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Design and Synthesis of Novel Human Epidermal Growth Factor Receptor 2 (HER2)/Epidermal Growth Factor Receptor (EGFR) Dual Inhibitors Bearing a Pyrrolo[3,2-*d*]pyrimidine Scaffold†

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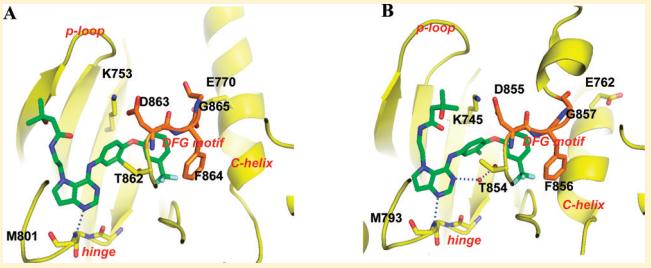
Supporting Information

ABSTRACT: Dual inhibitors of human epidermal growth factor receptor 2 (HER2) and epidermal growth factor receptor (EGFR) have been investigated for breast, lung, gastric, prostate, and other cancers; one, lapatinib, is currently approved for breast cancer. To develop novel HER2/EGFR dual kinase inhibitors, we designed and synthesized pyrrolo[3,2-*d*]pyrimidine derivatives capable of fitting into the receptors' ATP binding site. Among the prepared compounds, **34e** showed potent HER2 and EGFR (HER1) inhibitory activities as well as tumor growth inhibitory activity. The X-ray cocrystal structures of **34e** with both HER2 and EGFR demonstrated that **34e** interacts with the expected residues in their respective ATP pockets. Furthermore, reflecting its good oral bioavailability, **34e** exhibited potent in vivo efficacy in HER2-overexpressing tumor xenograft models. On the basis of these findings, we report **34e** (TAK-285) as a promising candidate for clinical development as a novel HER2/EGFR dual kinase inhibitor.



INTRODUCTION

Protein kinases play important roles in signal transduction pathways that regulate numerous cellular functions, including proliferation, differentiation, migration, apoptosis, and angiogenesis. Because signal transduction pathways are upregulated in many tumor cells, protein kinase inhibitors that target these upregulated pathways are attractive candidates for cancer therapy.[1] The targeting of human epidermal growth factor receptor (HER) or epidermal growth factor receptor (EGFR) by tyrosine kinase inhibitors (TKIs) represents one such therapeutic approach. The HER kinase family contains 4 members (EGFR (HER1 or ErbB-1), HER2 (ErbB-2 or neu), HER3 (ErbB-3), and HER4 (ErbB-4)) that are multidomain proteins consisting of an extracellular ligand binding domain, a single transmembrane domain, and an intracellular tyrosine kinase domain.[2] Several ligands that bind to the extracellular portion of EGFR, HER3, and HER4 have been identified. Upon ligand binding, these receptors form homo- or heterodimers to undergo autophosphorylation of each tyrosine residue within the intracellular kinase domain.[3] Although there is no known ligand for HER2, this receptor also undergoes spontaneous homo- or heterodimerization and activates downstream signaling.[4]

As for EGFR inhibitors, small molecules such as gefitinib[5] and erlotinib[6] (Figure 1), as well as anti-EGFR antibodies cetuximab[7] and panitumumab,[8] have been approved for the treatment of EGFR-overexpressing lung cancers and have been shown to be effective in a subset of patients. HER2 is overexpressed in a number of human cancers, including 20–40% of solid tumors, e.g., breast, ovarian, lung, gastric, and oral cancers, in which overexpression of this receptor correlates with poor prognosis.[9] In addition, because HER2 is only expressed at low levels in normal human tissues, it is an attractive target for tumor-specific therapies. On the basis of this, two approaches have been developed for blocking the upregulated HER2 signal pathway. One approach utilizes the anti-HER2 monoclonal antibody, trastuzumab,[10] which blocks the extracellular ligand-binding region of the receptor, thereby interfering with its activation and modulating the resultant intracellular signal cascade. The other approach for blocking the upregulated HER2 signal pathway involves the use of orally active small molecule TKIs several of which have been developed. Many types of malignancies are characterized by overexpression of HER2 and EGFR. Lapatinib,[11] a dual TKI for

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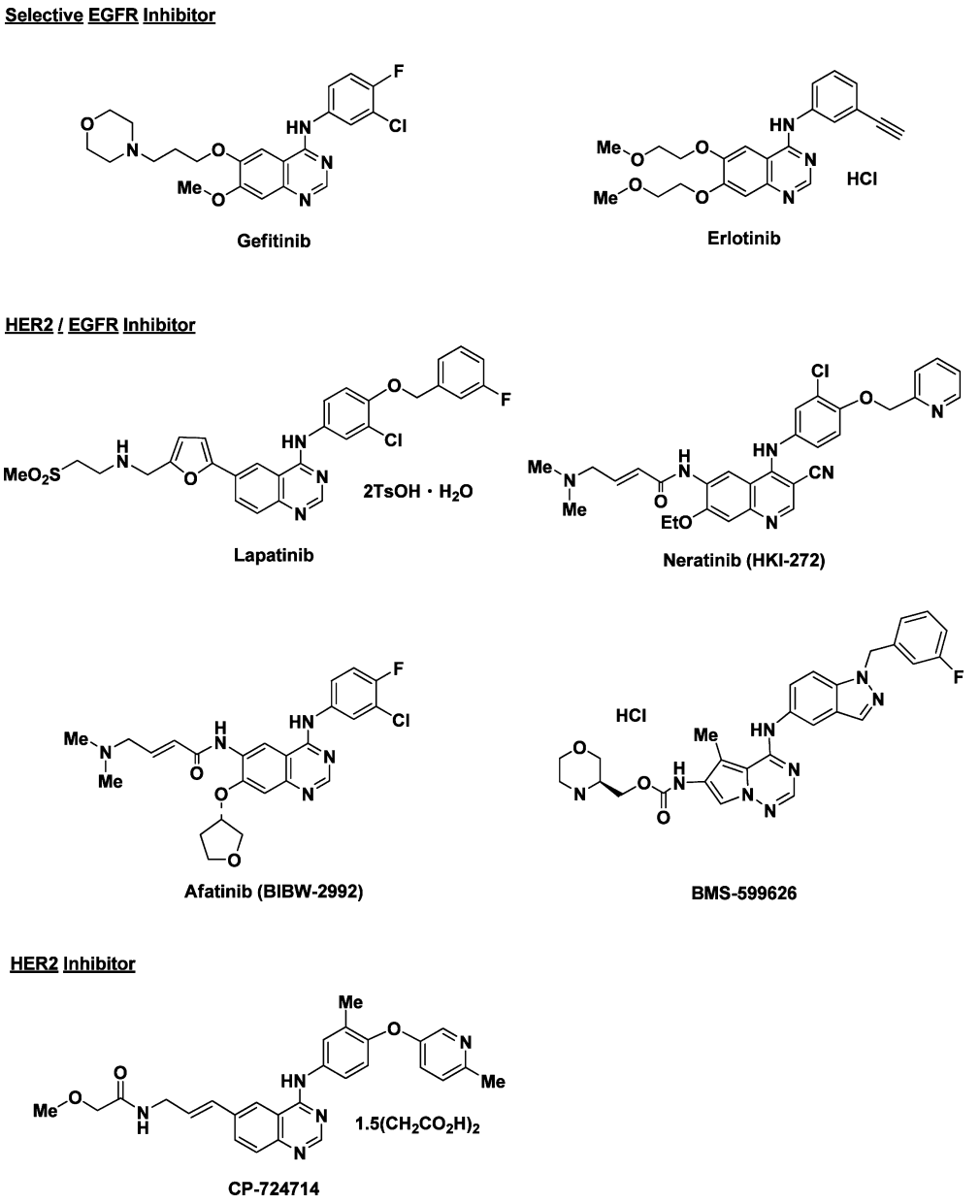

Figure 1. Structure of EGFR and/or HER2 small molecule inhibitors.

HER2 and EGFR, was approved in 2007 in combination with capecitabine or letrozole in patients with metastatic breast cancer that overexpress the HER2 receptor. Irreversible inhibitors (Neratinib/HKI-272[12] and Afatinib/BIBW-2992[13]) and reversible inhibitors (AEE-788[14] and BMS-599626[15]) have also been tested in clinical trials. In addition to breast cancer, these HER2/EGFR dual kinase inhibitors are being investigated in a number of solid tumors including lung, gastric, and prostate cancers.[9,11−15] In addition, CP-724714[16] has been reported as a HER2-specific TKI. All of these small molecule inhibitors share a common structure characterized by a pyrimidine or pyrimidine analogue at its core.

For the purpose of developing a novel HER2/EGFR TKI, we focused on the pyrrolo[3,2-*d*]pyrimidine scaffold, which contains a pyrimidine core that interacts with Met801 and Thr862 in the ATP-binding site of the HER2 hinge region (Figure 2). Nucleic acid research using pyrrolo[3,2-*d*]-pyrimidine derivatives has been conducted in our laboratories,[17] but this pyrimidine core had not been previously utilized for drug development. Molecular modeling studies using HER2 homology models based on the EGFR cocrystal structure

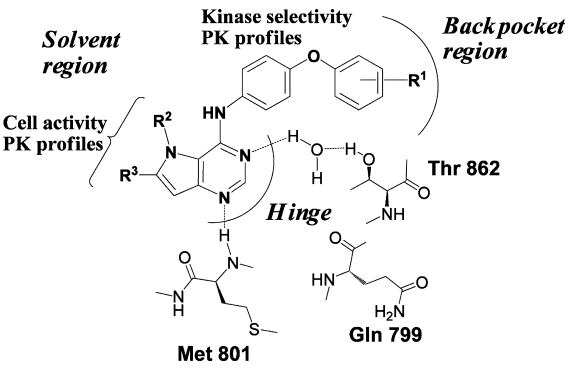

Figure 2. Design of pyrrolo[3,2-*d*]pyrimidine scaffold as HER2 kinase inhibitors.

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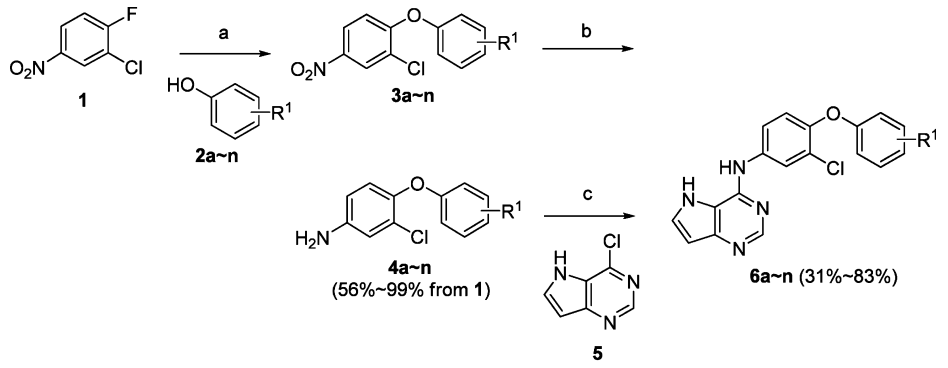
Scheme 1. Synthesis of Pyrrolo[3,2-*d*]pyrimidine Derivatives 6a−n[a]



[a]Reagents: (a) **2a−n**, K_2CO_3, DMF, 80 °C, 2 h; (b) H_2, Pt−C, AcOEt, rt, 2 h, or Fe, $CaCl_2$, EtOH, 100 °C, 15 h, 56−99% for 2 steps; (c) **5**, NMP, 100 °C, 3 h, 31−83%.

with erlotinib[6] suggested that the pyrrolo[3,2-*d*]pyrimidine scaffold would fit into the ATP-binding site of the HER2/EGFR protein. In this model, introduction of a phenoxy anilino group at the *C*-4 position could occupy a lipophilic back pocket and enhance anti-HER2/EGFR kinase activity. In addition, because the linkages at the *N*-5 or *C*-6 positions are directed toward the solvent contact region, we envisioned that chemical modification of the R^2 or R^3 groups at these positions could be utilized to further improve the cellular activity and pharmacokinetic properties of this chemotype.

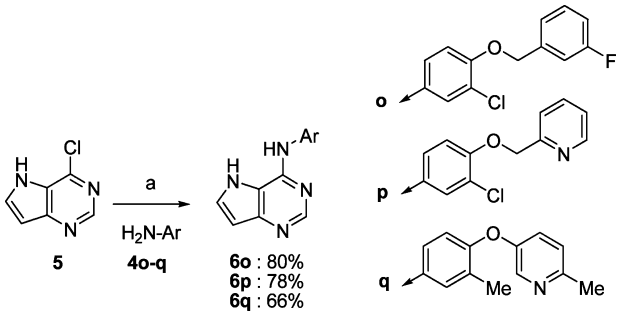
In this paper, we describe the structure−activity relationships (SAR) and in vivo efficacy of our pyrrolo[3,2-*d*]pyrimidine derivatives. Crystallographic studies of **34e** (TAK-285),[18] our candidate for clinical development, with HER2 and EGFR revealed key molecular details related to compound binding and specificity.

CHEMISTRY

With the aim of examining substituent effects on the terminal phenoxy rings, 4-substituted anilino-5*H*-pyrrolo[3,2-*d*]-pyrimidine derivatives (**6a−n**) were synthesized by the method shown in Scheme 1. Condensation of commercially available 2-chloro-1-fluoro-4-nitrobenzene (**1**) with phenols (**2a−n**) was carried out in the presence of potassium carbonate in dimethylformamide (DMF), followed by reduction of the nitro group with platinum on carbon under hydrogen atmosphere to afford substituted anilines (**4a−e**, **4i−n**) in good yields (61−99% in 2 steps). In the case of **4f−h** containing a cyano group, the nitro group was reduced using reduced-iron and calcium chloride in ethanol (EtOH) (56−71% in 2 steps). The obtained anilines **4a−n** were reacted with 4-chloro-5*H*-pyrrolo[3,2-*d*]-pyrimidine (**5**)[19] in 1-methyl-2-pyrrolidone (NMP) at 100 °C to afford **6a−n** in moderate yields (31−83%). In Scheme 2, the pyrrolo[3,2-*d*]pyrimidine derivatives **6o−q** were prepared from reported anilines **4o−q**[11c,12a,16c] in a similar manner to that shown in Scheme 1.

Next, we selected 3 anilino groups (**e**: −Cl; **j**: $-CF_3$; **l**: $-OCF_3$) and examined the introduction of methyl (**8e−l**), 2-hydroxyethyl (**10e−l**), and 2-(2-hydroxyethoxy)ethyl (**12e−l**) moieties as representative alkyl substituents at the *N*-5 position. The synthesis is shown in Scheme 3. The 4-chloro derivative **5** was reacted with iodomethane under basic conditions using potassium carbonate in DMF to afford 5-methyl intermediate **7** (93% yield). The methylated adduct **7** was converted into the desired products **8e−l** by coupling with aniline **4e** (92%), **4j** (75%), and **4l** (77%), respectively. Similarly, alkylation of

Scheme 2. Synthesis of Pyrrolo[3,2-*d*]pyrimidine Derivatives 6o−q[a]



[a]Reagents: (a) **4o−4q**, NMP, 100 °C, 3 h, 66−80%.

compound **5** with 2-iodoethyl benzoate or 2-(2-iodoethoxy)-ethyl benzoate in the presence of cesium carbonate in DMF gave the adducts **9** and **11** in 70% and 55% yield, respectively. After reaction of **9** or **11** with aniline (**4e**, **4j**, **4l**), the terminal benzoyl group was hydrolyzed by 1N aqueous sodium hydroxide (NaOH) to afford the desired target compounds (**10e−l**, **12e−l**) in 46−72% yields. A similar reaction was carried out for the synthesis of **12r**, which is a pyridine analogue of **12j**.

On the basis of this SAR study, we focused on the 2-(2-hydroxyethoxy)ethyl group of compound **12** at the *N*-5 position. We selected a trifluoromethyl group **j** as an anilino substituent on the terminal phenoxy ring, and carried out further optimization of the 2-(2-hydroxyethoxy)ethyl group at the *N*-5 position.

The synthesis of the *N*-5-hydroxypentyl derivative **15**, where the ether linkage in **12j** was replaced with a methylene bridge, is shown in Scheme 4. Alkylation of compound **5** with 5-chloropentyl acetate in the presence of cesium carbonate in DMF gave 5-(4-chloro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)pentyl acetate (**13**) in 69% yield. The obtained compound **13** was reacted with aniline **4j** in 2-propanol followed by hydrolysis of the acetate group with 1N aqueous NaOH to afford the desired compound **15** in 79% yield in two steps.

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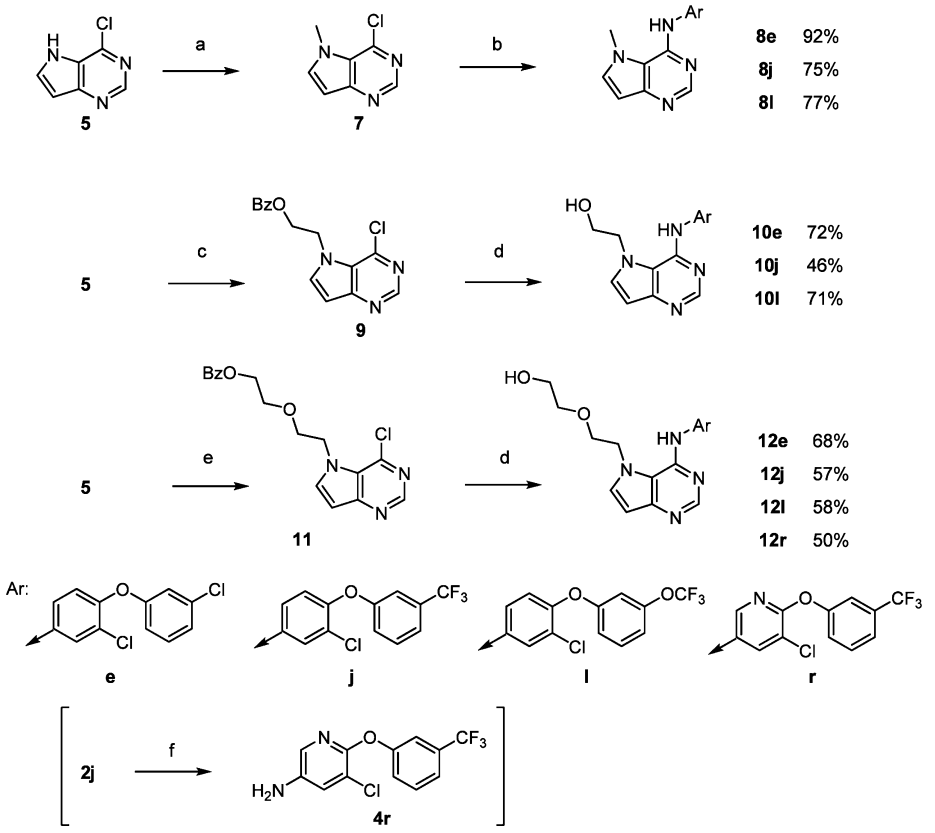
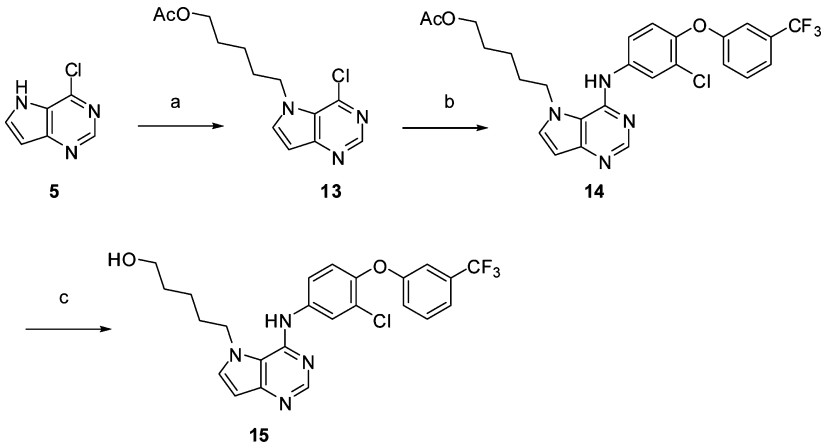
Scheme 3. Synthesis of Pyrrolo[3,2-*d*]pyrimidine Derivatives (8, 10, and 12)[a]



[a]Reagents: (a) iodomethane, K_2CO_3, DMF, rt, 3 h, 93%; (b) aniline **4e** or **4j** or **4l**, NMP, 140 °C, 4 h, 75−92%; (c) 2-iodoethyl benzoate, Cs_2CO_3, DMF, rt, 15 h, 70%; (d) (i) aniline **4e** or **4j** or **4l** NMP, 140 °C, 2.5 h, (ii) 1N NaOH, MeOH, rt, 46−72%; (e) 2-(2-iodoethoxy)ethyl benzoate, Cs_2CO_3, DMF, rt, 15 h, 55%; (f) (i) 2,3-dichloro-5-nitropyridine, NaH, THF, rt, 2.5 h, (ii) Fe, $CaCl_2$, EtOH, 80 °C, 8 h, 39% from **2j**.

