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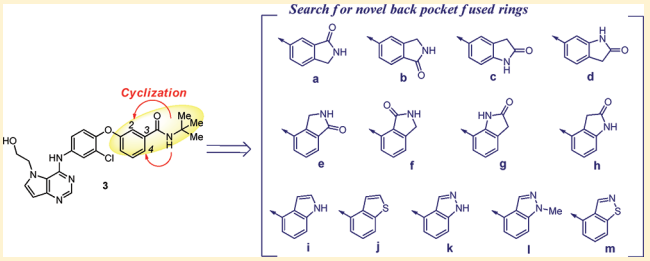
Design and Synthesis of Pyrrolo[3,2-*d*]pyrimidine Human Epidermal Growth Factor Receptor 2 (HER2)/Epidermal Growth Factor Receptor (EGFR) Dual Inhibitors: Exploration of Novel Back-Pocket Binders

Youichi Kawakita,* Hiroshi Banno, Tomohiro Ohashi, Toshiya Tamura, Tadashi Yusa, Akiko Nakayama, Hiroshi Miki, Hidehisa Iwata, Hidenori Kamiguchi, Toshimasa Tanaka, Noriyuki Habuka, Satoshi Sogabe, Yoshikazu Ohta, and Tomoyasu Ishikawa*

Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited: 26-1, Muraoka-Higashi 2-chome, Fujisawa, Kanagawa 251-8555, Japan

Supporting Information

ABSTRACT: To develop novel human epidermal growth factor receptor 2 (HER2)/epidermal growth factor receptor (EGFR) kinase inhibitors, we explored pyrrolo[3,2-*d*]pyrimidine derivatives bearing bicyclic fused rings designed to fit the back pocket of the HER2/EGFR proteins. Among them, the 1,2-benzisothiazole (**42m**) ring was selected as a suitable back pocket binder because of its potent HER2/EGFR binding and cell growth inhibitory (GI) activities and pseudoirreversibility (PI) profile as well as good bioavailability (BA). Ultimately, we arrived at our preclinical candidate **51m** by optimization of the *N*-5 side chain to improve CYP inhibition and metabolic stability profiles without a loss of potency (HER2/EGFR inhibitory activity, IC_{50}, 0.98/2.5 nM; and GI activity BT-474 cells, GI_{50}, 2.0 nM). Reflecting the strong *in vitro* activities, **51m** exhibited potent tumor regressive efficacy against both HER2- and EGFR-overexpressing tumor (4−1ST and CAL27) xenograft models in mice at oral doses of 50 mg/kg and 100 mg/kg.



INTRODUCTION

Protein tyrosine kinases play a key role in signal transduction pathways that regulate numerous cellular functions including proliferation, differentiation, migration, 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 2 (HER2 or ErbB-2/neu) and epidermal growth factor receptor (EGFR or HER1/ErbB-1) by tyrosine kinase inhibitors (TKIs) represents one such therapeutic approach. Indeed, there are several ATP-competitive HER2/EGFR TKIs currently in clinical trials or on the market for the treatment of cancer.[2]

We previously reported[3] pyrrolo[3,2-*d*]pyrimidine derivatives as potent dual TKIs against HER2/EGFR, and discovered a representative clinical candidate **1** (TAK-285, Figure 1), which showed significant *in vivo* antitumor efficacy based on HER2/EGFR inhibitory activities. In addition, we have determined X-ray cocrystal structures of **1** with both HER2 and EGFR.[3,4]

These studies proved that a pyrrolo[3,2-*d*]pyrimidine scaffold was an important central core for the creation of kinase inhibitors. Indeed, this scaffold was also effectively used as a hinge binder in our VEGFR2 program.[5] In addition, compound **1** exhibited a moderately slow off-rate, leading to a so-called "pseudo-irreversible" (PI) profile from the intracellular kinase

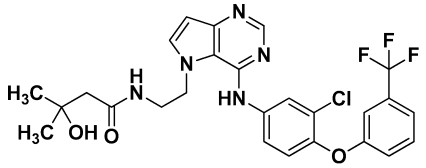


Figure 1. Chemical structure of **1** (TAK-285).

domains of HER2 and EGFR (*vide infra*). Some reports have indicated that a slow off-rate is related to high cellular activity.[6] On the basis of this knowledge, we continued our studies on the synthesis of HER2/EGFR kinase inhibitors using this unique scaffold to discover new candidates exhibiting potent antitumor activity with characteristic PI profiles. We hypothesized that a unique back pocket to accommodate the 3-trifluoromethylphenoxy group of **1**, created by the conformational shift in the *N*-terminal end of the *C*-helix, could be utilized to identify additional compounds with PI profiles. Our studies revealed that the PI profile correlates with the structure of the back pocket binder moiety.

Herein, we will describe the design, synthesis, and structure−activity relationships (SAR) of back pocket binding moieties

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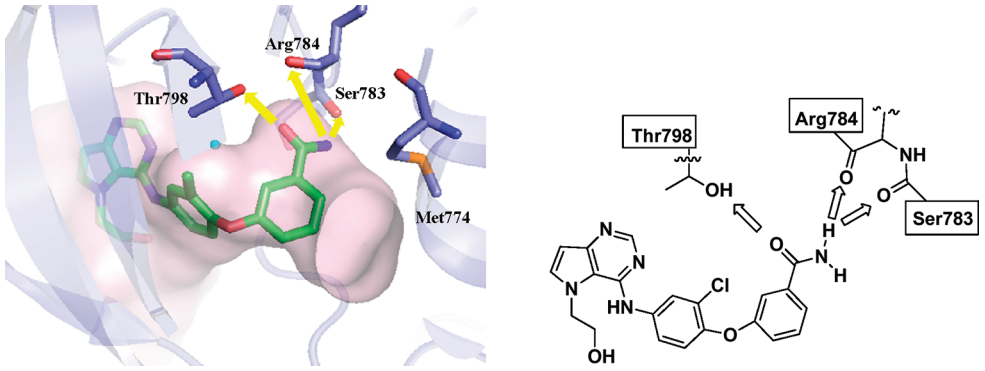
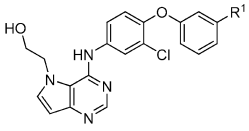


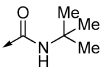
Figure 2. Docking model of the designed compound with HER2 protein.

that affect PI and cellular activity and describe the biological evaluation of these molecules.

