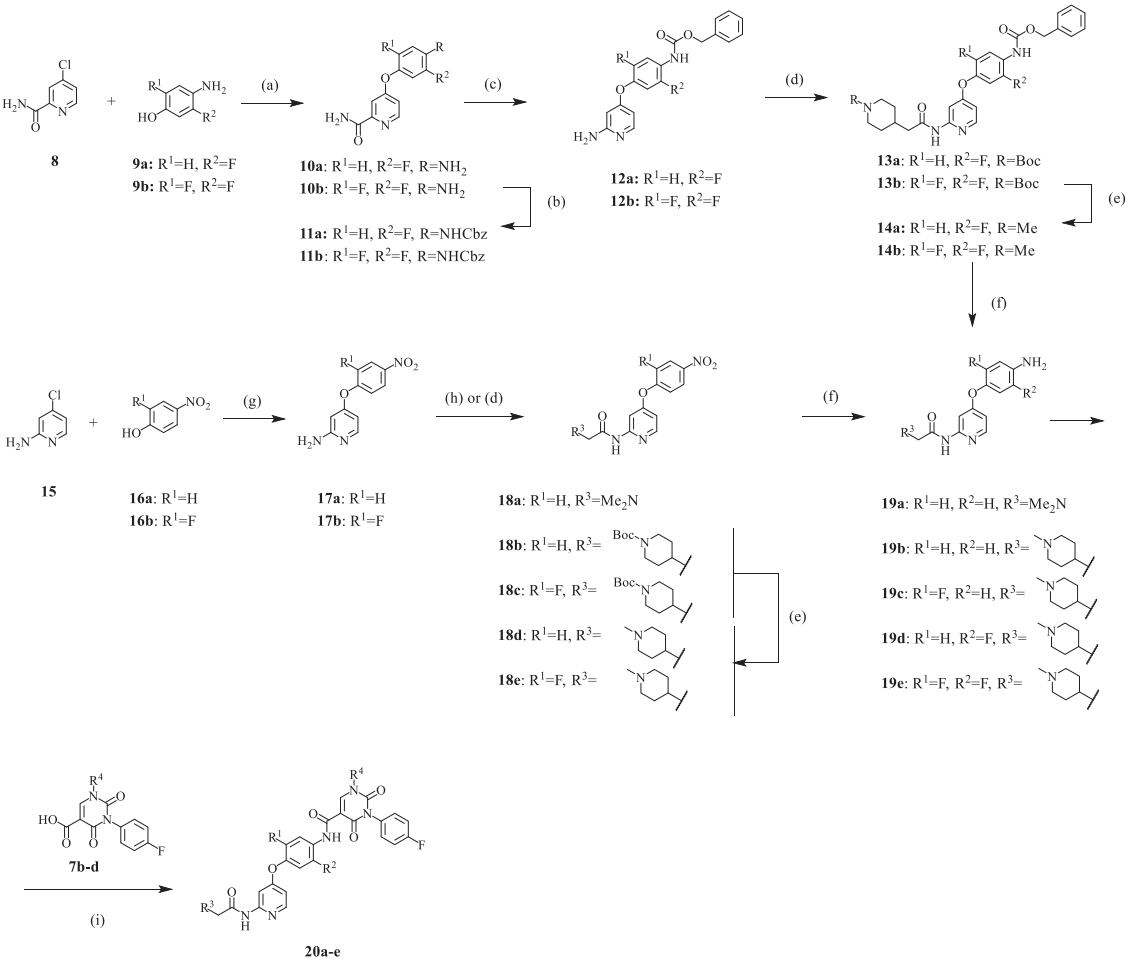


Fig. 1. Chemical structure of previously reported Axl selective inhibitors.



Scheme 1. Reagents and conditions: (a) cyclopropylamide, EtOH, RT; (b) 4-fluorophenyl isocyanate, K_2CO_3, DMF, 60 °C; (c) ammonia in MeOH, 0 °C to RT; (d) 4-fluorophenyl isocyanate, iPr$_2$NEt, 1,2-dichloroethane, reflux; (e) EtONa, EtOH, 0 °C to RT; (f) alkyl halide, K_2CO_3, DMF, 70 °C; (g) HCl in 1,4-dioxane/H_2O, 70–80 °C.

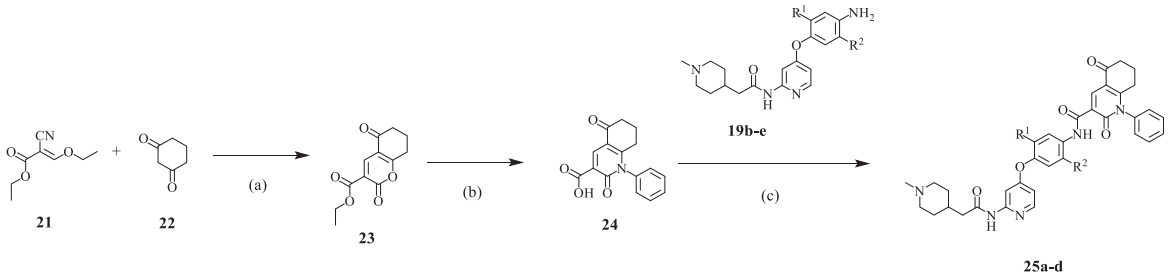


Scheme 2. Reagents and conditions: (a) *t*-BuOK, DMSO, 80 °C or 90 °C; (b) benzyl chloroformate, $NaHCO_3$, acetone, H_2O, RT; (c) [bis(trifluoroacetoxy)iodo] benzene, pyridine, DMF, H_2O, RT; (d) 1) benzotriazole, thionyl chloride, 1-Boc-piperidine-4-ylacetic acid, DCM, RT, 2) Et_3N, THF, 0 °C to RT; (e) TFA, DCM, RT, then formaldehyde, $NaBH(OAc)_3$, DCM, RT; (f) Palladium on carbon, hydrogen, MeOH, RT; (g) iPr$_2$NEt, NMP, 150 °C; (h) N,*N*-dimethylglycine, HATU, iPr$_2$NEt, DMF, 80 °C; (i) HATU, iPr$_2$NEt, DMF, RT to 70 °C.

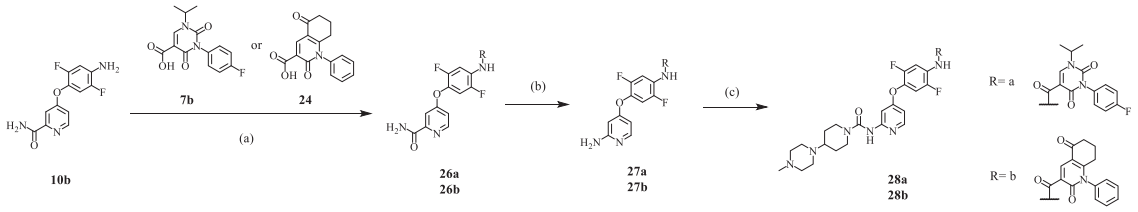
was not as high as that of the uracil derivatives (Table 1).

We then generated ureidopyridine derivatives in an attempt to increase solubility and microsomal stability while keeping potency (Table 3). Similar to the trend described above for pyridine derivatives containing a uracil moiety, **28a-b** respectively showed more than 47-fold and 19-fold greater selectivity for Axl than for Mer in the cell-free assay model, but no selectivity was observed in the cell-based assay.

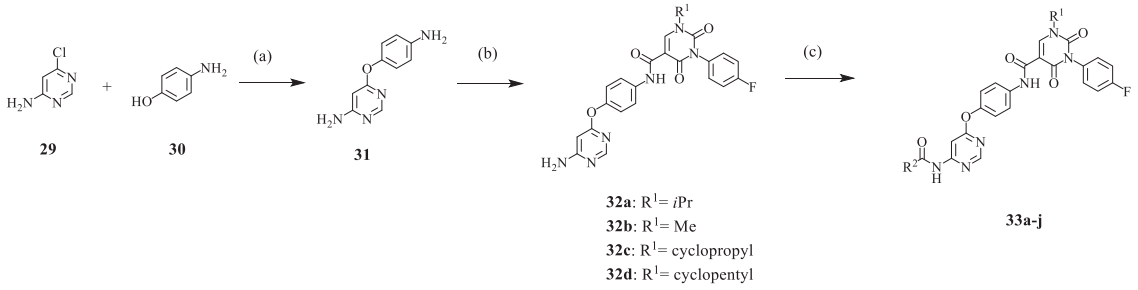
Of these two compounds, **28a** showed higher solubility and microsomal stability with potency against Axl and Mer like RXDX-106 and ONO-7475 (Table S2). We therefore, performed an in vivo test using **28a** to evaluate its retinal toxicity. Compound **28a** was administered once daily at 100 mg/kg for 14 days to BALB/c mice. There was no significant



Scheme 3. Reagents and conditions: (a) *t*BuOK, DMF, RT; (b) aniline, EtOH, RT; (c) HATU, *i*Pr$_2$NEt, DMF, RT or 80 °C.



Scheme 4. Reagents and conditions: (a) **7b** or **24**, HATU, *i*Pr$_2$NEt, RT to 75 °C; (b) [bis(trifluoroacetoxy)iodo]benzene, pyridine, DMF, H_2O, RT;(c) 1) phenyl chloroformate, *i*Pr$_2$NEt, THF, 0 °C to RT, 2) 1-methyl-4-(piperidin-4-yl)-piperazine, *i*Pr$_2$NEt, DMF, RT.



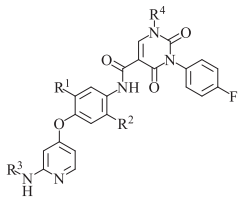
Scheme 5. Reagents and conditions: (a) *t*-BuOK, DMSO, 100 °C; (b) **7a-d**, HATU, *i*Pr$_2$NEt, DMF, RT or 70 °C; (c) cyclopropanecarbonyl chloride, *i*Pr$_2$NEt, THF, RT or 1) phenyl chloroformate, *i*Pr$_2$NEt, THF, RT, 2) amine, *i*Pr$_2$NEt, DMF, RT.

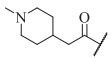
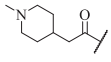
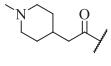
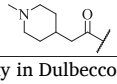
body weight loss during the dosing period (Fig. S1). The eyes were collected and submitted to the histopathologic examination. Similar to chronic inhibition of Mer,[22] retinal toxicity, characterized by degeneration or necrosis of photoreceptor cells in the outer nuclear layer throughout the retina, was observed in all treated mice (Fig. 2). We then performed cell-free kinase assays on a panel of 52 kinases to clarify the kinase selectivity of **28a** (Table S1). Compound **28a** showed inhibitory activity not only against Axl and Mer, but also against Tyro3, c-Met, and LCK. Although there are no reports of retinal toxicity based on inhibition of Tyro3, c-Met or LCK, we hypothesized that retinal toxicity led to Mer inhibition and could be avoided by increasing the inhibitor's selectivity against Axl over Mer.

In an attempt to improve the Axl selective of the inhibitor, we designed and synthesized pyrimidine derivatives containing a uracil moiety. Table 4 lists the result of the inhibitory activity, solubility, and microsomal stability assays for the resultant derivatives, **33a-j**. All of these compounds showed good potency in the cell-free Axl except for **33h**. The amide derivative **33a** did not show selectivity for Axl over Mer in the cell-based assay, but the ureidopyrimidine derivatives **33b-g** showed 3- to 6-fold more selective for Axl than for Mer. Compound **33c** showed good potency and selectivity in the cell-based assay, but solubility was low. Though **33e** and **33f** were unstable in mouse liver microsome, other compounds showed moderate to high microsomal stability. As for the effect of substituents on R^1, compounds with bulky moieties showed more potency and selectivity in the cell-free assay and cell-based assay (**33j** > **33g** > **33i** > **33h**).

Because **33g** showed good solubility and microsomal stability for oral administration with 4-fold Axl selectivity in the cell-based assay (Table 4), we explored this compound further. First, we evaluated pharmacokinetic profiles of **33g** after single oral administration of 10, 30 or 100 mg/kg in female Balb/c mice (Table 5). Plasma concentration of **33g** peaked at 2 h postdose, and C_{max} and $AUC_{(0\text{-}24h)}$ of **33g** increased dose-dependently in the dose range of 10–100 mg/kg. To evaluate the retinal toxicity, histopathologic examination was conducted in the eyes of BALB/c mice that were administered **33g** at 100 mg/kg once daily for 14 days. There was no significant body weight loss (Fig. S1) and no histological change in the retina (Fig. 2). We conclude that **33g** did not induce retinal toxicity. Next, we evaluated the antitumor effects of **33g** in the Ba/F3-Axl isogenic subcutaneous model (Fig. 3). Oral administration of **33g** at 1, 3 and 10 mg/kg once daily for 4 days inhibited tumor growth in this model without significant body weight loss. We then conducted kinome assay (as described above for **28a**) to clarify the kinase selectivity of **33g** (Table S1). Unlike **28a**, compound **33g** showed highly selective Axl inhibition. These results suggest that ureidopyrimidine derivatives may be highly selective Axl inhibitors. Further studies are needed to clarify the precise mechanism of ureidopyrimidine action.

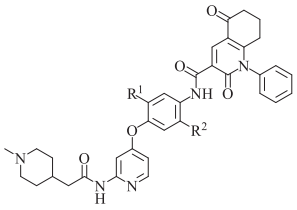
Table 1
Effect of substitution of R^1-R^4 on *in vitro* biological activity, solubility and microsomal stability of pyridine derivatives containing uracil moiety.



Entry	R^3	R^1	R^2	R^4	Cell free IC_{50} (nM)		Ba/F3 IC_{50} (nM)		PBS[*1] (μM)	Microsomal stability in mouse [*2] (residual, %)
					Axl	Mer	Axl	Mer		
20a		H	H		11	18	90	68	32	19
20b		H	H		0.65	8.0	5.9	9.8	80	81
20c		H	F		0.81	6.8	7.6	8.1	78	76
20d		F	H		2.5	22	23	44	82	78
20e		F	H		1.1	16	2.8	5.0	70	63

[*1] Solubility in Dulbecco's phosphate-buffered saline.
[*2] Microsomal stability in mouse liver microsomes. The ratio of peak area responses relative to the internal standard was determined, and the residual ratio of the test articles in the presence of NADPH relative to that in the absence of NADPH was calculated.

Table 2
Effect of substitution of R^1-R^2 on *in vitro* biological activity, solubility and microsomal stability of pyridine derivatives containing hexahydroquinoline-2,5-dione moiety.

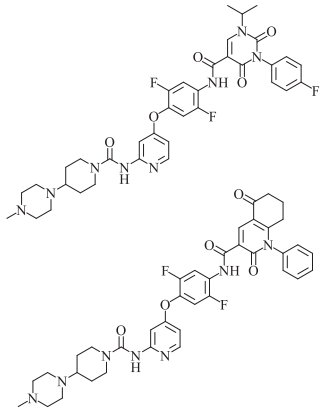


Entry	R^1	R^2	Cell free IC_{50} (nM)		PBS (μM)	Microsomal stability mouse residual (%)
			Axl	Mer		
25a	H	H	6.8	11	77	77
25b	F	H	6.9	14	75	71
25c	H	F	4.2	15	73	75
25d	F	F	2.3	10	50	72

4. Conclusions

We designed a novel series of pyridine derivatives as Axl/Mer inhibitors. Compound **28a** at a dose of 100 mg/kg showed retinal toxicity in mouse. However, subsequent derivatization led to the discovery of pyrimidine derivative **33g**, which at a dose of 100 mg/kg selectively inhibited the activity of Axl over Mer without retinal toxicity in mouse. Additionally, **33g** displayed in vivo anti-tumor effects without influencing body weight in the Ba/F3-Axl isogenic subcutaneous model.