Scheme 4. Synthesis of *N*-5 Hydroxypentyl Pyrrolo[3,2-*d*]pyrimidine derivative 15[a]



[a]Reagents: (a) 5-chloropentyl acetate, Cs_2CO_3, DMF, 40 °C, 4 days, 69%; (b) **4j**, 2-PrOH, 80 °C, 14 h; (c) 1N NaOH, rt, 1 h, MeOH, 79% for 2 steps.

The pyrrolo[3,2-*d*]pyrimidine derivative **20** with substitution of the nitrogen atom for *N*-5 ether oxygen in **12j** was synthesized as shown in Scheme 5. Reaction of compound **5** with 2-bromo-1,1-diethoxyethane gave alkylated product **16** in 72% yield. The obtained compound **16** was heated with phenol in the presence of potassium carbonate in NMP to afford phenoxy diethylacetal derivative (**17**) in excellent yield. After deprotecting the diethylacetal moiety with trifluoroacetic acid, reductive amination of the obtained ethanediol derivative (**18**) with 2-hydroxyethylamine provided 2-{[2-(4-phenoxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl]amino}ethanol (**19**) in good yield. Finally, compound **19** was coupled with aniline **4j** using pyridinium chloride as an acidic promoter in phenol to afford the desired product **20** in 19% yield. The low yield was due to formation of tricyclic compound **21**, resulting from intramolecular cyclization of molecule **19**, as the major product.

The pyrrolo[3,2-*d*]pyrimidine derivatives **28**−**30**, possessing a sulfur atom-containing side chain, were synthesized as shown in Scheme 6. The alkylbromide (**25**) was prepared from 2-iodoethyl benzoate derivative (**22**) and 2-mercaptoethanol

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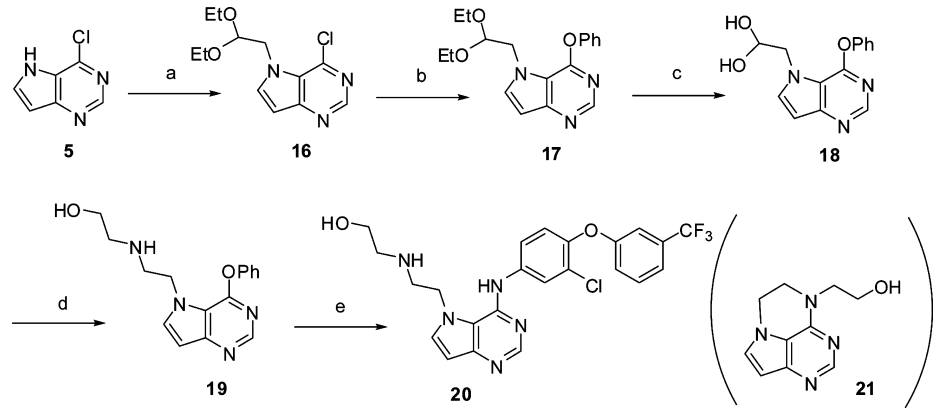
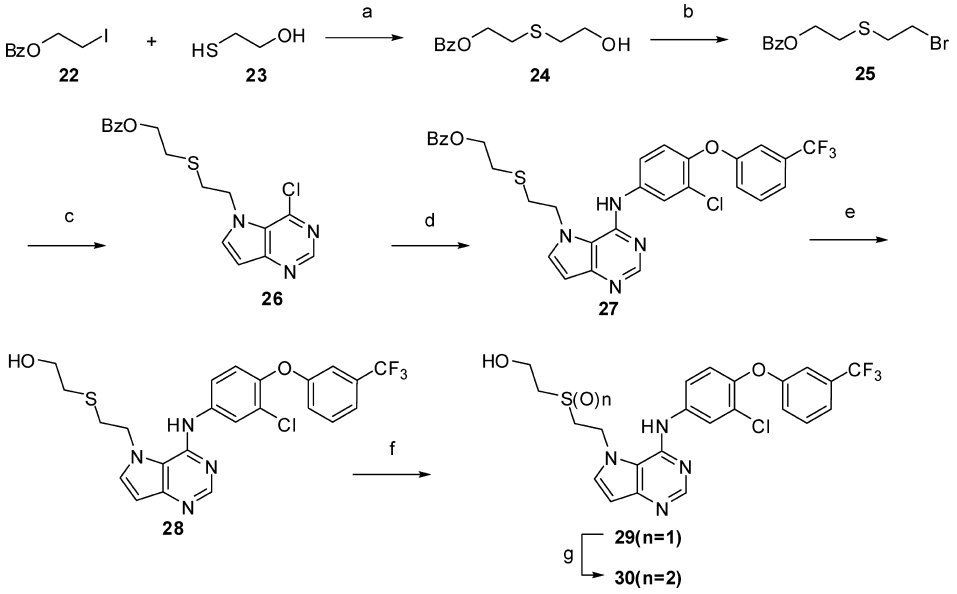
Scheme 5. Synthesis of a *N*-5 Hydroxyethylaminoethyl Pyrrolo[3,2-*d*]pyrimidine Derivative 20[a]



[a]Reagents: (a) 2-bromo-1,1-diethoxyethane, Cs_2CO_3, DMF, 80 °C, 4.5 h, 72%; (b) PhOH, K_2CO_3, NMP, 140 °C, 6 h, 95%; (c) TFA, CH_2Cl_2, rt, 16 h, 91%; (d) 2-aminoethanol, $NaB(OAc)_3H$, AcOH, DMF, rt, 16 h, 52%; (e) **4j**, pyridinium chloride, phenol, 140 °C, 16 h, 19%.

Scheme 6. Synthesis of a *N*-5 Sulfur-Containing Pyrrolo[3,2-*d*]pyrimidine Derivatives (28, 29, and 30)[a]



[a]Reagents: (a) *iso*-Pr_2EtN, 40 °C, 3 days, 77%; (b) PPh_3, CBr_4, rt, 3 days, 76%; (c) **5**, Cs_2CO_3, DMF, rt, 15 h, 80%; (d) **4j**, 2-PrOH, 80 °C, 15 h; (e) 1N NaOH, MeOH, rt, 1 h, 59% for 2 steps; (f) *m*CPBA, CH_2Cl_2, −78 °C, 1 h, 85%; (g) *tert*-BuO_2H, $Ti(O^{iso}Pr)_4$, MeOH, H_2O, rt, 2 days, 74%.

derivative (**23**), which were coupled in the presence of ethyldiisopropylamine to give the thioether derivative (**24**) in 77% yield. Bromination of compound **24** with carbon tetrabromide and triphenylphosphine provided the desired alkylbromide (**25**) in 76% yield. The obtained alkylbromide derivative (**25**) was introduced into the *N*-5 position of compound **5** in the usual manner to afford adduct **26** in 80% yield. Compound **26** was reacted with aniline derivative **4j** in 2-propanol followed by hydrolysis of the benzoyl ester with 1N aqueous NaOH to afford the thioether derivative **28** in 59% yield in 2 steps. The sulfoxide (**29**) was obtained by oxidization of compound **28** with 3-chloroperbenzoic acid (*m*CPBA) in 85% yield. Further oxidization of sulfoxide (**29**) with 70% aqueous *tert*-butyl hydroperoxide in the presence of titanium tetraisopropoxide provided its sulfone derivative (**30**) in 74% yield.

Preparation of the *N*-5 carboxamide derivatives **34a−e** has been summarized in Schemes 7 and 8. Alkylation of compound **5** with *tert*-butyl (2-bromoethyl)carbamate in the usual manner gave intermediate (**31a**) in 71% yield. Condensation of compound **31a** with aniline **4j** was carried out in 2-propanol, followed by deprotecting the terminal Boc group of the obtained molecule **32a** with 2N aqueous hydrochloric acid (HCl) to afford key intermediate (**33a**) in 85% yield in 2 steps. Amidation of the key intermediate **33a** with a variety of carboxylic acids in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (water-soluble carbodiimide, WSC) and 1-hydroxybenzotriazole monohydrate (HOBt) provided the corresponding desired carboxamides (**34a**, **34c**, and **34e**, Scheme 7). Carboxamide **34d** was isolated a methanesulfonic acid salt. The synthesis of carboxamide **34b** was achieved using 2-[(*tert*-butoxycarbonyl)(methyl)amino]-ethyl methanesulfonate as an *N*-5 alkylating agent in a similar manner, shown in Scheme 8.

Next, we examined the introduction of representative alkyl groups at the *C*-6 position in **12j**. The synthesis of the *C*-6 methyl derivative **40** is shown in Scheme 9. The Sonogashira cross-coupling reaction of 4-iodo-6-phenoxypyrimidine-5-amine (**35**) with trimethyl(prop-1-yn-1-yl)silane gave acetylene

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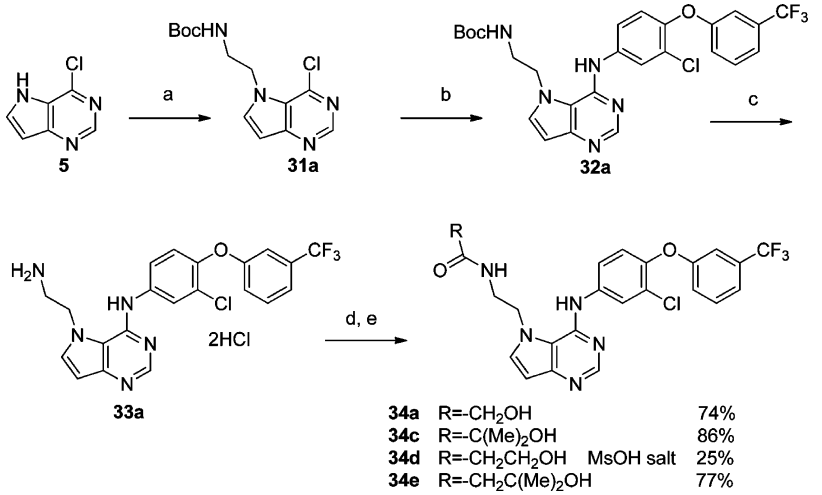
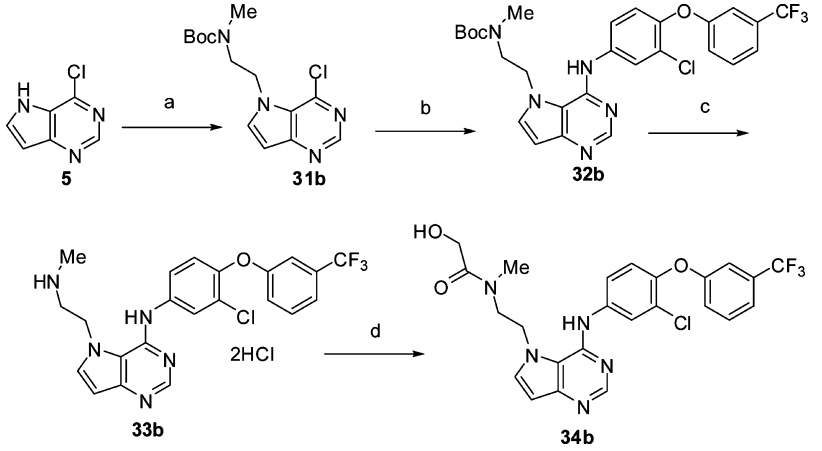
Scheme 7. Synthesis of a *N*-5 Carboxamido Pyrrolo[3,2-*d*]pyrimidine Derivatives (34a and 34c−34e)[a]



[a]Reagents: (a) *tert*-butyl (2-bromoethyl)carbamate, Cs_2CO_3, DMF, 40 °C, 4 days, 71%; (b) **4j**, 2-PrOH, 80 °C, 12 h, 85%; (c) 2N HCl, THF, 60 °C, 20 h, quant; (d) WSC, HOBt, Et_3N, carboxylic acid, DMF, 3 days, 74−86%; (e) MsOH, AcOEt, rt, 2 h, 25% from **33a**.

Scheme 8. Synthesis of a *N*-5 Carboxamido Pyrrolo[3,2-*d*]pyrimidine Derivative 34b[a]



[a]Reagents: (a) 2-[(*tert*-butoxycarbonyl)(methyl)amino]ethyl methanesulfonate, Cs_2CO_3, DMF, 40 °C, 4 days, 33%; (b) **4j**, 2-PrOH, 80 °C, 12 h, 76%; (c) 2N HCl, THF, 60 °C, 20 h, 91%; (d) WSC, HOBt, Et_3N, hydroxyacetic acid, DMF, 3 days, 62%.

derivative **36** in 73% yield. Intramolecular cyclization of compound **36** with sodium *tert*-butoxide afforded 6-methyl-4-phenoxy-5*H*-pyrrolo[3,2-*d*]pyrimidine (**37**) in 67% yield. The obtained molecule (**37**) was reacted with 2-{2-[(methylsulfonyl)-oxy]ethoxy}ethyl benzoate in the usual manner to afford adduct **38** in 93% yield. Reaction of compound **38** with aniline **4j** followed by hydrolysis of the benzoyl ester with 1N aqueous NaOH afforded the desired product **40** in good yield.

Scheme 10 shows the preparation of the 6-cyano derivative **49**. The Sonogashira reaction of compound **35** with 3,3-diethoxyprop-1-yne, followed by intramolecular cyclization using sodium *tert*-butoxide, provided intermediate **42**. After deprotecting the diethylacetal group in compound **42** with 1N aqueous HCl, the aldehyde group of the resulting product **43** was oxidized with sodium chlorite to provide carboxylic acid (**44**) in good yield. Conversion of compound **44** into the acid chloride using thionyl chloride was followed by reaction with ammonia to afford carboxamide (**45**) in 92% yield. Treatment of compound **45** with phosphorus oxychloride gave carbonitrile (**46**) in 61% yield. The obtained product **46** led to the desired 6-cyano pyrrolo[3,2-*d*]pyrimidine derivative (**49**) in a manner similar to that shown in Scheme 9.

BIOLOGICAL RESULTS AND DISCUSSION

To examine the potential of a pyrrolo[3,2-*d*]pyrimidine scaffold for use as a HER2/EGFR kinase inhibitor, a variety of substituted 4-anilino derivatives (**6a−q**) were evaluated. Table 1 shows their HER2/EGFR kinase and tumor cell growth (BT-474, HER2-overexpressing human breast cancer cell line) inhibitory activities as well as their in vitro human metabolic stability. Almost all of the derivatives showed good HER2/EGFR inhibitory activities with IC_{50} values less than 1 μM, especially against HER2. In addition, the reported anilino derivatives **6o** and **6p** showed HER2/EGFR dual inhibitory activity, and derivative **6q** showed HER2-selective activity similar to those of quinazoline analogues.[16] These results suggested that the *N*-1/*N*-3 nitrogen atoms of the pyrrolo[3,2-*d*]pyrimidine scaffold can interact with the Met801 and Thr862 residues in the hinge region of the HER2 ATP-binding pocket and that the 4-anilino group fits into the lipophilic back pocket as expected by our designs. All anilino substituents at the *C*-2 or

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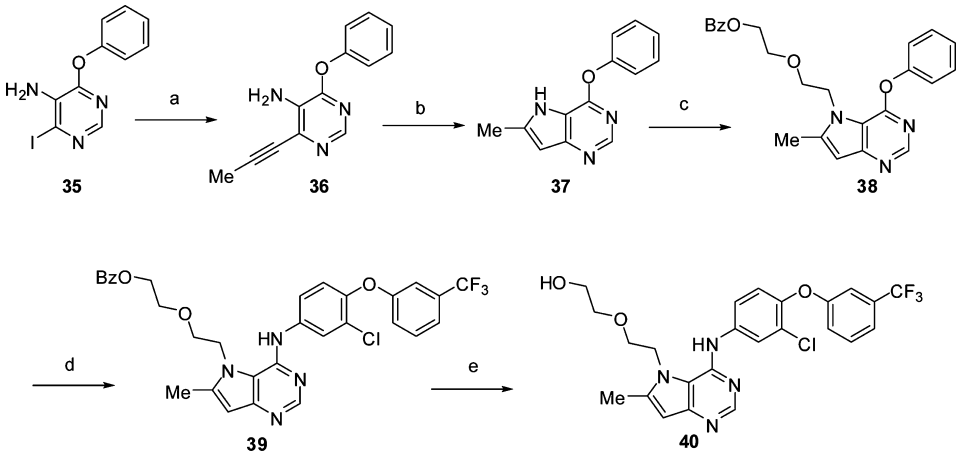
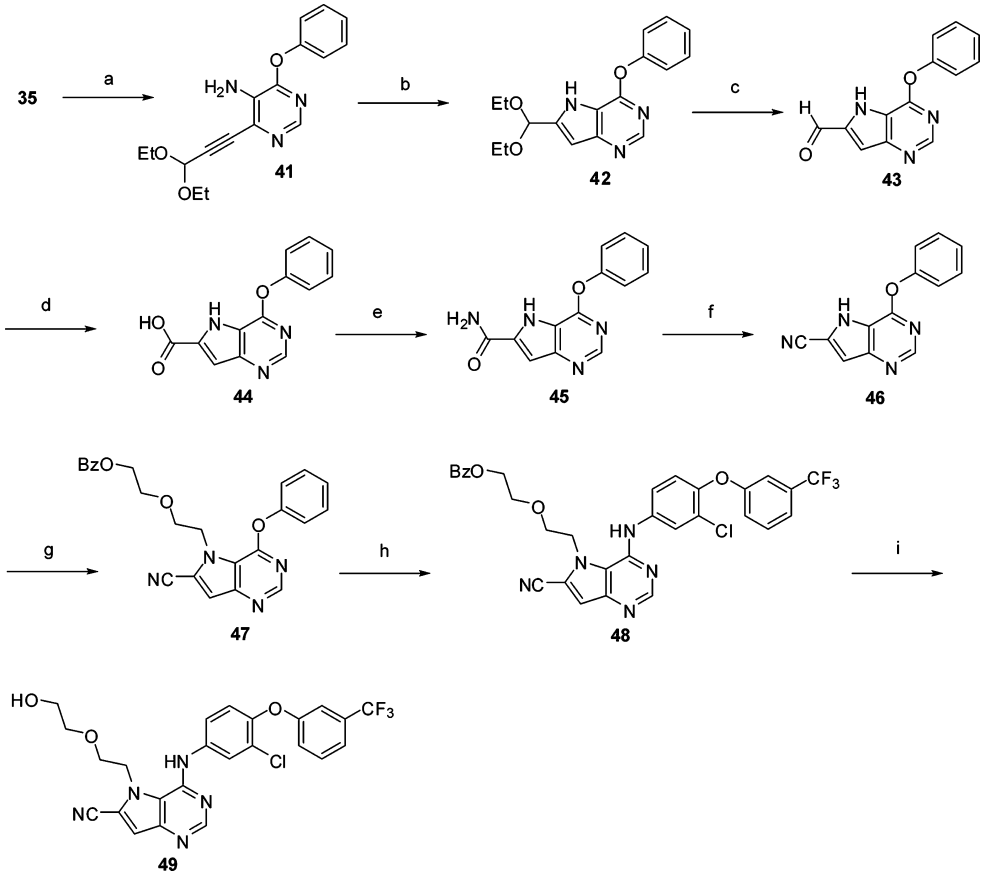
Scheme 9. Synthesis of a *C*-6 Methyl Pyrrolo[3,2-*d*]pyrimidine Derivative 40[a]



[a]Reagents: (a) trimethyl(prop-1-yn-1-yl)silane, $Pd(PPh_3)_2Cl_2$, CuI, KF, DMF, Et_3N, 60 °C, 16 h, 73%; (b) *tert*-BuOK, THF, 0 °C, 40 min, 67%; (c) 2-{2-[(methylsulfonyl)oxy]ethoxy}ethyl benzoate, K_2CO_3, DMF, 60 °C, 21 h, 93%; (d) **4j**, pyridinium chloride, phenol, 140 °C, 17 h, 58%; (e) 1N NaOH, MeOH, rt, 2 h, 90%.