A docking study suggested that a carboxamide moiety at the *C*-3 position on the terminal phenoxy ring can make hydrogen bonds with the OH of Thr798, the C═O of Ser783, and the C═O of Arg784 in the HER2 protein (Figure 2). In fact, our previously synthesized compound, *N*-*tert*-butylcarboxamide **3** revealed stronger HER2 inhibitory and growth inhibitory (GI) activities than those of **2**, which has the same anilino group as **1** (Table 1). In order to search for appropriate configurations

Table 1. Preliminary Biological Results for Pyrrolo[3,2-*d*]pyrimidine Derivatives



Compound	R^1	Enzyme IC_{50} (nM)[a]		Cell growth GI_{50} (nM)[a]
		HER2	EGFR	BT-474
2	CF_3	12 (11-14)	34 (26-44)	212 (179-249)
3		2.3 (2.0-2.6)	33 (23-48)	88 (57-123)

[a]95% Confidence interval.

of the carboxamide moiety to interact with Thr798/Thr790, Ser783/Cys775, and Arg784/Arg776 in the HER2/EGFR proteins, we designed bicyclic fused amide rings **a**–**h**, which were cyclized between the terminal phenoxy *C*-3 position and the *C*-2/*C*-4 position of **3**. Furthermore, on the basis of the activities observed for **a**–**h**, we designed several compounds possessing various bicyclic fused aromatic rings **i**–**m** (Figure 3).

CHEMISTRY

The *N*-*tert*-butylcarboxamide derivative **3** was synthesized as shown in Scheme 1. Condensation of commercially available 2-chloro-1-fluoro-4-nitrobenzene (**4**) with phenol **5** was carried out in the presence of potassium carbonate (K_2CO_3) in dimethylformamide (DMF), followed by reduction of the nitro group with platinum on carbon (Pt/C) under hydrogen atmosphere to afford substituted aniline **7** in two steps (95% yield). After reaction of the obtained aniline **7** with 2-(4-chloro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl benzoate (**8**),[3] the terminal benzoyl and methyl esters were hydrolyzed with 1 N aqueous sodium hydroxide (NaOH), resulting in an 88% yield of the corresponding carboxylic acid (**9**). Amidation of the carboxylic acid **9** with 2-methylpropan-2-amine in the presence of triethylamine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), and 1-hydroxybenzotriazole monohydrate (HOBt) provided the corresponding desired carboxamide in 66% yield.

Anilines with a 1,3-dihydro-2*H*-indol-2-one ring (**14c**, **d**, **g**, and **h**) were prepared as shown in Scheme 2. Reaction of **4** with the commercially available 1*H*-indol derivatives **10c**, **d**, **g**, and **h** gave the corresponding nitrobenzenes **11c**, **d**, **g** and **h**, respectively. After bromination of the obtained compounds **11c**, **d**, **g**, and **h** with *N*-bromosuccinimide (NBS), hydrolysis of the 3-bromo intermediates **12c**, **d**, **g**, and **h** with 6 N HCl provided 1,3-dihydro-2*H*-indol-2-one derivatives **13c**, **d**, **g**, and **h** in 45–80% yields over two steps. Finally, the nitro groups of the compounds **13c**, **d**, **g**, and **h** were reduced with Pt/C under hydrogen atmosphere to afford desired aniline compounds **14c**, **d**, **g**, and **h** in yields of 61–96%.

Synthesis of the aniline compounds **14a**, **b**, **e**, and **f**, bearing the corresponding 2,3-dihydro-1*H*-isoindol-1-one ring, is shown in Schemes 3 to 5. Reaction of the hydroxy methyl benzoates **15a** and **15e** with **4** in the presence of K_2CO_3 resulted in 92% to 96% yields of the condensed compounds **16a** and **16e**, which were reacted with NBS to provide 37% and 70% yields, respectively, of the methyl 2-(bromomethyl)benzoate derivatives **17a** and **17e**. Amination of the benzylbromides **17a** and **17e** with 28% aqueous ammonia solution was carried out in tetrahydrofuran (THF), followed by the subsequent cyclization of the amine intermediates *in situ* to afford 2,3-dihydro-1*H*-isoindol-1-one derivatives **18a** and **18e** in yields of 82% and 96%, respectively. The obtained products **18a** and **18e** were converted to the desired aniline compounds **14a** and **14e** in 50% to 70% yields under the usual hydrogenation conditions.

The 2,3-dihydro-1*H*-isoindol-1-one derivative **14b** was synthesized as shown in Scheme 4. Bromination of methyl 4-methoxy-2-methylbenzoate (**19**) with NBS in the presence of AIBN in trifluoromethylbenzene, followed by treatment with 28% aqueous ammonia solution, resulted in a 39% yield of cyclic compound **21**. After deprotection of the methyl group with aqueous hydrogen bromide (HBr) in 44% yield, reaction of phenol compound **22** with **4** provided coupled nitrobenzene **23** in 74% yield. Standard hydrogenation of **23** using Pt/C led to the desired aniline compound **14b** in quantitative yield.