Table 3
In vitro profile of biological activity, solubility and microsomal stability of ureidopyridine derivatives.

Entry		cell free IC_{50} (nM)		Ba/F3 IC_{50} (nM)		PBS (μM)	Microsomal stability mouse residual (%)
		Axl	Mer	Axl	Mer		
28a		0.76	36	1.9	3.2	76	86
28b		1.3	25	16	12	43	78

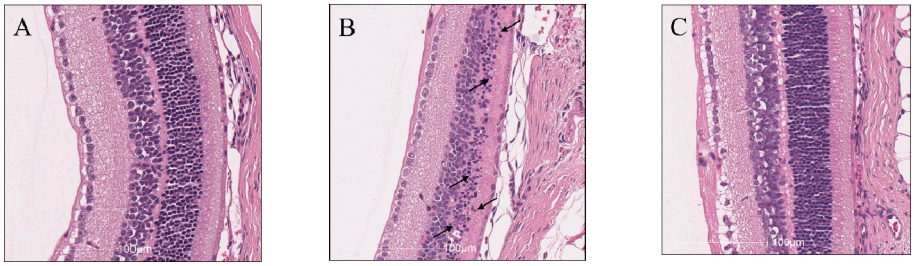


Fig. 2. Histological assessment of the retina. (A) Normal retina in control (untreated) mice, (B) Retina in mice treated with **28a** at 100 mg/kg. Degeneration/necrosis of photoreceptor cells in the outer granular layer (arrows), resulting in thinning of the retina, was observed, (C) Retina in mice treated with **33g** at 100 mg/kg. No histopathologic changes were detected. All slides were stained with hematoxylin-eosin (HE) stained slides. Scale bars, 100 μm. Images are representative of sections from 3 or 5 mice.

5. Experimental sections

5.1. Chemistry

5.1.1. General methods

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a JEOL spectrometer (operating at 500 MHz for ^{1}H and 126 MHz for ^{13}C or 400 MHz for ^{1}H and 101 MHz for ^{13}C). Chemical shifts were expressed in ppm (δ) from the residual $CHCl_3$ signal at δ_H 7.26 ppm and δ_C 77.0 ppm in $CDCl_3$ or the residual CHD_2SOCD_3 signal at δ_H 2.50 ppm and δ_C 39.5 ppm in CD_3SOCD_3 (s = singlet, d = doublet, t = triplet, q = qualtet, quin = quintet, sep = septet, m = multiplet, and br = broad). Coupling constants (*J*) are given in Hertz (Hz). High-resolution mass spectra (HRMS) were recorded on a Thermo Fisher LTQ Orbitrap XL spectrometer using electrospray ionization (ESI) and a Waters GCT Premier using electron ionization (EI). Column chromatography was carried out using silica gel 60 (spherical) (40–50 μm, Kanto Chemical Co., Inc.), Chromotorex (200–350 mesh, Fuji Silysia Chemical Ltd.), Isolera™ One (silica gel and NH-silica gel, Biotage) and a Hi-Flash™ column (silica gel and NH-silica gel, Yamazen Corporation). Chemicals and solvents used in the study were commercially available.

5.1.2. Diethyl 2-((cyclopropylamino)methylene)malonate (2)

To a solution of diethyl ethoxymethylenemalonate (**1**) (1.00 g, 4.63 mmol) in EtOH (20 mL) was added cyclopropylamine (290 mg, 5.09 mmol) in EtOH (2 mL) at room temperature. The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated and the residue was purified with column chromatography on NH silica gel (*n*-heptane/EtOAc = 9/1 ~ 1/1) to afford the title compound **2** as a colorless oil (1.03 g, 4.53 mmol, 98%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.71–0.72 (m, 2H), 0.79–0.81 (m, 2H), 1.28 (t, *J* = 7.0 Hz, 3H), 1.32 (t, *J* = 7.0 Hz, 3H), 2.84 (qd, *J* = 6.7, 3.7 Hz, 1H), 4.19 (dq, *J* = 18.5, 7.3 Hz, 4H), 8.09 (d, *J* = 14.1 Hz, 1H), 9.21 (br d, *J* = 12.2 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 6.6, 14.4, 14.5, 29.6, 59.6, 59.9, 90.5, 160.3, 166.0, 169.2; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{11}H_{18}NO_4^+$: 228.1230, found: 228.1226.

5.1.3. Diethyl 2-((3-(4-fluorophenyl)ureido)methylene)malonate (4)

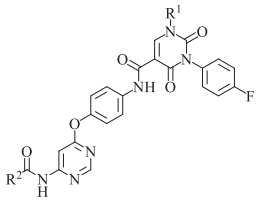
To a solution of 1,3-diethyl 2-(aminomethylidene)propanedioate[23] (**3**) (11.3 g, 60.3 mmol) in 1,2-dichloroethane (50 mL) were added 4-fluorophenyl isocyanate (6.86 mL, 60.3 mmol) and N,*N*-diisopropylethylamine (11.1 mL, 63.3 mmol) at room temperature. The reaction mixture was refluxed for 21 h. The mixture was cooled to room temperature, and the precipitate was collected by filtration, rinsed with TBME. The collected precipitate was purified with column chromatography on silica gel (*n*-heptane/ethyl acetate (EtOAc) = 1/1 ~ EtOAc) to afford the title compound **4** as a white solid (8.30 g, 25.6 mmol, 42.4%).

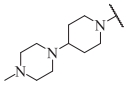
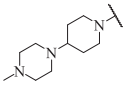
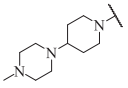
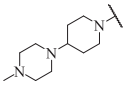
^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.10–1.33 (m, 6H), 4.11 (q, *J* = 7.3 Hz, 2H), 4.20 (q, *J* = 6.7 Hz, 2H), 7.15 (t, *J* = 8.9 Hz, 2H), 7.47 (dd, *J* = 8.6, 4.9 Hz, 2H), 8.33–8.53 (m, 1H), 10.26–10.44 (m, 1H), 10.46–10.72 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 14.6, 14.7, 60.5, 60.8, 98.4, 116.2 (d, J_{CF} = 22.7 Hz), 121.1 (d, J_{CF} = 7.6 Hz), 135.1, 148.3, 150.5, 158.7 (d, J_{CF} = 239.4 Hz), 164.8, 166.9; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{15}H_{18}FN_2O_5^+$: 325.1194, found: 325.1185.

5.1.4. Ethyl 3-(4-fluorophenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (5)

To a solution of diethyl 2-((3-(4-fluorophenyl)ureido)methylene) malonate (**4**) (8.27 g, 25.5 mmol) in EtOH (100 mL) was added 20% NaOEt in EtOH solution (19.7 mL, 51.0 mmol) at 0 °C and stirred at room temperature for 2 h. The reaction mixture was concentrated. The residue was poured into saturated aqueous NH_4Cl and extracted with DCM/*i*-PrOH = 10/1 solution, 5 times. The organic extract was dried over $MgSO_4$, filtered and then concentrated. The residue was purified

Table 4
Effect of substitution of R^1-R^2 on *in vitro* biological activity, solubility and microsomal stability pyrimidine derivatives containing uracil moiety.



Entry	R^1	R^2	cell free IC_{50} (nM)		Ba/F3 IC_{50} (nM)		PBS (μM)	Microsomal stability mouse residual (%)
			Axl	Mer	Axl	Mer		
33a			0.85	<4.1	9.0	13	6	83
33b			2.0	13	11	66	36	79
33c			2.1	28	15	86	1	59
33d			3.5	25	20	113	83	92
33e			6.4	64	40	150	86	0.30
33f			5.6	79	49	157	80	1.3
33g			2.0	38	15	61	89	88
33h	Me		32	157	173	321	90	99
33i			7.1	234	51	304	89	87
33j			1.5	11	6.3	27	80	82

with column chromatography on silica gel (*n*-heptane/EtOAc = 1/1 ~ EtOAc ~ EtOAc/MeOH = 9/1) to afford the title compound **5** as a white solid (6.15 g, 22.1 mmol, 87%).

^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.19$ (t, $J = 7.1$ Hz, 3H), 4.14 (q, $J = 6.9$ Hz, 2H), 7.13–7.36 (m, 4H), 8.21 (s, 1H), 11.76–12.15 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): $\delta = 14.7$, 60.6, 103.7, 116.2 (d, $J_{CF} = 22.7$ Hz), 131.5 (d, $J_{CF} = 8.8$ Hz), 132.0, 149.0, 151.3, 159.8, 162.1 (d, $J_{CF} = 244.4$ Hz), 163.2; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{13}H_{12}FN_2O_4^+$: 279.0776, found: 279.0768.

5.1.5. Ethyl 3-(4-fluorophenyl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6a)

To a solution of ethyl 3-(4-fluorophenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**5**) (300 mg, 1.08 mmol) in DMF (6 mL) were added iodomethane (134 μL, 2.16 mmol) and K_2CO_3 (298 mg, 2.16 mmol) at room temperature. The reaction mixture was stirred at 70 °C overnight, then cooled to room temperature. The reaction mixture was poured into water and extracted with EtOAc. The organic extract was washed with water and brine, dried over $MgSO_4$, filtered and then concentrated. The residue was purified with column chromatography on silica gel (*n*-heptane/EtOAc = 7/3 ~ EtOAc) to afford the title

Table 5
Pharmacokinetic parameters* of **33g** in mice.

Parameter		Dose (mg/kg)		
		10	30	100
C_{max}	(ng/mL)	1580	3790	13,500
t_{max}	(h)	2	2	2
$AUC_{(0-24h)}$	(ng·h/mL)	7290	18,100	72,700

* After a single oral administration of **33g** at 10, 30 and 100 mg/kg to female mice, blood was collected from retinal vein at 0.5, 1, 2, 8, and 24 h postdose and centrifuged to obtain plasma samples. Plasma concentrations of compound **33g** were measured using liquid chromatography-tandem mass spectrometry.

compound **6a** as a white solid (298 mg, 1.02 mmol, 95%).

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.31–1.37$ (m, 3H), 3.53 (s, 3H), 4.33 (q, $J = 6.9$ Hz, 2H), 7.16 (d, $J = 6.1$ Hz, 4H), 8.30 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 13.9$, 37.5, 61.1, 104.5, 116.1 (d, $J_{CF} = 22.7$ Hz), 129.8 (d, $J_{CF} = 7.6$ Hz), 130.2, 150.7, 150.9, 158.8, 162.2 (d, $J_{CF} = 249.5$ Hz), 162.8; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{14}H_{14}FN_2O_4^+$: 293.0932, found: 293.0925.

5.1.6. *Ethyl 3-(4-fluorophenyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6b)*

The title compound was prepared from **5** using a method analogous to that described for **6a** in 87% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.35$ (td, $J = 7.2$, 1.5 Hz, 3H), 1.43 (dd, $J = 6.7$, 1.2 Hz, 6H), 4.34 (qd, $J = 7.1$, 1.2 Hz, 2H), 4.84–4.97 (m, 1H), 7.10–7.21 (m, 4H), 8.34 (d, $J = 1.2$ Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 14.3$, 21.6, 49.9, 61.6, 105.4, 116.5 (d, $J_{CF} = 23.9$ Hz), 130.2 (d, $J_{CF} = 8.8$ Hz), 130.9, 146.8, 150.9, 158.7, 162.6 (d, $J_{CF} = 248.2$ Hz), 163.7; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{16}H_{18}FN_2O_4^+$: 321.1245, found: 321.1235.

5.1.7. *Ethyl 1-cyclopentyl-3-(4-fluorophenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6c)*

The title compound was prepared from **5** using a method analogous to that described for **6a** in 74.2% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.34$ (td, $J = 7.0$, 2.5 Hz, 3H), 1.54 (d, $J = 2.5$ Hz, 2H), 1.74 (br s, 2H), 1.89 (br s, 2H), 2.19 (br s, 2H), 4.33 (qd, $J = 7.0$, 2.1 Hz, 2H), 4.85–4.99 (m, 1H), 7.11–7.21 (m, 4H), 8.35 (d, $J = 2.5$ Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 14.3$, 24.1, 31.8, 59.4, 61.6, 105.3, 116.5 (d, $J_{CF} = 23.9$ Hz), 130.2 (d, $J_{CF} = 8.8$ Hz), 130.9 (d, $J_{CF} = 2.5$ Hz), 147.7, 151.2, 158.8, 162.6 (d, $J_{CF} = 248.2$ Hz), 163.7; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{18}H_{20}FN_2O_4^+$: 347.1402, found: 347.1391.

5.1.8. *Ethyl 1-cyclopropyl-3-(4-fluorophenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6d)*

To a solution of diethyl 2-((cyclopropylamino)methylene)malonate (**2**) (976 mg, 4.30 mmol) in DMF (20 mL) were added 4-fluorophenyl isocyanate (928 μL, 8.16 mmol) and K_2CO_3 (1.13 g, 8.16 mmol) at room temperature. The reaction mixture was stirred at 60 °C overnight under nitrogen, then cooled to room temperature. The reaction mixture was poured into water and extracted with EtOAc. The organic extract was washed with water and brine, dried over $MgSO_4$, filtered and then concentrated. The residue was purified with column chromatography on silica gel (*n*-heptane/EtOAc = 7/3 ~ EtOAc) to afford the title compound **6d** as a white solid (879 mg, 2.76 mmol, 64.3%).

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.96–0.98$ (m, 2H), 1.11–1.18 (m, 2H), 1.34 (t, $J = 7.0$ Hz, 3H), 3.24 (tt, $J = 7.3$, 3.7 Hz, 1H), 4.33 (q, $J = 6.9$ Hz, 2H), 7.14–7.16 (m, 4H), 8.33 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 7.2$, 14.3, 33.0, 61.6, 105.0, 116.5 (d, $J_{CF} = 25.2$ Hz), 130.2 (d, $J_{CF} = 10.1$ Hz), 130.5, 150.6, 151.5, 158.9, 162.6 (d, $J_{CF} = 249.5$ Hz), 163.3; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{16}H_{16}FN_2O_4^+$: 319.1089, found: 319.1079.

5.1.9. *3-(4-fluorophenyl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid (7a)*

4 M HCl in 1,4-dioxane (4.00 mL, 16.0 mmol) and water (2.00 mL) were added to ethyl 3-(4-fluorophenyl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**6a**) (290 mg, 992 μmol), and the reaction mixture was stirred at 80 °C for 29 h. The reaction mixture was cooled to room temperature, then poured into water. The reaction mixture was extracted with DCM, the organic extract was dried over $MgSO_4$, filtered and then concentrated. The residue was purified with column chromatography on silica gel (*n*-heptane/EtOAc = 1/1 ~ EtOAc ~ EtOAc/MeOH = 9/1) to afford the title compound **7a** as a white solid (245 mg, 0.927 mmol, 93%).

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 3.61$ (s, 3H), 7.16–7.25 (m, 4H), 8.53 (s, 1H), 12.26 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 38.6$, 102.0, 117.0 (d, $J_{CF} = 22.7$ Hz), 129.0 (d, $J_{CF} = 3.8$ Hz), 129.8 (d, $J_{CF} = 8.8$ Hz), 150.2, 152.3, 163.0 (d, $J_{CF} = 250.7$ Hz), 163.0, 165.4; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{12}H_{10}FN_2O_4^+$: 265.0619, found: 265.0614.