Scheme 10. Synthesis of a *C*-6 Cyano Pyrrolo[3,2-*d*]pyrimidine Derivative 49[a]



[a]Reagents: (a) 3,3-diethoxyprop-1-yne, $Pd(PPh_3)_2Cl_2$, CuI, MeCN, Et_3N, rt, 16.5 h, 89%; (b) *tert*-BuOK, THF, rt, 1.5 h, 51%; (c) 1N HCl, THF, rt, 2 h, 90%; (d) $NaClO_2$, NaH_2PO_4, DMSO, H_2O, rt, 2 h, quant; (e) (i) $SOCl_2$, 75 °C, 2 h, (ii) NH_3, THF, 0 °C, 92%; (f) $POCl_3$, 70 °C, 1.5 h, 61%; (g) 2-{2-[(methylsulfonyl)oxy]ethoxy}ethyl benzoate, K_2CO_3, DMF, 60 °C, 7 h, 61%; (h) **4j**, pyridinium chloride, phenol, 140 °C, 17 h, 74%; (i) 1N NaOH, MeOH, rt, 1 h, 61%.

C-3 positions of the phenoxy ring gave rise to better than 130 nM HER2 inhibitory activity, although the cyano group at the *C*-4 position in **6h** resulted in reduced HER2/EGFR inhibitory activity. Reflecting their potent inhibitory activities, especially against HER2, a variety of compounds (**6b**, **6e**, **6f**, **6g**, **6j**, **6l**, and **6m**) showed good growth inhibitory (GI) activity against BT-474 cells with GI_{50} values of 480−1100 nM, comparable to those of the reported anilino derivatives **6o−q**. Among them, we selected the 3-chloro (**6e**), 3-trifluoromethyl (**6j**), and 3-trifluoromethoxy (**6l**) anilino derivatives because

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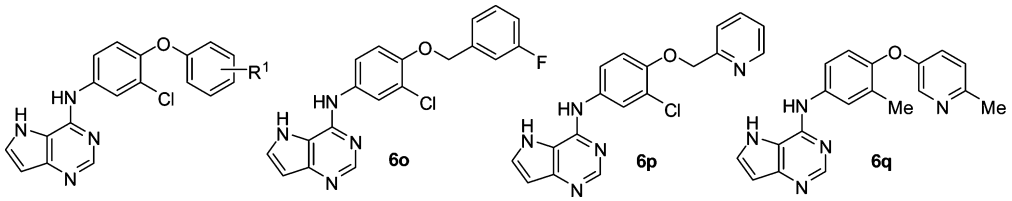
Table 1. Biological Data for *C*-4-Anilino Substituted Pyrrolo[3,2-*d*]pyrimidine Derivatives



		enzyme[a,b]		cell growth [a]	metabolic stability [a]
		IC_{50} (nM) (95% CI)		GI_{50} (nM) (95% CI)	μL/min/mg
compd	R^1	HER2	EGFR	BT-474	human
6a	H	16 (13−19)	110 (93−130)	869 (730−1034)	122
6b	2-F	9.3 (7.3−12)	61 (41−90)	737 (601−903)	87
6c	3-F	20 (15−26)	150 (94−250)	1267 (1070−1501)	56
6d	2-Cl	11 (7.2−17)	27 (18−42)	1413 (1181−1692)	66
6e	3-Cl	8.3 (6.4−11)	35 (23−51)	459 (385−544)	20
6f	2-CN	8.0 (5.9−11)	51 (43−60)	956 (835−1094)	97
6g	3-CN	8.2 (6.1−11)	150 (84−280)	787 (548−1131)	42
6h	4-CN	720 (590−880)	590 (460−740)	4473 (3795−5279)	
6i	2-CF_3	120 (110−130)	150 (110−220)	2328 (1918−2828)	
6j	3-CF_3	30 (23−39)	67 (40−110)	875 (750−1020)	7
6k	2-OCF_3	55 (40−76)	210 (110−390)	2543 (2198−2944)	22
6l	3-OCF_3	41(31−56)	220 (130−370)	1063 (905−1250)	15
6m	3-CH_3	4.6 (4−5.3)	20 (13−31)	762 (605−959)	187
6n	3-OCH_3	26 (19−36)	290 (220−400)	1216 (1067−1386)	150
6o		13 (9.8−17)	7.7 (5−12)	650 (426−994)	36
6p		22 (16−30)	18 (12−27)	1160 (890−1510)	23
6q		60 (49−73)	1900 (1300−2800)	2049 (1806−2324)	70

[a]These measurements were showed in the Experimental Section. [b]IC_{50} values and 95% confidence intervals (CI) were calculated by nonlinear regression analysis of the percentage inhibitions (n = 2).

these compounds showed good in vitro oxidative metabolic stability against human hepatic microsomes.

Next, with the aim of further enhancing their cellular activity, we examined the introduction of *N*-5 substituents into the pyrrolo[3,2-*d*]pyrimidine scaffold. As representative *N*-5 alkyl substituents, methyl (**8**), 2-hydroxyethyl (**10**), and 2-(2-hydroxyethoxy)ethyl (**12**) moieties were selected and their corresponding derivatives with a 3-chloro (**e**), 3-trifluoromethyl (**j**), or 3-trifluoromethoxy (**l**) anilino group were evaluated. These results are summarized in Table 2. Introduction of these substituents at the *N*-5 position maintained the potent HER2/EGFR inhibitory activities. This result was consistent with our design that *N*-5 chemical modifications would be directed toward the solvent contact region of the HER2/EGFR kinases. On the other hand, their cellular activity had a tendency to be higher when the molecule had a longer side chain. Among the prepared compounds, the 2-(2-hydroxyethoxy)ethyl derivatives **12e−l** showed significantly more potent cell growth inhibitory activity than those of the corresponding derivatives **8** and **10**. In addition, compounds **12e−l** showed acceptable metabolic stability and PK profiles in mice. On the basis of its potent GI activity and PK profile in mice, we selected the 3-trifluoromethyl derivative **12j** as lead compound for further optimization. Compared with **12j**, the pyridine analogue **12r** reduced EGFR inhibitory activity together with cellular GI activity. As cocrystal structure[20] of **12r** with HER2 was successfully obtained, we focused on structural discussions of EGFR.

Considering the in vivo oxidative metabolism in the ether linkage of *N*-5 2-(2-hydroxyethoxy)ethyl moiety in **12j**, we focused on replacement of its ether oxygen with a carbon, nitrogen, and sulfur atom. Furthermore, to investigate substituent effects at the *C*-6 position of the pyrrolo[3,2-*d*]pyrimidine scaffold, we introduced representative methyl (**40**) and cyano groups (**49**) to **12j**. These results are shown in Table 3. Similar to the 2-(2-hydroxyethoxy)ethyl moiety in **12j**, replacement of its ether oxygen with carbon (**15**), nitrogen (**20**), and sulfur (**28−30**) maintained good HER2/EGFR kinase inhibitory activities. On the other hand, these derivatives showed reduced GI activity compared with **12j**, although polar linkages such as sulfoxide (**29**) and sulfone (**30**) moieties improved the human metabolic stability. We believed that log *D* at pH 7.4 would be an important factor for enhancing their GI activity. In addition, introduction of the methyl (**40**) and cyano (**49**) group at the *C*-6 position led to a decrease of the cellular GI activities.

With the aim of pursuing compatibility between cellular activity and metabolic stability, we envisioned that replacement of the ether with a carboxamide linkage could provide a good profile with favorable polarity for our purpose (Table 4). These prepared derivatives (**34a−e**) were found to show potent HER2/EGFR inhibitory activities. Although the 2-hydroxyacetamide derivatives **34a,b** (log *D* at pH 7.4: 3.45−3.51) showed slightly reduced cellular activity, higher lipophilicity, comparable to that of **12j**, could be achieved by the addition of a methyl or methylene group to the side chain to recover cellular GI activity (**34c−e**, log *D* at pH 7.4: 3.54−4.18).

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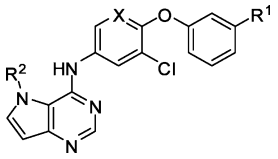
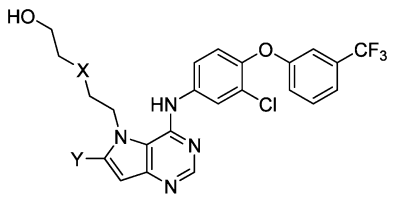
Table 2. Biological Data for *N*5- and *C*4-Substituted Pyrrolo[3,2-*d*]pyrimidine Derivatives



				enzyme [a,b]		cell growth[a]	metabolic stability[a]		PK
				IC_{50} (nM) (95% CI)		GI_{50} (nM) (95% CI)	μL/min/mg		$AUC_{0-8\ h}$[c] μg·h/mL
compd	X	R^1	R^2	HER2	EGFR	BT-474	mice	human	mice
8e	CH	Cl	CH_3	3.3 (2.6−4.3)	9.2 (5.9−14)	184 (142−232)	34	37	
8j	CH	CF_3		11 (8.9−14)	27 (20−37)	692 (565−846)	−13	14	4.545
8l	CH	OCF_3		17 (12−25)	50 (36−69)	1884 (1750−2028)	−9	1	
10e	CH	Cl	2- hydroxy ethyl	4.1 (3.5−4.7)	9.5 (5.9−15)	97 (81−115)	28	23	1.882
10j	CH	CF_3		12 (11−14)	34 (26−44)	212 (179−249)		35	6.381
10l	CH	OCF_3		29 (23−38)	110 (68−180)	582 (481−704)	9	16	11.546
12e	CH	Cl	2-(2- hydroxy ethoxy) ethyl	2.1 (1.8−2.5)	5.7 (4.1−8)	54 (46−63)	41	73	2.633
12j	CH	CF_3		5.1 (4.1−6.3)	15 (11−21)	27 (21−33)	39	63	3.324
12l	CH	OCF_3		8.4 (5.8−12)	49 (31−78)	116 (100−132)	11	53	3.342
12r	N	CF_3		11 (7.8−15)	420 (170−1000)	2170 (1978−2382)	64	96	3.996

[a]These measurements were showed in the Experimental Section. [b]IC_{50} values and 95% confidence intervals (CI) were calculated by nonlinear regression analysis of the percentage inhibitions (n = 2). [c]The animals used in the study were female BALB/cAJcl mice (7-weeks old; CLEA Japan, Inc.). A mixture of 5 test compounds was suspended in 0.5% (w/v) methylcellulose solution for oral administration at a dose of 10 mg each/10 mL/kg. The concentrations of compounds in the plasma were determined by LC/MS/MS.

Table 3. Biological Data for *C*6-, *N*5-, and *C*4-Substituted Pyrrolo[3,2-*d*]pyrimidine Derivatives



			enzyme[a,b]		cell growth[a]	metabolic stability[a]		PK	
			IC_{50} (nM) (95% CI)		GI_{50} (nM) (95% CI)	μL/min/mg		AUC_{0-8h}[c] μg·h/mL	
compd	X	Y	HER2	EGFR	BT-474	mice	human	mice	log *D* pH 7.4
12j	O	H	5.1 (4.1−6.3)	15 (11−21)	27 (21−33)	39	63	3.324	3.86
15	CH_2	H	15 (12−19)	33 (25−45)	406 (337−487)	16	34	0.856	4.90
20	NH	H	7.7 (5.3−11)	22 (15−32)	601 (561−643)	59	45	2.476	3.50
28	S	H	9.4 (7.4−12)	44 (26−73)	699 (604−807)	163	88	0.284	4.77
29	SO	H	12 (11−15)	31 (25−40)	1378 (1284−1479)		17	2.272	2.97
30	SO_2	H	12 (10−14)	40 (32−49)	1076 (991−1168)		17	2.080	3.41
40	O	CH_3	12 (10−15)	38 (26−56)	69 (37−105)	77	30	1.607	4.28
49	O	CN	19 (12−29)	230 (75−690)	1143 (1025−1274)	28	34	4.425	3.62

[a]These measurements were showed in the Experimental Section. [b]IC_{50} values and 95% confidence intervals (CI) were calculated by nonlinear regression analysis of the percentage inhibitions (n = 2). [c]The animals used in the study were female BALB/cAJcl mice (7-weeks old; CLEA Japan, Inc.). A mixture of 5 test compounds was suspended in 0.5% (w/v) methylcellulose solution for oral administration at a dose of 10 mg each/10 mL/kg. The concentrations of compounds in the plasma were determined by LC/MS/MS.

Compounds **34c−e**, together with **12j**, were selected for in vivo efficacy studies in a HER2-overexpressing BT-474 tumor xenograft mouse model (100 mg/kg, twice daily, orally for 14 days). Among these compounds, **34e** and **12j** exhibited significant in vivo efficacy (tumor/control ratio [T/C]): 29% and 48%, respectively,) without body weight loss (data not shown). On the basis of its potent in vivo efficacy and good in vitro profiles, **34e** was selected as a candidate for further investigation.

As shown in Figures 3 and 4, **34e** was evaluated using 4−1ST (HER2-overexpressing human gastric cancer tumor) xenograft models in mice and rats. Similar to the BT-474 model, **34e** exhibited dose-dependent tumor growth inhibition (T/C: 44% and 11% at 50 and 100 mg/kg, twice daily, respectively) without significant body weight loss in mice (Figure 3). Furthermore, **34e** showed potent in vivo efficacy in rats in a dose-dependent manner and inhibited the growth of 4−1ST

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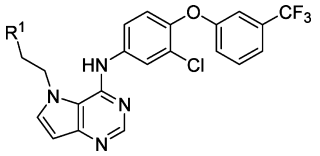
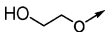
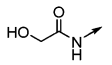
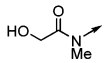
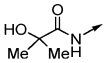
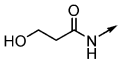
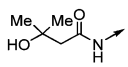
Table 4. Biological Data for *N*5- and *C*4-Substituted Pyrrolo[3,2-*d*]pyrimidine Derivatives



Compd	R^1	Enzyme [a, b] IC_{50} (nM) (95% CI)		Cell growth[a] GI_{50} (nM) (95% CI)	Metabolic Stability[a] μL/min/mg		PK [c] $AUC_{0\text{-}8h}$ μg·h/mL	Log D pH 7.4	*In vivo* efficacy [d] T/C (%)
		HER2	EGFR	BT-474	Mice	Human	Mice		Mice
12j		5.1 (4.1-6.3)	15 (11-21)	27 (21-33)	39	63	3.324	3.86	48
34a		4.3 (3.5-5.4)	10 (7.2-14)	124 (104-144)	11	16	0.680	3.51	-
34b		5.7 (4.4-7.2)	15 (9.8-23)	210 (176-246)	1	31	1.843	3.45	-
34c		15 (11-19)	35 (28-44)	50 (44-55)	-	18	2.497	4.13	75
34d		2.8 (2.2-3.6)	7.2 (5.5-9.4)	61 (49-74)	26	27	3.411	3.54	79
34e		17 (15-19)	23 (18-30)	17 (12-24)	28	35	1.923	4.18	29

[a]These measurements were showed in the Experimental Section. [b]IC_{50} values and 95% confidence intervals (CI) were calculated by nonlinear regression analysis of the percentage inhibitions (n = 2). [c]The animals used in the study were female BALB/cAJcl mice (7-weeks old; CLEA Japan, Inc.). A mixture of 5 test compounds was suspended in 0.5% (w/v) methylcellulose solution for oral administration at a dose of 10 mg each/10 mL/kg. The concentrations of compounds in the plasma were determined by LC/MS/MS. [d]Compounds suspended in a 0.5% (w/v) methylcellulose solution was administered orally twice daily (100 mg/kg) to the BT-474 xenograft mice for 14 days.

tumors with T/C of 38% and 14% at doses of 6.25 and 12.5 mg/kg and, particularly noteworthy, tumor regression with T/C of −12% and −16% at doses of 25 and 50 mg/kg (Figure 4). During the study, **34e** caused no significant body weight loss in rats (data not shown).

To clarify the potent efficacy of **34e**, we investigated the pharmacokinetic profile of **34e** in mice and rats, which is shown in Table 5. The oral bioavailability of **34e** was 97.7% in rats and 72.2% in mice at a dose of 50 mg/kg. In addition, we found that the concentration of **34e** in the tumor was much higher than that in the plasma of rats (Figure 5). Consequently, we concluded that the potent efficacy of **34e** was achieved by a combination of good PK profiles and high concentration levels of **34e** in the tumor. These results suggested that **34e** is a promising candidate for further evaluation.

