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N-Phenyl-*N*′-[4-(5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yloxy)phenyl]ureas as novel inhibitors of VEGFR and FGFR kinases

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ABSTRACT

We have recently reported the discovery of pyrrolo[3,2-*d*]pyrimidine derivatives **1a** and **1b** as potent triple inhibitors of vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), and Tie-2 kinases. To identify compounds having strong inhibitory activity against fibroblast growth factor receptor (FGFR) kinase, further modification was conducted using the co-crystal structure analysis of VEGFR2 and **1b**. Among the compounds synthesized, urea derivative **11l** having a piperazine moiety on the terminal benzene ring showed strong inhibitory activity against FGFR1 kinase as well as VEGFR2 kinase. A binding model of **11l** complexed with VEGFR2 suggested that the piperazine moiety forms additional interactions with Ile1025 and His1026.

1. Introduction

Angiogenesis, the formation of new blood vessels from preexisting vasculature, plays a critical role in the growth of tumors by supplying them with oxygen and nutrients.[1] Angiogenesis occurs in different cell types and is modulated by angiogenic factors such as members of the vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) families. VEGF, probably the most studied angiogenic factor, is upregulated by cancer-related changes, including proto-oncogene activation,[2] loss of tumor suppressor function,[3,4] growth factor stimuli,[5] and hypoxic status.[6,7] The biological effects of VEGF are mediated by three high-affinity tyrosine kinases, FLT1 (vascular endothelial growth factor receptor 1 (VEGFR1)), KDR (VEGFR2), and FLT4 (VEGFR3), which are specifically expressed on vascular endothelial cells or lymphendothelial cells.[8–10] Binding of VEGF to VEGFR induces conformational changes within VEGFR followed by receptor dimerization, autophosphorylation of tyrosine residues in the intracellular kinase domain, and potent mitogenic and chemotactic effects on endothelial cells.[11] Overexpression of VEGF correlates with poor prognosis and clinical staging in the majority of patients with solid tumors.[12–14] VEGF/VEGFR signaling is, therefore, regarded as an attractive therapeutic target for the inhibition of tumor angiogenesis. Bevacizumab, a recombinant humanized monoclonal antibody against VEGF, has been approved as first-line therapy for various conditions such as metastatic colorectal cancer.[15–17]

FGF, especially basic FGF (bFGF), is also a potent mitogen for vascular endothelial cells, which binds to specific FGF receptors (FGFR), including FGFR1 and has intrinsic tyrosine kinase activity.[18] On the other hand, gene amplification, and activated mutation or aberrant overexpression of FGFR family members are also described in a variety of cancers such as classic lobular carcinomas (CLC),[19] multiple myeloma (MM),[20] and breast cancer.[21] Therefore, inhibition of FGF-FGFR signaling pathways leads to potent antitumor activity through angiogenesis inhibition[22–24] or direct suppression of the growth of the FGF-dependent cancer cells. Several FGFR kinase inhibitors such as BIBF 1120,[25] TKI258,[26] and E7080[27] are under clinical trials.

We previously discovered a series of urea-containing pyrrolo[3,2-*d*] pyrimidine derivatives (e.g., **1a**,**b** in Fig. 1) as potent angiogenesis-related kinase inhibitors.[28] Representative compound **1a** exhibited inhibitory activities against VEGFR, PDGFR, and Tie-2 with IC_{50} values of <100 nM. However, **1a** was a weak inhibitor of FGFR (FGFR1 IC_{50} >10,000 nM). Since FGF-FGFR signaling has been described as a potential escape mechanism for tumors to anti-VEGF treatment,[29] we sought to develop compounds that would inhibit both VEGFR and FGFR to avoid the development of resistance over long-term treatment. In addition, these compounds will have to be more soluble than **1a** and **1b**, which have poor aqueous solubility. To address these concerns, we started to research a new type of compound.

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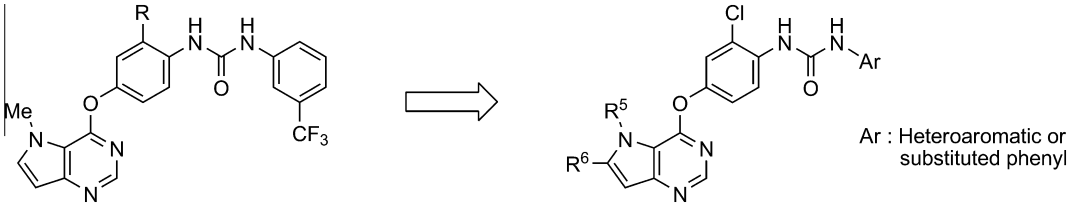


Figure 1. Design of compound (**I**) from pyrrolo[3,2-*d*]pyrimidine inhibitors **1a** and **1b**.

2. Design

The crystal structure of the complex between **1b** and VEGFR2 revealed **1b** was a so-called type-II kinase inhibitor (Fig. 2): **1b** induced an inactive conformation (DFG-out) of the kinase so that the urea portion occupies the back hydrophobic pocket with additional hydrogen-bonding interactions.[30] When **1b** is bound to VEGFR2, it forms hydrogen bonds with the backbone-NH of Cys919, side chain carboxylate of Glu885, and backbone-NH of Asp1046. The terminal phenyl moiety occupies the hydrophobic pocket created by rearrangement of the protein.

Analysis of the X-ray structure also suggested sufficient space was available for the introduction of various substituents around the terminal benzene ring and around the 5- or 6-position on the pyrrolo[3,2-*d*]pyrimidine core. Since this space was observed in the co-crystal structures of several kinases with their type-II inhibitors in common (e.g., imatinib/Bcr-Abl[31]), we hypothesized FGFR also might have similar space in this region although no structure of FGFR in complex with its type-II inhibitor has been solved. In addition, several amino acid residues which may favorably interact with polar substituents are present in this region. These amino acid residues are conserved between VEGFR2 and FGFR1. For instance, Asn923 (corresponding to Asn568 in FGFR1) was observed near the 5- or 6-position on the pyrrolo[3,2-*d*]pyrimidine core. Backbone-CO of Ile1025 (corresponding to Ile620 in FGFR1) and backbone-CO of His1026 (corresponding to His621 in FGFR1) were also found around the 4′- or 5′-position on the terminal benzene ring. Thus, these moieties appeared to be appropriate for modification in order to improve solubility as well as to enhance potency against FGFR1. On the basis of the above hypothesis, we designed and synthesized compounds (**I**) (Fig. 1). The major modifications pursued were as follows: (1) replacement of the external benzene ring with a polar heteroaromatic ring, aiming the interaction with backbone-CO of Ile and His through water molecule; (2) introduction of polar heteroatom-based substituents on the external benzene ring or pyrrolo[3,2-*d*]pyrimidine scaffold, aiming the direct interaction with Asn, Ile, and His. We also reasoned that the increased polarity by the introduction of heteroatom would likely make these compounds more soluble than **1a** and **1b**. In this paper, we report the synthesis, structure–activity relationships (SAR), and characterization of these new inhibitors.

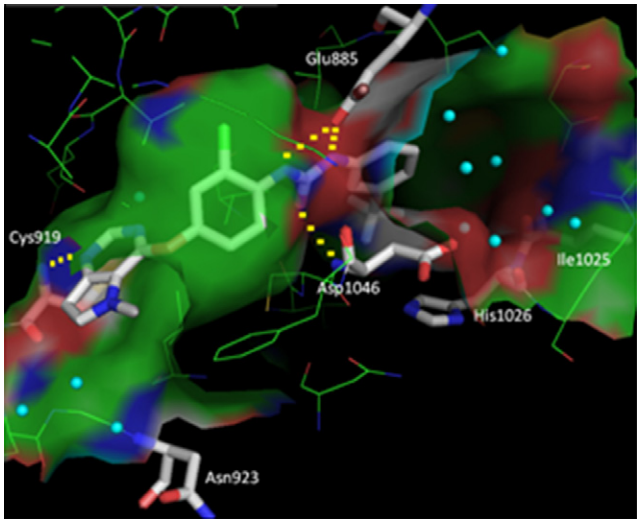


Figure 2. X-ray co-crystal structure of **1b** in complex with VEGFR2.

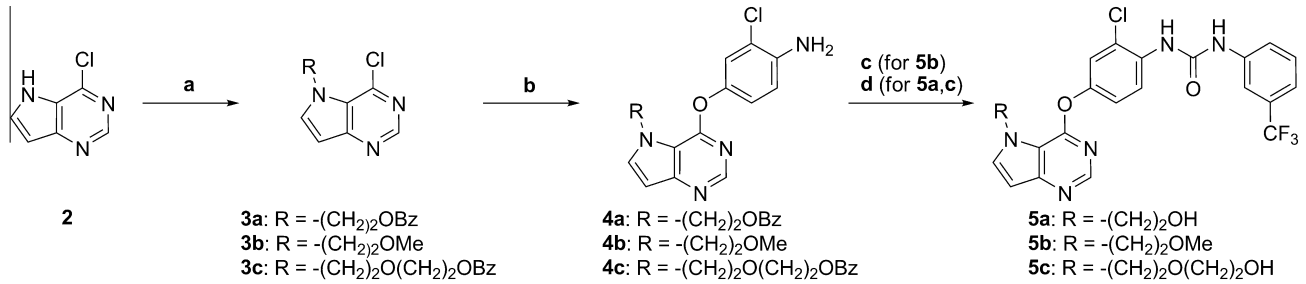
3. Chemistry

A general synthesis of C5-substituted pyrrolo[3,2-*d*]pyrimidine derivatives is shown in Scheme 1. Alkylation of 5*H*-pyrrolo[3,2-*d*]pyrimidine **2**[32,33] with the corresponding halides or tosylates gave **3a**–**c**, which were coupled with 3-chloro-4-aminophenol in the presence of K_2CO_3 to provide anilines **4a**–**c**. The anilines **4b** was converted to the corresponding urea derivative **5b** by reaction with 3-(trifluoromethyl)phenyl isocyanate, respectively. The aniline derivatives possessing benzoyl groups (**4a**,**c**) were also treated with 3-(trifluoromethyl)phenyl isocyanate followed by hydrolysis under basic conditions to provide the ureas **5a** and **5c**, respectively.

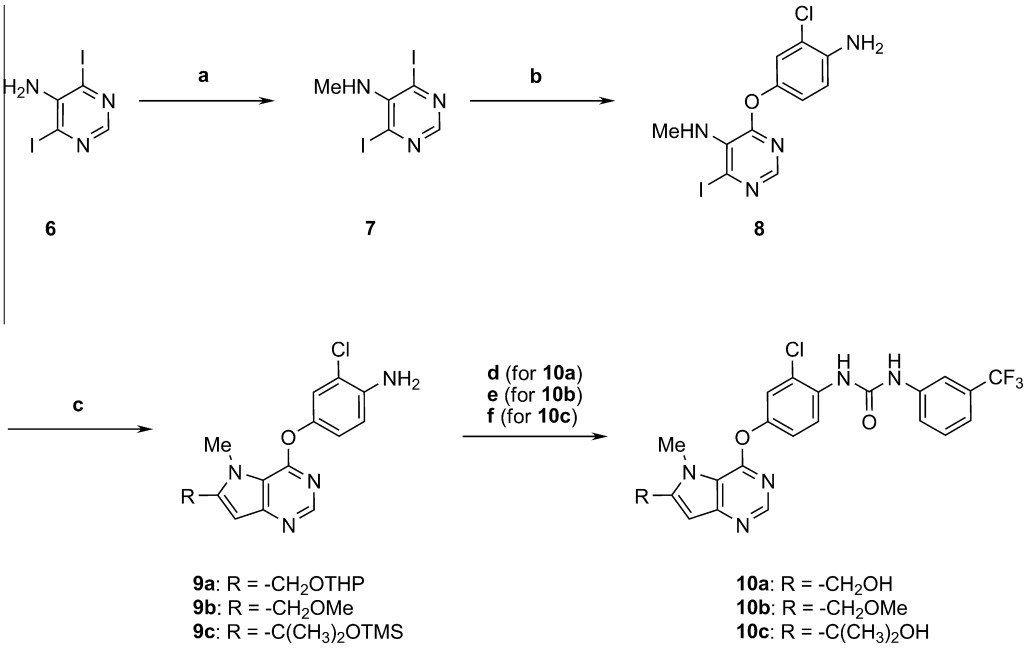
The synthesis of 6-substituted pyrrolo[3,2-*d*]pyrimidines was conducted as outlined in Scheme 2. Treatment of 5-aminopyrimidine **6** with methyl methanesulfonate selectively gave the 5-monomethylaminopyrimidine derivative **7**, which was coupled with 3-chloro-4-aminophenol to provide **8**. Sonogashira coupling of **8** with alkynes in the presence of $Pd(PPh_3)_2Cl_2$ and CuI followed by cyclization using CuI as catalyst to give **9a–c**, respectively.[34] The aniline **9b** was converted to the corresponding urea derivative **10b** in a manner similar to the synthesis of **5b**. The anilines possessing a tetrahydropyranyl (THP) group (**9a**) or trimethylsilyl (TMS) group (**9c**) were also converted to the corresponding ureas followed by deprotection to provide **10a** and **10c**, respectively.