5.1.10. *3-(4-fluorophenyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid (7b)*

4 M HCl in 1,4-dioxane (30 mL, 120 mmol) and water (10 mL) were added to ethyl 3-(4-fluorophenyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**6b**) (4.97 g, 15.5 mmol), and the reaction mixture was stirred at 70 °C for 45 h. The reaction mixture was cooled to room temperature, then concentrated. The residue was poured into water and the precipitate was collected by filtration, rinsed with water and dried to afford the title compound **7b** as a white solid (3.81 g, 13.0 mmol, 84%).

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.47$ (d, $J = 6.7$ Hz, 6H), 4.94 (spt, $J = 6.8$ Hz, 1H), 7.22 (d, $J = 6.7$ Hz, 4H), 8.57 (s, 1H), 12.34 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 21.6$, 51.2, 102.1, 116.9 (d, $J_{CF} = 23.9$ Hz), 129.4, 129.9 (d, $J_{CF} = 10.1$ Hz), 148.1, 150.0, 163.0 (d, $J_{CF} = 250.7$ Hz),

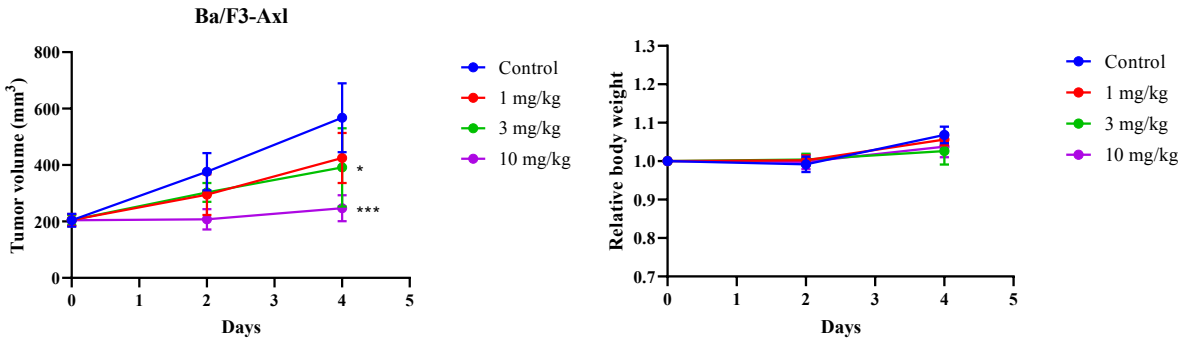


Fig. 3. Anti-tumor effect of **33g** in the Ba/F3-Axl isograft model. Mice were orally administered **33g** at the indicated dose once daily for 4 days. Data are means ± SD (n = 5). Tumor volumes were compared between the control (non-treated) and **33g**-treated group at the last time point. *$P < 0.05$; ***$P < 0.001$ (Dunnett's Multiple Comparison vs a Control).

163.3, 165.0; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{14}H_{14}FN_2O_4^+$: 293.0932, found: 293.0924.

5.1.11. 1-cyclopentyl-3-(4-fluorophenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid (7c)

The title compound was prepared from **6c** using a method analogous to that described for **7a** in 80% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.69–1.83 (m, 4H), 1.89–1.93 (m, 2H), 2.19–2.24 (m, 2H), 4.94 (quin, J = 8.0 Hz, 1H), 7.22 (d, J = 6.1 Hz, 4H), 8.56 (s, 1H), 12.35 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 24.0, 31.9, 60.3, 102.0, 116.9 (d, J_{CF} = 22.7 Hz), 129.4, 129.9, 148.8, 150.3, 163.0 (d, J_{CF} = 250.7 Hz), 163.3, 165.0; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{16}H_{16}FN_2O_4^+$: 319.1089, found: 319.1080.

5.1.12. 1-cyclopropyl-3-(4-fluorophenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid (7d)

The title compound was prepared from **6d** using a method analogous to that described for **7b** in 79% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.00–1.03 (m, 2H), 1.18–1.21 (m, 2H), 3.27–3.36 (m, 1H), 7.20–7.23 (m, 4H), 8.57 (s, 1H), 12.29 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 7.2, 33.7, 101.7, 116.9 (d, J_{CF} = 22.7 Hz), 129.1, 129.9 (d, J_{CF} = 10.1 Hz), 150.6, 151.7, 163.0 (d, J_{CF} = 249.5 Hz), 163.0, 165.2; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{14}H_{12}FN_2O_4^+$: 291.0776, found: 291.0769.

5.1.13. 4-(4-amino-3-fluorophenoxy)picolinamide (10a)

To a solution of 4-amino-3-fluorophenol (2.27 g, 17.9 mmol) in DMSO (20 mL) was added *t*-BuOK (2.15 g, 19.2 mmol) at room temperature, followed by stirring for 15 min. To the reaction mixture was added 4-chloropicolylamide (**8**) (2.00 g, 12.8 mmol) at room temperature, and stirred at 80 °C for 50 min. Then the reaction mixture was cooled to room temperature, poured into 1 N NaOH and extracted with EtOAc. The organic extract was washed with water and brine, dried over $MgSO_4$, filtered and then concentrated. The residue was purified with column chromatography on silica gel (*n*-heptane/EtOAc = 1/1 ~ EtOAc) and then NH silica gel (*n*-heptane/EtOAc = 1/1 ~ EtOAc ~ EtOAc/MeOH = 9/1) to afford the title compound **10a** as a white solid (1.87 g, 7.56 mmol, 59.1%).

^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.18 (s, 2H), 6.73–6.76 (m, 1H), 6.79–6.86 (m, 1H), 6.94–7.01 (m, 1H), 7.07 (dd, J = 5.5, 2.5 Hz, 1H), 7.33 (d, J = 3.1 Hz, 1H), 7.64–7.66 (m, 1H), 8.06 (br s, 1H), 8.43 (d, J = 5.5 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 109.1, 109.6 (d, J_{CF} = 21.4 Hz), 114.4, 117.0 (d, J_{CF} = 5.0 Hz), 117.9 (d, J_{CF} = 2.5 Hz), 135.3 (d, J_{CF} = 11.3 Hz), 142.7 (d, J_{CF} = 7.6 Hz), 150.7 (d, J_{CF} = 239.4 Hz), 150.8, 153.1, 166.0, 166.9; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{12}H_{11}FN_3O_2^+$: 248.0830, found: 248.0823.

5.1.14. 4-(4-amino-2,5-difluorophenoxy)picolinamide (10b)

The title compound was prepared from **8** and **9b** using a method analogous to that described for **10a** in 52.5% as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.53 (s, 2H), 6.72 (dd, J = 12.2, 8.6 Hz, 1H), 7.13 (dd, J = 5.5, 2.5 Hz, 1H), 7.21 (dd, J = 11.0, 7.3 Hz, 1H), 7.33 (d, J = 3.1 Hz, 1H), 7.68 (br s, 1H), 8.08 (br s, 1H), 8.47 (d, J = 5.5 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 103.4 (dd, J_{CF} = 23.5, 5.4 Hz), 108.4, 111.3 (d, J_{CF} = 22.7 Hz), 113.9, 127.8 (dd, J_{CF} = 14.5, 10.9 Hz), 136.7 (dd, J_{CF} = 15.7, 10.9 Hz), 146.3 (d, J_{CF} = 238.1 Hz), 151.02, 151.1 (d, J_{CF} = 240.7 Hz), 153.3, 165.9, 166.3; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{12}H_{10}F_2N_3O_2^+$: 266.0736, found: 266.0726.

5.1.15. Benzyl (4-((2-carbamoylpyridin-4-yl)oxy)-2-fluorophenyl) carbamate (11a)

To a solution of 4-(4-amino-3-fluorophenoxy)picolinamide (**10a**) (846 mg, 3.42 mmol) in acetone (20 mL) and H_2O (10 mL) was added benzyl chloroformate (733 μL, 5.13 mmol) at 0 °C, and stirred at room temperature for 20 h. The reaction mixture was poured into brine and extracted with EtOAc. The organic extract was washed with brine, dried over $MgSO_4$, filtered and then concentrated. To the residue was added TBME/EtOAc = 10/1 solution, and the precipitate was collected by filtration, rinsed with TBME to afford the title compound **11a** as a white solid (1.01 g, 2.65 mmol, 78%).

^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.14 (s, 2H), 7.03 (dd, J = 8.6, 1.8 Hz, 1H), 7.16 (dd, J = 5.5, 2.5 Hz, 1H), 7.23–7.28 (m, 1H), 7.29–7.33 (m, 1H), 7.34–7.42 (m, 5H), 7.62–7.77 (m, 2H), 8.08–8.09 (m, 1H), 8.49 (d, J = 5.5 Hz, 1H), 9.53 (br s, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 66.7, 109.7, 109.8 (d, J_{CF} = 21.4 Hz), 115.0, 117.5 (d, J_{CF} = 3.8 Hz), 124.4, 124.5, 126.2, 128.6 (d, J_{CF} = 7.6 Hz), 129.0, 137.0, 150.3, 151.1, 153.3, 154.5, 155.1 (d, J_{CF} = 247.0 Hz), 165.8, 165.9; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{20}H_{17}FN_3O_4^+$: 382.1198, found: 382.1183.

5.1.16. Benzyl (4-((2-carbamoylpyridin-4-yl)oxy)-2,5-difluorophenyl) carbamate (11b)

To a solution of 4-(4-amino-2,5-difluorophenoxy)picolinamide (583 mg, 2.20 mmol) in acetone (20 mL) and H_2O (5 mL) was added benzyl chloroformate (471 μL, 3.30 mmol) at room temperature, and stirred at room temperature for 31 h. The reaction mixture was poured into brine and extracted with EtOAc. The organic extract was washed with brine, dried over $MgSO_4$, filtered and then concentrated. The residue was purified with column chromatography on silica gel (*n*-heptane/EtOAc = 4/1 ~ 1/4) to afford the title compound **11b** as a white solid (684 mg, 1.71 mmol, 78%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.23 (s, 2H), 5.62 (br s, 1H), 6.93–6.99 (m, 2H), 7.00 (dd, J = 5.5, 2.5 Hz, 1H), 7.35–7.42 (m, 5H), 7.64 (d, J = 2.5 Hz, 1H), 7.81 (br s, 1H), 8.15 (br s, 1H), 8.43 (d, J = 5.5 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 66.9, 108.8, 111.8, 112.0 (d, J_{CF} = 23.9 Hz), 114.1, 125.9 (dd, J_{CF} = 13.9, 8.8 Hz), 128.58, 128.64, 129.0, 135.6 (dd, J_{CF} = 15.1, 10.8 Hz), 136.8, 150.0 (d, J_{CF} = 247.0 Hz), 150.2 (d, J_{CF} = 245.7 Hz), 151.3, 153.5, 154.2, 165.3, 165.8; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{20}H_{16}F_2N_3O_4^+$: 400.1103, found: 400.1088.

5.1.17. Benzyl (4-((2-aminopyridin-4-yl)oxy)-2-fluorophenyl)carbamate (12a)

To a solution of benzyl (4-((2-carbamoylpyridin-4-yl)oxy)-2-fluorophenyl)carbamate (**11a**) (1.01 g, 2.65 mmol) in DMF (20 mL), pyridine (859 μL, 10.6 mmol) and H_2O (239 μL, 13.3 mmol) was added [bis(trifluoroacetoxy)iodo]benzene (2.28 g, 5.31 mmol) at room temperature. The reaction mixture was stirred at room temperature for 6 h. The reaction mixture was poured into 1 N NaOH and H_2O and extracted with EtOAc. The organic extract was washed with H_2O and brine, dried over $MgSO_4$, filtered and then concentrated. The residue was purified with column chromatography on silica gel (*n*-heptane/EtOAc = 1/1 ~ EtOAc) to afford the title compound **12a** as a white solid (557 mg, 1.58 mmol, 59.4%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.40 (br s, 2H), 5.22 (s, 2H), 5.93 (s, 1H), 6.26 (dd, J = 6.1, 1.8 Hz, 1H), 6.77–6.94 (m, 3H), 7.30–7.48 (m, 5H), 7.93 (d, J = 5.5 Hz, 1H), 8.10 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 67.6, 95.5, 104.2, 108.6 (d, J_{CF} = 21.4 Hz), 117.0 (d, J_{CF} = 3.8 Hz), 121.5, 123.5, 123.6, 128.5, 128.6, 128.8, 135.8, 149.9, 152.6 (d, J_{CF} = 245.7 Hz), 153.3, 160.4, 166.1; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{19}H_{17}FN_3O_3^+$: 354.1248, found: 354.1234.

5.1.18. Benzyl (4-((2-aminopyridin-4-yl)oxy)-2,5-difluorophenyl) carbamate (12b)

The title compound was prepared from **11b** using a method analogous to that described for **12a** in 85% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.45 (br s, 2H), 5.22 (s, 2H), 5.91 (s, 1H), 6.24 (d, J = 5.5 Hz, 1H), 6.92 (dd, J = 10.4, 6.7 Hz, 1H), 6.98 (br s, 1H), 7.31–7.47 (m, 5H), 7.93 (d, J = 6.1 Hz, 1H), 8.09 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 67.8, 94.5, 103.2, 108.8, 109.0, 110.3 (d, J_{CF} = 23.9 Hz), 124.6 (dd, J_{CF} = 22.7, 11.3 Hz), 128.6, 128.8, 128.8, 135.5, 147.5 (d, J_{CF} = 241.9 Hz), 149.9, 150.9 (d, J_{CF} = 248.2 Hz), 152.9, 160.3, 165.8; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{19}H_{16}F_2N_3O_3^+$:

372.1154, found: 372.1138.

5.1.19. tert-butyl 4-(2-((4-(4-(((benzyloxy)carbonyl)amino)-3-fluorophenoxy)pyridin-2-yl)amino)-2-oxoethyl)piperidine-1-carboxylate (13a)

To a solution of benzotriazole (927 mg, 7.78 mmol) in DCM (10 mL) was added thionyl chloride (565 μL, 7.78 mmol) at room temperature. The reaction mixture was stirred at room temperature for 5 min. To the reaction mixture was added 1-Boc-piperidin-4-ylacetic acid (1.33 g, 5.45 mmol) at room temperature, and stirred at room temperature for 30 min. The precipitate was filtered off through Celite and washed with small amount of DCM. The filtrate was added dropwise to a solution of benzyl (4-((2-aminopyridin-4-yl)oxy)-2-fluorophenyl)carbamate (**12a**) (550 mg, 1.56 mmol) in THF (10 mL) at 0 °C, then *i*Pr$_2$NEt (4.08 mL, 23.3 mmol) was at 0 °C. The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was poured into H_2O and extracted with EtOAc. The organic extract was washed with brine, dried over $MgSO_4$, filtered and then concentrated. The residue was purified with column chromatography on NH silica gel (*n*-heptane/EtOAc = 1/1 ~ EtOAc) to afford the title compound **13a** as a white solid (824 mg, 1.42 mmol, 91%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.11–1.22 (m, 2H), 1.44 (s, 9H), 1.71 (d, J = 12.8 Hz, 2H), 2.02 (ttt, J = 11.2, 7.4, 3.7 Hz, 1H), 2.26 (d, J = 6.7 Hz, 2H), 2.70 (br s, 2H), 3.94–4.21 (m, 2H), 5.22 (s, 2H), 6.58 (dd, J = 5.8, 2.1 Hz, 1H), 6.84–6.91 (m, 3H), 7.31–7.44 (m, 5H), 7.80 (s, 1H), 7.94 (s, 1H), 8.09 (d, J = 6.1 Hz, 1H), 8.14 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 28.5, 31.9, 33.3, 44.0, 44.4, 67.5, 79.5, 102.4, 108.5 (d, J_{CF} = 21.4 Hz), 108.7, 116.9, 121.6, 123.9, 124.0, 128.4, 128.6, 128.7, 135.8, 149.0, 149.5, 152.5 (d, J_{CF} = 211.7 Hz), 153.5, 154.9, 166.4, 170.7; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{31}H_{36}FN_4O_6^+$: 579.2613, found: 579.2589.