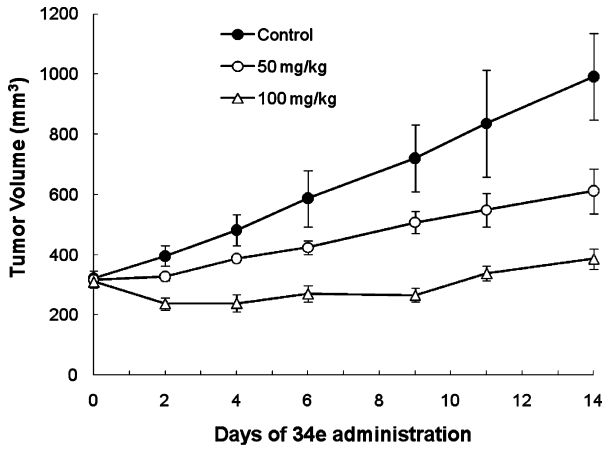


Figure 3. Antitumor efficacy of **34e** in 4−1ST xenograft models in mice. Dose levels 50 and 100 mg/kg; $P \leq 0.025$ vs control at day 14 (one-tailed Shirley−Williams test).

The inhibitory activity of **34e** against various kinases is summarized in Table 6. Besides HER2 and EGFR, **34e** exhibited HER4 inhibitory activity with an IC_{50} value of 260 nM. With these results, we confirmed that **34e** is a selective HER family inhibitor.

Structural Analysis and Discussion for Compound 34e and 12r. The crystal structures of a pyridine analogue **12r** in complex with HER2 and **34e** in complex with EGFR were described previously by Aertgeerts et al.[20] Although the resolution is still low (3.21 Å), we also determined the cocrystal structure of **34e** with HER2[21] and a cartoon presentation is shown in Figure 6A. This is the first time that crystal structures have been reported for both HER2 and EGFR in complex with the same inhibitor. From the two cocrystal structures of **34e** (Figure 6A,B), we confirmed that the *N*-1 nitrogen of the pyrrolo[3,2-*d*]pyrimidine scaffold was hydrogen-bonded to the main chain NH of the hinge region (Met801 in HER2 or Met793 in EGFR), whereas the *N*-3 nitrogen formed a water-mediated hydrogen bond network only with the side chain of Thr854 in EGFR. In the case of **34e** with HER2, this hydrogen bond network by the *N*-3 nitrogen was not observed, probably because of a moderate resolution level. The bulky 3-trifluoromethyl phenyl group of **34e** was not accommodated in the active form of both kinases and likely displaced the C-helix away from its position in the active conformation. Compared with the crystal structure of the pyridine analogue **12r** with HER2 (2.25 Å, Figure 6C), **34e** showed similar interactions to that of **12r**, which formed a water-mediated hydrogen bond network with the side chain of Thr862. In

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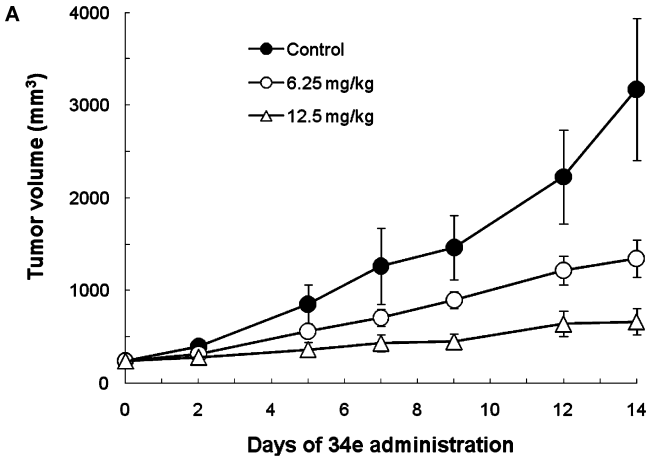

Figure 4. Antitumor efficacy of **34e** in 4−1ST xenograft models in rats. (A) Dose levels 6.25 mg/kg and 12.5 mg/kg; $P \leq 0.025$ vs control at day 14 (one-tailed Shirley−Williams test). (B) Dose levels 25 and 50 mg/kg; $P \leq 0.025$ vs control at day 14 (one-tailed Shirley−Williams test).

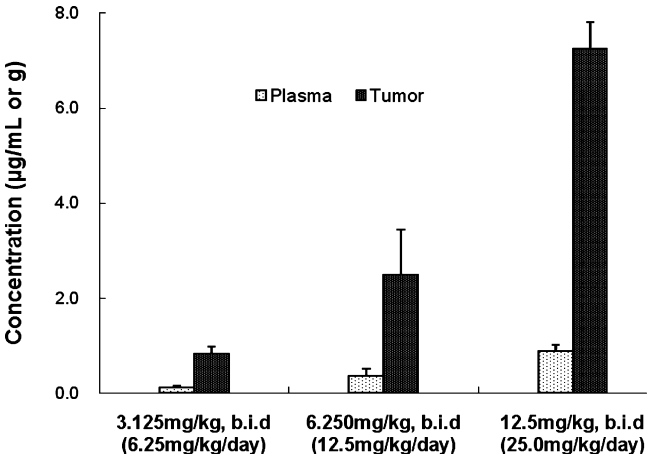

Figure 5. Concentration of **34e** in tumor (μg/g) and plasma (μg/mL) in rats.

addition, the 4-anilino groups of **34e** and **12r** occupied the same hydrophobic pocket formed by the side chain of amino acids near the DFG motif, and a remarkable difference was observed at the conformation of 3-trifluoromethyl phenyl group. The 3-trifluoromethyl group in **12r** was flipped by approximately 150° relative to its orientation in both the HER2/**34e** and EGFR/**34e** structures. In the costructure of **12r** with HER2, the nitrogen in the pyridine ring interacted with the main chain of Asp863 in the DFG motif. Furthermore, hydroxy group of R^2 side chain of **12r** was also hydrogen bonded with the side chain of Asp863. As a result, the conformation of the DFG motif in the HER2/**12r** was slightly different from those of the HER2/**34e** and EGFR/**34e** structures, which was considered to lead to the flip of 3-trifluoromethyl group. This difference may contribute to the difference between HER2 and EGFR inhibitory activity in **12r** (Table 2). It was noted that HER2/**34e** and EGFR/**34e** structures were inactive conformations with a DFG-in and C-helix-out conformation similar to that of the EGFR/Lapatinib structure (PDB 1XKK).

The *N*-5-substituents containing a carboxamide moiety of **34e** is suitably positioned at the solvent interface for both HER2 and EGFR with small conformational differences. Thus, a dramatic difference between the HER2 and EGFR cocrystal structures of **34e** was not observed. From the structural

Table 6. Kinase Selectivity of 34e

enzyme	IC_{50} (nM)	enzyme	IC_{50} (nM)
EGFR (HER1)	23	Lck	2400
HER2	17	CSK	4700
HER4	260	FAK	>10000
MEK1	1100	Lyn A	>10000
MEK5	5700	Lyn B	5200
c-Met	4200	ASK 1	>10000
Aurora B	1700	TAK 1	>10000
VEGFR 1	>10000	MEKK 1	>10000
VEGFR 2	>10000	IKK bata	>10000
FGFR1	>10000	JNK 1	>10000
FGFR 3	>10000	ERK 1	>10000
PDGFR α	>10000	P38 alpha	>10000
PDGFR β	>10000	PKA	>10000
IRK	>10000	PKC thete	>10000
TIE2	>10000	GSK3 beta	>10000
c-kit	>10000	B-raf	>10000
IGF-1R	>10000	TTK	>10000
Src	>10000	PLK 1	>10000

Table 5. Pharmacokinetic Parameters of 34e in Rats and Mice

	IV (5 mg/kg)			PO (50 mg/kg)			
	$C_{5\ min}$ (μg/mL)[a]	AUC_{0-24h} (μg·h/mL)[b]	MRT (h)[c]	C_{max} (μg/mL)[d]	T_{max} (h)[e]	$AUC_{0-24\ h}$ (μg·h/mL)[b]	BA (%)[f]
rat	2.277	5.63	3.39	4.157	4.00	55.03	97.7
mouse	4.060	2.72	0.96	4.364	0.50	19.64	72.2

[a]Plasma concentration at 5 min after dosing. [b]Area under the plasma concentration−time curve for 0−24 h after dosing. [c]Mean residual time of **34e** in the plasma. [d]Maximum plasma concentration after oral dosing. [e]Time to reach C_{max}. [f]Bioavailability.

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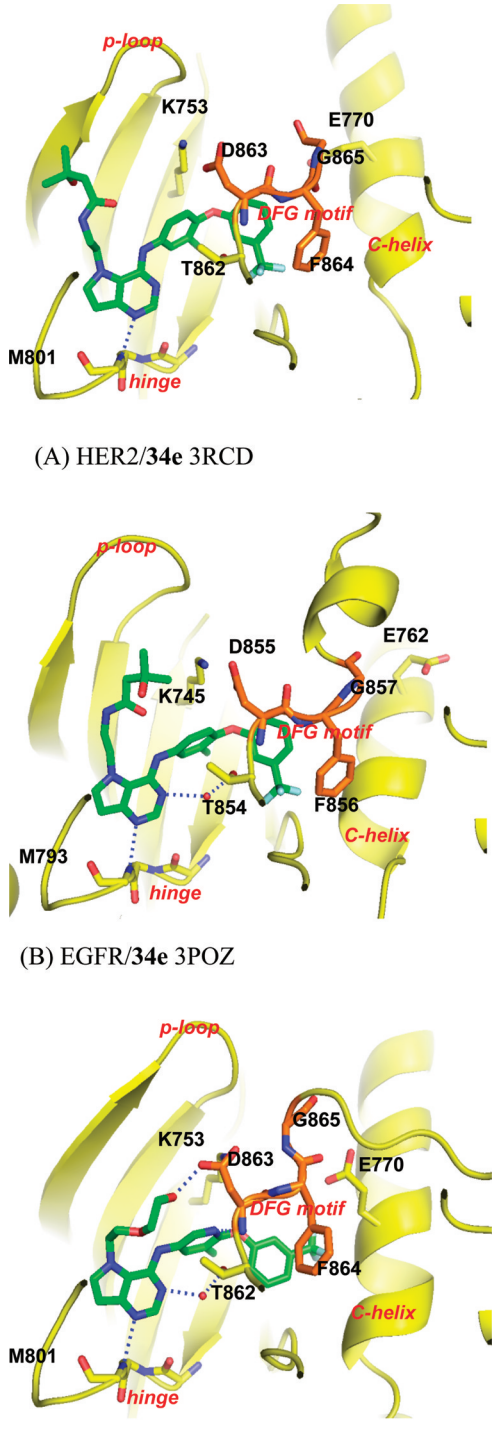

Figure 6. X-ray cocrystal structures of compound **34e** (TAK-285) with HER2 (A) and EGFR (B), together with that of compound **12r** with HER2 (C).[20]

similarities found for the binding mode of **34e** to the HER2 and EGFR kinases, we confirmed that **34e** is an ATP-competitive HER2/EGFR dual inhibitor.

CONCLUSION

We designed pyrrolo[3,2-*d*]pyrimidine derivatives and modified them chemically to develop novel HER2/EGFR TKIs. As expected, the prepared derivatives showed good HER2 and EGFR inhibitory activities. On the basis of results from *N*-5 side chain optimization studies, we selected **34e** for further evaluation. From the X-ray cocrystal structures of **34e** with both HER2 and EGFR, we confirmed that the pyrrolo[3,2-*d*]pyrimidine scaffold fits into the ATP pocket and that the *N*-1 nitrogen interact with Met801 of HER2 or Met793 of EGFR. In a further investigation, **34e** exhibited potent and selective kinase inhibitory profiles against the HER family as well as good cellular GI activity. Reflecting its desirable cellular activity, metabolic stability, and good PK profiles, **34e** showed potent in vivo antitumor efficacy in mice and rats. These data combined with our preliminary studies revealing that the HER2 inhibitory activity of **34e** compares favorably to that of lapatinib[11] and is superior to that of erlotinib[6] support the position that compound **34e** is a promising candidate for clinical development as a dual HER2/EGFR inhibitor. Compound **34e** (TAK-285) is currently in phase 1 clinical trials.

EXPERIMENTAL SECTION

Melting points were determined on a Yanagimoto micro melting point apparatus or SRS OptiMelt melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian Gemini-200 (200 MHz) spectrometer or Varian Mercury-300 (300 MHz) spectrometer. Chemical shifts are given in parts per million (ppm) with tetramethylsilane as an internal standard, and coupling constants (*J* values) are given in hertz (Hz). Splitting patterns and apparent multiplicities are designated as s (singlet), d (doublet), dd (double doublet), t (triplet), dt (double triplet), q (quartet), m (multiplet), br s (broad singlet). Elemental analyses were carried out by Takeda Analytical Research Laboratories, Ltd., and the results obtained were within ±0.4% of the theoretical values. MS spectra were collected with a Waters LC-MS system (ZMD-1) and were used to confirm ≥95% purity of each compound. The column used was an L-column 2 ODS (3.0 mm × 50 mm I.D., CERI, Japan) with a temperature of 40 °C and a flow rate of 1.2 mL/min. Mobile phase A was 0.05% TFA in ultrapure water. Mobile phase B was 0.05% TFA in acetonitrile, which was increased linearly from 5% to 90% over 2 min, 90% over the next 1.5 min, after which the column was equilibrated to 5% for 0.5 min.

Column chromatography was carried out on a silica gel column (Kieselgel 60, 63−200 mesh, Merck or Chromatorex NH-DM1020, 100−200 mesh, Fuji Silysia Chemical, Ltd., Japan). Yields were not optimized.

3-Chloro-4-phenoxyaniline (4a). To a mixture of 2-chloro-1-fluoro-4-nitrobenzene (**1**, 1.75 g, 10.0 mmol) and potassium carbonate (2.07 g, 10.0 mmol) in *N*,*N*-dimethylformamide (DMF) (20 mL) was added phenol (**2a**, 941 mg, 10.0 mmol). After being stirred at 80 °C for 2 h, water (40 mL) was added to the mixture and the mixture was extracted with ethyl acetate (EtOAc). The organic layer was washed with saturated brine (30 mL), dried over anhydrous magnesium sulfate ($MgSO_4$), and concentrated in vacuo to give **3a** (2.32 g) as brown oil. To a solution of **3a** (2.32 g, 9.31 mmol) in EtOAc (50 mL) was added 5% platinum−carbon (Pt−C) (200 mg), and the mixture was stirred under hydrogen atmosphere at room temperature for 2 h. After 5% Pt−C was filtered off, the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent, EtOAc:hexane = 1:9 to 5:5) to give 1.96 g (89%) of **4a** as brown oil. ^{1}H NMR ($CDCl_3$) δ 3.65 (br s, 2H), 6.56 (dd, 1H, J = 8.6, 2.7 Hz), 6.79 (d, 1H, J = 2.7 Hz), 6.84−6.95 (m, 3H), 6.98−7.06 (m, 1H), 7.19−7.35 (m, 2H).

The following compounds (**4b−4e**, **4i−4n**) were prepared from **1** and the corresponding phenols (**2b−2e**, **2i−2n**) by a method similar to that described for **4a**.

3-Chloro-4-(2-fluorophenoxy)aniline (4b). Yield 89%, brown oil. ^{1}H NMR ($CDCl_3$) δ 3.65 (br s, 2H), 6.55 (dd, 1H, J = 8.6, 2.7 Hz),

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6.70−6.82 (m, 2H), 6.86 (d, 1H, J = 8.6 Hz), 6.96−7.04 (m, 2H), 7.10−7.21 (m, 1H).

3-Chloro-4-(3-fluorophenoxy)aniline (4c). Yield 76%, white solid. ^{1}H NMR ($CDCl_3$) δ 3.69 (br s, 2H), 6.50−6.81 (m, 5H), 6.92 (d, 1H, J = 8.7 Hz), 7.14−7.28 (m, 1H).

3-Chloro-4-(2-chlorophenoxy)aniline (4d). Yield 97%, brown oil. ^{1}H NMR ($CDCl_3$) δ 3.69 (br s, 2H), 6.56 (dd, 1H, J = 8.7, 2.8 Hz), 6.68 (dd, 1H, J = 8.0, 1.5 Hz), 6.79 (d, 1H, J = 2.8 Hz), 6.86 (d, 1H, J = 8.7 Hz), 6.94−7.04 (m, 1H), 7.07−7.17 (m, 1H), 7.42 (dd, 1H, J = 8.0, 1.5 Hz).

3-Chloro-4-(3-chlorophenoxy)aniline (4e). Yield 65%, white solid. ^{1}H NMR ($CDCl_3$) δ 3.70 (br s, 2H), 6.58 (dd, 1H, J = 8.6, 2.7 Hz), 6.73−7.04 (m, 5H), 7.19 (t, 1H, J = 8.6 Hz).

3-Chloro-4-[2-(trifluoromethyl)phenoxy]aniline (4i). Yield 84%, yellow oil. ^{1}H NMR ($CDCl_3$) δ 3.70 (br s, 2H), 6.53−6.69 (m, 2H), 6.79 (d, 1H, J = 2.6 Hz), 6.92 (d, 1H, J = 8.7 Hz), 7.07 (t, 1H, J = 7.6 Hz), 7.34−7.42 (m, 1H), 7.64 (dd, 1H, J = 7.6, 0.9 Hz).

3-Chloro-4-[3-(trifluoromethyl)phenoxy]aniline (4j). Yield 72%, white solid. ^{1}H NMR ($CDCl_3$) δ 3.71 (br s, 2H), 6.59 (dd, 1H, J = 8.6, 2.7 Hz), 6.80 (d, 1H, J = 2.7 Hz), 6.92 (d, 1H, J = 8.6 Hz), 7.03 (dd, 1H, J = 8.0, 2.1 Hz), 7.10 (s, 1H), 7.23−7.32 (m, 1H), 7.38 (t, 1H, J = 8.0 Hz).

3-Chloro-4-[2-(trifluoromethoxy)phenoxy]aniline (4k). Yield 96%, brown oil. ^{1}H NMR ($CDCl_3$) δ 3.68 (br s, 2H), 6.57 (dd, 1H, J = 8.7, 2.6 Hz), 6.69 (dd, 1H, J = 8.2, 1.6 Hz), 6.79 (d, 1H, J = 2.6 Hz), 6.87 (d, 1H, J = 8.7 Hz), 6.97−7.08 (m, 1H), 7.11−7.20 (m, 1H), 7.28−7.35 (m, 1H).

3-Chloro-4-[3-(trifluoromethoxy)phenoxy]aniline (4l). Yield 61%, white solid. ^{1}H NMR ($CDCl_3$) δ 3.70 (br s, 2H), 6.59 (dd, 1H, J = 8.7, 2.8 Hz), 6.73 (s, 1H), 6.76−6.96 (m, 4H), 7.23−7.33 (m, 1H).

3-Chloro-4-(3-methylphenoxy)aniline (4m). Yield 92%, white solid. ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3H), 3.65 (br s, 2H), 6.56 (dd, 1H, J = 8.7, 2.6 Hz), 6.63−6.93 (m, 5H), 7.16 (t, 1H, J = 7.8 Hz).

3-Chloro-4-(3-methoxyphenoxy)aniline (4n). Yield 99%, brown oil. ^{1}H NMR ($CDCl_3$) δ 3.66 (br s, 2H), 3.77 (s, 3H), 6.42−6.50 (m, 2H), 6.53−6.61 (m, 2H), 6.78 (d, 1H, J = 2.6 Hz), 6.91 (d, 1H, J = 8.7 Hz), 7.17 (t, 1H, J = 8.1 Hz).