The urea derivatives **11a**–**p**, **12a**,**b**, and **14** were synthesized as shown in Scheme 3. The ureas **11a**–**p** were synthesized from **4d**[28] and the corresponding amines using phenyl chlorocarbonate or triphosgene. The reaction of **4d** with carbamates **15a**,**b** gave **12a**,**b**, respectively. In addition, conversion of carboxylic acid **13** to the corresponding isocyanate using diphenylphosphoryl azide (DPPA) followed by treatment with **4d** in a one-pot manner provided **14**.

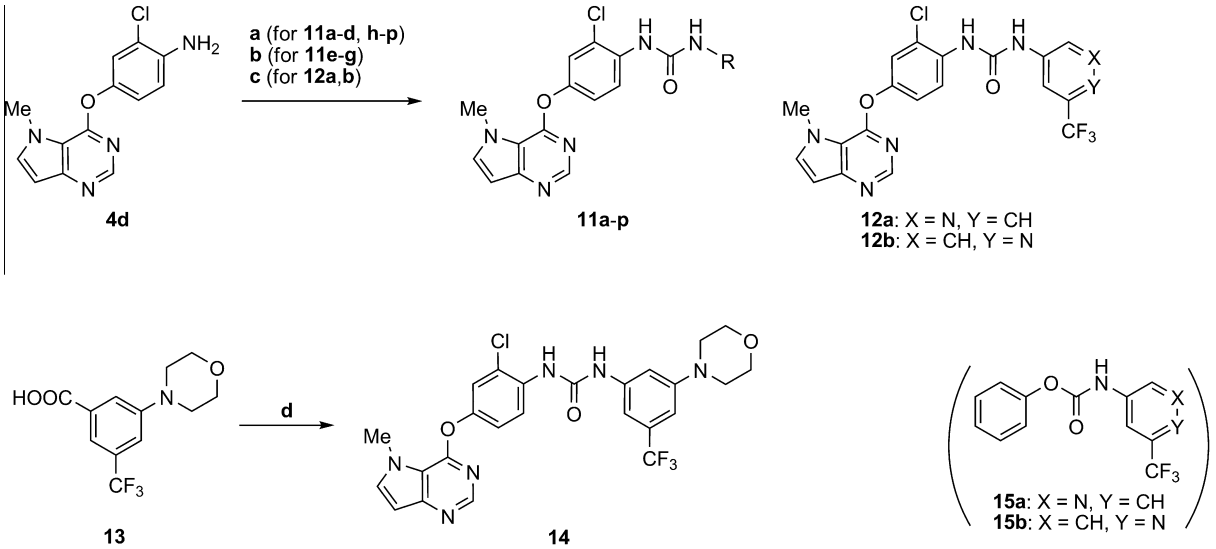
The required amine intermediates for the above synthesis were prepared as shown in Schemes 4 and 5. Anilines **17** and **18** were prepared from **16** by substitution of the fluorine with morpholine or 1-methylpiperidin-4-ol followed by hydrogenation using Pd/C. Anilines **21a**–**d** were similarly prepared by condensation of carboxylic acids **19a**,**b** with morpholine or 1-methylpiperazine followed by reduction using borane–dimethylsulfide complex.



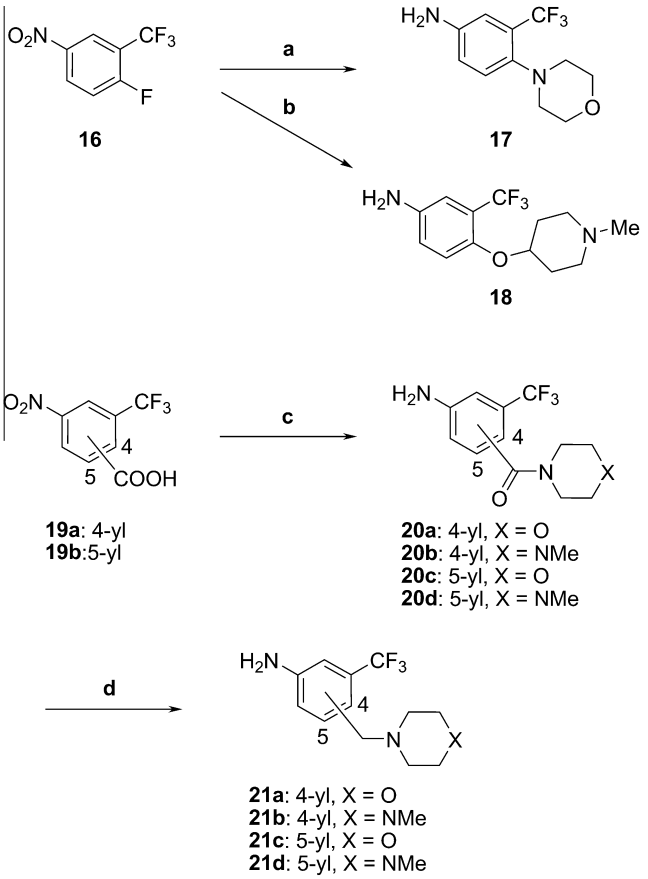
Scheme 1. Reagents and conditions: (a) R-X, Cs_2CO_3, DMF, rt; (b) 3-chloro-4-aminophenol, K_2CO_3, NMP, 120 °C; (c) 3-(trifluoromethyl)phenyl isocyanate, Et_3N, THF, rt; (d) (1) 3-(trifluoromethyl)phenyl isocyanate, Et_3N, THF, rt, (2) NaOH, H_2O/MeOH, rt.



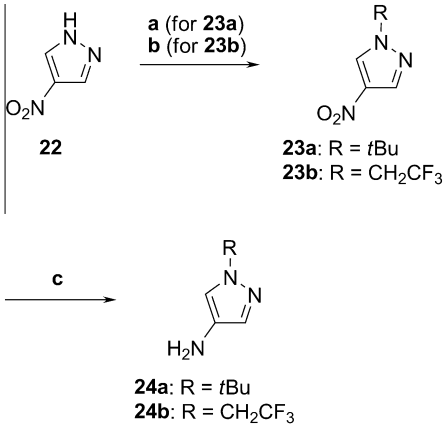
Scheme 2. Reagents and conditions: (a) MeOMs, NaH, DMF, 0 °C → rt; (b) 3-chloro-4-aminophenol, K_2CO_3, NMP, 100 °C; (c) (1) alkynes, $Pd(PPh_3)_2Cl_2$, CuI, CH_3CN, Et_3N, 60 °C, (2) CuI, DMF, 80 °C; (d) (1) 3-(trifluoromethyl)phenyl isocyanate, Et_3N, THF, rt, (2) *p*-TsOH, EtOH, rt; (e) 3-(trifluoromethyl)phenyl isocyanate, Et_3N, THF, rt; (f) (1) 3-(trifluoromethyl)phenyl isocyanate, Et_3N, THF, rt, (2) 1 M HCl aq, rt.



Scheme 3. Reagents and conditions: (a) amines, PhOCOCl, pyridine, DMA, 0 °C → 90 °C; (b) amines, triphosgene, Et_3N, THF, 0 °C → rt; (c) **15a** or **b**, pyridine, NMP, 90 °C; (d) (1) DPPA, Et_3N, toluene, reflux, (2) **4d**, rt.



Scheme 4. Reagents and conditions: (a) (1) morpholine, DMSO, 100 °C, (2) H_2, Pd/C, MeOH, rt; (b) (1) 1-methylpiperidin-4-ol, NaH, DMF, 0 °C → 100 °C, (2) H_2, Pd/C, MeOH, rt; (c) (1) $(COCl)_2$, DMF, CH_2Cl_2, 0 °C → rt, (2) morpholine or 1-methylpiperazine, CH_2Cl_2, 0 °C → rt, (3) H_2, Pd/C, MeOH, rt; (d) $Me_2S{\cdot}BH_3$, THF, 0 °C → rt.



Scheme 5. Reagents and conditions: (a) 2-bromo-2-methylpropane, K_2CO_3, DMF, 80 °C; (b) CF_3CH_2OTf, K_2CO_3, DMF, 80 °C; (c) H_2, Pd/C, MeOH, rt.

Alkylation of 3-nitropyrazole **22** with 2-bromo-2-methylpropane or 2,2,2-trifluoroethyl tosylate followed by hydrogenation of the nitro group gave **24a**,**b**, respectively.

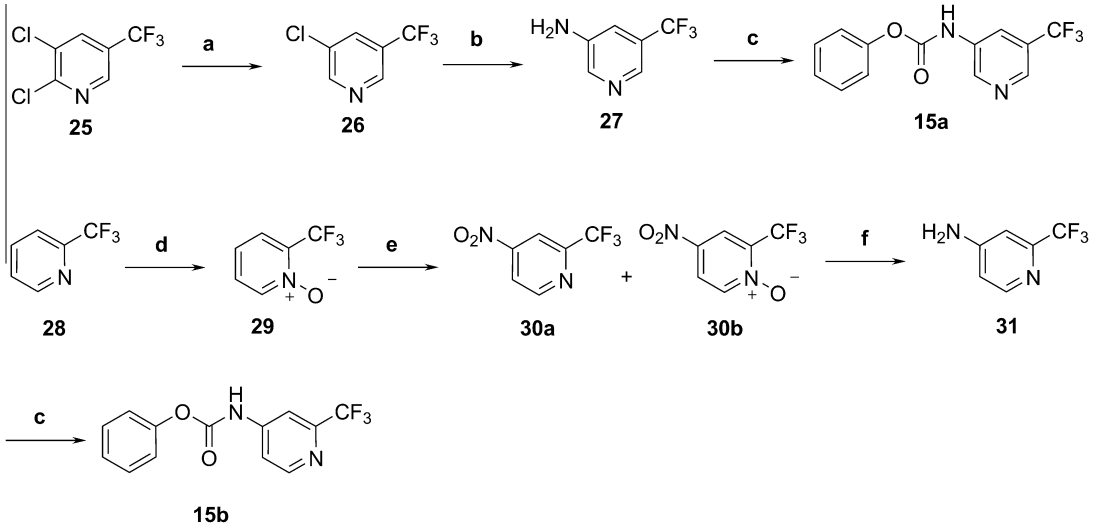
Carbamates **15a**,**b** were prepared as shown in Scheme 6. Selective dechlorination of **25** using Pd/C in the presence of ammonium formate gave 3-chloropyridine **26**, the chlorine of which was substituted with an amino group by treatment with aqueous ammonia and CuCl to yield **27**.[35] Acylation of **27** using phenyl chlorocarbonate provided the carbamate **15a**. Oxidation of **28** using *m*-chloroperbenzoic acid (*m*CPBA) followed by nitration provided a mixture of **30a** and **30b**, which was converted to 4-aminopyridine **31** by hydrogenation. Acylation of **31** with phenyl chlorocarbonate provided the carbamate **15b**.

4. Results and discussion

The pyrrolo[3,2-*d*]pyrimidine derivatives shown in Tables 1–3 were evaluated for their inhibitory activities against human VEGFR2 and FGFR1 kinases by a non-RI assay using the amplified luminescent proximity homogeneous assay (AlphaScreen®) system. AlphaScreen® is based on the transfer of energy from donor to acceptor microbeads brought together by a biomolecular interaction.[36] In this system, an anti-phosphotyrosine antibody is immobilized with acceptor beads, and the biotinylated poly-GluTyr (4:1) is conjugated with streptavidin donor beads. The amount of phosphorylated substrate is measured as a signal of AlphaScreen®.