Scheme 5 shows the synthesis of the aniline compound **14f**. Bromination of ethyl 2-methoxy-6-methylbenzoate (**24**) provided dibrominated benzoate **25** followed by conversion with

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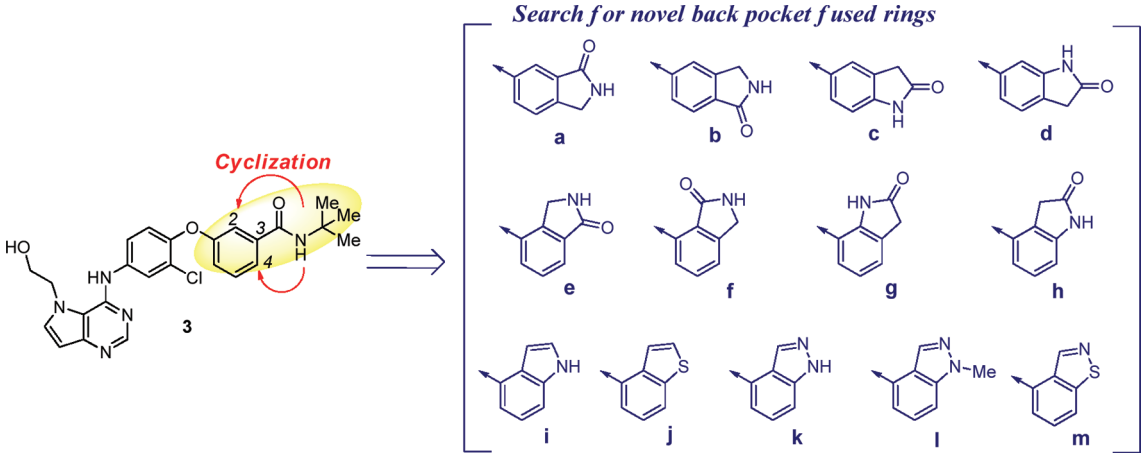
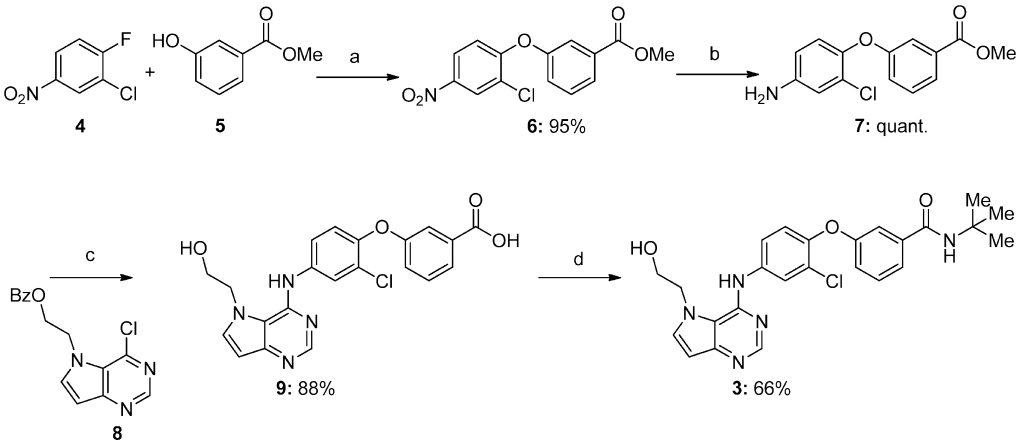


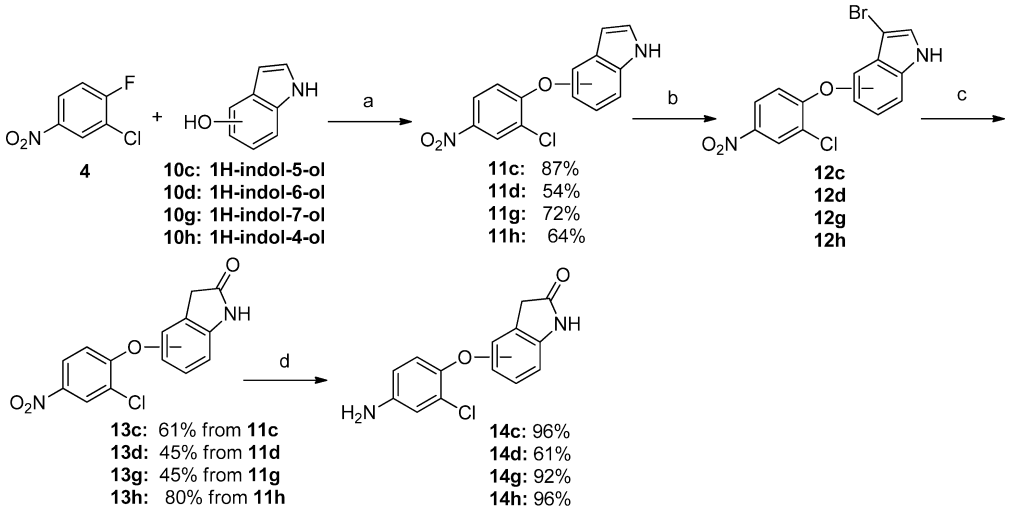
Figure 3. Design of new back pocket binders.

Scheme 1. Synthesis of the Pyrrolo[3,2-*d*]pyrimidine Derivative 3[a]



[a]Reagents: (a) K_2CO_3, DMF, rt; (b) H_2, Pt/C, EtOAc, rt; (c) (i) 2-propanol, 80 °C, (ii) 1 N NaOH, MeOH, rt; (d) EDC, HOBt, Et_3N, 2-methylpropan-2-amine, DMF, rt.

Scheme 2. Synthesis of Anilines Bearing a 1,3-Dihydro-2*H*-indol-2-one Ring[a]



[a]Reagents: (a) K_2CO_3, DMF, rt; (b) NBS, *tert*-BuOH, THF, rt; (c) 1 N HCl, THF, 70 °C; (d) H_2, Pt/C, MeOH, THF, rt.

aqueous ammonia to 4-bromo-7-methoxy-2,3-dihydro-1*H*-isoindol-1-one (**26**) in 40% yield over two steps. After removal of the methyl group in 71% yield using HBr, the obtained 4-bromo-7-hydroxy-2,3-dihydro-1*H*-isoindol-1-one (**27**) was hydrogenated with Pt/C in MeOH to give phenol derivative **28** in quantitative yield. Condensation of **28** with **4** in the presence of