2-(4-Amino-2-chlorophenoxy)benzonitrile (4f). To a mixture of **1** (4.40 g, 184 mmol) and potassium carbonate (5.20 g, 221 mmol) in DMF (60 mL) was added 2-hydroxybenzonitrile (**2f**, 3.00 g, 257 mmol). After being stirred at 100 °C for 2 h, water (300 mL) was added to the mixture under ice-cooling and the mixture was stirred at room temperature for 10 min. The resulting precipitate was collected by filtration. The precipitate was washed with *n*-hexane and dried under vacuum to give a yellow cake. To a suspension of the cake and reduced iron (8.80 g) in EtOH (90 mL) was added a solution of calcium chloride (1.50 g, 257 mmol) in water (10 mL), and the reaction mixture was stirred at 100 °C for 15 h. The solid was removed by filtration, and the resulting filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent, EtOAc:hexane = 2:8 to 10:0) to give 3.50 g (65%) of **4f** as brown oil. ^{1}H NMR ($CDCl_3$) δ 3.78 (br s, 2H), 6.58−6.65 (m, 2H), 6.76 (d, 1H, J = 2.7 Hz), 6.97 (d, 1H, J = 8.4 Hz), 7.04−7.10 (m, 1H), 7.39−7.45 (m, 1H), 7.62−7.65 (m, 1H).

The following compounds (**4g**,**4h**) were prepared from **1** and the corresponding phenols (**4g**,**4h**) by a method similar to that described for **4f**.

3-(4-Amino-2-chlorophenoxy)benzonitrile (4g). Yield 71%, yellow oil. ^{1}H NMR ($CDCl_3$) δ 3.75 (br s, 2H), 6.58−6.62 (m, 1H), 6.79 (d, 1H, J = 2.7 Hz), 6.92 (d, 1H, J = 8.4 Hz), 7.05−7.16 (m, 2H), 7.26−7.41 (m, 2H).

4-(4-Amino-2-chlorophenoxy)benzonitrile (4h). Yield 56%, beige solid. ^{1}H NMR ($CDCl_3$) δ 3.76 (br s, 2H), 6.60 (dd, 1H, J = 8.6, 2.7 Hz), 6.79 (d, 1H, J = 2.7 Hz), 6.84−7.00 (m, 3H), 7.51−7.62 (m, 2H).

5-Chloro-6-[3-(trifluoromethyl)phenoxy]pyridin-3-amine (4r). To a solution of **2j** (0.42 g, 2.59 mmol) in THF (8.0 mL) was added NaH (60% dispersion in mineral oil, 0.11 g, 2.75 mmol) under ice-cooling. After being stirred for 1 h, to the reaction mixture was added 2,3-dichloro-5-nitropyridine (0.50 g, 2.61 mmol). The reaction mixture was stirred at room temperature for 2.5 h. Water (100 mL) was added to the reaction mixture, and then the mixture was extracted with EtOAc (200 mL). The organic layer was washed with saturated brine (50 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (eluent, EtOAc:hexane = 1:9 to 3:1) to give colorless oil. To a suspension of the oil and reduced iron (0.65 g, 11.6 mmol) in a mixture of EtOH (19.5 mL) and water (3.5 mL) was added calcium chloride (0.13 g, 1.17 mmol), and the mixture was stirred at 80 °C for 8 h. The solid was removed by filtration, and filtrate was poured into water (100 mL). The organic layer was washed with saturated brine (100 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent, EtOAc:hexane = 1:4 to 1:1) to give 0.29 g (39%) of **4r** as brown oil. ^{1}H NMR ($CDCl_3$) δ 3.65 (br s, 2H), 7.20 (d, 1H, J = 2.9 Hz), 7.22−7.26 (m, 1H), 7.27−7.32 (m, 1H), 7.37−7.40 (m, 1H), 7.44−7.50 (m, 1H), 7.59 (d, 1H, J = 2.9 Hz).

***N*-(3-Chloro-4-phenoxyphenyl)-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine (6a).** A solution of **4a** (264 mg, 1.20 mmol) and 4-chloro-5*H*-pyrrolo[3,2-*d*]pyrimidine[18] (**5**, 154 mg, 1.00 mmol) in 1-methyl-2-pyrrolidone (3.0 mL) was stirred with heating at 100 °C for 3 h. After cooling to room temperature, the reaction mixture was diluted with EtOAc (80 mL) and partitioned with saturated sodium hydrogen carbonate (30 mL). The organic layer was washed with brine (30 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent, MeOH:EtOAc = 0:1 to 5:95) to give 105 mg (31%) of **6a** as colorless crystals, mp 247−250 °C. ^{1}H NMR (DMSO-d_6) δ 6.51 (br s, 1H), 6.94 (d, 2H, J = 7.9 Hz), 7.10 (t, 1H, J = 7.3 Hz), 7.20 (d, 1H, J = 8.9 Hz), 7.31−7.45 (m, 2H), 7.63−7.73 (m, 2H), 8.32−8.43 (m, 2H), 9.47 (br s, 1H), 11.17 (br s, 1H). Anal. Calcd for $C_{18}H_{13}ClN_4O$: C, 64.19; H, 3.89; N, 16.64. Found: C, 63.81; H, 3.91; N, 16.66.

The following compounds (**6b**−**6q**) were prepared from corresponding **4b**−**4q** and **5** by a method similar to that described for **6a**.

***N*-[3-Chloro-4-(2-fluorophenoxy)phenyl]-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine (6b).** Yield 51%, colorless crystals, mp 282−285 °C. ^{1}H NMR (DMSO-d_6) δ 6.50 (dd, 1H, J = 2.9, 2.0 Hz), 6.90−7.03 (m, 1H), 7.09−7.25 (m, 3H), 7.33−7.49 (m, 1H), 7.55−7.73 (m, 2H), 8.39 (s, 2H), 9.41 (br s, 1H), 11.08 (br s, 1H). Anal. Calcd for $C_{18}H_{12}ClFN_4O$: C, 60.94; H, 3.41; N, 15.79. Found: C, 61.03; H, 3.43; N, 15.65.

***N*-[3-Chloro-4-(3-fluorophenoxy)phenyl]-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine (6c).** Yield 74%, colorless crystals, mp 239−241 °C. ^{1}H NMR (DMSO-d_6) δ 6.46−6.54 (m, 1H), 6.70−6.85 (m, 2H), 6.89−7.02 (m, 1H), 7.29 (d, 1H, J = 8.9 Hz), 7.34−7.45 (m, 1H), 7.62−7.76 (m, 2H), 8.36−8.43 (m, 2H), 9.47 (s, 1H), 11.11 (br s, 1H). Anal. Calcd for $C_{18}H_{12}ClFN_4O$: C, 60.94; H, 3.41; N, 15.79. Found: C, 61.00; H, 3.41; N, 15.81.

***N*-[3-Chloro-4-(2-chlorophenoxy)phenyl]-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine (6d).** Yield 33%, colorless crystals, mp 251−254 °C. ^{1}H NMR (DMSO-d_6) δ 6.51 (dd, 1H, J = 2.8, 2.1 Hz), 6.88 (dd, 1H, J = 8.2, 1.4 Hz), 7.09−7.21 (m, 2H), 7.28−7.37 (m, 1H), 7.56−7.74 (m, 3H), 8.40 (s, 2H), 9.44 (br s, 1H), 11.09 (br s, 1H). Anal. Calcd for $C_{18}H_{12}Cl_2N_4O$: C, 58.24; H, 3.26; N, 15.09. Found: C, 58.31; H, 3.32; N, 14.98.

***N*-[3-Chloro-4-(3-chlorophenoxy)phenyl]-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine (6e).** Yield 71%, colorless crystals, mp 229−231 °C. ^{1}H NMR (DMSO-d_6) δ 6.51 (dd, 1H, J = 2.8, 2.2 Hz), 6.84−6.95 (m, 1H), 6.99 (t, 1H, J = 2.2 Hz), 7.17 (dd, 1H, J = 8.0, 1.1 Hz), 7.30 (d, 1H, J = 8.9 Hz), 7.40 (t, 1H, J = 8.0 Hz), 7.65−7.75 (m, 2H), 8.32−8.42 (m, 2H), 9.47 (br s, 1H), 11.11 (br s, 1H). Anal. Calcd for $C_{18}H_{12}Cl_2N_4O$: C, 58.24; H, 3.26; N, 15.09. Found: C, 58.25; H, 3.22; N, 15.17.

2-[2-Chloro-4-(5*H*-pyrrolo[3,2-*d*]pyrimidin-4-ylamino)-phenoxy]benzonitrile (6f). Yield 61%, yellow crystals, mp 264−266 °C. ^{1}H NMR (DMSO-d_6) δ 6.50 (s, 1H, s), 6.85 (d, 1H, J = 8.4 Hz), 7.22−7.60 (m, 2H), 7.65−7.74 (m, 3H), 7.88 (d, 1H, J = 7.8 Hz), 8.40 (s, 2H), 9.50 (br s, 1H), 11.09 (br s, 1H). Anal. Calcd for $C_{19}H_{12}ClN_5O$: C, 63.08; H, 3.34; N, 19.36. Found: C, 63.10; H, 3.25; N, 19.15.

3-[2-Chloro-4-(5*H*-pyrrolo[3,2-*d*]pyrimidin-4-ylamino)-phenoxy]benzonitrile (6g). Yield 71%, yellow crystals, mp 273−275 °C. ^{1}H NMR (DMSO-d_6) δ 6.53 (s, 1H), 7.26 (m, 1H), 7.32 (d, 1H, J = 8.7 Hz), 7.45 (s, 1H), 7.58 (d, 2H, J = 5.7 Hz), 7.70−7.73 (m, 2H), 8.41 (s, 2H), 9.50 (br s, 1H), 11.10 (br s, 1H). Anal. Calcd for $C_{19}H_{12}ClN_5O$: C, 63.08; H, 3.34; N, 19.36. Found: C, 62.97; H, 3.30; N, 19.48.

4-[2-Chloro-4-(5*H*-pyrrolo[3,2-*d*]pyrimidin-4-ylamino)-phenoxy]benzonitrile (6h). Yield 48%, yellow crystals, mp 318 °C. ^{1}H NMR (DMSO-d_6) δ 6.52 (dd, 1H, J = 3.0, 1.9 Hz), 6.99−7.18 (m, 2H), 7.37 (d, 1H, J = 8.9 Hz), 7.68−7.79 (m, 2H), 7.79−7.90 (m, 2H), 8.41 (s, 2H), 9.55 (br s, 1H), 11.15 (br s, 1H). Anal. Calcd for $C_{19}H_{12}ClN_5O{\cdot}0.3H_2O$: C, 62.15; H, 3.46; N, 19.07. Found: C, 62.25; H, 3.49; N, 18.84.

***N*-{3-Chloro-4-[2-(trifluoromethyl)phenoxy]phenyl}-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine (6i).** Yield 69%, colorless crystals, mp 273−276 °C. ^{1}H NMR (DMSO-d_6) 6.52 (dd, 1H, J = 3.0, 1.9 Hz), 6.83 (d, 1H, J = 8.5 Hz), 7.19−7.33 (m, 2H), 7.56−7.83 (m, 4H), 8.41 (s, 2H), 9.48 (br s, 1H), 11.10 (br s, 1H). Anal. Calcd for $C_{19}H_{12}ClF_3N_4O$: C, 56.38; H, 2.99; N, 13.84. Found: C, 56.58; H, 2.97; N, 13.98.

***N*-{3-Chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine (6j).** Yield 62%, colorless crystals, mp 258−260 °C. ^{1}H NMR (DMSO-d_6) δ 6.42−6.56 (m, 1H), 7.15−7.27 (m, 2H), 7.33 (d, 1H, J = 8.9 Hz), 7.47 (d, 1H, J = 8.0 Hz), 7.61 (t, 1H, J = 8.0 Hz), 7.66−7.78 (m, 2H), 8.41 (s, 2H), 9.48 (br s, 1H), 11.11 (br s, 1H). Anal. Calcd for $C_{19}H_{12}ClF_3N_4O$: C, 56.38; H, 2.99; N, 13.84. Found: C, 56.34; H, 2.95; N, 13.91.

***N*-{3-Chloro-4-[2-(trifluoromethoxy)phenoxy]phenyl}-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine (6k).** Yield 63%, colorless crystals, mp 228−230 °C. ^{1}H NMR (DMSO-d_6) 6.51 (dd, 1H, J = 2.9, 2.0 Hz), 6.90 (dd, 1H, J = 8.3, 1.5 Hz), 7.15−7.26 (m, 2H), 7.32−7.43 (m, 1H), 7.49−7.57 (m, 1H), 7.63−7.77 (m, 2H), 8.35−8.48 (m, 2H), 9.46 (br s, 1H), 11.10 (br s, 1H). Anal. Calcd for $C_{19}H_{12}ClF_3N_4O_2$: C, 54.23; H, 2.87; N, 13.32. Found: C, 54.43; H, 3.01; N, 13.13.

***N*-{3-Chloro-4-[3-(trifluoromethoxy)phenoxy]phenyl}-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine (6l).** Yield 63%, colorless crystals, mp 234−236 °C. ^{1}H NMR (DMSO-d_6) δ 6.52 (dd, 1H, J = 3.0, 1.9 Hz), 6.90−6.97 (m, 2H), 7.07−7.15 (m, 1H), 7.32 (d, 1H, J = 8.9 Hz), 7.49 (t, 1H, J = 8.9 Hz), 7.67−7.75 (m, 2H), 8.37−8.43 (m, 2H), 9.47 (br s, 1H), 11.10 (br s, 1H). Anal. Calcd for $C_{19}H_{12}ClF_3N_4O_2$: C, 54.23; H, 2.87; N, 13.32. Found: C, 54.23; H, 2.83; N, 13.33.

***N*-[3-Chloro-4-(3-methylphenoxy)phenyl]-5*H*-pyrrolo[3,2-*d*]-pyrimidin-4-amine (6m).** Yield 70%, colorless crystals, mp 232−234 °C. ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H), 6.51 (dd, 1H, J = 2.9, 2.0 Hz), 6.67−6.81 (m, 2H), 6.92 (d, 1H, J = 7.5 Hz), 7.12−7.31 (m, 2H), 7.56−7.74 (m, 2H), 8.28−8.44 (m, 2H), 9.42 (br s, 1H), 11.10 (br s, 1H). Anal. Calcd for $C_{19}H_{15}ClN_4O$: C, 65.05; H, 4.31; N, 15.97. Found: C, 64.88; H, 4.36; N, 15.84.

***N*-[3-Chloro-4-(3-methoxyphenoxy)phenyl]-5*H*-pyrrolo[3,2-*d*]-pyrimidin-4-amine (6n).** Yield 83%, colorless crystals, mp 204−207 °C. ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3H), 6.38−6.56 (m, 3H), 6.69 (dd, 1H, J = 8.2, 2.0 Hz), 7.13−7.34 (m, 2H), 7.58−7.74 (m, 2H), 8.32−8.43 (m, 2H), 9.42 (br s, 1H), 11.10 (br s, 1H). Anal. Calcd for $C_{19}H_{15}ClN_4O_2$: C, 62.21; H, 4.12; N, 15.27. Found: C, 62.11; H, 4.07; N, 15.33.

***N*-{3-Chloro-4-[(3-fluorobenzyl)oxy]phenyl}-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine (6o).** Yield 80%, colorless crystals, mp 269−270 °C. ^{1}H NMR ($CDCl_3$) δ: 5.15 (s, 2H), 6.52 (d, 1H, J = 3.0 Hz), 6.90−7.70 (m, 7H), 7.97 (d, 1H, J = 3.0 Hz), 8.44 (s, 1H), 8.84 (br s, 1H), 10.74 (br s, 1H). Anal. Calcd for $C_{19}H_{14}ClFN_4O$: C, 61.88; H, 3.83; N, 15.19. Found: C, 61.83; H, 3.89; N, 15.08.

***N*-[3-Chloro-4-(pyridin-2-ylmethoxy)phenyl]-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine (6p).** Yield 78%, yellow crystals, mp 236−238 °C. ^{1}H NMR (DMSO-d_6) δ 5.27 (s, 2H), 6.48 (d, 1H, J = 2.4 Hz), 7.25 (d, 1H, J = 8.7 Hz), 7.37 (dd, 1H, J = 7.5, 5.1 Hz), 7.55−7.60 (m, 2H), 7.66 (s, 1H), 7.89 (t, 1H, J = 7.5 Hz), 8.20 (dd, 1H, J = 2.4, 1.5 Hz), 8.35 (d, 1H, J = 1.5 Hz), 8.60 (dd, 1H, J = 4.8, 0.6 Hz), 9.25 (br s, 1H), 12.78 (br s, 1H). Anal. Calcd for $C_{18}H_{14}ClN_5O$: C, 61.46; H, 4.01; N, 19.91. Found: C, 61.20; H, 4.04; N, 19.66.

***N*-{3-Methyl-4-[(6-methylpyridin-3-yl)oxy]phenyl}-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine (6q).** Yield 66%, yellow crystals, mp 111−113 °C. ^{1}H NMR ($CDCl_3$) δ: 2.16 (s, 3H), 2.51 (s, 3H), 6.56 (d, 1H, J = 3.0 Hz), 6.80 (d, 1H, J = 9.0 Hz), 7.0−7.6 (m, 5H), 8 17 (m, 1H), 8.59 (s, 1H), 8.76 (br s, 1H), 11.08 (br s, 1H). Anal. Calcd for $C_{19}H_{17}N_5O{\cdot}0.2H_2O$: C, 68.13; H, 5.24; N, 20.91. Found: C, 68.18; H, 5.22; N, 20.82.

4-Chloro-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidine (7). To a suspension of **5** (0.32 g, 2.09 mmol) and potassium carbonate (0.45 g, 3.27 mmol) in DMF (2.0 mL) was added iodomethane (0.44 g, 3.13 mmol), and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was poured into water (25 mL), and extracted with EtOAc (90 mL). The organic layer was washed with brine (60 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (eluent, EtOAc:hexane = 1:4 to 9:1) to give 0.33 g (93%) of **7** as yellow solid. ^{1}H NMR ($CDCl_3$) δ 4.16 (s, 3H), 6.70 (d, 1H, J = 3.9 Hz), 7.42 (d, 1H, J = 3.9 Hz), 8.69 (s, 1H).

***N*-[3-Chloro-4-(3-chlorophenoxy)phenyl]-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine Hydrochloride (8e).** A solution of **4e** (100 mg, 0.597 mmol) and **7** (227 mg, 0.893 mmol) in 1-methyl-2-pyrrolidone (1.19 mL) was stirred at 140 °C for 4 h. After cooling to room temperature, the reaction mixture was diluted with EtOAc (30 mL). The resulting crystals were collected by filtration, washed with EtOAc (10 mL), and dried under vacuum to give 231 mg (92%) of **8e** as colorless hydrochloride salt crystals, mp 168−170 °C. ^{1}H NMR (DMSO-d_6) δ 4.31 (d, 3H, J = 4.0 Hz), 6.65 (dd, 1H, J = 3.0, 1.0 Hz), 6.95 (dd, 1H, J = 9.0, 2.0 Hz), 7.03 (m, 1H), 7.22 (m, 1H), 7.34 (d, 1H, J = 9.0 Hz), 7.44 (t, 1H, J = 9.0 Hz), 7.69 (m, 1H), 7.97 (m, 2H), 8.72 (s, 1H), 9.94 (br s, 1H). Anal. Calcd for $C_{19}H_{15}Cl_3N_4O{\cdot}0.5H_2O$: C, 52.98; H, 3.74; N, 13.01. Found: C, 53.00; H, 3.76; N, 13.26.