5.1.20. tert-butyl 4-(2-((4-(4-(((benzyloxy)carbonyl)amino)-2,5-difluorophenoxy)pyridin-2-yl)amino)-2-oxoethyl)piperidine-1-carboxylate (13b)

The title compound was prepared from **12b** using a method analogous to that described for **13a** in 56.7% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.17 (dd, J = 11.9, 4.0 Hz, 2H), 1.44 (s, 9H), 1.71 (d, J = 12.8 Hz, 2H), 1.95–2.08 (m, 1H), 2.25 (d, J = 7.3 Hz, 2H), 2.70 (br s, 2H), 4.07 (br s, 2H), 5.23 (s, 2H), 6.59 (dd, J = 5.8, 2.1 Hz, 1H), 6.92–6.96 (m, 2H), 7.35–7.42 (m, 5H), 7.78 (s, 1H), 7.85 (s, 1H), 8.07–8.20 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 28.5, 31.9, 33.3, 43.8, 44.3, 67.7, 79.4, 101.4, 107.9, 109.1, 110.3 (d, J_{CF} = 23.9 Hz), 125.1 (dd, J_{CF} = 20.2, 10.1 Hz), 128.4, 128.6, 128.7, 135.1, 135.6, 147.7 (d, J_{CF} = 244.4 Hz), 149.0, 150.7 (d, J_{CF} = 245.7 Hz), 153.0, 153.6, 154.9, 166.0, 170.9; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{31}H_{35}F_2N_4O_6^+$: 597.2519, found: 597.2496.

5.1.21. Benzyl (2-fluoro-4-((2-(2-(1-methylpiperidin-4-yl)acetamido)pyridin-4-yl)oxy)phenyl)carbamate (14a)

To a solution of *tert*-butyl 4-(2-((4-(4-(((benzyloxy)carbonyl)amino)-3-fluorophenoxy)pyridin-2-yl)amino)-2-oxoethyl)piperidine-1-carboxylate (**13a**) (803 mg, 1.39 mmol) in DCM (5 mL) was added TFA (5 mL) at room temperature. The reaction mixture was stirred at room temperature for 20 min and then concentrated. To a solution of the residue in THF (10 mL) were added 37% formaldehyde (205 μL, 2.78 mmol) and sodium triacetoxyborohydride (441 mg, 2.08 mmol) at room temperature. The reaction mixture was stirred at room temperature for 40 min, poured into saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic extract was washed with brine, dried over $MgSO_4$, filtered and then concentrated. The residue was purified with column chromatography on NH silica gel (*n*-heptane/EtOAc = 3/7 ~ EtOAc ~ EtOAc/MeOH = 9/1) to afford the title compound **14a** as a white solid (560 mg, 1.14 mmol, 82%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.33 (qd, J = 12.2, 3.7 Hz, 2H), 1.74 (d, J = 12.8 Hz, 2H), 1.79–1.89 (m, 1H), 1.93 (td, J = 11.8, 2.1 Hz, 2H), 2.24–2.26 (m, 5H), 2.81 (d, J = 11.6 Hz, 2H), 5.22 (s, 2H), 6.57 (dd, J = 5.8, 2.1 Hz, 1H), 6.84–6.90 (m, 3H), 7.31–7.45 (m, 5H), 7.80 (d, J = 1.8 Hz, 1H), 7.85 (br s, 1H), 8.09 (d, J = 5.5 Hz, 1H), 8.14 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 32.1, 32.7, 44.4, 46.4, 55.6, 67.4, 102.6, 108.5, 108.6, 116.8, 121.9, 123.9, 124.0, 128.4, 128.5, 128.7, 135.9, 148.9, 149.5, 152.7 (d, J_{CF} = 215.5 Hz), 153.8, 166.4, 171.3; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{27}H_{30}FN_4O_4^+$: 493.2246, found: 493.2230.

5.1.22. Benzyl (2,5-difluoro-4-((2-(2-(1-methylpiperidin-4-yl)acetamido)pyridin-4-yl)oxy)phenyl)carbamate (14b)

The title compound was prepared from **13b** using a method analogous to that described for **14a** in 74.4% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.27–1.39 (m, 2H), 1.71–1.75 (m, 2H), 1.78–1.88 (m, 1H) 1.92 (td,

J = 11.8, 2.1 Hz, 2H), 2.23–2.26 (m, 5H), 2.81 (d, J = 11.6 Hz, 2H), 5.23 (s, 2H), 6.58 (dd, J = 5.5, 2.5 Hz, 1H), 6.90–7.00 (m, 2H), 7.32–7.44 (m, 5H), 7.79 (d, J = 1.8 Hz, 1H), 8.01 (s, 1H), 8.10 (d, J = 6.1 Hz, 1H), 8.13 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 32.2, 32.7, 44.6, 46.4, 55.6, 67.7, 101.3, 107.9, 109.1 (d, J_{CF} = 25.2 Hz), 110.3 (d, J_{CF} = 23.9 Hz), 125.01 (d, J_{CF} = 10.1 Hz), 128.5, 128.7, 128.8, 135.2, 135.6, 147.6 (d, J_{CF} = 241.9 Hz), 149.0, 150.7 (d, J_{CF} = 245.7 Hz), 153.0, 153.62, 166.0, 171.2; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{27}H_{29}F_2N_4O_4^+$: 511.2151, found: 511.2133.

5.1.23. 4-(4-nitrophenoxy)pyridin-2-amine (17a)

To a solution of 2-amino-4-chloropyridine (**15**) (4.00 g, 31.1 mmol) in NMP (40.0 mL) were added *p*-nitrophenol (**16a**) (8.66 g, 62.2 mmol) and *i*Pr$_2$NEt (21.7 mL, 124 mmol) at room temperature. The reaction mixture was stirred at 150 °C for 137 h under nigrogen, then cooled to room temperature. The reaction mixture was poured into water and extracted with EtOAc. The organic extract was washed with water and brine, dried over $MgSO_4$, filtered and then concentrated. The residue was purified with column chromatography on silica gel (*n*-heptane/EtOAc = 7/3 ~ EtOAc) and then NH silica gel (*n*-heptane/EtOAc = 7/3 ~ EtOAc) to afford the title compound **17a** as a slightly yellow solid (3.50 g, 15.2 mmol, 48.7%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.51 (br s, 2H), 6.08–6.09 (m, 1H), 6.33 (dd, J = 6.4, 1.5 Hz, 1H), 7.15 (d, J = 9.2 Hz, 2H), 8.03 (d, J = 5.5 Hz, 1H), 8.25 (d, J = 9.2 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 97.5, 105.3, 119.6, 126.1, 144.1, 150.6, 160.5, 160.6, 164.2; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{11}H_{10}N_3O_3^+$: 232.0717, found: 232.0711.

5.1.24. 4-(2-fluoro-4-nitrophenoxy)pyridin-2-amine (17b)

The title compound was prepared from **15** and **16b** using a method analogous to that described for **17a** in 50.5% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.51 (br s, 2H), 6.04 (d, J = 2.5 Hz, 1H), 6.30 (dd, J = 5.8, 2.1 Hz, 1H), 7.19–7.30 (m, 1H), 8.02 (d, J = 6.11 Hz, 1H), 8.05–8.14 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 96.1, 104.0, 113.6 (d, J_{CF} = 23.9 Hz), 120.7, 122.0, 144.5, 148.0, 150.5, 153.4 (d, J_{CF} = 257.0 Hz), 160.6, 164.2; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{11}H_9FN_3O_3^+$: 250.0622, found: 250.0616.

5.1.25. 2-(dimethylamino)-N-(4-(4-nitrophenoxy)pyridin-2-yl)acetamide (18a)

To a solution of 4-(4-nitrophenoxy)pyridin-2-amine (**17a**) (500 mg, 2.16 mmol) in DMF (10 mL) was added *N*, *N*-dimethylglycine (335 mg, 3.24 mmol), HATU (1.23 g, 3.24 mmol) and *i*Pr$_2$NEt (1.13 mL, 6.49 mmol) at room temperature. The reaction mixture was stirred at 80 °C for 18 h, then cooled to room temperature. The reaction mixture was poured into water and extracted with EtOAc. The organic extract was washed with water and brine, dried over MgSO4, filtered and then concentrated. The residue was purified with column chromatography on silica gel (*n*-heptane/EtOAc = 9/1 ~ 2/8) to afford the title compound **18a** as a white solid (403 mg, 1.27 mmol, 58.9%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.36 (s, 6H), 3.07 (s, 2H), 6.71 (dd, J = 5.2, 2.1 Hz, 1H), 7.19 (d, J = 8.6 Hz, 2H), 7.96 (d, J = 2.5 Hz, 1H),

8.25 (d, $J = 6.1$ Hz, 1H), 8.28 (d, $J = 8.6$ Hz, 2H), 9.75 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 46.0, 63.6, 103.4, 110.2, 119.9, 126.2, 144.4, 145.0, 153.3, 160.1, 164.4, 170.0; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{15}H_{17}N_4O_4^+$: 317.1244, found: 317.1236.

5.1.26. tert-butyl 4-(2-((4-(4-nitrophenoxy)pyridin-2-yl)amino)-2-oxoethyl)piperidine-1-carboxylate (18b)

The title compound was prepared from **17a** using a method analogous to that described for **13a** in 81% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.15$–1.26 (m, 2H), 1.44 (s, 9H), 1.72 (d, $J = 12.8$ Hz, 2H), 1.94–2.11 (m, 1H), 2.28 (d, $J = 6.7$ Hz, 2H), 2.70 (br s, 2H), 4.08 (br s, 2H), 6.71 (dd, $J = 5.8$, 2.1 Hz, 1H), 7.19 (d, $J = 9.2$ Hz, 2H), 7.89–7.92 (m, 2H), 8.21 (d, $J = 5.5$ Hz, 1H), 8.28 (d, $J = 9.2$ Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 28.5, 31.9, 33.3, 43.8, 44.4, 79.5, 103.7, 110.2, 120.0, 126.2, 144.4, 149.6, 153.7, 154.9, 159.8, 164.5, 170.8; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{23}H_{29}N_4O_6^+$: 457.2082, found: 457.2068.

5.1.27. tert-butyl 4-(2-((4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)amino)-2-oxoethyl)piperidine-1-carboxylate (18c)

The title compound was prepared from **17b** using a method analogous to that described for **13a** in 55.3% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.17$ (qd, $J = 12.4$, 4.3 Hz, 2H), 1.43 (s, 9H), 1.71 (d, $J = 12.8$ Hz, 2H), 1.95–2.08 (m, 1H), 2.27 (d, $J = 7.3$ Hz, 2H), 2.70 (br s, 2H), 3.95–4.21 (m, 2H), 6.69 (dd, $J = 6.1$, 2.5 Hz, 1H), 7.28–7.34 (m, 1H), 7.86 (d, $J = 2.5$ Hz, 1H), 7.90 (s, 1H), 8.07–8.16 (m, 2H), 8.20 (d, $J = 6.1$ Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 28.5, 31.9, 33.3, 43.7, 44.3, 79.4, 102.3, 108.9, 113.8 (d, $J_{CF} = 22.7$ Hz), 120.9 (d, $J_{CF} = 2.5$ Hz), 122.6, 145.0, 147.1 (d, $J_{CF} = 11.3$ Hz), 149.4, 153.4 (d, $J_{CF} = 255.8$ Hz), 153.8, 154.8, 164.5, 171.0; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{23}H_{28}FN_4O_6^+$: 475.1987, found: 475.1974.

5.1.28. 2-(1-methylpiperidin-4-yl)-N-(4-(4-nitrophenoxy)pyridin-2-yl) acetamide (18d)

The title compound was prepared from **18b** using a method analogous to that described for **14a** in 83% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.34 (qd, $J = 12.2$, 3.7 Hz, 2H), 1.69–1.77 (m, 2H), 1.79–1.88 (m, 1H), 1.93 (td, $J = 11.9$, 2.5 Hz, 2H), 2.24 (s, 3H), 2.27 (d, $J = 6.7$ Hz, 2H), 2.81 (d, $J = 11.6$ Hz, 2H), 6.70 (dd, $J = 5.5$, 2.5 Hz, 1H), 7.16–7.21 (m, 2H), 7.92 (d, $J = 2.5$ Hz, 1H), 7.96 (s, 1H), 8.21 (d, $J = 5.5$ Hz, 1H), 8.26–8.31 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$):δ = 32.2, 32.7, 44.5, 46.4, 55.6, 103.8, 110.1, 112.0, 126.2, 144.4, 149.5, 153.9, 159.8, 164.5, 171.3; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{19}H_{23}N_4O_4^+$: 371.1714, found: 371.1708.

5.1.29. N-(4-(2-fluoro-4-nitrophenoxy)pyridin-2-yl)-2-(1-methylpiperidin-4-yl)acetamide (18e)

The title compound was prepared from **18c** using a method analogous to that described for **14a** in 86% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.33 (qd, $J = 12.2$, 3.7 Hz, 2H), 1.72–1.75 (m, 2H), 1.78–1.88 (m, 1H), 1.93 (td, $J = 11.8$, 2.1 Hz, 2H), 2.24 (s, 3H), 2.27 (d, $J = 6.7$ Hz, 2H), 2.81 (d, $J = 11.6$ Hz, 2H), 6.69 (dd, $J = 5.8$, 2.1 Hz, 1H), 7.27–7.34 (m, 1H), 7.87 (d, $J = 1.8$ Hz, 1H), 8.04 (s, 1H), 8.08–8.15 (m, 2H), 8.19 (d, $J = 5.5$ Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 32.2, 32.7, 44.7, 46.4, 55.6, 102.1, 109.0, 113.8 (d, $J_{CF} = 22.7$ Hz), 120.9 (d, $J_{CF} = 3.8$ Hz), 122.5, 145.0 (d, $J_{CF} = 7.6$ Hz), 147.2 (d, $J_{CF} = 11.3$ Hz), 149.6, 153.5 (d, $J_{CF} = 255.8$ Hz), 153.6, 164.5, 171.1; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{19}H_{22}FN_4O_4^+$: 389.1620, found: 389.1606.

5.1.30. N-(4-(4-aminophenoxy)pyridin-2-yl)-2-(dimethylamino) acetamide (19a)

To a solution of 2-(dimethylamino)-*N*-(4-(4-nitrophenoxy)pyridin-2-yl)acetamide (310 mg, 980 μmol) in MeOH (6 mL) was added 5% palladium on carbon (104 mg, 0.0490 mmol) at room temperature, and the reaction mixture was stirred at room temperature under a hydrogen (balloon) atmosphere for 1.5 h. The reaction mixture was filtered through Celite. The filtrate was concentrated. The residue was purified with column chromatography on NH silica gel (*n*-heptane/EtOAc = 1/1 ~ EtOAc) to afford the title compound **19a** as a white solid (255 mg, 891 μmol, 91%).