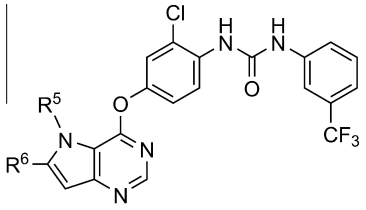
Starting from **1b**, we initially explored the SAR of substituents at the 5- and 6-positions of the pyrrolo[3,2-*d*]pyrimidine core (Table 1). As the crystallography data suggested (Fig. 2), even the introduction of larger substituents at the 5- and 6-positions (**5a**–**c**, **10a**–**c**) was well tolerated as far as VEGFR2 kinase inhibition is concerned. The C5 substituents possessing a hydroxy group (**5a**,**c**) exhibited stronger activity than that of the corresponding non-hydroxy substituents (**5b**). As we expected, these hydroxy groups may form additional interactions with Asn923. On the other hand, hydroxy-containing substituents did not enhance FGFR1 kinase inhibitory activities, indicating those substituents might not interact with Asn568 in FGFR1. As for the 6-position, larger substituents generally had lower potencies against VEGFR2 kinase, and no improvement in FGFR1 kinase inhibition. While the introduction of a hydroxymethyl group (**10a**) gave single-digit nanomolar IC_{50} value (VEGFR2 IC_{50} = 9.7 nM), the introduction of methoxymethyl (**10b**) or 2-hydroxypropan-2-yl (**10c**) groups resulted in derivatives that are about twofold less active (VEGFR2 IC_{50} = 14 nM for **10b**, 18 nM for **10c**).

Further, we replaced the benzene ring of **1b** with a variety of heterocyclic rings (Table 2). To determine the appropriate positions for the introduction of heteroatoms, we first synthesized the 2-, 3-, or 4-pyridine derivatives **11a**–**c**. The 2- or 3-pyridylurea derivatives **11a**,**b** (IC_{50} = 210 nM for **11a**, 230 nM for **11b**) showed stronger VEGFR2 kinase inhibitory activities than 4-pyridylurea derivative **11c** (IC_{50} = 650 nM), indicating that 2- or 3-position as optimal for VEGFR2 kinase inhibition. In contrast, essentially no difference in VEGFR2 kinase inhibitory potency was seen among the pyridylurea derivatives possessing a trifluoromethyl group (**11d**, **12a**,**b**), regardless of the position of pyridyl nitrogen. The compounds **11d** and **12a**,**b** all showed single-digit nanomolar IC_{50} values against VEGFR2 kinase. The activity gains found upon the introduction of a trifluoromethyl group into the pyridine ring were similar to those seen for derivatives containing a terminal benzene ring. As indicated by the crystal structure in Figure 2, the incorporation of lipophilic groups into the terminal aryl moiety may enhance VEGFR2 kinase inhibitory activity by occupying the small lipophilic cleft. On the other hand, replacement of benzene ring (**1b**) with pyridine ring (**11d** and **12a**,**b**) did not enhance inhibitory activity against FGFR1 kinase, suggesting effective water-mediated interaction between the pyridine nitrogen and the target amino acid residues such as Ile620 and His621 might not be formed. These observations moved our research to explore five-membered rings possessing lipophilic groups. As we expected, the azole derivatives **11e**–**h** thus synthesized retained strong VEGFR2 kinase inhibitory activity despite the change in terminal ring system. Interestingly, replacement of the benzene ring with an azole ring also seemed to affect the kinase selectivity. The screening of these compounds against FGFR1 kinase revealed that isoxaz-



Scheme 6. Reagents and conditions: (a) $HCOONH_4$, Pd/C, MeOH, rt; (b) aq NH_3, CuCl, 170 °C; (c) PhOCOCl, pyridine, THF, 0 °C → rt; (d) *m*CPBA, CH_2Cl_2, rt; (e) HNO_3, H_2SO_4, 0 °C → 130 °C; (f) H_2, Pd/C, MeOH, rt.

Table 1
Kinase inhibitory activity of compounds modified at the 5- and 6-positions[a]



Compd	R^5	R^6	VEGFR2 IC_{50} (nM)	FGFR1 IC_{50} (nM)
lb	Me	H	3.7 (3.4–4.1)	>10,000
5a	$(CH_2)_2OH$	H	4.0 (3.5–4.7)	>10,000
5b	$(CH_2)_2OCH_3$	H	9.0 (7.1–11)	2700 (2300–3100)
5c	$(CH_2)_2O(CH_2)_2OH$	H	2.7 (2.5–3.0)	6500 (4900–8700)
10a	Me	CH_2OH	9.7 (8.9–11)	>10,000
10b	Me	CH_2OCH_3	14 (12–17)	>10,000
10c	Me	$C(CH_3)_2OH$	18 (15–22)	>10,000

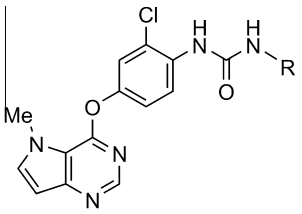
[a] Numbers in parentheses represent 95% confidence interval.

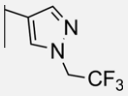
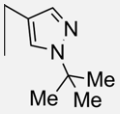
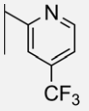
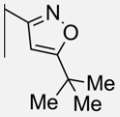
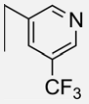
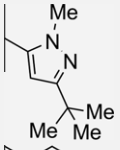
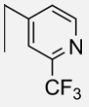
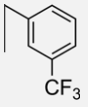
ole **11g** and the pyrazole **11h** showed substantial activity gains against FGFR1 kinase (FGFR1 IC_{50} = 220 nM for **11g**, 260 nM for **11h**). Further investigation revealed that FGFR1 kinase inhibition was depend on the nature of the ring system as well as the incorporated lipophilic substituent. Replacement of the isoxazole ring (**11g**) with pyrazole (**11f**) resulted in a fourfold loss of potency (IC_{50} = 940 nM for **11f**), while replacement of the *tert*-butyl group (**11f**) with trifluoroethyl (**11e**) brought a further drop in potency (IC_{50} >10,000 nM for **11e**). These results implied that adjustment of kinase selectivity may be achieved by further modification of the terminal aryl moiety.

In further studies, we also investigated the substituents at the 4′- and 5′-positions of the terminal benzene ring (Table 3). The initial substituent we examined was the methoxy group (**11i**,**n**), and the compounds thus synthesized retained strong VEGFR2 kinase inhibitory activities. These compounds, however, did not show enhanced FGFR1 kinase inhibition, indicating that the distance between the methoxy group and target amino acid residues was too far to interact. Subsequently, we found that the 4′- and 5′-positions were able to tolerate substantially large substituents and some of them affected kinase selectivity. As for VEGFR2 kinase inhibition, introduction of a morpholine group via a methylene linker (**11k**,**o**) was preferable for its direct attachment to the benzene ring (**11j**, **14**). Moreover, replacement of the morpholine moiety (**11k**,**o**) with the *N*-methylpiperazine moiety (**11l**,**p**) increased activity, indicating that the terminal amino groups may form additional interactions with VEGFR2. We found that 4′-(4″-methylpiperazin-1″-yl)methyl derivative **11l** strongly inhibited FGFR1 kinase with an IC_{50} value of 14 nM. The corresponding morpholine derivative **11k** showed much weaker FGFR1 kinase inhibition (IC_{50} = 4500 nM), revealing the importance of the terminal piperazine nitrogen for interaction with FGFR1. Replacement of the methylene linker of **11l** with an oxygen linker (**11m**) or moving the 4″-methylpiperazin-1″-ylmethyl group from the 4′-position (**11l**) to the 5′-position (**11p**) also resulted in 10-fold loss of potency (FGFR1 IC_{50} = 270 nM for **11m**, 390 nM for **11p**), although these modifications did not affect VEGFR2 kinase inhibition. The binding affinity for FGFR1 was much more sensitive to the nature of the benzene substituent than is observed for VEGFR2. One possible explanation for this is that the required interactions for stabilization of a compound-FGFR1 complex may be different from those of a compound-VEGFR2 complex. The SAR results suggested the interaction between the 4′-(4″-methylpiperazin-1″-yl)methyl group of **11l** and the target amino acid residues in FGFR1 might significantly contribute to stabilize a complex of **11l**-FGFR1 although the similar interaction might not be necessarily required for stabilization of a compound-VEGFR2 complex.

We selected compound **11l** for further profiling. As shown in Table 4, compounds **1b** and **11l** were evaluated for their ability to inhibit VEGF- or FGF-stimulated proliferation of human umbilical vein endothelial cells (HUVEC). The compound **11l** showed strong inhibitory activities against both VEGF- and FGF-stimulated HUVEC proliferation with IC_{50} values of 38 nM and 20 nM, respectively. In contrast, the potency of **1b** was 20-fold lesser in FGF-stimulated HUVEC proliferation (IC_{50} = 230 nM) than in VEGF-stimulated HUVEC proliferation (IC_{50} = 13 nM), probably due to its weak FGFR kinase inhibitory potency. A brief study on the aqueous solubility of **1b** and **11l** was also conducted. The measured solubilities for **1b** and **11l** in aqueous neutral solution (Japanese Pharmacopoeia second fluid (JP2), pH 6.8) were 0.09 μg/mL and 19 μg/mL, respectively. As expected, introduction of the piperazine group led to an improvement in aqueous solubility.

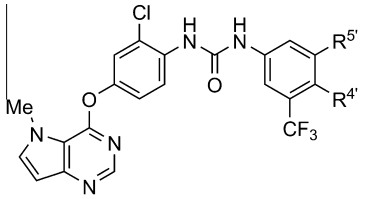
Table 2
Effect of modifications to the terminal heteroaromatic ring on kinase inhibitory activity[a]

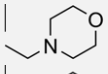
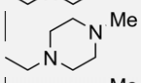
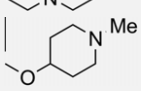
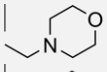
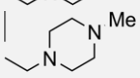


Compd	R	VEGFR2 IC_{50} (nM)	FGFR1 IC_{50} (nM)	Compd	R	VEGFR2 IC_{50} (nM)	FGFR1 IC_{50} (nM)
11a		210 (79–580)	>10,000	**11e**		4.0 (3.4–4.8)	>10,000
11b		230 (190–270)	>10,000	**11f**		7.6 (6.3–9.2)	940 (770–1100)
11c		650 (510–840)	>10,000				
11d		2.7 (2.2–3.3)	>10,000	**11g**		8.9 (7.0–11)	220 (210–240)
12a		2.9 (2.6–3.2)	>10,000	**11h**		19 (16–21)	260 (230–280)
12b		3.8 (3.2–4.6)	>10,000	**1b**		3.7 (3.4–4.1)	>10,000

[a] Numbers in parentheses represent 95% confidence interval.

Table 3
Effect of substitutions at the terminal benzene ring on kinase inhibitory activity[a]



Compd	$R^{4'}$	$R^{5'}$	VEGFR2 IC_{50} (nM)	FGFR1 IC_{50} (nM)
lb	H	H	3.7 (3.4–4.1)	>10,000
11i	OMe	H	9.4 (7.9–11)	>10,000
11j		H	61 (53–71)	610 (500–740)
11k		H	19 (16–22)	4500 (3200–6200)
11l		H	9.3 (8.3–10)	14 (12–16)
11m		H	12 (10–14)	270 (240–290)
11n	H	OMe	20 (18–22)	>10,000
14	H		60 (45–80)	>10,000
11o	H		23 (20–28)	>10,000
11p	H		11 (10–13)	390 (320–480)

[a] Numbers in parentheses represent 95% confidence interval.

Table 4
Activity and solubility of **1b** and **11l**[a]

Compd	IC_{50} (nM)				Solubility JP2[c](μg/mL)
	Kinase inhibition		Growth inhibition of HUVEC[b]		
	VEGFR2	FGFR1	VEGF	FGF	
lb	3.7 (3.4–4.1)	>10,000	13 (8.6–20)	230 (160–350)	0.09
11l	9.0 (8.3–10)	14 (12–16)	38 (30–48)	20 (16–26)	19

[a] Numbers in parentheses represent 95% confidence interval.
[b] Growth inhibition of human umbilical vein endothelial cells (HUVEC).
[c] Measured at pH 6.8 (Japanese Pharmacopoeia second fluid).