The following compounds (**8j**,**8l**) were prepared from corresponding **4j**,**4l** with **7** by a method similar to that described for **8e**.

***N*-{3-Chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine hydrochloride (8j).** Yield 75%, colorless crystals, mp 175−177 °C. ^{1}H NMR (DMSO-d_6) δ 4.31 (s, 3H), 6.64 (dd, 1H, J = 3.0, 1.0 Hz), 7.26 (m, 2H), 7.37 (d, 1H, J = 9.0 Hz), 7.51 (d, 1H, J = 8.0 Hz), 7.67 (m, 2H), 7.96 (m, 2H), 8.71 (s, 1H), 9.89 (br s, 1H). Anal. Calcd for $C_{20}H_{15}Cl_2F_3N_4O{\cdot}0.5H_2O$: C, 51.74; H, 3.47; N, 12.07. Found: C, 52.20; H, 3.37; N, 12.25.

***N*-{3-Chloro-4-[3-(trifluoromethoxy)phenoxy]phenyl}-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine Hydrochloride (8l).** Yield 77%, colorless crystals, mp 169−171 °C. ^{1}H NMR (DMSO-d_6) δ 4.30 (s, 3H), 6.63 (dd, 1H, J = 3.0, 2.0 Hz), 6.97 (m, 2H), 7.15 (d, 1H, J = 9.0 Hz), 7.35 (d, 1H, J = 9.0 Hz), 7.53 (t, 1H, J = 9.0 Hz), 7.68 (m, 1H), 7.96 (m, 2H), 8.70 (s, 1H), 9.87 (br s, 1H). Anal. Calcd for $C_{20}H_{15}Cl_2F_3N_4O_2$: C, 50.97; H, 3.21; N, 11.89. Found: C, 50.99; H, 3.13; N, 11.84.

2-(4-Chloro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl Benzoate (9). To a suspension of **5** (0.31 g, 2.01 mmol) and cesium carbonate (0.98 g, 5.08 mmol) in DMF (2.0 mL) was added 2-iodoethyl benzoate (1.45 g, 5.25 mmol), and the reaction mixture was stirred at room temperature for 15 h. The reaction mixture was poured into saturated sodium hydrogen carbonate (100 mL) and extracted with EtOAc (150 mL × 3). The organic layer was washed with brine (100 mL) and dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (eluent, EtOAc:hexane = 6:4 to 95:5) to give 0.42 g (70%) of **9** as white solid. ^{1}H NMR ($CDCl_3$) δ4.71 (t, 2H, J = 5.3 Hz), 4.91 (t, 2H, J = 5.3 Hz), 6.76 (d, 1H, J = 3.2 Hz), 7.35−7.67 (m, 4H), 7.84−7.97 (m, 2H), 8.73 (s, 1H).

2-(4-{[3-Chloro-4-(3-chlorophenoxy)phenyl]amino}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethanol (10e). A solution of **9** (100 mg, 0.331 mmol) and **4e** (126 mg, 0.496 mmol) in 1-methyl-2-pyrrolidone (0.66 mL) was stirred at 140 °C for 2.5 h. After cooling to room temperature, to the reaction mixture was added saturated sodium hydrogen carbonate (80 mL), and the mixture was extracted with EtOAc (80 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column

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chromatography (eluent, EtOAc:hexane = 1:9 to 1:0) to give white solid. To a solution of the solid in a mixture of MeOH (1.18 mL) and THF (1.18 mL) was added 1 N NaOH (0.27 mL), and the mixture was stirred at room temperature for 1.5 h. To the reaction mixture was added 1 N HCl (0.27 mL), and the mixture was extracted with a mixture of EtOAc (2.0 mL) and THF (1.8 mL). The organic layer was washed with brine (30 mL), dried over $MgSO_4$, and concentrated in vacuo. The crystallized residue was recrystallized from EtOAc/diisopropyl ether (2:1) to give 81 mg (72%) of **10e** as colorless crystals, mp 208−209 °C. ^{1}H NMR (DMSO-d_6) δ 3.87 (m, 2H), 4.53 (t, 2H, J = 4.5 Hz), 6.31 (br s, 1H,), 6.51 (d, 1H, J = 3.0 Hz), 6.88 (d, 1H, J = 9.0 Hz), 6.95 (s, 1H), 7.15 (d, 1H, J = 9.0 Hz), 7.28 (d, 1H, J = 9.0 Hz), 7.38 (t, 1H, J = 9.0 Hz), 7.60 (dd, 1H, J = 9.0, 2.0 Hz), 7.66 (d, 1H, J = 3.0 Hz), 7.97 (d, 1H, J = 2.0 Hz), 8.34 (s, 1H), 9.89 (br s, 1H). Anal. Calcd for $C_{20}H_{16}Cl_2N_4O_2$: C, 57.84; H, 3.88; N, 13.49. Found: C, 57.75; H, 3.66; N, 13.48.

The following compounds (**10j**,**10l**) were prepared from **9**,**11** with corresponding anilines (**4e**,**4j**,**4l**) by a method similar to that described for **10e**.

2-[4-({3-Chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}amino)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethanol (10j). Yield 46%, colorless crystals, mp 193−194 °C. ^{1}H NMR ($CDCl_3$) δ 4.16 (t, 2H, J = 4.4 Hz), 4.38 (t, 2H, J = 4.4 Hz), 6.12 (d, 1H, J = 3.2 Hz), 6.97 (d, 1H, J = 3.2 Hz), 7.09 (d, 1H, J = 8.8 Hz), 7.10−7.17 (m, 1H), 7.21 (s, 1H), 7.32 (d, 1H, J = 7.7 Hz), 7.43 (t, 1H, J = 8.0 Hz), 7.52 (dd, 1H, J = 8.8, 2.6 Hz), 7.84 (d, 1H, J = 2.6 Hz), 8.24 (s, 1H), 9.59 (br s, 1H). Anal. Calcd for $C_{21}H_{16}ClF_3N_4O_2$: C, 56.20; H, 3.59; N, 12.48. Found: C, 56.20; H, 3.54; N, 12.49.

2-[4-({3-Chloro-4-[3-(trifluoromethoxy)phenoxy]phenyl}amino)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethanol (10l). Yield 71%, colorless crystals, mp 168−170 °C. ^{1}H NMR ($CDCl_3$) δ 4.09−4.19 (m, 2H), 4.30−4.38 (m, 2H), 6.05 (d, 1H, J = 3.2 Hz), 6.79−6.97 (m, 5H), 7.10 (d, 1H, J = 8.9 Hz), 7.32 (t, 1H, J = 8.3 Hz), 7.51 (dd, 1H, J = 8.8, 2.5 Hz), 7.83 (d, 1H, J = 2.6 Hz), 8.18 (s, 1H), 9.67 (br s, 1H). Anal. Calcd for $C_{21}H_{16}ClF_3N_4O_3$: C, 54.26; H, 3.47; N, 12.05. Found: C, 54.56; H, 3.55; N, 12.06.

2-[2-(4-Chloro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethoxy]ethyl Benzoate (11). To a suspension of **5** (0.66 g, 4.31 mmol) and cesium carbonate (3.13 g, 9.61 mmol) in DMF (5.0 mL) was added 2-(2-iodoethoxy)ethyl benzoate (1.45 g, 4.53 mmol), and the reaction mixture was stirred at room temperature for 15 h. The reaction mixture was poured into saturated sodium hydrogen carbonate (100 mL) and extracted with EtOAc (450 mL). The organic layer was washed with brine (100 mL) and dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (eluent, EtOAc:hexane = 1:19 to 2:3) to give 0.82 g (55%) of **11** as colorless oil. ^{1}H NMR ($CDCl_3$) δ3.71 (dt, 2H, J = 6.6, 3.0 Hz), 3.89 (t, 2H, J = 5.1 Hz), 4.41 (dt, 2H, J = 6.6, 3.0 Hz), 4.68 (t, 2H, J = 5.1 Hz), 6.56 (d, 1H, J = 3.3 Hz), 7.40−7.46 (m, 2H), 7.54−7.60 (m, 2H), 7.94−7.98 (m, 2H), 8.66 (s, 1H).

The following compounds (**12e**−**12l**) were prepared from **11** with corresponding anilines (**4e**,**4j**,**4l**) by a method similar to that described for **10e**.

2-[2-(4-{[3-Chloro-4-(3-chlorophenoxy)phenyl]amino}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethoxy]ethanol (12e). Yield 68%, colorless crystals, mp 135−137 °C. ^{1}H NMR ($CDCl_3$) δ 2.05 (br s, 1H), 3.71−3.84 (m, 4H), 4.03 (t, 2H, J = 4.5 Hz), 4.57 (t, 2H, J = 4.5 Hz), 6.61 (d, 1H, J = 3.0 Hz), 6.83−6.88 (m, 1H), 6.92 (t, 1H, J = 2.2 Hz), 7.01−7.06 (m, 1H), 7.06 (d, 1H, J = 8.9 Hz), 7.19−7.27 (m, 2H), 7.61 (dd, 1H, J = 8.9, 2.6 Hz), 7.89 (d, 1H, J = 2.6 Hz), 8.52 (s, 1H), 8.82 (br s, 1H). Anal. Calcd for $C_{22}H_{20}Cl_2N_4O_3$: C, 57.53; H, 4.39; N, 12.20. Found: C, 57.28; H, 4.37; N, 12.16.

2-{2-[4-({3-Chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}amino)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethoxy}ethanol (12j). Yield 57%, colorless crystals, mp 130−132 °C. ^{1}H NMR ($CDCl_3$) δ 1.94 (br s, 1H), 3.71−3.85 (m, 4H), 4.03 (t, 2H, J = 4.4 Hz), 4.57 (t, 2H, J = 4.4 Hz), 6.63 (d, 1H, J = 3.2 Hz), 7.07 (d, 1H, J = 8.9 Hz), 7.08−7.14 (m, 1H), 7.19 (s, 1H), 7.22 (d, 1H, J = 3.2 Hz), 7.31 (d, 1H, J = 7.7 Hz), 7.42 (t, 1H, J = 8.0 Hz), 7.63 (dd, 1H, J = 8.9, 2.6 Hz), 7.91 (d, 1H, J = 3.0 Hz), 8.52 (s, 1H), 8.83 (br s, 1H). Anal. Calcd for $C_{23}H_{20}ClF_3N_4O_3$: C, 56.05; H, 4.09; N, 11.37. Found: C, 56.05; H, 4.09; N, 11.28.

2-{2-[4-({3-Chloro-4-[3-(trifluoromethoxy)phenoxy]phenyl}amino)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethoxy}ethanol (12l). Yield 58%, colorless crystals, mp 148−150 °C. ^{1}H NMR ($CDCl_3$) δ 1.95 (br s, 1H), 3.71−3.84 (m, 4H), 4.03 (t, 2H, J = 4.5 Hz), 4.57 (t, 2H, J = 4.5 Hz), 6.62 (d, 1H, J = 3.2 Hz), 6.80−6.95 (m, 3H), 7.08 (d, 1H, J = 8.8 Hz), 7.21 (d, 1H, J = 3.2 Hz), 7.30 (t, 1H, J = 8.2 Hz), 7.62 (dd, 1H, J = 8.8, 2.6 Hz), 7.90 (d, 1H, J = 2.6 Hz), 8.52 (s, 1H), 8.82 (br s, 1H). Anal. Calcd for $C_{23}H_{20}ClF_3N_4O_4$: C, 54.29; H, 3.96; N, 11.01. Found: C, 54.49; H, 3.93; N, 10.96.

2-{2-[4-({5-Chloro-6-[3-(trifluoromethyl)phenoxy]pyridin-3-yl}amino)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethoxy}ethanol (12r). Yield 58%, yellow crystals, mp 171−172 °C. ^{1}H NMR ($CDCl_3$) δ 3.69−3.80 (m, 4H), 4.00−4.04 (m, 2H), 4.54−4.59 (m, 2H), 6.65 (d, 1H, J = 3.3 Hz), 7.23 (d, 1H, J = 3.3 Hz), 7.31−7.36 (m, 1H), 7.40−7.55 (m, 3H), 8.24 (d, 1H, J = 2.7 Hz), 8.47 (d, 1H, J = 2.7 Hz), 8.51 (s, 1H), 8.83 (s, 1H). Anal. Calcd for $C_{22}H_{19}ClF_3N_5O_3$: C, 53.50; H, 3.88; N, 14.18. Found: C, 53.47; H, 3.80; N, 14.08.

5-(4-Chloro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)pentyl Acetate (13). To a suspension of **5** (0.50 g, 3.26 mmol) and cesium carbonate (1.59 g, 4.88 mmol) in DMF (5.0 mL) was added 5-chloropentyl acetate (0.71 mL, 3.26 mmol) at 40 °C, and the reaction mixture was stirred at 40 °C for 4 days. Water (150 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (200 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (eluent, EtOAc:hexane = 1:3 to 3:2) to give 0.64 g (69%) of **13** as white solid. ^{1}H NMR ($CDCl_3$) δ: 1.33−1.46 (m, 2H), 1.61−1.72 (m, 2H), 1.84−1.97 (m, 2H), 2.04 (s, 3H), 4.05 (t, 2H, J = 6.6 Hz), 4.48 (t, 2H, J = 7.5 Hz), 6.71 (d, 1H, J = 3.3 Hz), 7.46 (d, 1H, J = 3.3 Hz), 8.69 (s, 1H).

5-[4-({3-Chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}amino)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]pentan-1-ol (15). A mixture of **13** (0.20 g, 0.71 mmol) and **4j** (0.27 g, 0.94 mmol) in 2-propanol (3.5 mL) was stirred at 80 °C for 14 h. After cooling to 0 °C, to the reaction mixture was added 1 N NaOH (2.1 mL), and the mixture was stirred at room temperature for 1 h. To the reaction mixture was added 1 N HCl (2.1 mL) under ice-cooling, and the mixture was extracted with EtOAc (150 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent, MeOH:EtOAc = 0:1 to 1:19) to give 0.28 g (79%) of **15** as colorless crystals, mp 146−148 °C. ^{1}H NMR ($CDCl_3$) δ: 1.35 (t, 1H, J = 4.7 Hz), 1.50−1.69 (m, 4H), 1.92−2.05 (m, 2H), 3.63−3.71 (m, 2H), 4.32 (t, 2H, J = 7.4 Hz), 6.59 (d, 1H, J = 3.3 Hz), 6.70 (s, 1H), 7.08 (d, 1H, J = 8.7 Hz), 7.09−7.12 (m, 1H), 7.15−7.27 (m, 2H), 7.30−7.35 (m, 1H), 7.40−7.43 (m, 1H), 7.47 (dd, 1H, J = 8.7, 2.7 Hz), 7.82 (d, 1H, J = 2.7 Hz), 8.53 (s, 1H). Anal. Calcd for $C_{24}H_{22}ClF_3N_4O_2$: C, 58.72; H, 4.52; N, 11.41. Found: C, 58.73; H, 4.53; N, 11.40.

4-Chloro-5-(2,2-diethoxyethyl)-5*H*-pyrrolo[3,2-*d*]pyrimidine (16). To a suspension of **5** (1.00 g, 6.51 mmol) and cesium carbonate (6.37 g, 19.5 mmol) in DMF (13 mL) was added 2-bromo-1,1-diethoxyethane (2.9 mL, 19.5 mmol) at room temperature, and the reaction mixture was stirred at 80 °C for 4.5 h. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate (100 mL) and washed with water (80 mL). The organic layer was separated, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel chromatography (eluent, EtOAc:hexane = 1:1 to 1:0) to give 1.26 g (72%) of **16** as pale-yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 6H, J = 6.0 Hz), 3.40 (m, 2H), 3.72 (m, 2H), 4.08 (m, 1H), 4.56 (d, 2H, J = 5.0 Hz), 6.71 (d, 1H, J = 3.0 Hz), 7.55 (d, 1H, J = 3.0 Hz), 8.69 (s, 1H).

5-(2,2-Diethoxyethyl)-4-phenoxy-5*H*-pyrrolo[3,2-*d*]pyrimidine (17). A mixture of **16** (1.00 g, 3.71 mmol), phenol (0.42 g, 4.46 mmol), potassium carbonate (0.62 g, 4.46 mmol) in 1-methyl-2-pyrrolidone (6.7 mL) was stirred at 140 °C for 6 h. After cooling to room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with water (80 mL). The organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was

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purified by silica gel column chromatography (eluent, EtOAc:hexane = 1:9 to 6:4) to give 1.15 g (95%) of **17** as yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 6H, J = 7.0 Hz), 3.40 (m, 2H), 3.69 (m, 2H), 4.51 (d, 2H, J = 6.0 Hz), 4.76 (t, 1H, J = 6.0 Hz), 6.65 (d, 1H, J = 3.0 Hz), 7.20−7.50 (m, 6H), 8.45 (s, 1H).

2-(4-Phenoxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethane-1,1-diol (18). A solution of **17** (1.10 g, 3.36 mmol) in a mixture of dichloromethane (4.5 mL) and trifluoroacetic acid (4.5 mL) was stirred at room temperature for 16 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in EtOAc (100 mL). The solution was washed with saturated sodium hydrogen carbonate (80 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give 0.83 g (91%) of **18** as white solid. ^{1}H NMR (DMSO-d_6) δ 4.35 (d, 2H, J = 6.0 Hz), 5.17 (t, 1H, J = 6.0 Hz), 6.14 (d, 2H, J = 6.0 Hz), 6.59 (d, 1H, J = 3.0 Hz), 7.20−7.60 (m, 5H), 7.75 (d, 1H, J = 3.0 Hz), 8.28 (s, 1H).

2-{[2-(4-Phenoxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl]-amino}ethanol (19). A solution of **18** (0.50 g, 1.84 mmol) and 2-hydroxyethylamine (167 mg, 2.73 mmol) in a mixture of DMF (29 mL) and acetic acid (2.9 mL) was stirred at room temperature for 1.5 h. To the reaction mixture was added sodium triacetoxyborohydride (579 mg, 2.73 mmol), and the mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography (eluent, MeOH:EtOAc = 0:1 to 3:7) to give 286 mg (52%) of **19** as gel. ^{1}H NMR ($CDCl_3$) δ 2.80 (t, 2H, J = 5.0 Hz), 3.19 (t, 2H, J = 6.0 Hz), 3.63 (t, 2H, J = 5.0 Hz), 4.59 (t, 2H, J = 6.0 Hz), 6.68 (d, 1H, J = 3.0 Hz), 7.20−7.35 (m, 3H), 7.40−7.51 (m, 3H), 8.46 (s, 1H).