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.34$ (s, 6H), 3.03 (s, 2H), 3.64 (br s, 2H), 6.53–6.55 (m, 1H), 6.69 (d, $J = 8.6$ Hz, 2H), 6.89 (d, $J = 8.0$ Hz, 2H), 7.79 (s, 1H), 8.08 (d, $J = 6.1$ Hz, 1H), 9.60 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 46.0, 63.6, 101.3, 108.3, 116.3, 122.0, 144.2, 145.9, 149.1, 152.7, 167.6, 169.7; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{15}H_{19}N_4O_2^+$: 287.1503, found: 287.1495.

5.1.31. N-(4-(4-aminophenoxy)pyridin-2-yl)-2-(1-methylpiperidin-4-yl) acetamide (19b)

The title compound was prepared from **18d** using a method analogous to that described for **19a** in 94% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.27–1.39 (m, 2H), 1.72–1.76 (m, 2H), 1.78–1.88 (m, 1H), 1.92 (td, $J = 11.9$, 2.5 Hz, 2H), 2.23–2.25 (m, 5H), 2.81 (d, $J = 11.6$ Hz, 2H), 3.64 (s, 2H), 6.52 (s, 1H), 6.68–6.70 (m, 2H), 6.87–6.90 (m, 2H), 7.74–7.78 (m, 1H), 7.81 (br s, 1H), 8.03 (d, $J = 5.5$ Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 32.2, 32.8, 44.7, 46.5, 55.6, 101.8, 108.3, 116.4, 122.0, 144.2, 145.9, 148.7, 153.3, 167.7, 170.9; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{19}H_{25}N_4O_2^+$: 341.1972, found: 341.1966.

5.1.32. N-(4-(4-amino-2-fluorophenoxy)pyridin-2-yl)-2-(1-methylpiperidin-4-yl)acetamide (19c)

The title compound was prepared from **18e** using a method analogous to that described for **19a** in 89% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.33$ (qd, $J = 12.2$, 3.7 Hz, 2H), 1.74 (d, $J = 12.8$ Hz, 2H), 1.78–1.88 (m, 1H), 1.92 (t, $J = 11.6$ Hz, 2H), 2.23–2.25 (m, 5H), 2.81 (d, $J = 11.0$ Hz, 2H), 3.67–3.78 (m, 2H), 6.43–6.45 (m, 1H), 6.50 (d, $J = 12.2$ Hz, 1H), 6.56–6.58 (m, 1H), 6.94 (t, $J = 8.6$ Hz, 1H), 7.77 (s, 1H), 7.84 (br s, 1H), 8.06 (d, $J = 5.5$ Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$):δ = 32.2, 32.7, 44.6, 46.4, 55.6, 101.3, 103.6 (d, $J_{CF} = 21.4$ Hz), 107.8, 111.0 (d, $J_{CF} = 2.5$ Hz), 124.1, 132.0 (d, $J_{CF} = 13.9$ Hz), 145.9 (d, $J_{CF} = 10.1$ Hz), 148.7, 153.5, 155.0 (d, $J_{CF} = 249.5$ Hz), 167.1, 171.2; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{19}H_{24}FN_4O_2^+$: 359.1878, found: 359.1868.

5.1.33. N-(4-(4-amino-3-fluorophenoxy)pyridin-2-yl)-2-(1-methylpiperidin-4-yl)acetamide (19d)

The title compound was prepared from **14a** using a method analogous to that described for **19a** in 90% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.27–1.39 (m, 2H), 1.73 (d, $J = 12.8$ Hz, 2H), 1.83 (ttt, $J = 11.3$, 7.4, 3.6 Hz, 1H), 1.92 (td, $J = 11.8$, 2.1 Hz, 2H), 2.23–2.25 (m, 5H), 2.81 (d, $J = 11.6$ Hz, 2H), 3.62–3.75 (m, 2H), 6.55 (dd, $J = 5.8$, 2.1 Hz, 1H), 6.70–6.73 (m, 1H), 6.76–6.80 (m, 2H), 7.77 (br s, 1H), 7.96 (br s, 1H), 8.06 (d, $J = 6.1$ Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 32.2, 32.7, 44.5, 46.4, 55.6, 102.0, 108.3, 109.1 (d, $J_{CF} = 20.2$ Hz), 117.1 (d, $J_{CF} = 3.8$ Hz), 117.3 (d, $J_{CF} = 5.0$ Hz), 132.5 (d, $J_{CF} = 13.9$ Hz), 145.2, 148.7, 151.4 (d, $J_{CF} = 241.9$ Hz), 153.6, 167.2, 171.2; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{19}H_{24}FN_4O_2^+$: 359.1878, found: 359.1868.

5.1.34. N-(4-(4-amino-2,5-difluorophenoxy)pyridin-2-yl)-2-(1-methylpiperidin-4-yl)acetamide (19e)

The title compound was prepared from **14b** using a method analogous to that described for **19a** in 81% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.33$ (qd, $J = 12.1$, 3.4 Hz, 2H), 1.73 (d, $J = 12.8$ Hz, 2H), 1.78–1.88 (m, 1H), 1.92 (t, $J = 11.6$ Hz, 2H), 2.23–2.26 (m, 5H), 2.81 (d, $J = 11.0$ Hz, 2H), 3.79 (s, 2H), 6.53–6.67 (m, 2H), 6.85 (dd, $J = 10.4$, 7.3 Hz, 1H), 7.78 (s, 1H), 7.96 (br s, 1H), 8.08 (d, $J = 6.1$ Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 32.2, 32.7, 44.6, 46.4, 55.6, 101.14, 104.5 (dd, $J_{CF} = 23.5$, 4.2 Hz), 107.8, 110.7 (d, $J_{CF} = 22.7$ Hz), 130.7 (dd, $J_{CF} = 14.5$, 9.7 Hz), 133.6 (dd, $J_{CF} = 14.5$, 9.7 Hz),

146.7 (d, J_{CF} = 238.1 Hz), 148.8, 151.1 (d, J_{CF} = 247.0 Hz), 153.5, 166.7, 171.2; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{19}H_{23}F_2N_4O_2^+$: 377.1784, found: 377.1772.

5.1.35. N-(4-((2-(2-(dimethylamino)acetamido)pyridin-4-yl)oxy) phenyl)-3-(4-fluorophenyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (20a)

The title compound was prepared from **19a** and **7b** using a method analogous to that described for **18a** in 89% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.49 (d, J = 6.7 Hz, 6H), 2.35 (s, 6H), 3.04 (s, 2H), 4.89–5.04 (m, 1H), 6.57 (dd, J = 6.1, 1.8 Hz, 1H), 7.06 (d, J = 8.6 Hz, 2H), 7.24 (s, 4H), 7.67 (d, J = 9.2 Hz, 2H), 7.85 (d, J = 1.8 Hz, 1H), 8.11 (d, J = 6.1 Hz, 1H), 8.67 (s, 1H), 9.63 (s, 1H), 10.80 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 21.6, 46.0, 50.5, 63.6, 101.9, 105.8, 108.6, 116.9 (d, J_{CF} = 23.9 Hz), 121.4, 121.8, 130.0 (d, J_{CF} = 7.6 Hz), 130.4 (d, J_{CF} = 2.5 Hz), 135.4, 146.2, 149.3, 150.4, 150.5, 152.8, 159.9, 162.8 (d, J_{CF} = 248.2 Hz), 163.0, 166.7, 169.8; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{29}H_{30}FN_6O_5^+$: 561.2256, found: 561.2241.

5.1.36. 3-(4-fluorophenyl)-1-isopropyl-N-(4-((2-(2-(1-methylpiperidin-4-yl)acetamido)pyridin-4-yl)oxy)phenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (20b)

The title compound was prepared from **19b** and **7b** using a method analogous to that described for **18a** in 81% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.32 (qd, J = 12.1, 3.4 Hz, 2H), 1.48 (d, J = 6.1 Hz, 6H), 1.73 (d, J = 12.8 Hz, 2H), 1.78–1.88 (m, 1H), 1.92 (t, J = 11.6 Hz, 2H), 2.23–2.25 (m, 5H), 2.81 (d, J = 11.0 Hz, 2H), 4.88–5.04 (m, 1H), 6.50–6.60 (m, 1H), 7.05 (dd, J = 9.2, 1.2 Hz, 2H), 7.23–7.25 (m, 4H), 7.67 (dd, J = 9.2, 1.2 Hz, 2H), 7.81 (s, 1H), 7.92 (s, 1H), 8.06 (dd, J = 6.1, 1.2 Hz, 1H), 8.67–8.68 (m, 1H), 10.81 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 21.7, 32.2, 32.7, 44.8, 46.4, 50.5, 55.6, 102.2, 105.8, 108.7, 116.9 (d, J_{CF} = 22.7 Hz), 121.4, 121.9, 130.0 (d, J_{CF} = 7.6 Hz), 130.3 (d, J_{CF} = 3.8 Hz), 135.4, 146.2, 149.0, 150.3, 150.5, 153.2, 159.9, 162.8 (d, J_{CF} = 249.5 Hz), 163.0, 166.8, 170.8; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{33}H_{36}FN_6O_5^+$: 615.2726, found: 615.2713.

5.1.37. N-(2-fluoro-4-((2-(2-(1-methylpiperidin-4-yl)acetamido)pyridin-4-yl)oxy)phenyl)-3-(4-fluorophenyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (20c)

The title compound was prepared from **19d** and **7b** using a method analogous to that described for **18a** in 71.2% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.33 (qd, J = 12.2, 3.7 Hz, 2H), 1.49 (d, J = 6.7 Hz, 6H), 1.74 (d, J = 12.8 Hz, 2H), 1.79–1.89 (m, 1H), 1.89–1.97 (m, 2H), 2.24–2.26 (m, 5H), 2.81 (d, J = 11.6 Hz, 2H), 4.95 (spt, J = 6.8 Hz, 1H), 6.59 (dd, J = 6.1, 2.5 Hz, 1H), 6.84–6.94 (m, 2H), 7.21–7.25 (m, 4H), 7.83 (d, J = 2.5 Hz, 1H), 7.90 (s, 1H), 8.10 (d, J = 5.5 Hz, 1H), 8.41–8.49 (m, 1H), 8.66 (s, 1H), 10.98–10.99 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 21.7, 32.2, 32.7, 44.9, 46.4, 50.5, 55.6, 102.3, 105.8, 108.5 (d, J_{CF} = 21.4 Hz), 108.9, 116.5 (d, J_{CF} = 2.5 Hz), 116.9 (d, J_{CF} = 22.7 Hz), 123.1, 124.0 (d, J_{CF} = 8.8 Hz), 130.1 (d, J_{CF} = 10.1 Hz), 130.26, 146.3, 149.2, 150.3, 150.6, 153.2, 153.3 (d, J_{CF} = 250.7 Hz), 160.2, 161.8, 162.8, 166.2, 170.7; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{33}H_{36}F_2N_6O_5^+$: 633.2632, found: 633.2611.

5.1.38. 1-cyclopropyl-N-(3-fluoro-4-((2-(2-(1-methylpiperidin-4-yl) acetamido)pyridin-4-yl)oxy)phenyl)-3-(4-fluorophenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (20d)

The title compound was prepared from **19c** and **7d** using a method analogous to that described for **18a** in 81% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.99–1.06 (m, 2H), 1.16–1.23 (m, 2H), 1.32 (qd, J = 12.2, 3.7 Hz, 2H), 1.70–1.74 (m, 2H), 1.77–1.87 (m, 1H), 1.88–1.97 (m, 2H), 2.23–2.25 (m, 5H), 2.80 (d, J = 11.6 Hz, 2H), 3.27–3.35 (m, 1H), 6.57 (dd, J = 5.8, 2.1 Hz, 1H), 7.06–7.14 (m, 1H), 7.20–7.24 (m, 5H), 7.76–7.87 (m, 2H), 7.99 (s, 1H), 8.08 (d, J = 5.5 Hz, 1H), 8.64 (s, 1H), 10.84 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 7.2, 32.2, 32.7, 33.4, 44.9, 46.4, 55.6, 101.1, 105.1, 107.9, 109.4 (d, J_{CF} = 22.7 Hz), 116.3, 116.9 (d, J_{CF} = 22.7 Hz), 123.6, 130.0 (d, J_{CF} = 8.8 Hz), 136.6 (d, J_{CF} = 10.1 Hz), 136.9, 137.0, 149.0, 150.3, 151.1, 153.2, 154.3 (d, J_{CF} = 250.7 Hz), 159.8, 162.9 (d, J_{CF} = 250.7 Hz), 163.2, 166.3, 170.8; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{33}H_{33}F_2N_6O_5^+$: 631.2475, found: 631.2454.

5.1.39. 1-cyclopentyl-N-(3-fluoro-4-((2-(2-(1-methylpiperidin-4-yl) acetamido)pyridin-4-yl)oxy)phenyl)-3-(4-fluorophenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (20e)

The title compound was prepared from **19c** and **7c** using a method analogous to that described for **18a** in 80% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.32 (qd, J = 12.2, 3.7 Hz, 2H), 1.66–1.86 (m, 9H), 1.88–1.98 (m, 4H), 2.21–2.25 (m, 5H), 2.80 (d, J = 11.6 Hz, 2H), 4.98 (quin, J = 7.8 Hz, 1H), 6.57 (dd, J = 5.8, 2.1 Hz, 1H), 7.06–7.14 (m, 1H), 7.20–7.25 (m, 5H), 7.80 (d, J = 1.8 Hz, 1H), 7.83 (dd, J = 12.2, 2.5 Hz, 1H), 8.06 (s, 1H), 8.08 (d, J = 6.1 Hz, 1H), 8.65 (s, 1H), 10.89 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 24.2, 31.9, 32.2, 32.7, 44.8, 46.4, 55.6, 59.8, 101.2, 105.4, 107.9, 109.7 (d, J_{CF} = 22.7 Hz), 116.3, 116.9 (d, J_{CF} = 22.7 Hz), 123.6, 130.0 (d, J_{CF} = 8.8 Hz), 130.3, 136.7 (d, J_{CF} = 10.1 Hz), 136.9 (d, J_{CF} = 11.3 Hz), 147.3, 149.0, 150.7, 153.2, 154.3 (d, J_{CF} = 249.5 Hz), 160.1, 162.8 (d, J_{CF} = 250.7 Hz), 163.0, 166.4, 170.8; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{35}H_{37}F_2N_6O_5^+$: 659.2788, found: 659.2766.

5.1.40. Ethyl 2,5-dioxo-5,6,7,8-tetrahydro-2H-chromene-3-carboxylate (23)

To a solution of 1,3-cyclohexanedione (**22**) (1.00 g, 8.92 mmol) and ethyl (ethoxymethylene)cyanoacetate (**21**) (1.51 g, 8.92 mmol) in DMF (15 mL) was added potassium *tert*-butoxide (1.00 g, 8.92 mmol) at room temperature. The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was poured into 1 N HCl (10.7 mL) and water (40 mL), then extracted with EtOAc. The organic extract was washed with water and brine, dried over Na_2SO_4, filtered and then concentrated. The residue was purified with column chromatography on silica gel (*n*-heptane/EtOAc = 4/1 ~ 3/7) to afford the title compound **23** as a red oil (1.08 g, 4.58 mmol, 51.4%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.36 (t, J = 7.1 Hz, 3H), 2.09–2.26 (m, 2H), 2.50–2.61 (m, 2H), 2.81–2.96 (m, 2H), 4.26–4.44 (m, 2H), 8.62 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 14.2, 20.0, 28.5, 36.5, 61.9, 114.2, 115.4, 144.9, 155.9, 162.3, 178.4, 193.1; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{12}H_{13}O_5^+$: 237.0757, found: 237.0748.