A binding model of compound **11l** and VEGFR2 was developed using the co-crystal structure of **1b** and VEGFR2. Figure 3 depicts the proposed binding mode of **11l** in the back hydrophobic pocket and the surrounding region. The piperazine moiety is oriented towards the protein exterior, and the terminal nitrogen forms hydrogen-bonding interactions with the backbone carbonyls of Ile1025 and His1026. This observation was consistent with the experimental SAR results in which replacement of the external nitrogen (**11l**) with oxygen (**11k**) led to a twofold loss of potency. These Ile and His residues are known to be conserved among several kinases. Indeed, it was reported the piperazine moiety of imatinib form hydrogen bonding with the carbonyls of Ile360 (corresponding to Ile1025 in VEGFR2) and His361 (corresponding to Ile1026 in VEGFR2) of Bcr-Abl.[29] Since FGFR1 also conserves Ile and His in this region (Ile620 and His621 in FGFR1), **11l** might have exhibited strong potency against FGFR1 kinase by the similar interaction.

5. Conclusion

On the basis of the information derived from co-crystal structure analysis of VEGFR2 and **1b**, a series of pyrrolo[3,2-*d*]pyrimidine derivatives was designed and synthesized with the goal of obtaining strong inhibitory activity against FGFR kinase and improved solubility. As a result, the urea **11l**, possessing a piperazine moiety on the terminal benzene ring, strongly inhibited FGFR1 kinase while retaining potent VEGFR2 kinase inhibitory activity. Further evaluation revealed that **11l** showed strong inhibitory activities against both VEGF- and FGF-stimulated HUVEC proliferation and improved solubility. Since FGF-FGFR signaling has been shown to not only influence tumor angiogenesis but also directly contribute to tumor growth and survival, simultaneous inhibition of these receptor tyrosine kinases by **11l** may enhance antitumor activity.

6. Experimental

Melting points were determined on a BÜCHI Melting Point B-545, and were not corrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Varian Mercury 300 (300 MHz) or Bruker DPX300 (300 MHz) instruments. Chemical shifts are reported as δ values (ppm) downfield from internal tetramethylsilane of the indicated organic solution. Peak multiplicities are expressed as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; ddd, doublet of doublet of doublets; dt, doublet of triplet; br s, broad singlet; m, multiplet. Coupling constants (J values) are given in hertz (Hz). Elemental analyzes were carried out by Takeda Analytical Laboratories. Reaction progress was determined by thin layer chromatography (TLC) analysis on silica gel 60 F254 plate (Merck) or NH TLC plates (Fuji Silysia Chemical Ltd). Chromatographic purification was carried on silica gel 60 (0.063–0.200 mm or 0.040–0.063 mm, Merck), basic silica gel (Chromatorex-NH, 100–200 mesh, Fuji Silysia Chemical Ltd) or Purif-Pack (SI 60 lM or NH 60 lM, Fuji Silysia Chemical Ltd). Commercial reagents and solvents were used without additional purification. Abbreviations are used as follows: $CDCl_3$, deuterated chloroform; DMSO-d_6, dimethyl sulfoxide-d_6; AcOEt, ethyl acetate; DMF, *N*,*N*-dimethylformamide; MeOH, methanol; THF, tetrahydrofuran; EtOH, ethanol; DMSO, dimethyl sulfoxide; NMP, *N*-methylpyrrolidone; IPE, diisopropyl ether; DMA, *N*,*N*-dimethylacetamide.

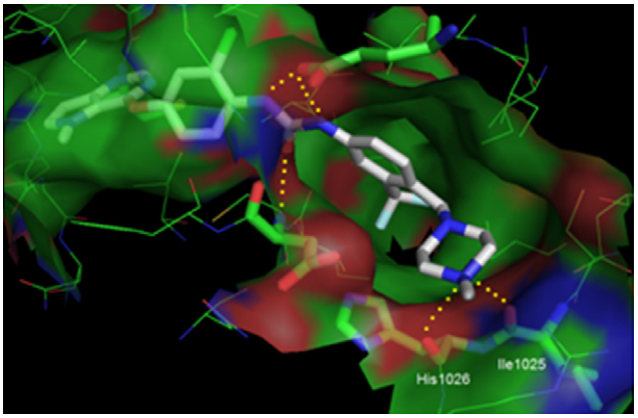


Figure 3. Binding model of **11l** in complex with VEGFR2.

6.1. 2-(4-Chloro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl benzoate (3a)

A mixture of **2** (3.00 g, 19.5 mmol), 2-bromoethyl benzoate (4.70 g, 20.5 mmol), cesium carbonate (9.53 g, 25.3 mmol), and DMF (25 mL) was stirred at room temperature for 18 h. The mixture was diluted with water and extracted with AcOEt. The extract was washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane) to give **3a** (5.50 g, 93%) as a pale yellow solid: ^{1}H NMR (DMSO-d_6) δ 4.65 (2H, t, J = 5.0 Hz), 4.90 (2H, t, J = 5.0 Hz), 6.73 (1H, d, J = 3.3 Hz), 7.45 (2H, t, J = 7.7 Hz), 7.61 (1H, t, J = 7.7 Hz), 7.78–7.81 (2H, m), 8.12 (1H, d, J = 3.3 Hz), 8.60 (1H, s).

6.2. 4-Chloro-5-(2-methoxyethyl)-5*H*-pyrrolo[3,2-*d*]pyrimidine (3b)

The compound **3b** was prepared in a similar manner to the procedure described for the preparation of **3a** to yield a pale yellow solid (96%): ^{1}H NMR (DMSO-d_6) δ 3.19 (3H, s), 3.68 (2H, t, J = 5.4 Hz), 4.65 (2H, t, J = 5.4 Hz), 6.71–6.72 (1H, m), 7.97 (1H, d, J = 3.3 Hz), 8.60 (1H, s).

6.3. 2-[2-(4-Chloro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethoxy]-ethyl benzoate (3c)

The compound **3c** was prepared in a similar manner to the procedure described for the preparation of **3a** to yield a pale yellow solid (78%): ^{1}H NMR (DMSO-d_6) δ 3.66–3.69 (2H, m), 3.82–3.86 (2H, m), 4.27–4.30 (2H, m), 4.65–4.68 (2H, m), 6.60 (1H, d, J = 3.0 Hz),

7.46–7.51 (2H, m), 7.61–7.66 (1H, m), 7.80–7.84 (2H, m), 7.95 (1H, d, J = 3.0 Hz), 8.56 (1H, s).

6.4. 2-[4-(4-Amino-3-chlorophenoxy)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethyl benzoate (4a)

A mixture of **3a** (2.00 g, 6.63 mmol), 4-amino-3-chlorophenol (1.05 g, 7.29 mmol), potassium carbonate (2.02 g, 14.6 mmol), and NMP (10 mL) was stirred at 120 °C for 18 h. The mixture was diluted with water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography (AcOEt/hexane) to give **4a** (2.15 g, 79%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 4.69–4.70 (2H, m), 4.78–4.80 (2H, m), 5.31 (2H, br s), 6.61 (1H, d, J = 3.2 Hz), 6.79 (1H, d, J = 8.9 Hz), 6.87 (1H, dd, J = 8.9, 2.6 Hz), 7.01 (1H, d, J = 2.6 Hz), 7.45 (2H, t, J = 7.8 Hz), 7.60–7.65 (1H, m), 7.76–7.79 (2H, m), 7.91 (1H, d, J = 3.2 Hz), 8.26 (1H, s).

6.5. 2-Chloro-4-{[5-(2-methoxyethyl)-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl]oxy}aniline (4b)

The compound **4b** was prepared in a similar manner to the procedure described for the preparation of **4a** to yield a pale yellow solid (56%): ^{1}H NMR (DMSO-d_6) δ 3.19 (3H, s), 3.72 (2H, t, J = 5.4 Hz), 4.55 (2H, t, J = 5.4 Hz), 5.31 (2H, s), 6.56 (1H, d, J = 3.2 Hz), 6.83 (1H, d, J = 8.9 Hz), 6.97 (1H, dd, J = 8.9, 2.7 Hz), 7.20 (1H, d, J = 2.7 Hz), 7.75 (1H, d, J = 3.2 Hz), 8.26 (1H, d, J = 0.6 Hz).

6.6. 2-{2-[4-(4-Amino-3-chlorophenoxy)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethoxy}ethyl benzoate (4c)

The compound **4c** was prepared in a similar manner to the procedure described for the preparation of **4a** to yield a pale yellow solid (91%): ^{1}H NMR (DMSO-d_6) δ 3.68–3.71 (2H, m), 3.86–3.89 (2H, m), 4.29–4.32 (2H, m), 4.55–4.59 (2H, m), 5.30 (2H, br s), 6.48 (1H, d, J = 3.0 Hz), 6.80 (1H, d, J = 8.4 Hz), 6.94 (1H, dd, J = 8.4, 2.4 Hz), 7.18 (1H, d, J = 2.4 Hz), 7.44–7.99 (2H, m), 7.60–7.66 (1H, m), 7.75 (1H, d, J = 3.0 Hz), 7.80–7.83 (2H, m), 8.23 (1H, s).

6.7. 1-(2-Chloro-4-{[5-(2-hydroxyethyl)-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl]oxy}phenyl)-3-[3-(trifluoromethyl)phenyl]urea (5a)

To a solution of **4a** (300 mg, 0.734 mmol), triethylamine (1.00 mL, 7.34 mmol) in THF (5 mL) was added 3-(trifluoromethyl)phenyl isocyanate (130 mg, 0.807 mmol), and the mixture stirred at room temperature for 15 h. The mixture was diluted with AcOEt and washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was dissolved in 0.5 M NaOH in MeOH (2 mL), and the solution was stirred at room temperature for 30 min. The mixture was diluted with AcOEt and washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography (AcOEt/hexane) followed by recrystallization from AcOEt to give **5a** (93.4 mg, 26%) as a white solid: mp 170–174 °C; ^{1}H NMR (DMSO-d_6) δ 3.76–3.81 (2H, m), 4.45 (2H, t, J = 5.4 Hz), 4.91 (1H, t, J = 5.4 Hz), 6.59–6.60 (1H, m), 7.27 (1H, dd, J = 9.2, 2.7 Hz), 7.32–7.33 (1H, m), 7.49–7.57 (3H, m), 7.79 (1H, d, J = 3.0 Hz), 8.03 (1H, br s), 8.15 (1H, d, J = 9.2 Hz), 8.29 (1H, s), 8.43 (1H, s), 9.71 (1H, br s). Anal. Calcd for $C_{22}H_{17}ClF_3N_5O_3$: C, 53.72; H, 3.48; N, 14.24. Found: C, 53.61; H, 3.50; N, 14.25.

6.8. 1-(2-Chloro-4-{[5-(2-methoxyethyl)-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl]oxy}phenyl)-3-[3-(trifluoromethyl)phenyl]urea (5b)

To a solution of **4b** (100 mg, 0.314 mmol), triethylamine (998 μL, 7.21 mmol) in THF (3 mL) was added 3-(trifluoromethyl)phenyl isocyanate (127 mg, 0.793 mmol), and the mixture stirred at room temperature for 18 h. The mixture was diluted with AcOEt and washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane) followed by recrystallization from AcOEt–IPE to give **5b** (74.5 mg, 47%) as a white solid: mp 177–178 °C; ^{1}H NMR (DMSO-d_6) δ 3.22 (3H, s), 3.74 (2H, t, J = 5.3 Hz), 4.57 (2H, t, J = 5.3 Hz), 6.60 (1H, d, J = 3.2 Hz), 7.27–7.34 (2H, m), 7.49–7.55 (3H, m), 7.80 (1H, d, J = 3.2 Hz), 8.03 (1H, br s), 8.15 (1H, d, J = 9.0 Hz), 8.30 (1H, s), 8.43 (1H, s), 9.71 (1H, br s). Anal. Calcd for $C_{23}H_{19}ClF_3N_5O_3{\cdot}0.5H_2O$: C, 53.65; H, 3.92; N, 13.60. Found: C, 53.61; H, 3.67; N, 13.57.

6.9. 1-[2-Chloro-4-({5-[2-(2-hydroxyethoxy)ethyl]-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl}oxy)phenyl]-3-[3-(trifluoromethyl)phenyl]urea (5c)

The compound **5c** was prepared in a similar manner to the procedure described for the preparation of **5a** to yield a pale yellow solid (17%): mp 170–174 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 3.40–3.41 (4H, m), 3.81–3.85 (2H, m), 4.56–4.59 (3H, m), 6.60 (1H, d, J = 3.0 Hz), 7.27–7.34 (2H, m), 7.49–7.56 (3H, m), 7.83 (1H, d, J = 3.0 Hz), 8.03 (1H, br s), 8.14 (1H, d, J = 8.7 Hz), 8.29 (1H, s), 8.44 (1H, s), 9.71 (1H, br s). Anal. Calcd for $C_{24}H_{21}ClF_3N_5O_4$: C, 53.79; H, 3.95; N, 13.07. Found: C, 53.76; H, 4.01; N, 13.00.