2-({2-[4-({3-Chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}-amino)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethyl}amino)ethanol (20). A mixture of **19** (154 mg, 0.516 mmol), **4j** (223 mg, 0.775 mmol), pyridinium chloride (179 mg, 1.55 mmol), and phenol (396 mg, 4.21 mmol) was heated at 140 °C for 16 h. After cooling to room temperature, the reaction mixture was diluted with dichloromethane (40 mL), and washed with saturated sodium hydrogen carbonate (30 mL). The organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent, EtOAc:methanol = 1:0 to 7:3) to give 48 mg (19%) of **20** as colorless crystals, mp 188−189 °C. ^{1}H NMR (DMSO-d_6) δ 2.48 (m, 2H), 3.05 (m, 2H), 3.34 (m, 2H), 4.45 (m, 2H), 4.56 (t, 1H, J = 5.0 Hz), 6.49 (d, 1H, J = 3.0 Hz), 7.20 (m, 2H), 7.29 (d, 1H, J = 9.0 Hz), 7.45 (d, 1H, J = 8.0 Hz), 7.66 (m, 3H, m), 8.05 (d, 1H, J = 3.0 Hz), 8.32 (s, 1H). Anal. Calcd for $C_{23}H_{21}ClF_3N_5O_2$: C, 56.16; H, 4.30; N, 14.24. Found: C, 56.35; H, 4.30; N, 14.03.

2-(5,6-Dihydro-4*H*-pyrrolo[3,2,1-de]pteridin-4-yl)ethanol (21). **21** was obtained as a main product (29 mg, 28%) in this reaction. ^{1}H NMR ($CDCl_3$) δ 3.78 (t, 2H, J = 4.0 Hz), 3.84 (t, 2H, J = 5.0 Hz), 3.94 (t, 2H, J = 4.0 Hz), 4.26 (t, 2H, J = 5.0 Hz), 6.49 (d, 1H, J = 3.0 Hz), 7.14 (d, 1H, J = 3.0 Hz), 8.35 (s, 1H). LC-MS 205.09 (MH).

2-[(2-Hydroxyethyl)thio]ethyl benzoate (24). A solution of 2-iodoethyl benzoate (**22**, 6.00 g, 21.7 mmol), 2-sulfanylethanol (**23**, 1.5 mL, 22.0 mmol), and ethyldiisopropylamine (4.5 mL) in DMF (60 mL) was stirred at 40 °C for 3 days. Water (300 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (300 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent, EtOAc:hexane = 1:4 to 7:3) to give 3.77 g (77%) of **24** as orange oil. ^{1}H NMR ($CDCl_3$) δ: 2.15 (t, 1H, J = 6.0 Hz), 2.83 (t, 2H, J = 5.9 Hz), 2.92 (t, 2H, J = 6.8 Hz), 3.79 (dt, 2H, J = 6.0, 6.0 Hz), 4.50 (t, 2H, J = 6.8 Hz), 7.43−7.48 (m, 2H), 7.55−7.61 (m, 1H), 8.03−8.08 (m, 2H).

2-[(2-Bromoethyl)thio]ethyl Benzoate (25). To a solution of **24** (1.00 g, 4.42 mmol) and carbon tetrabromide (2.78 g, 8.38 mmol) in THF (30 mL) was added dropwise a solution of triphenylphosphine (2.20 g, 8.38 mmol) in THF (10 mL) under ice-cooling. After stirring at room temperature for 3 days, water (200 mL) was added, and then the mixture was extracted with EtOAc (300 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent, EtOAc:hexane = 1:9 to 4:6) to give 0.98 g (76%) of **25** as colorless oil. ^{1}H NMR ($CDCl_3$) δ: 2.95 (t, 2H, J = 6.8 Hz), 3.02−3.08 (m, 2H), 3.50−3.56 (m, 2H), 4.49 (t, 2H, J = 6.8 Hz), 7.43−7.48 (m, 2H), 7.55−7.61 (m, 1H), 8.03−8.06 (m, 2H).

2-{[2-(4-Chloro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl]thio}-ethyl benzoate (26). Compound **26** was obtained as colorless oil from **25** by a method similar to that described for **11**. Yield 80%. ^{1}H NMR ($CDCl_3$) δ: 2.81 (t, 2H, J = 6.8 Hz), 3.08 (t, 2H, J = 6.9 Hz), 4.45 (t, 2H, J = 6.8 Hz), 4.69 (t, 2H, J = 6.9 Hz), 6.73 (d, 1H, J = 3.3 Hz), 7.39−7.46 (m, 2H), 7.53−7.62 (m, 2H), 7.96−8.06 (m, 2H), 8.71 (s, 1H).

2-{[2-(4-{3-Chloro-4-[3-(trifluoromethyl)phenoxy]benzyl}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl]thio}ethanol (28). Compound **28** was obtained as colorless crystals in 59% yield from **26** by a method similar to that described for **15**; mp 108−109 °C. ^{1}H NMR ($CDCl_3$) δ: 1.92−2.00 (m, 1H), 2.52 (t, 2H, J = 5.6 Hz), 3.13 (t, 2H, J = 6.5 Hz), 3.65−3.75 (m, 2H), 4.61 (t, 2H, J = 6.5 Hz), 6.67 (d, 1H, J = 3.3 Hz), 7.08 (d, 1H, J = 8.7 Hz), 7.09−7.13 (m, 1H), 7.18−7.23 (m, 1H), 7.29 (d, 1H, J = 3.3 Hz), 7.32−7.35 (m, 1H), 7.41−7.46 (m, 1H), 7.51 (dd, 1H, J = 8.7, 2.7 Hz), 7.77 (d, 1H, J = 2.7 Hz), 7.80 (s, 1H), 8.55 (s, 1H). Anal. Calcd for $C_{23}H_{20}ClF_3N_4O_2S$: C, 54.28; H, 3.96; N, 11.01. Found: C, 54.35; H, 3.94; N, 11.02.

2-{[2-(4-{3-Chloro-4-[3-(trifluoromethyl)phenoxy]benzyl}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl]sulfinyl}ethanol (29). To a solution of **28** (0.10 g, 0.20 mmol) in dichloromethane (10 mL) was added dropwise a 70% solution of 3-chloroperbenzoic acid (0.058 g, 0.34 mmol) in dichloromethane (5.0 mL) at −78 °C. The mixture was stirred at −78 °C for 1 h, and aqueous sodium thiosulfate (100 mL) was added. After stirring at room temperature for 30 min, the mixture was extracted with EtOAc (200 mL). The organic layer was washed with brine (50 mL) and dried over $MgSO_4$. After concentration in vacuo, the residue was purified by silica gel column chromatography (eluent, MeOH:EtOAc = 0:1 to 1:4) to give 87 mg (85%) of **29** as colorless crystals, mp 158−159 °C. ^{1}H NMR (DMSO-d_6) δ: 2.78−3.01 (m, 2H), 3.27−3.40 (m, 1H), 3.42−3.58 (m, 1H), 3.71−3.79 (m, 2H), 4.80−4.90 (m, 2H), 5.02−5.09 (m, 1H), 6.58−6.63 (m, 1H), 7.16−7.25 (m, 2H), 7.27−7.31 (m, 1H), 7.44−7.50 (m, 1H), 7.59−7.64 (m, 1H), 7.66−7.72 (m, 1H), 7.74−7.82 (m, 1H), 7.96−8.03 (m, 1H), 8.37 (s, 1H), 9.38 (s, 1H). Anal. Calcd for $C_{23}H_{20}ClF_3N_4O_3S$: C, 52.62; H, 3.84; N, 10.67. Found: C, 52.62; H, 3.82; N, 10.67.

2-{[2-(4-{3-Chloro-4-[3-(trifluoromethyl)phenoxy]benzyl}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl]sulfonyl}ethanol (30). A mixture of **29** (0.15 g, 0.29 mmol), titanium tetraisopropoxide (0.043 mL), methanol (0.024 mL), and water (0.010 mL) in dichloromethane (10 mL) was stirred at room temperature for 30 min. 70% aqueous *tert*-butyl hydroperoxide (0.12 mL) was added to the reaction mixture, and the mixture was stirred at room temperature for 2 days. Aqueous sodium thiosulfate (100 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (200 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent, MeOH:EtOAc = 0:1 to 1:4) to give 0.12 g (74%) of **30** as colorless crystals, mp 196−197 °C. ^{1}H NMR (DMSO-d_6) δ: 3.09−3.15 (m, 2H), 3.62−3.75 (m, 4H), 4.92−5.02 (m, 2H), 5.09−5.15 (m, 1H), 6.50−6.57 (m, 1H), 7.16−7.32 (m, 3H), 7.45−7.48 (m, 1H), 7.58−7.74 (m, 3H), 7.91−7.97 (m, 1H), 8.37 (br s, 1H), 8.69−8.79 (m, 1H). Anal. Calcd for $C_{23}H_{20}ClF_3N_4O_4S$: C, 51.07; H, 3.73; N, 10.36. Found: C, 51.10; H, 3.63; N, 10.34.

***tert*-Butyl [2-(4-Chloro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)-ethyl]carbamate (31a).** To a suspension of **5** (0.50 g, 3.26 mmol) and cesium carbonate (1.59 g, 8.24 mmol) in DMF (10 mL) was added *tert*-butyl (2-bromoethyl)carbamate (1.09 g, 4.89 mmol) at 40 °C, and the mixture was stirred at 40 °C for 4 days. Water (100 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (200 mL). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (eluent, EtOAc:hexane = 1:3 to 3:2) to give 0.69 g (71%) of **31a** as white solid. ^{1}H NMR ($CDCl_3$) δ: 1.31−1.46 (m, 9H), 3.55 (dt, 2H, J = 6.0 Hz), 4.51−4.68 (m, 3H), 6.74 (d, 1H, J = 3.2 Hz), 7.47 (d, 1H, J = 3.2 Hz), 8.71 (s, 1H).

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***tert*-Butyl{2-[4-({3-chloro-4-[3-(trifluoromethyl)phenoxy]-phenyl}amino)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethyl}-carbamate (32a).** A mixture of **31a** (0.71 g, 2.39 mmol) and **4j** (0.83 g, 2.79 mmol) in 2-propanol (7.1 mL) was stirred at 80 °C for 12 h. To the reaction mixture was added saturated sodium hydrogen carbonate (50 mL) under ice-cooling, and the mixture was extracted with EtOAc (200 mL). The organic layer was washed with brine and dried over $MgSO_4$. The solvent was concentrated in vacuo, and the residue was purified by silica gel column chromatography (eluent, EtOAc:hexane = 1:1 to 1:0) to give 1.12 g (85%) of **32a** as white solid. ^{1}H NMR ($CDCl_3$) δ: 1.49 (s, 9H), 3.43−3.54 (m, 2H), 4.43−4.51 (m, 2H), 5.10 (t, 1H, J = 5.6 Hz), 6.60 (d, 1H, J = 3.3 Hz), 7.07 (m, 1H), 7.09−7.14 (m, 1H), 7.16−7.22 (m, 2H), 7.25−7.30 (m, 1H), 7.37−7.45 (m, 1H), 7.89 (dd, 1H, J = 8.7, 2.4 Hz), 8.02 (d, 1H, J = 2.4 Hz), 8.50 (s, 1H), 8.64 (br s, 1H).

5-(2-Aminoethyl)-*N*-{3-chloro-4-[3-(trifluoromethyl)-phenoxy]phenyl}-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine dihydrochloride (33a). A mixture of **32a** (1.12 g, 2.04 mmol), 2 N HCl (15 mL), and THF (30 mL) was stirred at 60 °C for 20 h. After the mixture was concentrated under reduced pressure, ethanol (50 mL) was added to the concentrate, and then the mixture was further concentrated in vacuo. The residual crystals were collected by filtration and washed with ethyl acetate to give 1.07 g (quant) of **33a** as pale-yellow solid. ^{1}H NMR (DMSO-d_6) δ: 3.21−3.35 (m, 2H), 4.92−5.02 (m, 2H), 6.71−6.76 (m, 1H), 7.24−7.32 (m, 2H), 7.37 (d, 1H, J = 9.0 Hz), 7.50−7.56 (m, 1H), 7.64−7.71 (m, 2H), 7.91−7.97 (m, 1H), 7.98−8.06 (m, 1H), 8.13−8.26 (m, 3H), 8.71 (br s, 1H), 9.90 (br s, 1H).

***N*-{2-[4-({3-Chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}-amino)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethyl}-2-hydroxyacetamide (34a).** A mixture of **33a** (0.11 g, 0.21 mmol), glycolic acid (0.044 g, 0.58 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC) (0.17 g, 0.89 mmol), 1-hydroxybenzotriazole monohydrate (HOBt) (0.13 g 0.96 mmol), and triethylamine (0.40 mL) in DMF (5.0 mL) was stirred at room temperature for 3 days. Water (100 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (200 mL). The organic layer was washed with water (50 mL) and brine (50 mL), dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent, MeOH:EtOAc = 0:1 to 1:9) to give 0.11 g (74%) of **34a** as colorless crystals, mp 145−147 °C. 1H-NMR ($CDCl_3$) δ: 2.93−3.09 (m, 1H), 3.59−3.73 (m, 2H), 4.24 (s, 2H), 4.43−4.53 (m, 2H), 6.59 (d, 1H, J = 3.3 Hz), 7.07 (d, 1H, J = 8.7 Hz), 7.09−7.46 (m, 6H), 7.72 (dd, 1H, J = 8.7, 2.4 Hz), 8.06 (d, 1H, J = 2.4 Hz), 8.49 (s, 1H), 8.57 (1H, s). Anal. Calcd for $C_{23}H_{19}ClF_3N_5O_3$: C, 54.61; H, 3.79; N, 13.84. Found: C, 54.77; H, 3.82; N, 13.89.

The following compounds (**34c**,**34e**) were prepared from **33a** with the corresponding carboxylic acid by a method similar to that described for **34a**.

***N*-{2-[4-({3-Chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}-amino)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethyl}-2-hydroxy-2-methylpropanamide (34c).** Yield 86%, colorless crystals, mp 183−185 °C. ^{1}H NMR ($CDCl_3$) δ: 1.49 (s, 6H), 2.12−2.27 (m, 1H), 3.56−3.67 (m, 2H), 4.42−4.52 (m, 2H), 6.61 (d, 1H, J = 3.3 Hz), 7.06 (d, 1H, J = 9.0 Hz), 7.08−7.14 (m, 1H), 7.15−7.43 (m, 5H), 7.86 (dd, 1H, J = 9.0, 2.7 Hz), 8.10 (d, 1H, J = 2.7 Hz), 8.51 (s, 1H), 8.72 (s, 1H). Anal. Calcd for $C_{25}H_{23}ClF_3N_5O_3$: C, 56.24; H, 4.34; N, 13.12. Found: C, 56.33; H, 4.38; N, 13.10.

***N*-{2-[4-({3-Chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}-amino)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethyl}-3-hydroxy-3-methylbutanamide (34e).** Yield 77%, colorless crystals, mp 167−169 °C. ^{1}H NMR ($CDCl_3$) δ: 1.33 (s, 6H), 2.49 (s, 2H), 2.65−2.77 (m, 1H), 3.57−3.68 (m, 2H), 4.44−4.53 (m, 2H), 6.61 (d, 1H, J = -3.0 Hz), 6.93−7.01 (m, 1H), 7.07 (d, 1H, J = 9.0 Hz), 7.09−7.15 (m, 1H), 7.19 (d, 1H, J = 3.0 Hz), 7.23−7.35 (m, 2H), 7.40−7.45 (m, 1H), 7.77 (dd, 1H, J = 9.0, 2.7 Hz), 8.08 (d, 1H, J = 2.7 Hz), 8.52 (s, 1H), 8.66 (s, 1H). Anal. Calcd for $C_{26}H_{25}ClF_3N_5O_3$: C, 56.99; H, 4.60; N, 12.78. Found: C, 57.22; H, 4.61; N, 12.80.

***N*-{2-[4-({3-Chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}-amino)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethyl}-3-hydroxypropanamide methanesulfonate (34d).** A solution of **33a** (3.50 g, 6.72 mmol), 3.6 M aqueous 3-hydroxypropanoic acid (5.6 mL, 20.1 mmol), WSC (10.1 g, 52.7 mmol), HOBt (4.56 g 33.7 mmol), and triethylamine (0.80 mL) in THF (17 mL) and DMF (17 mL) was stirred at room temperature for 3 days. Water (300 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (400 mL). The organic layer was washed with water (100 mL) and brine (100 mL) successively, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent, MeOH:EtOAc = 0:1 to 1:9) to give white solid. To a solution of the white solid in EtOAc (50 mL) was added methanesulfonic acid (0.16 mL), and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure, and the residue was recrystallized from EtOAc to give 1.04 g (25%) of **34d** as colorless crystals, mp 154−155 °C. ^{1}H NMR (DMSO-d_6) δ: 2.22 (t, 2H, J = 6.3 Hz), 2.31 (s, 3H), 3.41−3.51 (m, 4H), 3.56 (t, 2H, J = 6.5 Hz), 6.67 (d, 1H, J = 3.0 Hz), 7.25−7.32 (m, 2H), 7.37 (d, 1H, J = 8.8 Hz), 7.50−7.56 (m, 1H), 7.62−7.74 (m, 2H), 7.98 (d, 1H, J = 2.8 Hz), 8.33 (t, 1H, J = 5.5 Hz), 8.75 (s, 1H), 10.11 (br s, 1H). Anal. Calcd for $C_{25}H_{25}ClF_3N_5O_6S$: C, 48.74; H, 4.09; N, 11.37. Found: C, 48.60; H, 4.08; N, 11.24.

***tert*-Butyl [2-(4-chloro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)-ethyl]methylcarbamate (31b).** To a solution of 2-(methylamino)-ethanol (1.00 g, 13.3 mmol) in THF (10 mL) was added di-*tert*-butyl dicarbonate (3.6 mL) at room temperature. After stirring at room temperature for 2 h, the mixture was concentrated under reduced pressure. The residue was dissolved in THF (50 mL), and to the solution was added dropwise triethylamine (3.7 mL) and methanesulfonyl chloride (1.6 mL) at 0 °C successively, and the reaction mixture was stirred at 0 °C for 1 h. Then, saturated sodium hydrogen carbonate (10 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (200 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated in vacuo to give colorless oil. To a suspension of **5** (1.34 g, 8.76 mmol) and cesium carbonate (5.69 g, 29.5 mmol) in DMF (20 mL) was added the obtained colorless oil, and the reaction mixture was stirred at 40 °C for 4 days. Water (100 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (300 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (eluent, EtOAc:hexane = 1:3 to 3:2) to give 0.90 g (33%) of **31b** as pale-yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.12 (s, 4.5H), 1.43 (m, 4.5H), 2.55 (s, 1.5H), 2.81 (s, 1.5H), 3.58−3.60 (m, 2H), 4.54−4.69 (m, 2H), 6.73 (d, 1H, J = 3.0 Hz), 7.29−7.35 (m, 0.5H), 7.38−7.46 (m, 0.5H), 8.71 (s, 1H).

***tert*-Butyl{2-[4-({3-chloro-4-[3-(trifluoromethyl)phenoxy]-phenyl}amino)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethyl}-methylcarbamate (32b).** Compound **32b** was obtained as white solid in 76% yield from **31b** by a method similar to that described for **32a**. ^{1}H NMR ($CDCl_3$) δ: 1.51 (s, 9H), 3.01 (s, 3H), 3.51−3.59 (m, 2H), 4.41−4.51 (m, 2H), 6.60 (d, 1H, J = 3.0 Hz), 7.06 (d, 1H, J = 8.7 Hz), 7.08−7.13 (m, 1H), 7.15−7.24 (m, 2H), 7.30 (d, 1H, J = 8.4 Hz), 7.38−7.44 (m, 1H), 7.85−7.93 (m, 1H), 7.99−8.04 (m, 1H), 8.50 (s, 1H), 8.82 (s, 1H).