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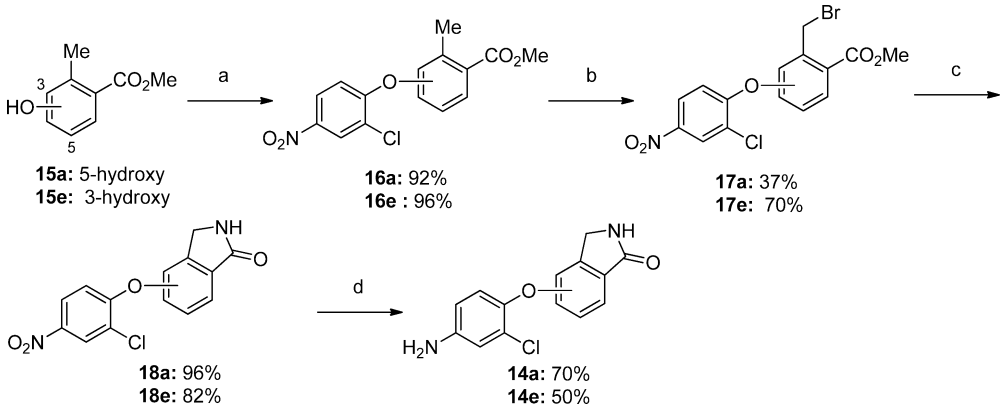
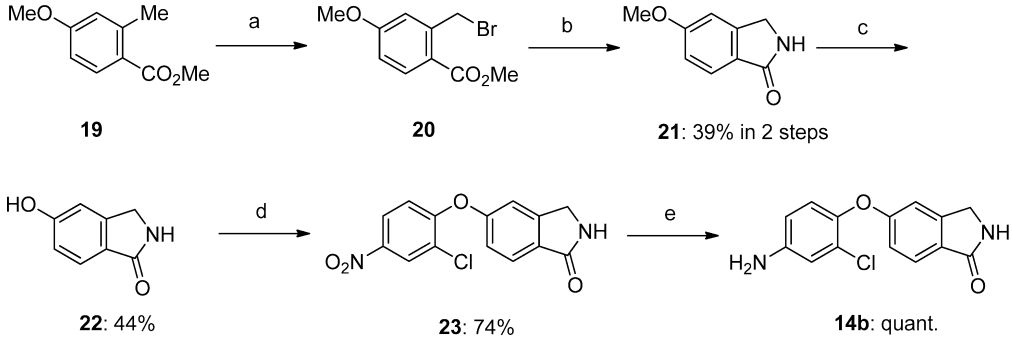
Scheme 3. Synthesis of Anilines Bearing a 2,3-Dihydro-1*H*-isoindol-1-one Ring (I)[a]



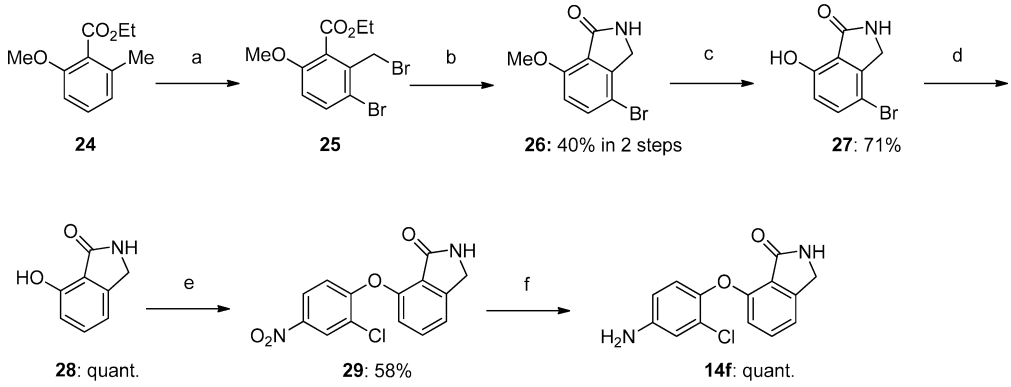
[a]Reagents: (a) **4**, K_2CO_3, DMF, rt; (b) NBS, AIBN, CF_3–Ph, 100 °C; (c) 28% aq. NH_3, THF, rt; (d) H_2, Pt/C, MeOH, THF, rt.

Scheme 4. Synthesis of Aniline Bearing a 2,3-Dihydro-1*H*-isoindol-1-one Ring (II)[a]



[a]Reagents: (a) NBS, AIBN, CF_3–Ph, 100 °C; (b) 28% aq. NH_3, THF, rt; (c) 48% aq. HBr, 100 °C; (d) **4**, K_2CO_3, DMF, rt; (e) H_2, Pt/C, MeOH, THF, rt.

Scheme 5. Synthesis of Aniline Bearing a 2,3-Dihydro-1*H*-isoindol-1-one Ring (III)[a]



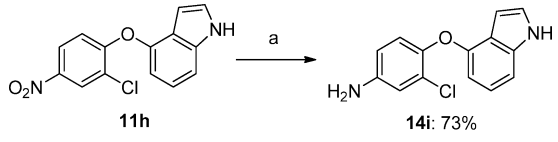
[a]Reagents: (a) NBS, AIBN, CF_3–Ph, 100 °C; (b) 28% aq. NH_3, THF, rt; (c) 48% aq. HBr, 100 °C; (d) H_2, Pt/C, MeOH, rt; (e) **4**, K_2CO_3, DMF, rt; (f) H_2, Pt/C, MeOH, THF, rt.

K_2CO_3 provided a coupled product **29**, and subsequent reduction of the nitro group of **29** afforded the desired aniline **14f** in quantitative yield.

As shown in Scheme 6, preparation of the aniline compound **14i** bearing an 1*H*-indole ring was achieved by reduction of the nitro group of **11h** using reduced iron and calcium chloride in EtOH (73% yield).

The aniline compound **14j** bearing the 1-benzothiophene ring was synthesized as shown in Scheme 7. Aromatization of 6,7-dihydro-1-benzothiophen-4(5*H*)-one (**30**) was carried out with phenyltrimethylammonium tribromide in THF, followed by treatment with lithium carbonate and lithium bromide in DMF to provide 1-benzothiophene-4-ol **31** in 91% yield.[7] The

Scheme 6. Synthesis of Aniline Bearing a 1*H*-Indole Ring[a]



[a]Reagents: (a) Fe, $CaCl_2$, aq. EtOH, 100 °C.