6.10. 4,6-Diiodo-*N*-methylpyrimidin-5-amine (7)

To a suspension of sodium hydride (60% in oil, 5.20 g, 130 mmol) in THF (300 mL) was added 4,6-diiodopyrimidin-5-amine (30.0 g, 86.5 mmol) at 0 °C, and the mixture was stirred. After 30 min, methyl methanesulfonate (13.2 mL, 156 mmol) was added to the mixture, and the resulting mixture was stirred at room temperature for 4 h. The mixture was diluted with AcOEt and washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane) to give **7** (21.9 g, 70%) as a brown solid: ^{1}H NMR ($CDCl_3$) δ 3.03 (3H, d, J = 6.0 Hz), 3.78 (1H, br s), 8.04 (1H, s).

6.11. 4-(4-Amino-3-chlorophenoxy)-6-iodo-*N*-methylpyrimidin-5-amine (8)

To a solution of **7** (9.60 g, 55.4 mmol), in NMP (200 mL) were added potassium carbonate (18.4 g, 133 mmol) and 4-amino-3-chlorophenol (8.8 g, 61 mmol), and the resulting mixture stirred at 110 °C for 1 h. The mixture was diluted with water and extracted with AcOEt. The extract was washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane) to give **8** (9.6 g, 46%) as a brown solid: ^{1}H NMR (DMSO-d_6) δ 3.02 (3H, d, J = 5.3 Hz), 4.80 (1H, q, J = 5.3 Hz), 5.31 (2H, s), 6.82 (1H, d, J = 8.7 Hz), 6.90 (1H, dd, J = 8.7, 2.7 Hz), 7.13 (1H, d, J = 2.7 Hz), 7.77 (1H, s).

6.12. 2-Chloro-4-({5-methyl-6-[(tetrahydro-2*H*-pyran-2-yloxy)methyl]-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl}oxy)aniline (9a)

To a solution of **8** (3.00 g, 7.97 mmol), in acetonitrile (90 mL) and triethylamine (90 mL) were added 2-(prop-2-yn-1-yloxy)tet-

rahydro-2*H*-pyran (1.34 g, 9.56 mmol), $Pd(PPh_3)_2Cl_2$ (0.28 g, 0.40 mmol), and CuI (0.15 g, 0.80 mmol), and the resulting mixture stirred at 60 °C for 1 h. The mixture was diluted with water and extracted with AcOEt. The extract was washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane) to give 4-(4-amino-3-chlorophenoxy)-*N*-methyl-6-[3-(tetrahydro-2*H*-pyran-2-yloxy)prop-1-yn-1-yl]pyrimidin-5-amine (2.11 g, 68%) as a brown oil. To a solution of the oil thus obtained (2.10 g, 5.40 mmol) in DMF (42 mL) was added CuI (152 mg, 0.80 mmol), and the mixture stirred at 80 °C for 1 h. The mixture was diluted with water and extracted with AcOEt. The extract was washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography (AcOEt/hexane) to give **9a** (1.35 g, 64%) as a brown solid: ^{1}H NMR (DMSO-d_6) δ 1.48–1.86 (6H, m), 3.56–3.64 (1H, m), 3.85–3.97 (1H, m), 4.06 (2H, s), 4.13 (3H, s), 4.71 (1H, d, J = 12.8 Hz), 4.73–4.76 (1H, m), 4.93 (1H, d, J = 12.8 Hz), 6.66 (1H, s), 6.83 (1H, d, J = 8.7 Hz), 6.99 (1H, dd, J = 8.7, 2.6 Hz), 7.19 (1H, d, J = 2.6 Hz), 8.43 (1H, s).

6.13. 2-Chloro-4-{[6-(methoxymethyl)-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl]oxy}aniline (9b)

The compound **9b** was prepared in a similar manner to the procedure described for the preparation of **9a** to yield a pale yellow solid (29%): ^{1}H NMR (DMSO-d_6) δ 2.34 (3H, s), 4.02 (3H, s), 4.66 (2H, s), 5.34 (2H, s), 6.62 (1H, s), 6.84 (1H, d, J = 8.7 Hz), 7.00 (1H, dd, J = 8.7, 2.7 Hz), 7.22 (1H, d, J = 2.7 Hz), 8.25 (1H, s).

6.14. 2-Chloro-4-[(5-methyl-6-{1-methyl-1-[(trimethylsilyl)oxy]ethyl}-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]aniline (9c)

The compound **9c** was prepared in a similar manner to the procedure described for the preparation of **9a** to yield a pale yellow solid (60%): ^{1}H NMR (DMSO-d_6) δ 0.07 (9H, s), 1.76 (6H, s), 4.05 (2H, s), 4.31 (3H, s), 6.56 (1H, br s), 6.83 (1H, d, J = 8.7 Hz), 6.99 (1H, dd, J = 8.7, 2.7 Hz), 7.20 (1H, d, J = 2.7 Hz), 8.45 (1H, br s).

6.15. 1-(2-Chloro-4-{[6-(hydroxymethyl)-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl]oxy}phenyl)-3-[3-(trifluoromethyl)phenyl]urea (10a)

To a solution of **9a** (1.35 g, 3.47 mmol) and triethylamine (1.45 mL, 10.4 mmol) in THF (27 mL) was added 3-(trifluoromethyl)phenyl isocyanate (543 μL, 3.89 mmol), and the mixture stirred at room temperature for 5 h. The mixture was diluted with AcOEt and washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography (AcOEt/hexane) to give a white solid. The solid thus obtained was dissolved in EtOH (21 mL), and *p*-toluenesulfonic acid monohydrate (509 mg, 2.67 mmol) was added to the solution. The mixture was stirred at room temperature for 3 h. The mixture was concentrated under reduced pressure, and the residue was partitioned with saturated aqueous $NaHCO_3$ solution and AcOEt/THF. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography (AcOEt/EtOH) followed by recrystallization from AcOEt–hexane to give **10a** (105 mg, 8%) as a white solid: mp 219–220 °C; ^{1}H NMR (DMSO-d_6) δ 4.05 (3H, s), 4.73 (2H, d, J = 5.4 Hz), 5.55 (1H, t, J = 5.4 Hz), 6.57 (1H, s), 7.29–7.37 (2H, m), 7.51–7.58 (3H, m), 8.06 (1H, s), 8.17 (1H, d, J = 9.0 Hz), 8.28 (1H, s), 8.47 (1H, s), 9.75 (1H, s). Anal. Calcd for $C_{23}H_{19}ClF_3N_5O_3$: C, 53.72; H, 3.48; N, 14.24. Found: C, 53.81; H, 3.56; N, 14.27.

6.16. 1-(2-Chloro-4-{[6-(methoxymethyl)-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl]oxy}phenyl)-3-[3-(trifluoromethyl) phenyl]-urea (10b)

The compound **10b** was prepared in a similar manner to the procedure described for the preparation of **5b** to yield a white solid (37%): mp 179–181 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 3.33 (3H, s), 4.04 (3H, s), 4.69 (2H, s), 6.66 (1H, s), 7.29–7.35 (2H, m), 7.51–7.58 (3H, m), 8.05 (1H, s), 8.16 (1H, d, J = 9.0 Hz), 8.29 (1H, s), 8.47 (1H, s), 9.74 (1H, s). Anal. Calcd for $C_{23}H_{19}ClF_3N_5O_3{\cdot}H_2O$: C, 52.73; H, 4.04; N, 13.37. Found: C, 52.75; H, 3.92; N, 13.36.

6.17. 1-(2-Chloro-4-{[6-(1-hydroxy-1-methylethyl)-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl]oxy}phenyl)-3-[3-(trifluoromethyl)phenyl]urea (10c)

To a solution of **9c** (0.95 g, 2.35 mmol) and triethylamine (1.31 mL, 9.38 mmol) in THF (19 mL) was added 3-(trifluoromethyl)phenyl isocyanate (0.39 mL, 2.82 mmol), and the mixture stirred at room temperature for 5 h. The mixture was diluted with AcOEt and washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography (AcOEt/hexane) to give a white solid. The solid thus obtained was dissolved in EtOH (12.3 mL), and 1 M HCl (2.43 mL) was added to the solution. The mixture was stirred at room temperature for 15 min. The mixture was concentrated under reduced pressure, and the residue was partitioned with saturated aqueous $NaHCO_3$ solution and AcOEt/THF. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane) followed by recrystallization from AcOEt–hexane to give **10c** (550 mg, 45%) as a white solid: mp 158–165 °C; ^{1}H NMR (DMSO-d_6) δ 1.64 (6H, s), 4.31 (3H, s), 5.55 (1H, s), 6.51 (1H, s), 7.29 (1H, dd, J = 9.0, 2.7 Hz), 7.32–7.35 (1H, m), 7.53–7.56 (3H, m), 8.05 (1H, s), 8.16 (1H, d, J = 9.0 Hz), 8.25 (1H, s), 8.45 (1H, s), 9.73 (1H, s). Anal. Calcd for $C_{24}H_{21}ClF_3N_5O_3{\cdot}H_2O$: C, 53.59; H, 4.31; N, 13.02. Found: C, 53.87; H, 4.18; N, 13.07.

6.18. 1-{2-Chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}-3-pyridin-2-ylurea (11a)

To a solution of **4d** (275 mg, 1.00 mmol) and pyridine (633 μL, 8.00 mmol) in DMA (2 mL) was added phenyl chlorocarbonate (251 μL, 2.00 mmol) at 0 °C. After being stirred at room temperature for 1 h, 2-aminopyridine (188 mg, 2.00 mmol) was added and the resulting mixture was stirred at 90 °C for 15 h. The mixture was diluted with water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography (AcOEt/hexane) followed by recrystallization from AcOEt to give **11a** (299 mg, 76%) as a white solid: mp 259–260 °C; ^{1}H NMR (DMSO-d_6) δ 4.11 (3H, s), 6.61 (1H, d, J = 3.0 Hz), 7.03–7.07 (1H, m), 7.25 (1H, d, J = 8.4 Hz), 7.33 (1H, dd, J = 9.2, 2.6 Hz), 7.60 (1H, d, J = 2.6 Hz), 7.77–7.82 (1H, m), 7.80 (1H, d, J = 3.0 Hz), 8.31 (1H, s), 8.31–8.34 (1H, m), 8.40 (1H, d, J = 9.2 Hz), 10.03 (1H, s), 11.83 (1H, br s). Anal. Calcd for $C_{19}H_{15}ClN_6O_2$: C, 57.80; H, 3.83; N, 21.29. Found: C, 57.66; H, 3.91; N, 21.01.

The following compounds **11b–d** and **11h–p** were prepared using a procedure similar to that described for **11a** from **4d**.

6.19. 1-{2-Chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}-3-pyridin-3-ylurea (11b)

Yield 23%, white solid: mp 233–237 °C (AcOEt); ^{1}H NMR (DMSO-d_6) δ 4.11 (3H, s), 6.61 (1H, d, J = 3.0 Hz), 7.32 (1H, dd, J = 9.0, 2.7 Hz), 7.32–7.37 (1H, m), 7.57 (1H, d, J = 2.7 Hz), 7.80 (1H, d, J = 3.0 Hz), 7.98 (1H, ddd, J = 8.2, 2.5, 1.5 Hz), 8.17 (1H, d, J = 9.0 Hz), 8.22 (1H, dd, J = 4.5, 1.5 Hz), 8.31 (1H, s), 8.49 (1H, br s), 8.63 (1H, d, J = 2.5 Hz), 9.56 (1H, br s). Anal. Calcd for $C_{19}H_{15}ClN_6O_2$: C, 57.80; H, 3.83; N, 21.29. Found: C, 57.81; H, 3.88; N, 21.11.

6.20. 1-{2-Chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}-3-pyridin-4-ylurea (11c)

Yield 22%, white solid: mp 233–237 °C (AcOEt); ^{1}H NMR (DMSO-d_6) δ 4.11 (3H, s), 6.61 (1H, d, J = 3.2 Hz), 7.33 (1H, dd, J = 9.0, 2.6 Hz), 7.46 (2H, d, J = 6.3 Hz), 7.58 (1H, d, J = 2.6 Hz), 7.80 (1H, d, J = 3.2 Hz), 8.15 (1H, d, J = 9.0 Hz), 8.31 (1H, s), 8.39 (2H, d, J = 6.3 Hz), 8.56 (1H, br s), 9.78 (1H, br s). Anal. Calcd for $C_{19}H_{15}ClN_6O_2{\cdot}H_2O$: C, 55.28; H, 4.15; N, 20.36. Found: C, 55.39; H, 4.17; N, 20.08.