***N*-{3-Chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}-5-[2-(methylamino)ethyl]-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine Dihydrochloride (33b).** Compound **33b** was obtained as white solid in 91% yield from **32b** by a method similar to that described for **33a**. ^{1}H NMR (DMSO-d_6) δ: 2.54 (t, 3H, J = 5.3 Hz), 3.32−3.44 (m, 2H), 5.01−5.15 (m, 2H), 6.74 (d, 1H, J = 3.3 Hz), 7.22−7.27 (m, 2H), 7.36 (d, 1H, J = 8.7 Hz), 7.51 (d, 1H, J = 8.4 Hz), 7.60−7.69 (m, 2H), 7.91−7.96 (m, 1H), 8.01−8.07 (m, 1H), 8.72 (s, 1H), 9.00−9.18 (m, 2H), 10.06 (br s, 1H).

***N*-{2-[4-({3-Chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}-amino)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethyl}-2-hydroxy-*N*-methylacetamide (34b).** Compound **34b** was obtained as colorless crystals in 62% yield from **33b** by a method similar to that described for **34a**; mp 176−178 °C. ^{1}H NMR ($CDCl_3$) δ: 3.02 (s, 3H), 3.29 (t, 1H, J = 4.5 Hz), 3.72−3.81 (m, 2H), 4.26 (d, 2H, J = 4.5 Hz), 4.45−4.53 (m, 2H), 6.64 (d, 1H, J = 3.3 Hz), 7.08 (d, 1H, J = 8.7 Hz), 7.09−7.15 (m, 1H), 7.23−7.28 (m, 1H), 7.32−7.37 (m, 1H), 7.30−7.35 (m, 1H), 7.39−7.46 (m, 1H), 7.74 (dd, 1H, J = 8.7, 2.7 Hz), 8.05 (d, 1H,

J = 2.7 Hz), 8.39 (s, 1H), 8.53 (s, 1H). Anal. Calcd for $C_{24}H_{21}ClF_3N_5O_3$: C, 55.44; H, 4.07; N, 13.47. Found: C, 55.54; H, 4.09; N, 13.36.

4-Iodo-6-phenoxypyrimidine-5-amine (35). To a solution of 4,6-diiodo-pyrimidine-5-amine (40.0 g, 115 mmol) in 1-methyl-2-pyrrolidone (200 mL) were added phenol (11.9 g, 127 mmol) and potassium carbonate (17.5 g, 127 mmol), and the reaction mixture was stirred at 100 °C for 12 h. After cooling to room temperature, the reaction mixture was diluted with EtOAc (600 mL), and washed with water (700 mL) and brine (500 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent, EtOAc:hexane = 1:4 to 1:1) to give 33.0 g (91%) of **35**. ^{1}H NMR ($CDCl_3$) δ 4.34 (br s, 2H), 7.14–7.46 (m, 5H), 7.87 (s, 1H).

4-Phenoxy-6-prop-1-yn-1-ylpyrimidin-5-amine (36). A mixture of 4-iodo-6-phenoxypyrimidine-5-amine (**35**, 5.00 g, 16.0 mmol), 1-(trimethylsilyl)-1-propyne (3.3 mL, 22.3 mmol), dichlorobis(triphenylphosphine)palladium(II) (558 mg, 0.795 mmol), triphenylphosphine (421 mg, 0.161 mmol), copper iodide (303 mg, 0.159 mmol), potassium fluoride (1.29 g, 22.2 mmol), and triethylamine (50 mL) in DMF (100 mL) was stirred at 60 °C for 16 h. The reaction mixture was diluted with diethyl ether (50 mL) and quenched with saturated sodium hydrogen carbonate (100 mL). The mixture was extracted with diethyl ether (100 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (eluent, EtOAc:hexane = 1:4 to 1:1) to give 2.64 g (73%) of **36** as orange solid. ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H), 4.37 (br s, 2H), 7.15–7.19 (m, 2H), 7.24–7.29 (m, 1H), 7.40–7.46 (m, 2H), 8.08 (s, 1H).

6-Methyl-4-phenoxy-5*H*-pyrrolo[3,2-*d*]pyrimidine (37). To a solution of **36** (2.56 g, 11.4 mmol) in THF (100 mL) was added dropwise 1 N potassium *tert*-butoxide in THF solution (12 mL, 12.0 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 40 min. The reaction mixture was diluted with EtOAc (100 mL) and quenched with water (100 mL) at 0 °C. The mixture was extracted with EtOAc (100 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (eluent, EtOAc:hexane = 4:1 to 1:1) to give 1.71 g (67%) of **37** as pale-orange solid. ^{1}H NMR ($CDCl_3$) δ 2.54 (d, 3H, J = 0.9 Hz), 6.44 (q, 1H, J = 0.9 Hz), 7.21–7.30 (m, 3H), 7.42–7.47 (m, 2H), 8.46 (m, 2H).

2-[2-(6-Methyl-4-phenoxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)-ethoxy]ethyl benzoate (38). A mixture of **37** (300 mg, 1.33 mmol), 2-{2-[(methylsulfonyl)oxy]ethoxy}ethyl benzoate (464 mg, 1.61 mmol), and potassium carbonate (431 mg, 3.19 mmol) in DMF (7 mL) was stirred at 60 °C for 21 h. The reaction mixture was diluted with EtOAc (10 mL), quenched with water (10 mL), and extracted with EtOAc (10 mL). The organic layer was washed with brine (10 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (eluent: EtOAc:hexane = 1:4 to 4:1) to give 518 mg (93%) of **38** as yellow oil. ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3H), 3.62–3.74 (m, 2H), 3.92 (t, 2H, J = 5.3 Hz), 4.33–4.44 (m, 2H), 4.57 (t, 2H, J = 5.1 Hz), 6.36 (s, 1H), 7.15–7.34 (m, 3H), 7.34–7.51 (m, 4H), 7.51–7.65 (m, 1H), 7.87–8.00 (m, 2H), 8.40 (s, 1H).

2-{2-[4-({3-Chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}-amino)-6-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethoxy}ethyl benzoate (39). A mixture of **38** (270 mg, 0.65 mmol), **4j** (279 mg, 0.97 mmol), pyridinium chloride (235 mg, 2.03 mmol), and phenol (496 mg) was stirred at 140 °C for 17 h. After cooling to room temperature, the mixture was diluted with dichloromethane (50 mL), and the organic layer was washed with saturated sodium hydrogen carbonate (30 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (eluent, EtOAc:hexane = 1:4 to 1:0) to give 228 mg (58%) of **39** as yellow oil. ^{1}H NMR ($CDCl_3$) δ 2.44 (s, 3H), 3.93–4.12 (m, 4H), 4.47–4.51 (m, 4H), 6.40 (s, 1H), 6.77 (d, 1H, J = 9.0 Hz), 7.01–7.03 (m, 1H), 7.15 (s, 1H), 7.20–7.50 (m, 5H), 7.75–7.86 (m, 3H), 8.44 (s, 1H), 8.72 (br s, 1H).

2-{2-[4-({3-Chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}-amino)-6-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethoxy}-ethanol (40). To a suspension of **39** (220 mg, 0.36 mmol) in MeOH (1.6 mL) was added 1 N NaOH (0.5 mL), and the reaction mixture was stirred at room temperature for 2 h. The mixture was neutralized with 1 N HCl (0.5 mL) and extracted with EtOAc (50 mL). The organic layer was washed with brine (30 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (eluent, MeOH:EtOAc = 0:1 to 1:4) and crystallized from diisopropylether to give 164 mg (90%) of **40** as colorless crystals, mp 168–169 °C. ^{1}H NMR (DMSO-d_6) δ 2.46 (s, 3H), 3.49–3.51 (m, 4H), 3.84 (t, 2H, J = 4.0 Hz), 4.55 (br s, 2H), 4.76 (t, 2H, J = 4.0 Hz), 7.17–7.21 (m, 2H), 7.27 (d, 1H, J = 9.0 Hz), 7.44 (d, 1H, J = 8.0 Hz), 7.50–7.70 (m, 2H), 8.01 (d, 1H, J = 2.0 Hz), 8.29 (s, 1H), 8.98 (br s, 1H). Anal. Calcd for $C_{24}H_{22}ClF_3N_4O_3$: C, 56.87; H, 4.37; N, 11.05. Found: C, 56.89; H, 4.34; N, 11.05.

4-(3,3-Diethoxyprop-1-yn-1-yl)-6-phenoxypyrimidin-5-amine (41). A mixture of **35** (7.00 g, 22.4 mmol), 3,3-diethoxyprop-1-yne (3.8 mL, 26.5 mmol), dichlorobis(triphenylphosphine)-palladium(II) (783 mg, 1.12 mmol), copper iodide (255 mg, 1.34 mmol), and triethylamine (120 mL) in acetonitrile (160 mL) was stirred at room temperature for 16.5 h. The mixture was concentrated in vacuo, and the residue was purified by column chromatography (eluent, EtOAc:hexane = 1:9 to 1:1) to give 6.20 g (89%) of **41** as brown oil. ^{1}H NMR ($CDCl_3$) δ 1.29 (t, 6H, J = 7.2 Hz), 3.62–3.77 (m, 2H), 3.77–3.91 (m, 2H), 4.48 (br s, 2H), 5.56 (s, 1H), 7.14–7.21 (m, 2H), 7.27–7.33 (m, 1H), 7.39–7.50 (m, 2H), 8.11 (s, 1H).

6-(Diethoxymethyl)-4-phenoxy-5*H*-pyrrolo[3,2-*d*]pyrimidine (42). To a solution of **41** (6.20 g, 19.8 mmol) in THF (100 mL) was added 1 N potassium *tert*-butoxide in THF (25 mL) at 0 °C, and the reaction mixture was stirred at room temperature for 1.5 h. The mixture was diluted with EtOAc (100 mL), quenched with saturated aqueous ammonium chloride (100 mL), and extracted with EtOAc (100 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (eluent, EtOAc:hexane = 1:9 to 1:1) to give 3.15 g (51%) of **42** as orange solid. ^{1}H NMR ($CDCl_3$) δ 1.20–1.37 (m, 6H), 3.55–3.79 (m, 4H), 5.81 (d, 1H, J = 0.6 Hz), 6.69 (dd, 1H, J = 2.2, 0.6 Hz), 7.18–7.36 (m, 3H), 7.39–7.52 (m, 2H), 8.50 (s, 1H), 8.92 (br s, 1H).

4-Phenoxy-5*H*-pyrrolo[3,2-*d*]pyrimidine-6-carbaldehyde (43). To a solution of **42** (3.15 g, 10.1 mmol) in THF (40 mL) was added 1 N HCl (40 mL), and the reaction mixture was stirred at room temperature for 2 h. The mixture was neutralized with 1 N NaOH (40 mL) and extracted with a mixture of EtOAc (30 mL)/THF (30 mL). The organic layer was washed with brine (20 mL), dried over $MgSO_4$, and concentrated in vacuo. The residual solid was collected by filtration and washed with diisopropylether to give 2.17 g (90%) of **43** as khaki powder. ^{1}H NMR ($CDCl_3$) δ 7.25–7.40 (m, 3H), 7.43–7.58 (m, 3H), 8.44 (s, 1H), 10.06 (s, 1H), 13.26 (br s, 1H).

4-Phenoxy-5*H*-pyrrolo[3,2-*d*]pyrimidine-6-carboxylic Acid (44). To a mixture of **43** (2.17 g, 9.07 mmol) in DMSO (21 mL) and sodium dihydrogen phosphate (5.45 g, 45.4 mmol) in water (14 mL) was added dropwise a solution of sodium chlorite (2.06 g, 22.8 mmol) in water (14 mL), and the reaction mixture was stirred at room temperature for 2 h. The mixture was quenched with saturated sodium hydrogen carbonate (10 mL) gradually, and the pH was adjusted to pH 2 by addition of 1 N HCl. The resulting precipitate was collected by filtration and washed with diisopropylether to give 2.40 g (quant) of **44** as white powder. ^{1}H NMR ($CDCl_3$) δ 7.09 (s, 1H), 7.23–7.36 (m, 3H), 7.41–7.54 (m, 2H), 8.36 (s, 1H), 12.82 (br s, 1H).

4-Phenoxy-5*H*-pyrrolo[3,2-*d*]pyrimidine-6-carboxamide (45). A mixture of **44** (465 mg, 1.82 mmol) and thionyl chloride (7 mL) was stirred at 75 °C for 2 h. After cooling to room temperature, the mixture was concentrated in vacuo and the residue was suspended in THF (10 mL). The suspension was poured into 28% ammonia (20 mL) at 0 °C, and the resulting precipitate was collected by filtration. The filtrate was extracted with a mixture of EtOAc (10 mL)/THF (10 mL). The organic layer was washed with brine (5 mL), dried over $MgSO_4$, and concentrated in vacuo. The residual solid was collected by filtration,

and combined with the above precipitate to give 427 mg (92%) of **45** as pale-yellow powder. ^{1}H NMR (DMSO-d_6) δ 7.25 (s, 1H), 7.27−7.35 (m, 3H), 7.39−7.57 (m, 2H), 7.75 (s, 1H), 8.17 (s, 1H), 8.36 (s, 1H), 12.58 (br s, 1H).

4-Phenoxy-5*H*-pyrrolo[3,2-*d*]pyrimidine-6-carbonitrile (46). A mixture of **45** (427 mg, 1.68 mmol) in phosphoryl chloride (5 mL) was stirred at 70 °C for 1.5 h. After cooling to room temperature, the mixture was concentrated in vacuo. The residue was dissolved in THF (10 mL), and to the solution were added water (5 mL) and 28% ammonia (10 mL) at 0 °C. The mixture was extracted with a mixture of EtOAc (10 mL)/THF (10 mL). The organic layer was washed with brine (5 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (eluent, EtOAc:hexane = 1:9 to 1:1) to give 240 mg (61%) of **46** as pale-yellow powder. ^{1}H NMR (DMSO-d_6) δ 7.29−7.37 (m, 3H), 7.45−7.54 (m, 2H), 7.58 (s, 1H), 8.46 (s, 1H), 13.77 (br s, 1H).

2-[2-(6-Cyano-4-phenoxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)-ethoxy]ethyl benzoate (47). A mixture of **46** (240 mg, 1.02 mmol), 2-{2-[(methylsulfonyl)oxy]ethoxy}ethyl benzoate (354 mg, 1.23 mmol), and potassium carbonate (355 mg, 2.57 mmol) in DMF (5 mL) was stirred at 60 °C for 7 h. To the mixture was added additional 2-{2-[(methylsulfonyl)oxy]ethoxy}ethyl benzoate (133 mg, 0.462 mmol), and the reaction mixture was stirred at 60 °C for 16 h. After cooling to room temperature, the mixture was diluted with EtOAc (10 mL), quenched with aqueous ammonium chloride (20 mL), and extracted with EtOAc (20 mL). The organic layer was washed with brine (5 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (eluent, EtOAc:hexane = 1:4 to 4:1) to give 267 mg (61%) of **47** as colorless oil. ^{1}H NMR ($CDCl_3$) δ 3.73−3.79 (m, 2H), 3.96 (t, 2H, J = 4.9 Hz), 4.37−4.43 (m, 2H), 4.83 (t, 2H, J = 4.9 Hz), 7.17 (s, 1H), 7.18−7.23 (m, 2H), 7.27−7.35 (m, 1H), 7.36−7.49 (m, 4H), 7.51−7.58 (m, 1H), 7.85−7.92 (m, 2H), 8.49 (s, 1H).

2-{2-[4-({3-Chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}-amino)-6-cyano-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethoxy}ethyl Benzoate (48). A mixture of **47** (262 mg, 0.610 mmol), **4j** (264 mg, 0.919 mmol), pyridinium chloride (222 mg, 1.92 mmol), and phenol (462 mg) was stirred at 140 °C for 17 h. After cooling to room temperature, the mixture was diluted with dichloromethane (10 mL), washed with brine (5 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (eluent, EtOAc:hexane = 1:4 to 1:1) to give 283 mg (74%) of **48** as yellow oil. ^{1}H NMR ($CDCl_3$) δ 3.96−4.06 (m, 2H), 4.16−4.22 (m, 2H), 4.45−4.54 (m, 2H), 4.68−4.79 (m, 2H), 6.80 (d, 1H, J = 8.8 Hz), 7.01−7.09 (m, 1H), 7.14−7.20 (m, 1H), 7.24 (s, 1H), 7,27−7.53 (m, 6H), 7.68−7.76 (m, 2H), 7.92 (d, 1H, J = 2.5 Hz), 8.53 (s, 1H), 8.95 (s, 1H).

4-({3-Chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}amino)-5-[2-(2-hydroxyethoxy)ethyl]-5*H*-pyrrolo[3,2-*d*]pyrimidine-6-carbonitrile (49). To a solution of **48** (283 mg, 0.454 mmol) in MeOH (3 mL) was added 1 N NaOH (0.6 mL), and the reaction mixture was stirred at room temperature for 1 h. The mixture was neutralized with 1 N HCl (0.6 mL) and extracted with EtOAc (5 mL). The organic layer was washed with brine (20 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (eluent, EtOAc:hexane = 1:2 to 4:1) and crystallized from diisopropylether to give 143 mg (61%) of **49** as colorless crystals, mp 143−144 °C. ^{1}H NMR ($CDCl_3$) δ 3.74−3.88 (m, 4H), 4.08−4.16 (m, 2H), 4.70−4.80 (m, 2H), 7.05−7.15 (m, 2H), 7.16−7.21 (m, 1H), 7.25 (s, 1H), 7.30−7.36 (m, 1H), 7.43 (t, 1H, J = 7.8 Hz), 7.67 (dd, 1H, J = 8.8, 2.8 Hz), 7.96 (d, 1H, J = 2.8 Hz), 8.58 (s, 1H), 9.03 (br s, 1H). Anal. Calcd for $C_{24}H_{19}ClF_3N_5O_3$: C, 55.66; H, 3.70; N, 13.52. Found: C, 55.70; H, 3.54; N, 13.33.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Methods used in molecular modeling, enzyme assays, cell line, and animal models, pharmacokinetics, metabolic stability, and structural analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

†Protein Data Bank accession code: 3RCD.

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■ ABBREVIATIONS USED

^{1}H NMR, proton nuclear magnetic resonance; DMF, *N*,*N*-dimethylformamide; EGFR, epidermal growth factor receptor; EtOAc, ethyl acetate; EtOH, ethanol; GI, growth inhibitory; HCl, hydrochloric acid; HER2, human epidermal growth factor receptor 2; HOBt, 1-hydroxybenzotriazole monohydrate; mCPBA, 3-chloroperbenzoic acid; $MgSO_4$, magnesium sulfate; NaOH, sodium hydroxide; NMP, 1-methyl-2-pyrrolidone; ppm, parts per million; Pt−C, platinum−carbon; SAR, structure−activity relationships; TKIs, tyrosine kinase inhibitors; WSC, water-soluble carbodiimide

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(21) The coordinates and structure factors have been deposited with the Protein Data Bank with accession code 3RCD.

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