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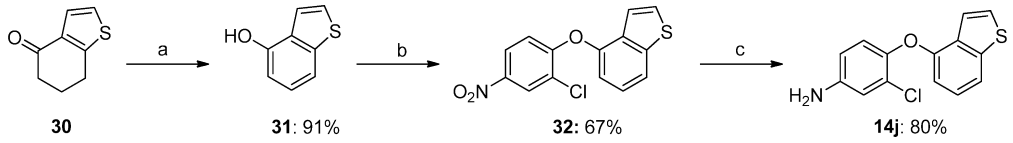
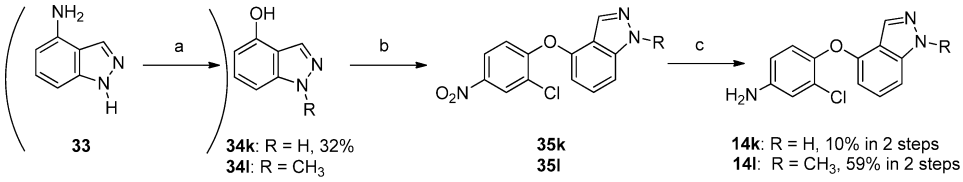
Scheme 7. Synthesis of Aniline Bearing a 1-Benzothiophene Ring[a]



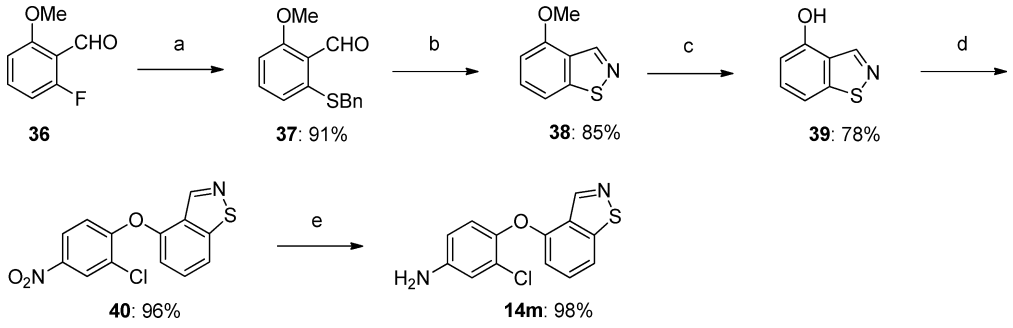
[a]Reagents: (a) (i) $PhMe_3NBr_3$, THF, rt, (ii) Li_2CO_3, LiBr, DMF, 150 °C; (b) **4**, K_2CO_3, DMF, rt; (c) Fe, $CaCl_2$, aq. EtOH, 100 °C.

Scheme 8. Synthesis of Aniline Bearing a 1*H*-Indazole Ring[a]



[a]Reagents: (a) $NaNO_2$, H_2SO_4, 100 °C; (b) **4**, K_2CO_3, DMF, rt; (c) H_2, Pt/C, AcOEt, rt.

Scheme 9. Synthesis of Aniline Bearing a 1,2-Benzisothiazole Ring[a]



[a]Reagents: (a) BnSH, *tert*-BuONa, DMF, rt; (b) H_2NOSO_3H, Thioanisole, CH_3CN, H_2O, rt; (c) PyHCl, 195 °C; (d) **4**, K_2CO_3, DMF, rt; (e) Fe, 1 N HCl, EtOH, 80 °C.

obtained phenol compound **31** was coupled with **4** in the presence of K_2CO_3 to provide **32** in 67% yield. Reduction of the nitro group of **32** with reduced iron powder gave the desired aniline compound **14j** in 80% yield.

The synthesis of the aniline compounds **14k** and **14l** bearing an 1*H*-indazole ring is shown in Scheme 8. Conversion of commercially available 1*H*-indazol-4-amine **33** into 1*H*-indazol-4-ol **34k** using sodium nitrite, followed by the reaction of **34k** with **4**, afforded a coupled nitrobenzene compound **35k**. The nitro group of **35k** was reduced with Pt/C under hydrogen atmosphere to provide the desired aniline compound **14k**. Another methylated aniline derivative **14l** was synthesized in 59% yield from commercially available 1-methyl-1*H*-indazol-4-ol (**34l**) in a manner similar to the synthesis of **14k**.

The aniline compound **14m** bearing an 1,2-benzisothiazole ring was synthesized as shown in Scheme 9. The coupling of commercially available 2-fluoro-6-methoxybenzaldehyde (**36**) with phenylmethanethiol was carried out in the presence of sodium *tert*-butoxide in DMF (91% yield), followed by cyclization[8] of the obtained compound **37** with hydroxylamine-*O*-sulfonic acid in the presence of thioanisole in aqueous acetonitrile to afford 1,2-benzisothiazole **38** in 85% yield. Subsequently, removal of the methyl group from **38** was achieved using pyridine hydrochloride with heating at 195 °C for 2 h in 78% yield. Attempts at demethylation of **38** and **39** using HBr resulted in decomposition to form complex mixtures. The obtained phenol derivative **39** was coupled with **4** in DMF to give nitrobenzene derivative **40** in 96% yield. Finally, the nitro group of **40** was reduced with reduced iron powder under acidic conditions to afford the desired aniline derivative **14m** in 98% yield.

The synthesis of the 4,5-substituted pyrrolo[3,2-*d*]-pyrimidines **42a−m** was conducted as shown in Scheme 10. The aniline derivatives **14a−m** were reacted with pyrrolo[3,2-*d*]pyrimidine **8** in 2-propanol at 80 °C, followed by hydrolysis of the benzoyl group to afford the desired compounds **42a−m** in 27−98% yield over two steps.