5.1.41. 2,5-dioxo-1-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (24)

To a solution of ethyl 2,5-dioxo-5,6,7,8-tetrahydro-2H-chromene-3-carboxylate (**23**) (917 mg, 3.88 mmol) in EtOH (15 mL) was added aniline (362 mg, 3.88 mmol) at room temperature. The reaction mixture was stirred at room temperature for 16 h. The precipitate was collected by filtration, then rinsed with EtOH to afford the title compound **24** as a white solid (585 mg, 2.06 mmol, 53.2%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.05–2.15 (m, 2H), 2.53–2.65 (m, 4H), 7.23 (s, 1H), 7.48–7.74 (m, 4H), 9.19 (s, 1H), 12.98–13.50 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 21.0, 29.4, 36.5, 116.4, 116.7, 127.2, 130.6, 130.7, 135.9, 143.8, 160.9, 164.1, 165.4, 192.5; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{16}H_{14}NO_4^+$: 284.0917, found: 284.0909.

5.1.42. N-(4-((2-(2-(1-methylpiperidin-4-yl)acetamido)pyridin-4-yl)oxy) phenyl)-2,5-dioxo-1-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (25a)

The title compound was prepared from **19b** and **24** using a method analogous to that described for **18a** in 73.6% as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.25–1.40 (m, 2H), 1.69–1.77 (m, 2H), 1.78–1.87 (m, 1H), 1.88–1.97 (m, 2H), 2.04–2.14 (m, 2H), 2.18–2.30 (m, 5H), 2.48–2.65 (m, 4H), 2.80 (d, J = 11.9 Hz, 2H), 6.53 (dd, J = 5.5, 2.3 Hz, 1H), 7.03 (d, J = 7.7 Hz, 2H), 7.24–7.30 (m, 2H), 7.55–7.66 (m, 3H), 7.74 (d, J = 7.7 Hz, 2H), 7.78–7.89 (m, 2H), 8.05 (d,

$J = 5.5$ Hz, 1H), 9.31 (s, 1H), 11/39 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 21.2, 29.4, 32.2, 32.7, 36.6, 44.8, 46.4, 55.6, 102.3, 108.5, 115.7, 120.0, 121.3, 122.0, 127.4, 130.2, 130.6, 135.7, 137.0, 142.0, 149.0, 150.1, 153.2, 159.6, 160.7, 163.5, 166.9, 170.8, 193.0; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{35}H_{36}N_5O_5^+$: 606.2711, found: 606.2696.

5.1.43. N-(3-fluoro-4-((2-(2-(1-methylpiperidin-4-yl)acetamido)pyridin-4-yl)oxy)phenyl)-2,5-dioxo-1-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (25b)

The title compound was prepared from **19c** and **24** using a method analogous to that described for **18a** in 65.3% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.27–1.39 (m, 2H), 1.68–1.76 (m, 2H), 1.78–1.88 (m, 1H), 1.89–1.96 (m, 2H), 2.06–2.14 (m, 2H), 2.20–2.27 (m, 5H), 2.51–2.65 (m, 4H), 2.80 (d, J = 11.6 Hz, 2H), 6.55 (dd, J = 5.5, 2.5 Hz, 1H), 7.06–7.12 (m, 1H), 7.25–7.28 (m, 2H), 7.29–7.34 (m, 1H), 7.57–7.70 (m, 3H), 7.78–7.92 (m, 3H), 8.07 (d, J = 6.1 Hz, 1H), 9.31 (s, 1H), 11.49 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 21.2, 29.4, 32.2, 32.7, 36.6, 44.8, 46.4, 55.6, 101.3, 107.7, 109.7 (d, J_{CF} = 22.7 Hz), 115.7, 116.4 (d, J_{CF} = 2.5 Hz), 119.7, 123.5, 127.4, 130.3, 130.6, 136.8 (d, J_{CF} = 22.7 Hz), 136.9 (d, J_{CF} = 10.1 Hz), 137.0 (d, J_{CF} = 10.1 Hz), 142.2, 149.0, 153.2, 154.6 (d, J_{CF} = 249.5 Hz), 159.9, 160.9, 163.5, 166.4, 170.8, 192.9; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{35}H_{35}FN_5O_5^+$: 624.2617, found: 624.2602.

5.1.44. N-(2-fluoro-4-((2-(2-(1-methylpiperidin-4-yl)acetamido)pyridin-4-yl)oxy)phenyl)-2,5-dioxo-1-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (25c)

The title compound was prepared from **19d** and **24** using a method analogous to that described for **18a** in 66.1% as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.26–1.39 (m, 2H), 1.68–1.77 (m, 2H), 1.79–1.89 (m, 1H), 1.89–1.99 (m, 2H), 2.04–2.16 (m, 2H), 2.20–2.29 (m, 5H), 2.45–2.55 (m, 2H), 2.57–2.64 (m, 2H), 2.81 (d, J = 11.9 Hz, 2H), 6.56 (dd, J = 6.0, 2.3 Hz, 1H), 6.80–6.95 (m, 2H), 7.20–7.30(m, 2H), 7.54–7.70 (m, 3H), 7.83 (d, J = 2.3 Hz, 2H), 8.08 (d, J = 6.0 Hz, 1H), 8.52–8.63 (m, 1H), 9.30 (s, 1H), 11.55 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 21.2, 29.4, 32.2, 32.7, 36.6, 44.8, 46.4, 55.6, 102.4, 108.4 (d, J_{CF} = 22.2 Hz), 108,7, 115.5, 116.6, 119.9, 123.3, 124.3 (d, J_{CF} = 11.1 Hz), 127.5, 130.1, 130.6, 136.9, 142.1, 149.1, 150.1 (d, J_{CF} = 11.1 Hz), 153.2, 153.2 (d, J_{CF} = 249.5 Hz), 160.0, 161.0, 163.4, 166.3, 170.8, 193.0; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{35}H_{35}FN_5O_5^+$: 624.2617, found: 624.2598.

5.1.45. N-(2,5-difluoro-4-((2-(2-(1-methylpiperidin-4-yl)acetamido) pyridin-4-yl)oxy)phenyl)-2,5-dioxo-1-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (25d)

The title compound was prepared from **19e** and **24** using a method analogous to that described for **18a** in 34.8% as a pink-colored solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.26–1.40 (m, 2H), 1.68–1.78 (m, 2H), 1.78–1.88 (m, 1H), 1.88–1.98 (m, 2H), 2.04–2.15 (m, 2H), 2.19–2.30 (m, 5H), 2.48–2.67 (m, 4H), 2.81 (d, J = 11.4 Hz, 2H), 6.57 (dd, J = 5.7, 2.5 Hz, 1H), 6.88–6.98 (m, 1H), 7.24–7.27 (m, 2H), 7.50–7.68 (m, 3H), 7.77–7.96 (m, 2H), 8.09 (d, J = 5.5 Hz, 1H), 8.53–8.67 (m, 1H), 9.29 (s, 1H), 11.68 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 21.2, 29.5, 32.2, 32.7, 36.6, 44.9, 46.4, 55.6, 101.3, 107.8, 110.0 (d, J_{CF} = 23.2 Hz), 110.7 (d, J_{CF} = 27.3 Hz), 115.6, 119.6, 125.2 (dd, J_{CF} = 22.2, 11.1 Hz), 127.4, 130.2, 130.6, 135.8 (dd, J_{CF} = 14.5, 10.6 Hz), 136.8, 142.4, 148.5 (d, J_{CF} = 244.4 Hz), 149.2, 150.4 (d, J_{CF} = 240.4 Hz), 153.1, 160.2, 161.1, 163.3, 165.9, 170.7, 192.9; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{35}H_{34}F_2N_5O_5^+$: 642.2523, found: 642.2505.

5.1.46. N-(4-((2-carbamoylpyridin-4-yl)oxy)-2,5-difluorophenyl)-3-(4-fluorophenyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (26a)

The title compound was prepared from **7b** and **10b** using a method analogous to that described for **18a** in 79% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.47 (d, J = 7.3 Hz, 6H), 4.94 (spt, J = 6.8 Hz, 1H), 6.06–6.09 (m, 1H), 6.96 (dd, J = 10.1, 7.0 Hz, 1H), 7.00 (dd, J = 5.5, 2.5 Hz, 1H), 7.19–7.24 (m, 4H), 7.64 (d, J = 2.5 Hz, 1H), 7.82 (d, J = 4.3 Hz, 1H), 8.41 (d, J = 5.5 Hz, 1H), 8.47 (dd, J = 12.2, 7.3 Hz, 1H), 8.66 (s, 1H) 11.14 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 21.6, 50.7, 105.4, 109.6, 110.3 (d, J_{CF} = 22.7 Hz), 110.6 (d, J_{CF} = 25.2, Hz), 114.0, 116.9 (d, J_{CF} = 22.7 Hz), 125.3 (dd, J_{CF} = 22.7, 12.6 Hz), 130.1 (d, J_{CF} = 8.8 Hz), 130.2 (d, J_{CF} = 3.8 Hz), 135.5 (dd, J_{CF} = 14.5, 9.7 Hz), 146.7, 148.5 (d, J_{CF} = 245.7 Hz), 150.2, 150.3 (d, J_{CF} = 243.2 Hz), 150.5, 152.1, 160.4, 162.8 (d, J_{CF} = 250.7 Hz), 162.8, 165.5, 166.2; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{26}H_{21}F_3N_5O_5^+$: 540.1489, found: 540.1468.

5.1.47. N-(4-((2-carbamoylpyridin-4-yl)oxy)-2,5-difluorophenyl)-2,5-dioxo-1-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (26b)

The title compound was prepared from **10b** and **24** using a method analogous to that described for **18a** in 82% as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.09 (quin, J = 6.4 Hz, 2H), 2.54 (t, J = 6.2 Hz, 2H), 2.58–2.62 (m, 2H), 5.50 (d, J = 3.2 Hz, 1H), 6.93–7.00 (m, 2H), 7.26–7.28 (m, 1H), 7.52–7.67 (m, 4H), 7.69 (d, J = 2.3 Hz, 1H), 7.80 (d, J = 3.2 Hz, 1H), 8.43 (d, J = 5.5 Hz, 1H), 8.61 (dd, J = 12.4, 6.9 Hz, 1H), 9.31 (s, 1H), 11.71 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6): δ = 21.0, 29.6, 36.5, 108.9, 110.0 (d, J_{CF} = 26.3 Hz), 111.8 (d, J_{CF} = 23.2 Hz), 114.1, 115.3, 117.9, 125.7 (dd, J_{CF} = 11.6, 11.6 Hz), 128.3, 130.2, 130.4, 135.2 (dd, J_{CF} = 13.0, 13.0 Hz), 137.5, 141.1, 148.6 (d, J_{CF} = 242.4 Hz), 150.2 (d, J_{CF} = 243.4 Hz), 151.3, 153.4, 161.7, 163.2, 163.5, 165.3, 165.8, 194.1; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{28}H_{21}F_2N_4O_5^+$: 531.1475, found: 531.1461.

5.1.48. N-(4-((2-aminopyridin-4-yl)oxy)-2,5-difluorophenyl)-3-(4-fluorophenyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (27a)

The title compound was prepared from **26a** using a method analogous to that described for **12a** in 61.1% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.48 (d, J = 6.7 Hz, 6H), 4.46 (br s, 2H), 4.95 (spt, J = 6.8 Hz, 1H), 5.94 (d, J = 1.8 Hz, 1H), 6.26 (dd, J = 5.8, 2.1 Hz, 1H), 6.93 (dd, J = 10.4, 6.7 Hz, 1H), 7.21–7.25 (m, 4H), 7.93 (d, J = 5.5 Hz, 1H), 8.41 (dd, J = 11.9, 7.0 Hz, 1H), 8.65 (s, 1H), 11.08 (br s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 21.6, 50.6, 94.6, 103.3, 105.4, 110.2 (d, J_{CF} = 22.7 Hz), 110.5 (d, J_{CF} = 26.5 Hz), 116.9 (d, J_{CF} = 22.7 Hz), 124.5 (dd, J_{CF} = 22.7, 11.3 Hz), 130.0 (d, J_{CF} = 8.8 Hz), 130.2 (d, J_{CF} = 3.8 Hz), 136.4 (dd, J_{CF} = 14.5, 9.7 Hz), 146.6, 148.5 (d, J_{CF} = 247.0 Hz), 149.9, 150.4, 150.4 (d, J_{CF} = 249.5 Hz), 160.3, 160.4, 162.8, 162.8 (d, J_{CF} = 250.7 Hz), 165.7; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{25}H_{21}F_3N_5O_4^+$: 512.1540, found: 512.1521.

5.1.49. N-(4-((2-aminopyridin-4-yl)oxy)-2,5-difluorophenyl)-2,5-dioxo-1-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (27b)

The title compound was prepared from **26b** using a method analogous to that described for **12a** in 85% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.07–2.10 (m, 2H), 2.53 (t, J = 6.1 Hz, 2H), 2.57–2.63 (m, 2H), 4.39 (s, 2H), 5.93 (d, J = 2.5 Hz, 1H), 6.26–6.28 (m, 1H), 6.91 (dd, J = 10.7, 7.0 Hz, 1H), 7.24–7.26 (m, 2H), 7.55–7.66 (m, 3H), 7.93 (d, J = 6.1 Hz, 1H), 8.53 (dd, J = 11.9, 7.0 Hz, 1H), 9.29 (s, 1H), 11.65 (s, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 21.0, 29.6, 36.5, 93.1, 101.5, 109.8 (d, J_{CF} = 26.5 Hz), 111.8 (d, J_{CF} = 22.7 Hz), 115.3, 117.9, 125.0 (dd, J_{CF} = 11.3, 11.3 Hz), 128.3, 130.2, 130.5, 136.0 (dd, J_{CF} = 15.1, 10.1 Hz), 137.4, 141.1, 148.5 (d, J_{CF} = 241.9 Hz), 150.2, 150.4 (d, J_{CF} = 240.7 Hz), 161.7, 162.2, 163.1, 163.4, 165.2, 194.1; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{27}H_{21}F_2N_4O_4^+$: 503.1525, found: 503.1513.