6.21. 1-{2-Chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}-3-[4-(trifluoromethyl)pyridin-2-yl]urea (11d)

Yield 21%, white solid: mp 259–260 °C (AcOEt); ^{1}H NMR (DMSO-d_6) δ 4.09 (3H, s), 6.58–6.59 (1H, m), 7.31–7.38 (2H, m), 7.59 (1H, d, J = 2.1 Hz), 7.77–7.80 (2H, m), 8.28–8.31 (2H, m), 8.57 (1H, d, J = 5.7 Hz), 10.36 (1H, s), 10.61 (1H, br s). Anal. Calcd for $C_{20}H_{14}ClF_3N_6O_2{\cdot}0.2H_2O$: C, 51.50; H, 3.11; N, 18.02. Found: C, 51.48; H, 2.98; N, 18.16.

6.22. 1-(3-*tert*-Butyl-1-methyl-1*H*-pyrazol-5-yl)-3-{2-chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}urea (11h)

Yield 6%, white solid: mp 194–196 °C (AcOEt); ^{1}H NMR (DMSO-d_6) δ 1.22 (9H, s), 3.65 (3H, s), 4.10 (3H, s), 6.11 (1H, s), 6.60 (1H, d, J = 3.0 Hz), 7.30 (1H, dd, J = 9.3, 2.7 Hz), 7.56 (1H, d, J = 2.7 Hz), 7.79 (1H, d, J = 3.0 Hz), 8.16 (1H, d, J = 9.3 Hz), 8.30 (1H, s), 8.61 (1H, s), 9.22 (1H, s). Anal. Calcd for $C_{22}H_{24}ClN_7O_2$: C, 58.21; H, 5.33; N, 21.60. Found: C, 57.89; H, 5.31; N, 21.35.

6.23. 1-{2-Chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}-3-[4-methoxy-3-(trifluoromethyl)phenyl]urea (11i)

Yield 33%, white solid: mp 233–237 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 3.86 (3H, s), 4.10 (3H, s), 6.61 (1H, d, J = 3.0 Hz), 7.24 (1H, d, J = 9.2 Hz), 7.30 (1H, dd, J = 9.0, 2.7 Hz), 7.56 (1H, d, J = 2.7 Hz), 7.59 (1H, dd, J = 9.2, 2.7 Hz), 7.80 (1H, d, J = 3.0 Hz), 7.88 (1H, d, J = 2.7 Hz), 8.17 (1H, d, J = 9.0 Hz), 8.30 (1H, s), 8.37 (1H, br s), 9.51 (1H, br s). Anal. Calcd for $C_{22}H_{17}ClF_3N_5O_3$: C, 53.72; H, 3.48; N, 14.24. Found: C, 53.63; H, 3.47; N, 14.22.

6.24. 1-{2-Chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}-3-[4-morpholin-4-yl-3-(trifluoromethyl) phenyl]urea (11j)

Yield 34%, white solid: mp 147–150 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 2.80–2.83 (4H, s), 3.68–3.71 (4H, s), 4.10 (3H, s), 6.60 (1H, d, J = 3.0 Hz), 7.30 (1H, dd, J = 9.1, 2.7 Hz), 7.54–7.63 (3H, m), 7.79 (1H, d, J = 3.0 Hz), 7.94 (1H, J = 2.1 Hz), 8.16 (1H, d, J = 9.1 Hz), 8.29 (1H, s), 8.42 (1H, s), 9.67 (1H, s). Anal. Calcd for $C_{25}H_{22}ClF_3N_6O_2$: C, 54.90; H, 4.05; N, 15.37. Found: C, 54.67; H, 4.14; N, 15.17.

6.25. 1-{2-Chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}-3-[4-(morpholin-4-ylmethyl)-3-(trifluoromethyl)phenyl]urea (11k)

Yield 29%, white solid: mp 149–151 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 2.30–2.40 (4H, m), 3.55–3.60 (6H, m), 4.10 (3H, s), 6.60 (1H, d, J = 2.9 Hz), 7.31 (1H, dd, J = 8.9, 2.6 Hz), 7.56–7.59 (2H, m), 7.68 (1H, d, J = 8.7 Hz), 7.79 (1H, d, J = 2.9 Hz), 8.00 (1H, d, J = 2.1 Hz), 8.18 (1H, d, J = 8.9 Hz), 8.30 (1H, s), 8.42 (1H, s), 9.70 (1H, s). Anal. Calcd for $C_{26}H_{24}ClF_3N_6O_2{\cdot}H_2O$: C, 53.94; H, 4.53; N, 14.52. Found: C, 53.86; H, 4.50; N, 14.27.

6.26. 1-{2-Chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}-3-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl}urea (11l)

Yield 23%, white solid: mp 138–139 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 2.16 (3H, s), 2.20–2.45 (8H, m), 3.54 (2H, s), 4.10 (3H, s), 6.61 (1H, d, J = 2.9 Hz), 7.31 (1H, dd, J = 8.8, 2.6 Hz), 7.56–7.67 (3H, m), 7.80 (1H, d, J = 2.9 Hz), 8.00 (1H, s), 8.18 (1H, d, J = 8.8 Hz), 8.30 (1H, s), 8.43 (1H, s), 9.70 (1H, s). Anal. Calcd for $C_{27}H_{27}ClF_3N_7O_2{\cdot}1.2H_2O$: C, 54.45; H, 4.98; N, 16.46. Found: C, 54.73; H, 4.98; N, 16.16.

6.27. 1-{2-Chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}-3-{4-[(1-methylpiperidin-4-yl)oxy]-3-(trifluoromethyl)phenyl}urea (11m)

Yield 11%, white solid: mp 137–139 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 1.65–1.75 (2H, m), 1.85–1.95 (2H, m), 2.19–2.35 (5H, m), 2.50–2.60 (2H, m), 4.10 (3H, s), 4.45–4.51 (1H, m), 6.60 (1H, d, J = 3.0 Hz), 7.26–7.31 (2H, m), 7.52–7.55 (2H, m), 7.79 (1H, d, J = 3.0 Hz), 7.85 (1H, d, J = 2.4 Hz), 8.17 (1H, d, J = 9.6 Hz), 8.30 (1H, s), 8.33 (1H, s), 9.47 (1H, s). Anal. Calcd for $C_{27}H_{26}ClF_3N_6O_3{\cdot}2H_2O$: C, 53.07; H, 4.95; N, 13.75. Found: C, 53.43; H, 4.80; N, 13.48.

6.28. 1-{2-Chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}-3-[3-methoxy-5-(trifluoromethyl)phenyl]urea (11n)

Yield 21%, white solid: mp 205–208 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 3.83 (3H, s), 4.11 (3H, s), 6.61 (1H, d, J = 3.0 Hz), 6.88 (1H, br s), 7.26 (1H, br s), 7.32 (1H, dd, J = 9.0, 2.7 Hz), 7.51 (1H, br s), 7.57 (1H, d, J = 2.7 Hz), 7.80 (1H, d, J = 3.0 Hz), 8.15 (1H, d, J = 9.0 Hz), 8.30 (1H, s), 8.47 (1H, br s), 9.76 (1H, br s). Anal. Calcd for $C_{22}H_{17}ClF_3N_5O_2{\cdot}H_2O$: C, 51.82; H, 3.76; N, 13.74. Found: C, 51.98; H, 3.75; N, 13.80.

6.29. 1-{2-Chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}-3-[3-(morpholin-4-ylmethyl)-5-(trifluoromethyl)phenyl]urea (11o)

Yield 23%, white solid: mp 134–136 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 2.30–2.45 (4H, m), 3.30–3.61 (6H, m), 4.10 (3H, s), 6.60 (1H, d, J = 3.3 Hz), 7.26 (1H, s), 7.28 (1H, dd, J = 8.8, 2.6 Hz), 7.54 (1H, s), 7.56 (1H, d, J = 2.6 Hz), 7.78 (1H, d, J = 3.3 Hz), 7.94 (1H, s), 8.16 (1H, d, J = 8.8 Hz), 8.30 (1H, s), 8.40 (1H, s), 9.74 (1H, s). Anal. Calcd for $C_{26}H_{24}ClF_3N_6O_3{\cdot}H_2O$: C, 53.94; H, 4.53; N, 14.52. Found: C, 53.77; H, 4.39; N, 14.32.

6.30. 1-{2-Chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}-3-{3-[(4-methylpiperazin-1-yl)methyl]-5-(trifluoromethyl)phenyl}urea (11p)

Yield 7%, white solid: mp 131–132 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 2.15 (3H, s), 2.20–2.50 (8H, m), 3.52 (2H, s), 4.10 (3H, s), 6.60 (1H, d, J = 3.0 Hz), 7.23 (1H, s), 7.30 (1H, dd, J = 9.0, 2.7 Hz), 7.50 (1H, s), 7.56 (1H, d, J = 2.7 Hz), 7.79 (1H, d, J = 3.0 Hz), 7.94 (1H, s), 8.17 (1H, d, J = 9.0 Hz), 8.29 (1H, s), 8.40 (1H, s), 9.75 (1H, s). Anal. Calcd for $C_{27}H_{27}ClF_3N_7O_2\cdot1.5H_2O$: C, 53.96; H, 5.03; N, 16.31. Found: C, 53.92; H, 4.97; N, 16.01.

6.31. 1-{2-Chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}-3-[1-(2,2,2-trifluoroethyl)-1*H*-pyrazol-4-yl]urea (11e)

To a solution of **4d** (275 mg, 1.0 mmol) and triethylamine (2.79 mL, 20 mmol) in dichloromethane (10 mL) was added triphosgene (297 mg, 1.0 mmol) at 0 °C. After being stirred at room temperature for 1 h, 4-amino-1-(2,2,2-trifluoroethyl)pyrazole (330 mg, 2.0 mmol) was added, and the resulting mixture stirred at room temperature for 15 h. The mixture was diluted with water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane) followed by recrystallization from AcOEt to give **11e** (227 mg, 49%) as a white solid: mp 227–231 °C; ^{1}H NMR (DMSO-d_6) δ 4.10 (3H, s), 5.09 (2H, q, J = 9.2 Hz), 6.61 (1H, d, J = 3.2 Hz), 7.29 (1H, dd, J = 9.0, 2.6 Hz), 7.54 (1H, d, J = 2.6 Hz), 7.56 (1H, s), 7.79 (1H, d, J = 3.2 Hz), 7.97 (1H, s), 8.18 (1H, d, J = 9.0 Hz), 8.30 (1H, s), 8.32 (1H, br s), 9.25 (1H, br s). Anal. Calcd for $C_{19}H_{15}ClF_3N_7O_2$: C, 48.99; H, 3.25; N, 21.05. Found: C, 49.04; H, 3.14; N, 21.02.

The following compounds **11f**,**g** were prepared using a procedure similar to that described for **11e** from **4d**.

6.32. 1-(1-*tert*-Butyl-1*H*-pyrazol-4-yl)-3-{2-chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}urea (11f)

Yield 7%, white solid: mp 137–140 °C (AcOEt); ^{1}H NMR (DMSO-d_6) δ 1.51 (9H, s), 4.10 (3H, s), 6.60 (1H, d, J = 3.0 Hz), 7.28 (1H, dd, J = 9.0, 2.7 Hz), 7.43 (1H, s), 7.53 (1H, d, J = 2.7 Hz), 7.79 (1H, d, J = 3.0 Hz), 7.85 (1H, s), 8.21 (1H, d, J = 9.0 Hz), 8.28 (1H, s), 8.30 (1H, s), 9.08 (1H, s). Anal. Calcd for $C_{21}H_{22}ClN_7O_2$: C, 57.34; H, 5.04; N, 22.29. Found: C, 57.23; H, 5.06; N, 22.14.