Preparation of the *N*-5 carboxamide derivatives **45m−51m** is summarized in Scheme 11. Condensation of **43**[3] with the aniline **14m** was carried out in 2-propanol, followed by the removal of the Boc protecting group with 4 N HCl in EtOAc to afford the key intermediate **44** in 80% yield over two steps. Amidation of **44** with a variety of carboxylic acids in the presence of EDC and HOBt provided the corresponding carboxamide derivatives (**45−48**, **50−51**)**m** in 41−80% yield. The carboxamide compound **46m** was isolated as a salt of methanesulfonic acid. In the case of **50m** and **51m**, the Boc groups of the intermediates were removed by treatment with 4 N HCl in EtOAc, and **50m** was isolated as an HCl salt in 41% yield. However, **51m** was isolated as the free form in 64% yield by neutralization. The synthesis of the carboxamide derivative **49m** was achieved using 2,2-dimethylpropanoic acid anhydride and the product isolated as a salt of methanesulfonic acid in 63% yield.

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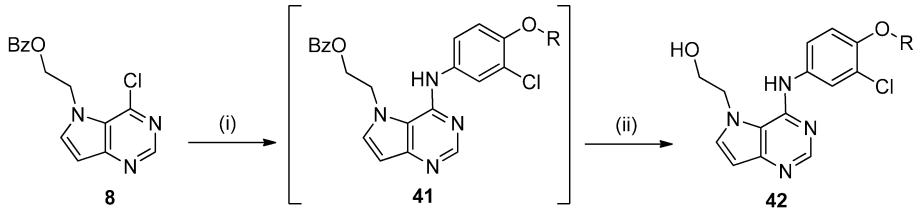
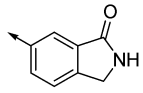
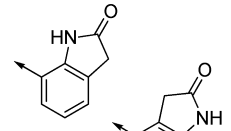
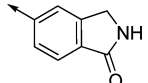
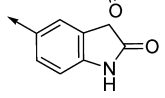
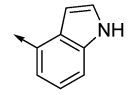
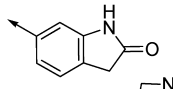
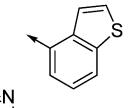
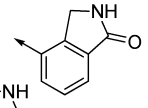
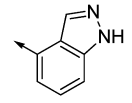
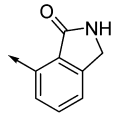
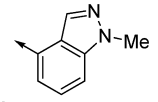
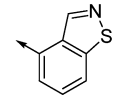
Scheme 10. Introduction of Various Anilino Groups to the Pyrrolo[3,2-*d*]pyrimidine Scaffold[a]



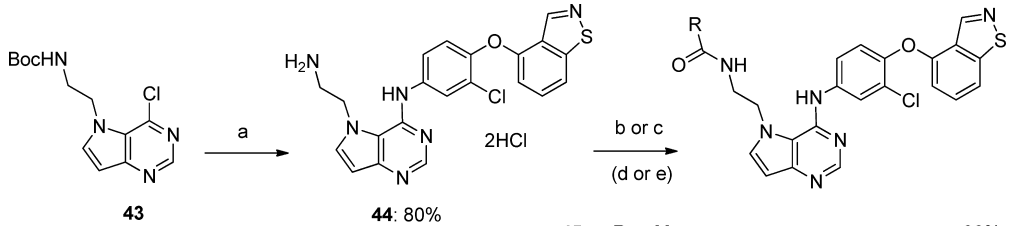
Reagents: (a) (i) aniline **14a-m**, 2-propanol, 80°C, (ii) 1N NaOH, MeOH, rt.

Compound	R	Yield[a]	Compound	R	Yield[a]
42a		71%	**42g**		50%
42b		54%	**42h**		27%
42c		45%	**42i**		86%
42d		52%	**42j**		62%
42e		98%	**42k**		39%
42f		36%	**42l**		57%
			42m		67%

[a] Yield was not optimized

[a]Reagents: (a) (i) aniline **14a–m**, 2-propanol, 80 °C, (ii) 1 N NaOH, MeOH, rt.

Scheme 11. Optimization of 5-Caboxamido Group[a]



45m: R = -Me			80%
46m: R = -C(Me)$_2$OH	MsOH salt		45%
47m: R = -C(Me)$_2$CH$_2$OH			52%
48m: R = -C(Me)$_2$ SO$_2$Me			75%
49m: R = -C(Me)$_3$	MsOH salt		63%
50m: R = 	HCl salt		41%
51m: R = -C(Me)$_2$NH$_2$			64%

[a]Reagents: (a) (i) **14m**, 2-propanol, 80 °C, (ii) 4 N HCl in EtOAc, EtOH, 80 °C; (b) RCO$_2$H or 1-(*tert*-butoxycarbonyl)proline or *N*-(*tert*-butoxycarbonyl)-2-methylalanine, EDC, HOBt, Et$_3$N, DMF, rt; (c) 2,2-dimethylpropanoic acid anhydride, Et$_3$N, DMF, rt; (d) 4N HCl in EtOAc, EtOH, 80 °C or 1 N HCl, EtOH, 80 °C; (e) MsOH, EtOAc or DMSO, rt.

RESULTS AND DISCUSSION

Our designed pyrrolo[3,2-*d*]pyrimidine derivatives **42a–h**, containing indolone and isoindolone rings in the 4-anilino moiety, were evaluated, and their activities are shown in Table 2. Among the compounds cyclized at the *C*-3 and *C*-4 positions (**42a–d**), the 6-substituted-2,3-dihydro-1*H*-isoindol-1-one

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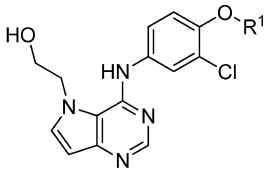
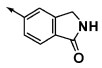
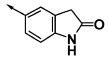
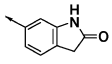
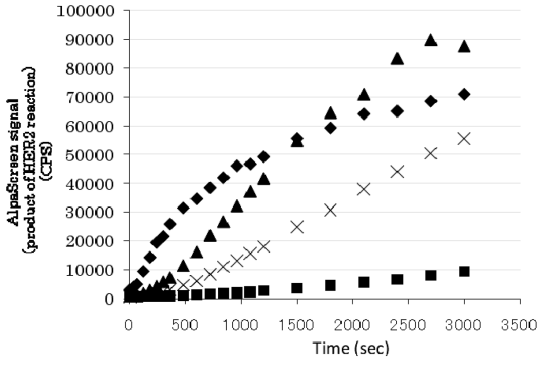
Table 2. Biological Profiles for Indolone and Isoindolone Derivatives