5.1.50. N-(2,5-difluoro-4-((2-(4-(4-methylpiperazin-1-yl)piperidine-1-carboxamido)pyridin-4-yl)oxy)phenyl)-3-(4-fluorophenyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (28a)

To a solution of *N*-(4-((2-aminopyridin-4-yl)oxy)-2,5-difluorophenyl)-3-(4-fluorophenyl)-1-isopropyl-2,4-dioxo-1,2,3,4-

tetrahydropyrimidine-5-carboxamide (**27a**) (34.0 mg, 66 μmol) in THF (2 mL) were added *i*Pr_2NEt (35 μL, 199 μmol) and phenyl chloroformate (25.0 mg, 160 μmol) at 0 °C. The reaction mixture was stirred for 30 min at room temperature. The reaction mixture was poured into water and extracted with EtOAc. The organic extract was washed with brine, dried over $MgSO_4$, filtered and then concentrated. To a solution of the residue in DMF (2 mL) were added *i*Pr_2NEt (35 μL, 199 μmol) and 1-methyl-4-(piperidine-4-yl)-piperazine (24.4 mg, 133 μmol) at room temperature. The reaction mixture was stirred for 17 h at room temperature. The reaction mixture was poured into water and extracted with EtOAc. The organic extract was washed with water and brine, dried over $MgSO_4$, filtered and then concentrated. The residue was purified with column chromatography on NH silica gel (EtOAc ~ EtOAc/MeOH = 4/1) to afford the title compound **28a** as a white solid (33 mg, 46 μmol, 69%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.49 (d, J = 7.3 Hz, 8H), 1.87 (d, J = 11.0 Hz, 2H), 2.25–2.30 (m, 4H), 2.38–2.50 (m, 4H), 2.50–2.65 (m, 4H), 2.80–2.93(m, 2H), 4.08 (d, J = 13.5 Hz, 2H), 4.89–5.02 (m, 1H), 6.52 (dd, J = 6.1, 2.5 Hz, 1H), 6.95 (dd, J = 10.4, 7.3 Hz, 1H), 7.19–7.25 (m, 5H), 7.62 (d, J = 2.5 Hz, 1H), 8.04 (d, J = 5.5 Hz, 1H), 8.45 (dd, J = 11.9, 7.0 Hz, 1H), 8.65 (s, 1H), 11.09 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 21.7, 28.3, 43.7, 46.1, 49.1, 50.6, 55.5, 61.5, 100.1, 105.5, 107.0, 110.1 (d, J_{CF} = 22.7 Hz), 110.5 (d, J_{CF} = 26.5 Hz), 116.9 (d, J_{CF} = 22.7 Hz), 124.7, 130.1 (d, J_{CF} = 7.6 Hz), 130.2, 136.3, 146.5, 148.4 (d, J_{CF} = 242.6 Hz), 149.0, 150.4 (d, J_{CF} = 242.6 Hz), 150.5, 153.5, 154.8, 160.2, 162.8, 162.8 (d, J_{CF} = 249.5 Hz), 165.7; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{36}H_{40}F_3N_8O_5^+$: 721.3068, found: 721.3041.

5.1.51. *N-(2,5-difluoro-4-((2-(4-(4-methylpiperazin-1-yl)piperidine-1-carboxamido)pyridin-4-yl)oxy)phenyl)-2,5-dioxo-1-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (28b)*

The title compound was prepared from **27b** using a method analogous to that described for **28a** in 59.3% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.43–1.53 (m, 2H), 1.87 (d, J = 11.6 Hz, 2H), 2.09 (quin, J = 6.4 Hz, 2H), 2.27 (s, 3H), 2.35–2.48 (m, 5H), 2.50–2.61 (m, 8H), 2.82–2.92 (m, 2H), 4.08 (d, J = 13.5 Hz, 2H), 6.49 (dd, J = 6.1, 2.5 Hz, 1H), 6.93 (dd, J = 10.4, 7.3 Hz, 1H), 7.19 (s, 1H), 7.24–7.30 (m, 2H), 7.58–7.60 (m, 1H), 7.61–7.65 (m, 3H), 8.03 (d, J = 5.5 Hz, 1H), 8.57 (dd, J = 11.9, 7.0 Hz, 1H), 9.29 (s, 1H), 11.66 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 21.2, 28.3, 29.5, 36.6, 43.7, 46.1, 49.1, 55.5, 61.6, 100.2, 106.8, 110.0 (d, J_{CF} = 22.7 Hz), 110.7 (d, J_{CF} = 26.5 Hz), 115.6, 119.6, 125.0 (dd, J_{CF} = 11.5, 11.5 Hz), 127.4, 130.2, 130.6, 136.1 (d, J_{CF} = 13.9, 10.1 Hz), 136.9, 142.4, 148.5 (d, J_{CF} = 241.9 Hz), 148.9, 150.4 (d, J_{CF} = 244.4 Hz), 153.5, 154.8, 160.1, 161.1, 163.3, 165.7, 192.9; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{38}H_{40}F_2N_7O_5^+$: 712.3054, found: 712.3040.

5.1.52. *6-(4-aminophenoxy)pyrimidin-4-amine (31)*

The title compound was prepared from **29** and **30** using a method analogous to that described for **10a** in 67.7% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.64 (br s, 2H), 4.75 (br s, 2H), 5.74 (s, 1H), 6.68–6.70 (m, 2H), 6.89–6.92 (m, 2H), 8.28 (s, 1H); ^{13}C NMR (126 MHz, methanol-d_4): δ = 86.0, 116.1, 121.7, 144.3, 145.6, 157.8, 166.2, 171.0; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{10}H_{11}N_4O^+$: 203.0927, found: 203.0921.

5.1.53. *N-(4-((6-aminopyrimidin-4-yl)oxy)phenyl)-3-(4-fluorophenyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (32a)*

The title compound was prepared from **31** and **7b** using a method analogous to that described for **18a** in 93% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.47 (d, J = 6.7 Hz, 6H), 4.92–4.97 (m, 3H), 5.79 (s, 1H), 7.07 (d, J = 9.2 Hz, 2H), 7.20–7.28 (m, 4H), 7.65 (d, J = 8.6 Hz, 2H), 8.25 (s, 1H), 8.66 (s, 1H), 10.79 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 21.6, 50.5, 87.8, 105.8, 116.9 (d, J_{CF} = 22.7 Hz), 121.7, 122.2, 130.0 (d, J_{CF} = 8.8 Hz), 130.3 (d, J_{CF} = 3.8 Hz), 135.4, 146.3, 149.1, 150.5, 158.8, 159.9, 162.8 (d, J_{CF} = 250.7 Hz), 163.0, 165.1, 170.5; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{24}H_{22}FN_6O_4^+$: 477.1681, found: 477.1669.

5.1.54. *N-(4-((6-aminopyrimidin-4-yl)oxy)phenyl)-3-(4-fluorophenyl)-1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (32b)*

The title compound was prepared from **31** and **7a** using a method analogous to that described for **18a** in 92% as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.49 (s, 3H), 5.67 (s, 1H), 6.79 (s, 2H), 7.08 (d, J = 8.6 Hz, 2H), 7.28–7.44 (m, 4H), 7.66 (d, J = 8.6 Hz, 2H), 8.02 (s, 1H), 8.81 (s, 1H), 10.81 (s, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 37.9, 86.8, 104.5, 116.5 (d, J_{CF} = 23.9 Hz), 121.6, 122.6, 131.3 (d, J_{CF} = 8.8 Hz), 131.9, 135.7, 149.1, 151.1, 152.4, 158.7, 160.6, 162.3 (d, J_{CF} = 245.7 Hz), 163.9, 166.5, 167.0; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{22}H_{18}FN_6O_4^+$: 449.1368, found: 449.1355.

5.1.55. *N-(4-((6-aminopyrimidin-4-yl)oxy)phenyl)-1-cyclopropyl-3-(4-fluorophenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (32c)*

The title compound was prepared from **31** and **7d** using a method analogous to that described for **18a** in 91% as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.94–1.02 (m, 4H), 3.20–3.26 (m, 1H), 5.67 (s, 1H), 6.79 (s, 2H), 7.07 (d, J = 9.2 Hz, 2H), 7.29–7.40 (m, 4H), 7.66 (d, J = 9.2 Hz, 2H), 8.02 (s, 1H), 8.45 (s, 1H), 10.77 (s, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 7.0, 33.5, 86.9, 104.5, 116.5 (d, J_{CF} = 23.9 Hz), 121.6, 122.6, 131.4 (d, J_{CF} = 8.8 Hz), 131.9, 135.7, 149.2, 151.0, 151.3, 158.7, 160.4, 162.3 (d, J_{CF} = 245.7 Hz), 163.5, 166.5, 169.9; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{24}H_{20}FN_6O_4^+$: 475.1525, found: 475.1512.

5.1.56. *N-(4-((6-aminopyrimidin-4-yl)oxy)phenyl)-1-cyclopentyl-3-(4-fluorophenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (32d)*

The title compound was prepared from **31** and **7c** using a method analogous to that described for **18a** in 94% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.70–1.86 (m, 4H), 1.88–2.01 (m, 2H), 2.17–2.27 (m, 2H), 4.80 (s, 2H), 4.98 (quin, J = 7.8 Hz, 1H), 5.81 (s, 1H), 7.08 (d, J = 8.6 Hz, 2H), 7.23 (s, 4H), 7.67 (d, J = 9.2 Hz, 2H), 8.27 (s, 1H), 8.66 (s, 1H), 10.79 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 24.2, 31.9, 59.7, 87.8, 105.7, 117.0 (d, J_{CF} = 23.9 Hz), 121.6, 122.2, 130.0 (d, J_{CF} = 7.6 Hz), 130.4, 135.4, 147.1, 149.1, 150.8, 158.7, 159.9, 162.8 (d, J_{CF} = 250.7 Hz), 163.1, 165.1, 170.5; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{26}H_{24}FN_6O_4^+$: 503.1838, found: 503.1824.

5.1.57. *N-(4-((6-(cyclopropanecarboxamido)pyrimidin-4-yl)oxy)phenyl)-3-(4-fluorophenyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (33a)*

To a solution of *N*-(4-((6-aminopyrimidin-4-yl)oxy)phenyl)-3-(4-fluorophenyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**32a**) (40 mg, 84 μmol) in THF (2 mL) was added *i*Pr_2NEt (73 μL, 0.42 mmol) and cyclopropanecarbonyl chloride (31 μL, 0.34 mmol) at room temperature. The reaction mixture was stirred at room temperature for 3 days. The reaction mixture was poured into water and extracted with EtOAc. The organic extract was washed with brine, dried over $MgSO_4$, filtered and then concentrated. The residue was purified with column chromatography on NH silica gel (heptane/EtOAc = 1/1 ~ EtOAc) to afford the title compound **33a** as a white solid (35 mg, 64 μmol, 77%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.90–0.96 (m, 2H), 1.08–1.14 (m, 2H), 1.48 (d, J = 6.7 Hz, 6H), 1.53–1.55 (m, 1H), 4.95 (spt, J = 6.8 Hz, 1H), 7.08–7.10 (m, 2H), 7.23–7.25 (m, 4H), 7.65 (d, J = 1.2 Hz, 1H), 7.67–7.72 (m, 2H), 8.18 (s, 1H), 8.43 (s, 1H), 8.67 (s, 1H), 10.81 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 9.14, 16.0, 21.6, 50.5, 95.5, 105.9, 116.9 (d, J_{CF} = 22.7 Hz), 121.5, 122.1, 130.0 (d, J_{CF} = 7.6 Hz), 130.4 (d, J_{CF} = 3.8 Hz), 135.7, 146.3, 148.8, 150.5, 157.8, 158.9, 159.9, 162.8 (d, J_{CF} = 250.7 Hz), 163.0, 171.3, 173.3; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{28}H_{26}FN_6O_5^+$: 545.1943, found: 545.1929.

5.1.58. N-(4-((6-(azetidine-1-carboxamido)pyrimidin-4-yl)oxy)phenyl)-3-(4-fluorophenyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (33b)

The title compound was prepared from **32a** using a method analogous to that described for **28a** in 89% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.48$ (d, $J = 6.7$ Hz, 6H), 2.33 (quin, $J = 7.5$ Hz, 2H), 4.10 (t, $J = 7.6$ Hz, 4H), 4.95 (spt, $J = 6.8$ Hz, 1H), 6.85 (s, 1H), 7.07–7.13 (m, 2H), 7.20–7.28 (m, 4H), 7.53–7.54 (m, 1H), 7.66–7.71 (m, 2H), 8.35 (s, 1H), 8.67 (s, 1H), 10.79 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 15.2$, 21.6, 49.2, 50.5, 93.7, 105.9, 116.9 (d, $J_{CF} = 22.7$ Hz), 121.5, 122.1, 130.0 (d, $J_{CF} = 7.6$ Hz), 130.4 (d, $J_{CF} = 3.8$ Hz), 135.6, 146.2, 148.9, 150.5, 154.1, 157.7, 159.8 (2C), 162.8 (d, $J_{CF} = 249.5$ Hz), 163.0, 171.0; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{28}H_{27}FN_7O_5^+$: 560.2052, found: 560.2035.

5.1.59. 3-(4-fluorophenyl)-1-isopropyl-2,4-dioxo-N-(4-((6-(pyrrolidine-1-carboxamido)pyrimidin-4-yl)oxy)phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide (33c)

The title compound was prepared from **32a** using a method analogous to that described for **28a** in 89% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.48$ (d, $J = 6.7$ Hz, 6H), 1.97 (br s, 4H), 3.46 (br s, 4H), 4.88–5.02 (m, 1H), 7.09–7.11 (m, 3H), 7.20–7.28 (m, 4H), 7.58–7.59 (m, 1H), 7.67–7.69 (m, 2H), 8.36 (s, 1H), 8.67 (s, 1H), 10.79 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 21.6$, 25.6, 46.0, 50.5, 93.9, 105.9, 116.9 (d, $J_{CF} = 22.7$ Hz), 121.5, 122.1, 130.0 (d, $J_{CF} = 7.6$ Hz), 130.4 (d, $J_{CF} = 2.5$ Hz), 135.6, 146.2, 149.0, 150.5, 152.2, 157.7, 159.8, 160.2, 162.8 (d, $J_{CF} = 248.2$ Hz), 163.0, 171.0; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{29}H_{29}FN_7O_5^+$: 574.2209, found: 574.2192.

5.1.60. 3-(4-fluorophenyl)-N-(4-((6-(3-hydroxyazetidine-1-carboxamido)pyrimidin-4-yl)oxy)phenyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (33d)

The title compound was prepared from **32a** using a method analogous to that described for **28a** in 85% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.48$ (d, $J = 6.7$ Hz, 6H), 2.25 (d, $J = 6.1$ Hz, 1H), 3.96 (dd, $J = 10.4$, 4.3 Hz, 2H), 4.31 (dd, $J = 9.4$, 7.0 Hz, 2H), 4.67–4.75 (m, 1H), 4.90–5.00 (m, 1H), 6.90 (s, 1H), 7.06–7.14 (m, 2H), 7.24 (d, $J = 2.5$ Hz, 4H), 7.53 (d, $J = 1.2$ Hz, 1H), 7.65–7.74 (m, 2H), 8.36–8.36 (m, 1H), 8.67 (s, 1H), 10.80 (s, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): $\delta = 21.2$, 51.0, 60.2, 79.7, 93.0, 105.2, 116.4 (d, $J_{CF} = 22.9$ Hz), 121.5, 122.7, 131.4 (d, $J_{CF} = 8.8$ Hz), 132.1, 136.0, 147.5, 148.8, 150.6, 155.8, 158.1, 160.6, 161.6, 162.3 (d, $J_{CF} = 247.0$ Hz), 163.3, 170.6; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{28}H_{27}FN_7O_6^+$: 576.2001, found: 576.1983.

5.1.61. N-(4-((6-(3-(dimethylamino)azetidine-1-carboxamido)pyrimidin-4-yl)oxy)phenyl)-3-(4-fluorophenyl)-1-isopropyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (33e)

The title compound was prepared from **32a** using a method analogous to that described for **28a** in 89% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.48$ (d, $J = 6.7$ Hz, 6H), 2.18 (d, $J = 1.2$ Hz, 6H), 3.10–3.18 (m, 1H), 3.88–3.95 (m, 2H), 4.02–4.09 (m, 2H), 4.95 (spt, $J = 6.6$ Hz, 1H), 6.91 (s, 1H), 7.09 (d, $J = 7.3$ Hz, 2H), 7.20–7.27 (m, 4H), 7.53 (s, 1H), 7.68 (d, $J = 8.0$ Hz, 2H), 8.35 (s, 1H), 8.67 (d, $J = 1.2$ Hz, 1H), 10.79 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 21.6$, 41.8, 50.5, 53.4, 54.9, 93.8, 105.9, 116.9 (d, $J_{CF} = 22.7$ Hz), 121.5, 122.1, 130.0 (d, $J_{CF} = 7.6$ Hz), 130.4 (d, $J_{CF} = 3.8$ Hz), 135.6, 146.2, 148.9, 150.5, 154.2, 157.7, 159.8 (2C), 162.8 (d, $J_{CF} = 249.5$ Hz), 163.0, 171.0; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{30}H_{32}FN_8O_5^+$: 603.2474, found: 603.2456.