6.33. 1-(5-*tert*-Butylisoxazol-3-yl)-3-{2-chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}urea (11g)

Yield 36%, amorphous solid: ^{1}H NMR (DMSO-d_6) δ 1.30 (9H, s), 4.10 (3H, s), 6.47 (1H, s), 6.61 (1H, d, J = 3.0 Hz), 7.32 (1H, dd, J = 9.2, 2.7 Hz), 7.58 (1H, d, J = 2.7 Hz), 7.80 (1H, d, J = 3.0 Hz), 8.19 (1H, d, J = 9.2 Hz), 8.30 (1H, s), 8.74 (1H, br s), 10.21 (1H, br s). Anal. Calcd for $C_{21}H_{21}ClN_5O_3\cdot0.4H_2O$: C, 56.29; H, 4.90; N, 18.76. Found: C, 56.47; H, 4.90; N, 18.52.

6.34. 1-{2-Chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}-3-[5-(trifluoromethyl)pyridin-3-yl]urea (12a)

A mixture of **15a** (282 mg, 1.0 mmol), **4d** (275 mg, 1.0 mmol), pyridine (633 mg, 8.0 mmol), and NMP (2 mL) was stirred at 90 °C for 7 h. The mixture was diluted with water and extracted with AcOEt. The extract was washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography (AcOEt/hexane) followed by recrystallization from AcOEt–MeOH to give **12a** (228 mg, 49%) as a white solid: mp 220–224 °C; ^{1}H NMR (DMSO-d_6) δ 4.11 (3H, s), 6.61 (1H, d, J = 3.0 Hz), 7.34 (1H, dd, J = 9.0, 2.7 Hz), 7.59 (1H, d, J = 2.7 Hz), 7.80 (1H, d, J = 3.0 Hz), 8.14 (1H, d, J = 9.0 Hz), 8.31 (1H, s), 8.48 (1H, m), 8.60 (1H, m), 8.65 (1H, br s), 8.77 (1H, m), 9.94 (1H, br s). Anal. Calcd for $C_{20}H_{14}ClF_3N_6O_2$: C, 51.90; H, 3.05; N, 18.16. Found: C, 51.92; H, 2.95; N, 18.17.

6.35. 1-{2-Chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}-3-[2-(trifluoromethyl)pyridin-4-yl]urea (12b)

The compound **12b** was prepared in a similar manner to the procedure described for the preparation of **12a** to yield a white solid (54%): mp 227–231 °C (AcOEt); ^{1}H NMR (DMSO-d_6) δ 4.11 (3H, s), 6.61 (1H, d, J = 3.0 Hz), 7.35 (1H, dd, J = 9.0, 2.7 Hz), 7.57 (1H, dd, J = 5.7, 1.7 Hz), 7.60 (1H, d, J = 2.7 Hz), 7.80 (1H, d, J = 3.0 Hz), 8.09 (1H, d, J = 1.7 Hz), 8.12 (1H, d, J = 9.0 Hz), 8.31 (1H, s), 8.57 (1H, d, J = 5.7 Hz), 8.68 (1H, br s), 10.16 (1H, br s). Anal. Calcd for $C_{20}H_{14}ClF_3N_6O_2\cdot0.25H_2O$: C, 51.40; H, 3.13; N, 17.98. Found: C, 51.38; H, 3.07; N, 17.88.

6.36. 1-{2-Chloro-4-[(5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-yl)oxy]phenyl}-3-[3-morpholin-4-yl-5-(trifluoromethyl)-phenyl]urea (14)

To a solution of **13** (1.00 g, 3.63 mmol) and triethylamine (2.50 mL, 18.2 mmol) in toluene (30 mL) was added diphenylphosphoryl azide (860 μL, 3.99 mmol), and the mixture heated at reflux for 4 h. After cooling to room temperature, **4d** (907 mg, 3.30 mmol) was added and the resulting mixture stirred at room temperature for 2 h. The mixture was diluted with water and extracted with AcOEt. The extract was washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane) followed by recrystallization from AcOEt–MeOH to give **14** (572 mg, 32%) as a white solid: mp 211–215 °C; ^{1}H NMR (DMSO-d_6) δ 3.16–3.19 (4H, m), 3.74–3.77 (4H, m), 4.10 (3H, s), 6.60 (1H, d, J = 3.0 Hz), 6.88 (1H, s), 7.18 (1H, s), 7.29–7.35 (2H, m), 7.56 (1H, d, J = 2.7 Hz), 7.79 (1H, d, J = 3.0 Hz), 8.17 (1H, d, J = 8.7 Hz), 8.30 (1H, s), 8.39 (1H, s), 9.60 (1H, s). Anal. Calcd for $C_{25}H_{22}ClF_3N_6O_2$: C, 54.90; H, 4.05; N, 15.37. Found: C, 54.66; H, 4.16; N, 15.15.

6.37. Phenyl [5-(trifluoromethyl)pyridin-3-yl]carbamate (15a)

To a solution of **27** (780 mg, 4.8 mmol) and pyridine (1.56 mL, 19.3 mmol) in THF (10 mL) was added phenyl chloroformate (664 μL, 5.3 mmol), and the mixture stirred at room temperature for 2 h. The mixture was diluted with AcOEt and washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane) to give **15a** (712 mg, 52%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 7.17–7.23 (3H, m), 7.28–7.32 (1H, m), 7.39–7.47 (2H, m), 8.39 (1H, br s), 8.63 (1H, s), 8.72 (1H, d, J = 2.4 Hz).

6.38. Phenyl [2-(trifluoromethyl)pyridin-4-yl]carbamate (15b)

The compound **15b** was prepared in a similar manner to the procedure described for the preparation of **15a** to yield a white solid (46%): ^{1}H NMR (DMSO-d_6) δ 7.16–7.22 (2H, m), 7.26–7.33 (1H, m), 7.33 (1H, br s), 7.39–7.47 (2H, m), 7.58 (1H, dd, J = 5.7, 2.1 Hz), 7.85 (1H, d, J = 2.1 Hz), 8.62 (1H, d, J = 5.7 Hz).

6.39. 4-Morpholin-4-yl-3-(trifluoromethyl)aniline (17)

A mixture of 1-fluoro-4-nitro-2-(trifluoromethyl)benzene (564 mg, 2.70 mmol), morpholine (2.35 g, 27.0 mmol), and DMSO (20 mL) was stirred at 100 °C for 7 h. The mixture was diluted with water and extracted with AcOEt. The extract was washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was dissolved in MeOH (15 mL) and 10% palladium on carbon (water ~50%, 15 mg) was added. The resulting mixture was stirred under a hydrogen atmosphere at room temperature for 3 h. The catalyst was filtered off, and the filtrate concentrated in vacuo. The residual solid was collected and washed with AcOEt–hexane to give **17** (668 mg, 99%) as a pale yellow solid: ^{1}H NMR (DMSO-d_6) δ 2.69–2.72 (4H, m), 3.62–3.65 (4H, m), 5.37 (2H, br s), 6.75–6.81 (2H, m), 7.24 (1H, d, J = 8.7 Hz).

6.40. 4-[(1-Methylpiperidin-4-yl)oxy]-3-(trifluoromethyl) aniline (18)

To a suspension of sodium hydride (60% in oil, 398 mg, 9.94 mmol) in DMF (10 mL) was added l-methylpiperidin-4-ol (1.26 g, 6.03 mmol) at 0 °C, and the mixture stirred at 0 °C. After 30 min, **16** was added to the mixture, and the resulting mixture stirred at 100 °C for 7 h. The mixture was diluted with AcOEt and washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was dissolved in MeOH (15 mL) and 10% palladium on carbon (water ~50%, 10 mg) was added. The resulting mixture was stirred under a hydrogen atmosphere at room temperature for 3 h. The catalyst was filtered off, and the filtrate was concentrated in vacuo to give **18** (998 mg, 60%) as a black solid: ^{1}H NMR (DMSO-d_6) δ 1.56–2.67 (2H, m), 1.81–1.92 (2H, m), 1.99–2.20 (5H, m), 2.50–2.60 (2H, m), 4.24–4.30 (1H, m), 5.02 (2H, s), 6.75 (1H, dd, J = 8.5, 2.8 Hz), 6.81 (1H, d, J = 2.8 Hz), 6.97 (1H, d, J = 8.5 Hz).

6.41. 4-(Morpholin-4-ylcarbonyl)-3-(trifluoromethyl)aniline (20a)

To a solution of **19a** (2.00 g, 8.51 mmol) in dichloromethane (55 mL) were added oxalyl chloride (3.65 mL, 42.4 mmol) and DMF (100 μL), and the mixture stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure, and the residue was dissolved in dichloromethane. To the solution was added morpholine (2.50 mL, 22.7 mmol) at 0 °C, and the mixture was stirred at room temperature for 5 h. The mixture was diluted with water and extracted with AcOEt. The extract was washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was dissolved in MeOH (15 mL) and 10% palladium on carbon (water ~50%, 100 mg) was added. The resulting mixture was stirred under a hydrogen atmosphere at room temperature for 3 h. The catalyst was filtered off, and the filtrate was concentrated in vacuo. The residual solid was collected and washed with AcOEt–hexane to give **20a** (1.68 g, 72%) as a pale yellow solid: ^{1}H NMR (DMSO-d_6) δ 2.10–2.40 (4H, m), 3.05–3.20 (2H, m), 3.50–3.65 (2H, m), 5.78 (2H, s), 6.75–6.79 (1H, m), 6.88 (1H, d, J = 1.8 Hz), 7.01 (1H, d, J = 7.8 Hz).

6.42. 4-[(4-Methylpiperazin-1-yl)carbonyl]-3-(trifluoromethyl) aniline (20b)

The compound **20b** was prepared in a similar manner to the procedure described for the preparation of **20a** to yield a white solid (84%): ^{1}H NMR (DMSO-d_6) δ 2.10–2.40 (7H, m), 3.05–3.20 (2H, m), 3.50–3.65 (2H, m), 5.78 (2H, s), 6.75–6.79 (1H, m), 6.88 (1H, d, J = 1.8 Hz), 7.01 (1H, d, J = 7.8 Hz).

6.43. 3-(Morpholin-4-ylcarbonyl)-5-(trifluoromethyl)aniline (20c)

The compound **20c** was prepared in a similar manner to the procedure described for the preparation of **20a** to yield a white solid (61%): ^{1}H NMR (DMSO-d_6) δ 3.30–3.70 (8H, m), 5.80 (2H, s), 6.74 (1H, s), 6.78 (1H, s), 6.80 (1H, s).

6.44. 3-[(4-Methylpiperazin-1-yl)carbonyl]-5-(trifluoromethyl) aniline (20d)

The compound **20d** was prepared in a similar manner to the procedure described for the preparation of **20a** to yield a white solid (75%): ^{1}H NMR (DMSO-d_6) δ 2.21 (3H, s), 2.25–2.40 (4H, m), 3.25–3.40 (4H, m), 5.80 (2H, s), 6.69 (1H, s), 6.76 (1H, s), 6.89 (1H, s).

6.45. 4-(Morpholin-4-ylmethyl)-3-(trifluoromethyl)aniline (21a)

To a solution of **20a** (1.00 g, 3.65 mmol) in THF (25 mL) was added 1.9 M dimethylsulfide–borane in THF (9.60 mL, 18.2 mmol) dropwise at 0 °C. The mixture was stirred at room temperature for 1 h, and then heated at reflux for 3 h. After cooling to room temperature, 6 M HCl (10 mL) was added and the resulting mixture was stirred for 30 min, then cooled to 0 °C. To the mixture was added 8 M NaOH (10 mL) and the mixture was partitioned between water and AcOEt. The organic layer was separated and washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure to give **21a** (1.88 g, 99%) as a pale yellow oil: ^{1}H NMR (DMSO-d_6) δ 2.30–2.40 (4H, m), 3.39 (2H, s), 3.50–3.60 (4H, m), 5.45 (2H, s), 6.72–6.75 (1H, m), 6.85 (1H, d, J = 2.1 Hz), 7.30 (1H, d, J = 8.4 Hz).

6.46. 4-[(4-Methylpiperazin-1-yl)methyl]-3-(trifluoromethyl) aniline (21b)

The compound **21b** was prepared in a similar manner to the procedure described for the preparation of **21a** to yield a white solid (99%): ^{1}H NMR (DMSO-d_6) δ 2.25 (3H, s), 2.50–2.65 (4H, m), 2.90–3.05 (4H, m), 3.45 (2H, s), 5.48 (2H, s), 6.58 (1H, s), 6.72 (1H, d, J = 9.0 Hz), 6.85 (1H, d, J = 9.0 Hz).