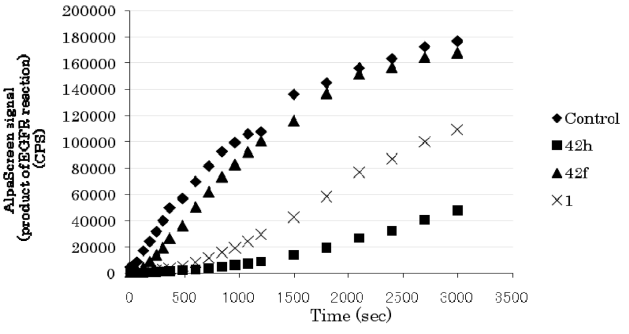


Comp.	R^1	Enzyme IC_{50} (nM)[b]		Cell growth GI_{50} (nM)[b]	HER2 Pseudo-irreversibility	EGFR Pseudo-irreversibility	PK $AUC_{0\text{-}8h}$[a] µg·h/ml
		HER2	EGFR	BT-474	** or *		Mouse
42a		1.3 (2.0-2.6)	7.8 (5.5-11)	43 (37-50)	**	*	0.783
42b		18 (15-21)	510 (320-800)	4083 (3468-5037)	*	*	0.967
42c		28 (24-33)	960 (560-1600)	1352 (1151-1603)	*	*	-
42d		130 (110-150)	2300 (2000-2600)	1875 (1373-2750)	*	*	-
42e		150 (110-190)	890 (770-1000)	3363 (2828-4163)	*	*	0.979
42f		2.5 (1.9-3.2)	21 (15-30)	768 (719-821)	*	*	1.152
42g		1.5 (1.4-1.7)	2.8 (2.4-3.4)	73 (57-91)	**	**	1.097
42h		1.6 (1.3-2.0)	3.0 (2.2-3.9)	13 (10-16)	**	**	0.448

[a]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. [b]95% confidence interval.



(A) Pyrrolo[3,2-*d*]pyrimidine compounds/HER2



(B) Pyrrolo[3,2-*d*]pyrimidine compounds/EGFR

**** > Compound 1 (TAK-285) > ***

Figure 4. Dissociation profiles of pyrrolo[3,2-*d*]pyrimidines from HER2 (A) and EGFR (B). Phosphorylation of peptide substrate as a function of time is shown. The reaction was initiated by diluting a preformed enzyme−inhibitor complex into reaction buffer. To determine the dissociation kinetics of the inhibitors from HER2 and EGFR, the recovery of enzyme activity from a preformed enzyme−inhibitor complex was evaluated using the Alphascreen system (PerkinElmer, USA). The PI strengths of compounds, compared with **1**, are indicated by 2 asterisks (**) when the compounds showed obviously strong PI profiles and by a single asterisk (*) when the PI was weak compared with that of **1**.

derivative **42a** showed the most potent HER2/EGFR inhibitory activities and growth inhibition (GI) activity in BT-474 cells. The other compounds, **42b−d**, showed reduced potencies against HER2/EGFR kinases as well as reduced GI activity. It was confirmed that suitable interaction of the carboxamide moiety in the bicyclic amide ring with the HER2/EGFR proteins is important for high-level inhibitory activity.

Among the compounds cyclized at the *C*-3 and *C*-2 positions (**42e−h**), compound **42e** exhibited reduced potency against the HER2/EGFR kinases, but **42f−h** showed potent HER2/EGFR kinase inhibitory activities similar to those of **42a**. Interestingly,

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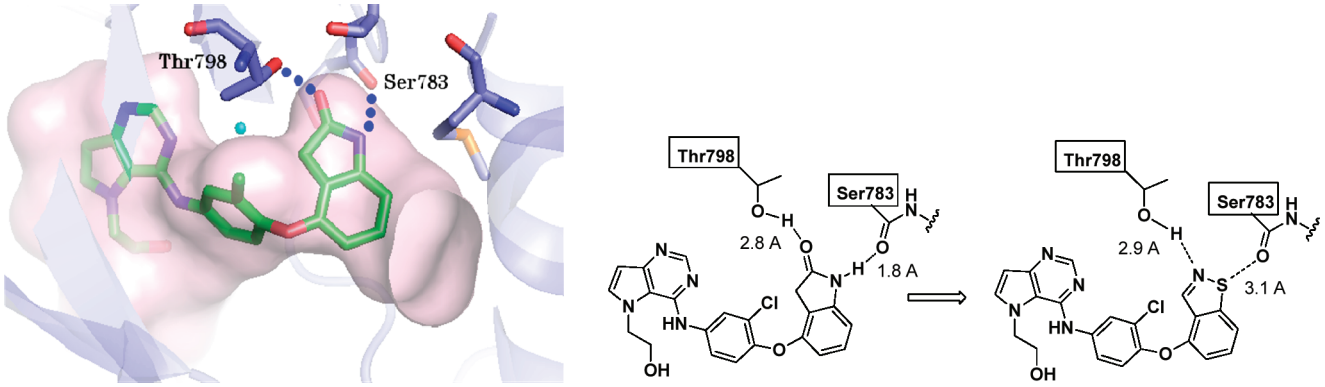
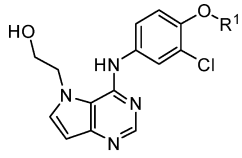
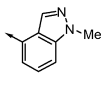


Figure 5. Docking model of indolone derivative **42h** with HER2.