5.1.62. 3-(4-fluorophenyl)-1-isopropyl-2,4-dioxo-N-(4-((6-(3-(pyrrolidin-1-yl)azetidine-1-carboxamido)pyrimidin-4-yl)oxy)phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide (33f)

The title compound was prepared from **32a** using a method analogous to that described for **28a** in 87% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.48$ (d, $J = 7.3$ Hz, 6H), 1.77–1.87 (m, 4H), 2.48–2.51 (m, 4H), 3.36 (tt, $J = 7.0$, 4.9 Hz, 1H), 3.98 (dd, $J = 8.6$, 4.9 Hz, 2H), 4.07–4.15 (m, 2H), 4.88–5.02 (m, 1H), 6.89 (s, 1H), 7.06–7.14 (m, 2H), 7.22–7.28 (m, 4H), 7.53 (s, 1H), 7.64–7.72 (m, 2H), 8.35 (s, 1H), 8.67 (s, 1H), 10.79 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 21.6$, 23.6, 50.5, 50.7, 52.3, 53.7, 93.8, 105.9, 116.9 (d, $J_{CF} = 22.7$ Hz), 121.5, 122.1, 130.0 (d, $J_{CF} = 7.6$ Hz), 130.4 (d, $J_{CF} = 2.5$ Hz), 135.6, 146.2, 148.9, 150.5, 154.2, 157.7, 159.8 (2C), 162.8 (d, $J_{CF} = 249.5$ Hz), 163.0, 170.9; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{32}H_{34}FN_8O_5^+$: 629.2631, found: 629.2614.

5.1.63. 3-(4-fluorophenyl)-1-isopropyl-N-(4-((6-(4-(4-methylpiperazin-1-yl)piperidine-1-carboxamido)pyrimidin-4-yl)oxy)phenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (33 g)

The title compound was prepared from **32a** using a method analogous to that described for **28a** in 80% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.45$–2.03 (m, 2H), 1.48 (d, $J = 6.7$ Hz, 6H), 1.90 (d, $J = 11.6$ Hz, 2H), 2.27 (s, 3H), 2.32–2.52 (m, 5H), 2.58 (br s, 4H), 2.92 (t, $J = 11.6$ Hz, 2H), 4.09 (d, $J = 12.8$ Hz, 2H), 4.89–5.01 (m, 1H), 7.09 (d, $J = 9.2$ Hz, 2H), 7.20–7.28 (m, 4H), 7.35 (s, 1H), 7.49 (s, 1H), 7.68 (d, $J = 8.6$ Hz, 2H), 8.35 (s, 1H), 8.67 (s, 1H), 10.79 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 21.6$, 28.3, 43.8, 46.1, 49.1, 50.5, 55.4, 61.4, 94.2, 105.9, 116.9 (d, $J_{CF} = 22.7$ Hz), 121.5, 122.1, 130.0 (d, $J_{CF} = 7.6$ Hz), 130.4 (d, $J_{CF} = 3.8$ Hz), 135.6, 146.2, 148.9, 150.5, 152.8, 157.6, 159.8, 160.5, 162.8 (d, $J_{CF} = 248.2$ Hz), 163.0, 170.9; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{35}H_{41}FN_9O_5^+$: 686.3209, found: 686.3193.

5.1.64. 3-(4-fluorophenyl)-1-methyl-N-(4-((6-(4-(4-methylpiperazin-1-yl)piperidine-1-carboxamido)pyrimidin-4-yl)oxy)phenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (33 h)

The title compound was prepared from **32b** using a method analogous to that described for **28a** in 76% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.42$–1.57 (m, 2H), 1.90 (d, $J = 12.2$ Hz, 2H), 2.24–2.32 (m, 4H), 2.44 (t, $J = 10.7$ Hz, 4H), 2.58 (br s, 4H), 2.91 (t, $J = 12.5$ Hz, 2H), 3.60 (s, 3H), 4.09 (d, $J = 12.8$ Hz, 2H), 7.09 (d, $J = 7.3$ Hz, 2H), 7.20–7.30 (m, 4H), 7.36 (s, 1H), 7.49 (s, 1H), 7.67 (d, $J = 7.3$ Hz, 2H), 8.35 (s, 1H), 8.60 (s, 1H), 10.73 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 28.3$, 38.2, 43.8, 46.1, 49.1, 55.4, 61.4, 94.1, 105.7, 117.0 (d, $J_{CF} = 22.7$ Hz), 121.6, 122.2, 130.0 (d, $J_{CF} = 8.8$ Hz), 130.1 (d, $J_{CF} = 3.8$ Hz), 135.5, 149.0, 150.6, 150.8, 152.8, 157.6, 159.6, 160.5, 162.9 (d, $J_{CF} = 249.5$ Hz), 163.5, 170.9; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{33}H_{37}FN_9O_5^+$: 658.2896, found: 658.2883.

5.1.65. 1-cyclopropyl-3-(4-fluorophenyl)-N-(4-((6-(4-(4-methylpiperazin-1-yl)piperidine-1-carboxamido)pyrimidin-4-yl)oxy)phenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (33i)

The title compound was prepared from **32c** using a method analogous to that described for **28a** in 76% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.99$–1.05 (m, 2H), 1.15–1.22 (m, 2H), 1.50 (qd, $J = 12.1$, 4.0 Hz, 2H), 1.90 (d, $J = 11.0$ Hz, 2H), 2.27 (s, 3H), 2.38–2.48 (m, 5H), 2.58 (br s, 4H), 2.85–2.98 (m, 2H), 3.30 (tt, $J = 7.3$, 3.7 Hz, 1H), 4.09 (d, $J = 13.5$ Hz, 2H), 7.08–7.10 (m, 2H), 7.20–7.27 (m, 4H), 7.33 (s, 1H), 7.49 (s, 1H), 7.66–7.69 (m, 2H), 8.35 (d, $J = 1.2$ Hz, 1H), 8.64 (s, 1H), 10.74 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 7.2$, 28.3, 33.3, 43.8, 46.1, 49.1, 55.4, 61.4, 94.2, 105.5, 116.9 (d, $J_{CF} = 22.7$ Hz), 121.5, 122.1, 129.3, 130.0 (d, $J_{CF} = 8.8$ Hz), 135.5, 148.9, 150.0, 151.2, 152.8, 157.6, 159.6, 160.5, 162.8 (d, $J_{CF} = 249.5$ Hz), 163.2, 170.9; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{35}H_{39}FN_9O_5^+$: 684.3053, found: 684.3037.

5.1.66. 1-cyclopentyl-3-(4-fluorophenyl)-N-(4-((6-(4-(4-methylpiperazin-1-yl)piperidine-1-carboxamido)pyrimidin-4-yl)oxy)phenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (33j)

The title compound was prepared from **32d** using a method analogous to that described for **28a** in 70.6% as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.50$ (qd, $J = 12.1$, 4.0 Hz, 2H),

1.74–1.83 (m, 4H), 1.88–1.95 (m, 4H), 2.19–2.23 (m, 2H), 2.27 (s, 3H), 2.36–2.52 (m, 5H), 2.58 (br s, 4H), 2.86–2.97 (m, 2H), 4.05–4.14 (m, 2H), 4.97 (quin, $J = 7.8$ Hz, 1H), 7.08–7.10 (m, 2H), 7.23 (d, $J = 2.5$ Hz, 4H), 7.34 (s, 1H), 7.49 (d, $J = 1.2$ Hz, 1H), 7.65–7.71 (m, 2H), 8.35 (s, 1H), 8.66 (s, 1H), 10.79 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 24.2$, 28.3, 31.9, 43.8, 46.1, 49.1, 55.4, 59.7, 61.4, 94.2, 105.8, 116.9 (d, $J_{CF} = 22.7$ Hz), 121.5, 122.1, 130.0 (d, $J_{CF} = 7.6$ Hz), 130.4 (d, $J_{CF} = 3.8$ Hz), 135.6, 147.0, 148.9, 150.8, 152.8, 157.6, 159.8, 160.5, 162.8 (d, $J_{CF} = 249.5$ Hz), 163.0, 170.9; HRMS–ESI *m/z* $[M+H]^+$ calcd. for $C_{37}H_{43}FN_9O_5^+$: 712.3366, found: 712.3349.

5.2. Biology

5.2.1. Cells and compounds

Recombinat human Axl-expressing or Mer-expressing mouse Ba/F3 cells were used for cell-based Axl inhibition assay (Carna Biosciences, Inc.), and cells were cultured in RPMI1640 (WAKO) containing 10% (v/v) FBS (Sigma-Aldrich), penicillin–streptomycin (WAKO). Cells were cultured at 37 °C under a 5% CO_2 atmosphere.

5.2.2. Cell free Axl/Mer assay

GST fused human Axl or Mer cytoplasmic domain was incubated with serial dilution of compound Km concentration of ATP, and respective substrates in appropriate buffer for reaction at room temperature for 1 h in <0.1% DMSO solution. For Axl kinase assay, after reaction with ADP-Glo™ kinase assay kit, activities of kinase were measured using luminometer. For Mer kinase assay, phosphorylation of biotinylated substrates was measured by TR-FRET system using Eu-labeled anti-phosphotyrosine antibody and APC-labeled streptavidin. All experiments were performed in duplicate wells for each condition (n = 2), and conducted once or twice independently. IC_{50} values were calculated from plots of concentration versus percent inhibition by using Microsoft Excel.

5.2.3. Cell based Ba/F3 Axl/Mer assay

Transformation of Ba/F3 cells required the kinase activity of the TEL-Axl fusion or TEL-Mer fusionprotein. If a compound inhibits the activity of the activity of the recombinant kinase, the proliferation of transformed cells will suppress. Ba/F3-Axl or -Mer cells (500 cells) were seeded in a 384-well cell culture plate in RPMI1640 (WAKO) containing 10% (v/v) FBS (Sigma-Aldrich), 1% penicillin–streptomycin (WAKO). And, various concentrations of compounds were added, and the cells were incubated at 37 °C for 2 days in culture medium containing 0.1% DMSO. Then, Cell Titer-Glo-2.0 (Promega) was added to each well, viable cell number was measured with a EnVision or ARVO (Perkin Elmer) microplate reader. All experiments were performed in quadruplicate wells for each condition (n = 4), and conducted once or twice independently. IC_{50} values were calculated from plots of concentration versus percent inhibition by using Microsoft Excel.

5.2.4. Solubility assay

Kinetic solubility was determined in Dulbecco's phosphate-buffered saline (pH 7, Invitrogen Corporation) containing 1% (v/v) DMSO. Firstly, DMSO stock solution was prepared at the concentration of 10 mM. The stock solution was diluted by 100 times with test fluid and then stirred for 15 min at room temperature. After vacuum suction filtration, the concentration of the filtrate was measured by using HPLC-UV method (wavelength: 254 nm).

5.2.5. Microsomal stability screening in liver microsomes

Test articles were incubated with mouse liver microsomes (0.5 mg/mL) for 30 min at 37 °C in the reaction mixture containing 0.1 M potassium phosphate (pH 7.4), 0.1 mM EDTA, 0.33 mM β-$NADP^+$, 8 mM glucose-6-phosphate, 0.1 unit/mL glucose-6-phosphate dehydrogenase, and 6 mM $MgCl_2$. After adding acetonitrile/methanol (7:3, v/v) mixture containing propranolol as the internal standard to the reaction mixture, the resulting supernatant was subjected to an LC-MS/MS analysis. The ratio of peak area responses relative to the internal standard was determined, and the residual ratio of the test articles in the presence of NADPH relative to that in the absence of NADPH was calculated.

5.2.6. Evaluation of pharmacokinetic profile in mice

Compound **33g** was formulated for oral administrations at 10, 30, and 100 mg/kg in DMSO/Tween80/5% glucose (7:13:80, v/v/v) and administered to female CAnN.Cg-Foxn1nu/CrlCrlj mice. After administration, blood was collected from retinal vein at 0.5, 1, 2, 8, and 24 h postdose and centrifuged to obtain plasma samples. Plasma concentrations of compound **33g** were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Plasma samples were precipitated with 20 volumes of acetonitrile/methanol (7:3, v/v) containing internal standards (10 ng/mL propranolol and 500 ng/mL niflumic acid). Following vortex mixing and centrifugation, the supernatant was filtered, and the resulting filtrate was injected into an LC-MS/MS. For samples above the upper limit of quantification, the resulting filtrate was 100-fold diluted with 50% acetonitrile and subjected to an LC-MS/MS analysis. Detection was accomplished by multiple reaction monitoring in positive ionization mode. The ratio of the peak area responses relative to the internal standard were used to construct a standard curve using linear least squares regression with a $1/x^2$ weighting. PK parameters, maximum drug concentration (C_{max}), area under the concentration–time curve ($AUC_{(0\text{-}24h)}$) from zero to 24 h postdose, and time to reach maximum (peak) concentration following drug administration were determined based on the mean plasma concentration.

5.2.7. Ba/F3-Axl isogenic subcutaneous model

Mice were maintained under specific pathogen-free conditions and housed in barrier facilities on a 12-hour light/dark cycle, with food and water ad libitum. Animal experiments were conducted in accordance with the Institutional Animal Care and Use Committee guidelines of Eisai Co., Ltd.

Cultured Ba/F3-Axl cells were prepared in Hank's balanced salt solution (Thermo Fisher Scientific Inc.) to yield a suspension of 2.0×10^6 cells/mL. A 0.1 mL aliquot of the cell suspension was transplanted subcutaneously into the right flank region of female C3H mice (7 weeks old, CLEA Japan or Japan SLC). When tumor volumes reached around 200 mm^3, mice were selected on the basis of their tumor volumes and general condition, and were randomly divided into groups according to their tumor volumes (n = 5 per group). The oral administration was started on day 0, and the administration continued once daily for 4 days. The differences in tumor volume between the non-treated and **33g**-treated groups were analyzed by one-way ANOVA followed by the Dunnett's multiple comparison test. A value of $P < 0.05$ (two-sided) was considered statistically significant. Statistical analyses were performed using GraphPad Prism.

5.2.8. In vivo study in mice to detect the retinal toxicity

28a and **33g** were orally administrated once a daily for 14 days to female BALB/cAJcl mice (n = 3 or 5/group) at 30 and 100 mg/kg or 100 mg/kg, respectively. Control animals were not treated during the dosing period. All animals were sacrificed according to the guideline for animal experiment of Eisai Co., Ltd. and necropsied after a 14-day dosing period. Eyes collected from all animals were fixed in a mixture of 3% glutaraldehyde and 2.5% formalin. Tissues were embedded in paraffin, sectioned and stained with hematoxylin-eosin (HE) slide and were submitted for histopathologic examination.

5.2.9. Statistical analysis

All data are presented as means ± SD. The differences between the means of groups were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. P values less than 0.05 were considered statistically significant. Statistical analyses were performed using GraphPad Prism version 8.3.1.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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