6.47. 3-(Morpholin-4-ylmethyl)-5-(trifluoromethyl)aniline (21c)

The compound **21c** was prepared in a similar manner to the procedure described for the preparation of **21a** to yield a white solid (72%): ^{1}H NMR (DMSO-d_6) δ 2.30–2.40 (4H, m), 3.36 (2H, s), 3.50–3.60 (4H, m), 5.54 (2H, s), 6.69 (1H, s), 6.72 (1H, s), 6.77 (1H, s).

6.48. 3-[(4-Methylpiperazin-1-yl)methyl]-5-(trifluoromethyl) aniline (21d)

The compound **21d** was prepared in a similar manner to the procedure described for the preparation of **21a** to yield a white solid (50%): ^{1}H NMR (DMSO-d_6) δ 2.28 (3H, s), 2.60–2.70 (4H, m), 2.95–3.05 (4H, m), 3.43 (2H, s), 5.56 (2H, s), 6.66–6.76 (3H, m).

6.49. 1-*tert*-Butyl-4-nitro-1*H*-pyrazole (23a)

A mixture of 4-nitropyrazole (1.13 g, 10 mmol), 2-bromo-2-methylpropane (17.8 g, 130 mmol), potassium carbonate (21.6 g, 156 mmol), and DMF (50 mL) was stirred at 80 °C for 3 days. The mixture was diluted with water and extracted with AcOEt. The ex-

tract was washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane) to give **23a** (1.13 g, 67%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.63 (9H, s), 8.09 (1H, s), 8.24 (1H, s).

6.50. 4-Nitro-1-(2,2,2-trifluoroethyl)-1*H*-pyrazole (23b)

The compound **23b** was prepared in a similar manner to the procedure described for the preparation of **23a** to yield a white solid (96%): ^{1}H NMR (DMSO-d_6) δ 4.77 (2H, q, J = 8.1 Hz), 8.16 (1H, s), 8.29 (1H, s).

6.51. 1-*tert*-Butyl-1*H*-pyrazol-4-amine (24a)

A mixture of **23a** (1.10 g, 6.5 mmol), 10% palladium on carbon (water ~50%, 100 mg), and MeOH (20 mL) was stirred under hydrogen atmosphere at room temperature for 3 h. The mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane) to give **24a** (838 mg, 92%) as a pale yellow oil: ^{1}H NMR (DMSO-d_6) δ 1.53 (9H, s), 2.86 (2H, br s), 7.15 (1H, s), 7.19 (1H, s).

6.52. 1-(2,2,2-Trifluoroethyl)-1*H*-pyrazol-4-amine (24b)

The compound **24b** was prepared in a similar manner to the procedure described for the preparation of **24a** to yield a pale yellow oil (89%): ^{1}H NMR (DMSO-d_6) δ 2.98 (2H, br s), 4.57 (2H, q, J = 8.4 Hz), 7.09 (1H, s), 7.25 (1H, s).

6.53. 5-(Trifluoromethyl)pyridin-3-amine (27)

A mixture of **25** (6.48 g, 30.0 mmol), ammonium formate (3.78 g, 60.0 mmol), 10% palladium on carbon (water ~50%, 2.59 g), and MeOH (15 mL) was stirred at room temperature for 2 h. The mixture was diluted with diethyl ether (60 mL) and the resulting solid was filtered off. The careful removal of excess diethyl ether by evaporation provided a solution of **26** in MeOH. To the solution of **26** in MeOH thus obtained was added 28% aqueous ammonia (45 mL) and CuCl (1.64 g, 16.6 mmol), and the mixture was stirred in an autoclave at 170 °C for 48 h. The mixture was cooled to room temperature and extracted with dichloromethane (40 mL, 30 mL × 2). The combined extracts were washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane) to give **27** (786 mg, 16%) as a pale yellow solid: ^{1}H NMR (DMSO-d_6) δ 3.92 (2H, br s), 7.15 (1H, s), 8.24 (1H, d, J = 2.3 Hz), 8.27 (1H, s).

6.54. 2-(Trifluoromethyl)pyridine 1-oxide (29)

To a solution of **28** (12.1 g, 82.0 mmol) in dichloromethane (96 mL) was added *m*-chloroperbenzoic acid (70%, 22.2 g, 90.2 mmol), and the mixture stirred at room temperature for 24 h. A 5% sodium thiosulfate solution (100 mL) and saturated aqueous $NaHCO_3$ solution (100 mL) were added to the mixture, and the resulting mixture stirred at room temperature for 1 h. The organic layer was separated, and the water layer was extracted with dichloromethane (100 mL × 3). The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography (AcOEt/hexane) to give **29** (6.31 g, 47%) as a colorless oil: ^{1}H NMR (DMSO-d_6) δ 7.34 (1H, t, J = 7.9 Hz), 7.40–7.50 (1H, m), 7.70 (1H, dd, J = 7.9, 1.9 Hz), 8.31 (1H, d, J = 6.6 Hz).

6.55. 2-(Trifluoromethyl)pyridin-4-amine (31)

Concd H_2SO_4 (20 mL) was added to **29** (6.30 g, 38.6 mmol) at 0 °C followed by addition of fuming HNO_3 (d1.52, 20 mL), and the mixture stirred at 130 °C for 5 h. The mixture was cooled to room temperature and poured into ice-water (100 g), neutralized with 8 M NaOH, and extracted with dichloromethane (100 mL × 3). The extracts were concentrated under reduced pressure and the residue was purified by silica gel column chromatography (AcOEt/hexane) to give **30a** (1.05 g, 14%) and **30b** (968 mg, 12%). A mixture of **30a** (1.05 g, 5.46 mmol), MeOH (20 mL), and 10% palladium on carbon (water ~50%, 420 mg) was stirred under a hydrogen atmosphere at room temperature for 6 h. The catalyst was filtered off, and the filtrate was concentrated in vacuo to give **31** (908 mg, quant.) as a colorless oil. A mixture of **30b** (966 mg, 4.64 mmol), MeOH (20 mL), and 10% palladium on carbon (water ~50%, 386 mg) was stirred under a hydrogen atmosphere at room temperature for 6 h. The catalyst was filtered off, and the filtrate was concentrated in vacuo to give **31** (772 mg, quant.) as a colorless oil.

Compound **30a**: ^{1}H NMR (DMSO-d_6) δ 8.27 (1H, dd, J = 5.1, 2.1 Hz), 8.43 (1H, d, J = 2.1 Hz), 9.09 (1H, d, J = 5.1 Hz).

Compound **30b**: ^{1}H NMR (DMSO-d_6) δ 8.28 (1H, dd, J = 7.1, 3.0 Hz), 8.36 (1H, d, J = 7.1 Hz), 8.53 (1H, d, J = 3.0 Hz).

Compound **31**: ^{1}H NMR (DMSO-d_6) δ 4.40 (2H, br s), 6.64 (1H, dd, J = 5.6, 2.4 Hz), 6.89 (1H, d, J = 2.4 Hz), 8.31 (1H, d, J = 5.6 Hz).

6.56. Expression, purification, crystallization, and structure determination

The gene for human VEGFR2 (residue 806–1171 except a kinase insertion domain (KID) region (940–989)) was cloned into a pFastBacHT (Invitrogen, USA) baculovirus expression vector with an N-terminal 6-Histidine tag containing an recombinant Tobacco Etch Virus (rTEV) protease cleavage site. The protein was expressed in Sf9 insect cells (Invitrogen) and purified by immobilized metal-chelate affinity chromatography (IMAC). Protein bound to the IMAC column was eluted with a buffer containing 20 mM Tris–HCl pH 8.0, 0.5 M NaCl, 10% glycerol, 5 mM DTT, and 250 mM imidazole. The eluent from the IMAC column was loaded onto a Sephacryl S-200 size exclusion column pre-equilibrated in 20 mM Tris–HCl pH 8.0, 50 mM NaCl, 5 mM DTT, and 10% glycerol and eluted with the same buffer. Fractions containing the monomeric protein species were pooled and then the tag was removed by cleavage with rTEV protease, followed by a Mono-Q anion exchange column pre-equilibrated in 20 mM Tris–HCl pH 8.0, 50 mM NaCl, and 5 mM DTT and eluted using a linear gradient to 20 mM Tris–HCl pH 8.0, 300 mM NaCl, and 5 mM DTT. Fractions containing the non-phosphorylated protein species were pooled and their buffer was exchanged to 50 mM HEPES pH 7.5, 25 mM NaCl, 10 mM DTT, and 10% glycerol by a HiPrep desalting column. Inhibitor in DMSO solution was added to a solution of approximately 8 mg/mL protein with gentle stirring to give a final concentration of 0.5 mM, and the enzyme inhibitor complex was then incubated for 3 h on ice.

Crystallization experiments were performed at 4 °C by mixing 100 nL of enzyme/inhibitor (**1b**) solution with 100 nL of precipitant solution containing 1.3 M tri-sodium citrate. Crystals were harvested by mixing precipitant solution with the cryoprotectant glycerol to a final concentration of 24% and flash-frozen by direct immersion in liquid nitrogen. X-ray diffraction data of the crystals were collected at 100 K with RIGAKU in-house Cu Kα X-ray generator and RAXIS image-plate system, and at SPring-8 in Harima, Japan on beam-line BL32B2 at a wavelength of 1 Å, respectively. The crystals belong to the monoclinic space group *C*2 with approximate cell dimensions 135 × 56 × 52 Å, β = 95°, diffracting to 1.65 Å resolution. The structure was solved by molecular replace-

ment with MOLREP in the CCP4 program suite using the apo structure of VEGFR2 and refined with CNX2002. The final refined crystallographic statistics for the co-crystal structure with **1b** are R = 19.7% (R_{free} = 22.4%) with root-mean-square deviations (RMSD) in bond lengths and angles of 0.008 Å and 1.2°, respectively.

6.57. VEGFR2 and FGFR1 kinase assays

VEGFR2 and FGFR1 kinase activities were determined by use of an anti-phosphotyrosine antibody with quantitation performed through the AlphaScreen® system (PerkinElmer, USA). For VEGFR2, enzyme reactions were performed in 50 mM Tris–HCl pH 7.5, 5 mM $MnCl_2$, 5 mM $MgCl_2$, 0.01% Tween-20, and 2 mM DTT, containing 10 μM ATP, 0.1 μg/mL biotinylated poly-GluTyr (4:1), and 0.1 nM of VEGFR2 (Millipore, UK). For FGFR1, kinase assay was performed as described above with 0.1 nM of FGFR1 (ProQinase GmbH, Germany) and 0.2 μM of ATP.

Prior to catalytic initiation with ATP, compound and enzyme were incubated for 5 min at room temperature. The reactions were quenched by the addition of 25 μL of 100 mM EDTA, 10 μg/mL AlphaScreen streptavidine donor beads, and 10 μg/mL acceptor beads in 62.5 mM HEPES pH 7.4, 250 mM NaCl, and 0.1% BSA. Plates were incubated in the dark overnight and then read by EnVision 2102 Multilabel Reader (PerkinElmer). Wells containing the substrate and the enzyme without compound were used as total reaction control. Wells containing biotinylated poly-GluTyr (4:1) and enzyme without ATP were used as basal control. The concentration of inhibitor producing 50% inhibition of the kinase activities (IC_{50} values) and 95% confidence intervals (95% CI) for VEGFR2 and FGFR1 were analyzed using GraphPad Prism version 5.01, GraphPad Software (USA). Sigmoidal dose–response (variable slope) curves were fitted using non-linear regression analysis, with the top and bottom of the curve constrained at 100 and 0, respectively.

6.58. Cell proliferation assay

HUVECs (Cambrex, USA) were seeded into a 96-well plate at 3000 cells/well in Human Endothelial-SFM Growth Medium (Invitrogen) containing 3% fetal bovine serum (FBS) (Hyclone, USA) and were incubated overnight at 37 °C in a 5% CO_2 incubator. Various concentrations of the test compounds were added in the presence of 60 ng/mL VEGF (R&D systems, USA) or 10 ng/mL bFGF (Invitrogen), and the cells were cultured for a further 5 days. Cellular proliferation was determined by the WST-8 formazan assay using Cell Counting Kit-8 (DOJINDO Laboratories, Japan). Briefly, 10 μL/well of Cell Counting Kit-8 was added and the cells were cultured for several hours. Then, the absorbance value at 450 nm was measured using a Benchmark Plus Microplatereader (Bio-Rad Labs., USA). The IC_{50} values and 95% confidence intervals (95% CI) were calculated from a dose–response curve generated by least-squares linear regression of the response using NLIN procedure of the SAS software (SAS Institute Japan, Inc., Japan).

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