among the compounds **42f–h**, only **42f** exhibited a reduction of GI activity in BT-474 cells. However, 4-substituted-1,3-dihydro-2*H*-indol-2-one (**42h**) showed the most potent GI activity against BT-474 cells. To understand the reason for the difference in the GI activities of **42f** and **42h**, we examined their PI profiles against HER2 and EGFR; the results are shown in Figure 4. In Figure 4, we present data regarding the dissociation rate of compounds **42f** and **42h** from HER2 and EGFR compared with a standard compound **1** in a 100-fold dilution assay.[6d,9] Enzyme and inhibitor were incubated with an inhibitor concentration of 10 times the IC_{50} value to allow formation of an enzyme–inhibitor complex. This complex was diluted to the inhibitor concentration of 0.1 times IC_{50} value by 100-fold dilution with the addition of reaction buffer containing high concentration ATP (1 mmol/L). The rate of the product formation in the kinase reaction with this 100-fold dilution assay reflects the PI profile based on the dissociation of the enzyme–inhibitor complex. Compound **42h** showed strong PI effects on both HER2 and EGFR kinases, which corresponds to a characteristic slow-off rate profile. However, **42f** had a weaker PI profile than that of the standard compound **1**, which corresponds to a tendency to dissociate easily from the HER2 and EGFR proteins. Similarly, we determined the PI profiles of **42a–g**, shown in Table 2. The PI strengths of compounds **42a–h**, compared with **1**, are indicated by 2 asterisks (**) when the compounds showed obviously strong PI profiles and by a single asterisk (*) when the PI was weak compared with that of **1**. Our results confirmed that the GI activities of the compounds **42a–h** were well correlated with their PI profiles.

Table 3. Biological Profiles for Fused Hetero Aromatic Derivatives



Comp.	R^1	Enzyme IC_{50} (nM)		Cell growth GI_{50} (nM)[b]	HER2 EGFR PI	Metabolic Stability μL /min/mg		PK $AUC_{0\text{-}8h}$[a] μg·h/ml
		HER2	EGFR	BT-474	** or *	Mouse	Human	Mouse
42h		1.6 (1.3-2.0)	3.0 (2.2-3.9)	13 (10-16)	** **	66	141	0.448
42i		2.5 (2.1-3.1)	3.1 (2.5-3.9)	36 (33-40)	** **		204	0.495
42j		6.0 (5.4-6.6)	8.8 (8.0-9.8)	15 (11-20)	** **	24	144	1.445
42k		1.7 (1.4-2.1)	1.9 (1.3-2.8)	5.5 (4.7-6.3)	** **	123	114	0.021
42l		2.1 (1.7-2.5)	36 (26-50)	164 (150-179)	* *	106	219	2.629
42m		2.1 (1.9-2.4)	4.1 (3.6-4.6)	3.6 (2.5-4.9)	** **	142	129	1.860

[a]See corresponding footnotes in Table 2. [b]95% confidence interval.

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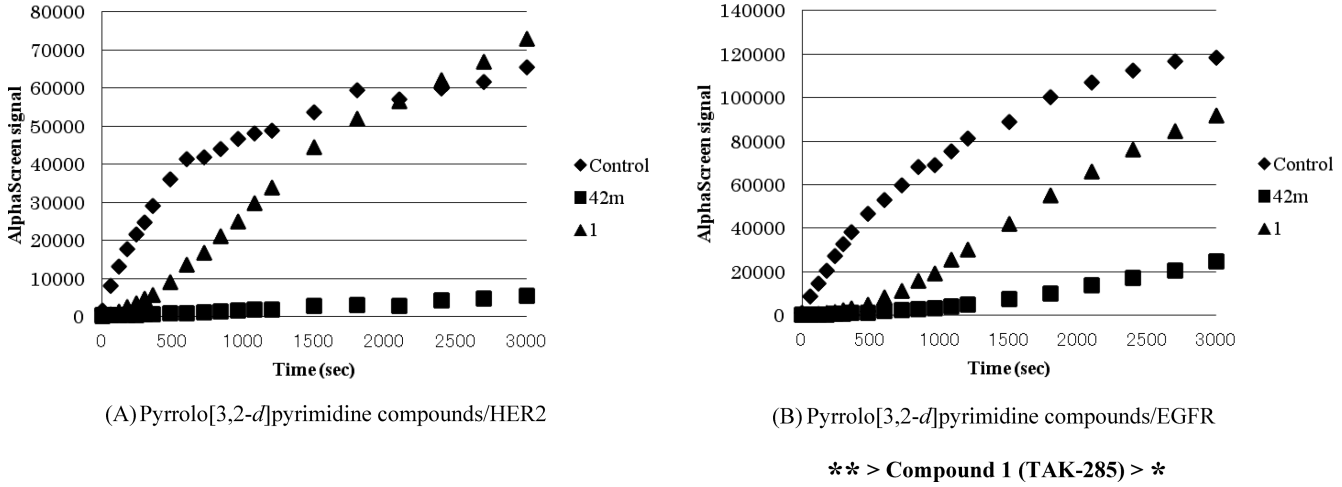


(A) Pyrrolo[3,2-*d*]pyrimidine compounds/HER2

(B) Pyrrolo[3,2-*d*]pyrimidine compounds/EGFR

**** > Compound 1 (TAK-285) > ***

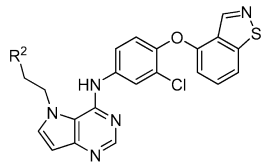
Figure 6. Dissociation profiles of pyrrolo[3,2-*d*]pyrimidines from HER2 (A) and EGFR (B).

On the basis of the strength of the PI profile for **42h**, we constructed a docking model of this compound with the HER2 protein using Gold (ver. 2.0, the Cambridge crystallographic data center, UK). In the modeling study, the aniline portion of **42h** is oriented deeply in the back pocket of the ATP binding site, and the amide group on the terminal 1,3-dihydro-2*H*-indol-2-one ring (**h**) makes two direct hydrogen bonding interactions with the hydroxy group of Thr798 and the carbonyl group of Ser783. The two direct interactions may be an important factor for the substantial PI activity of inhibitors. On the basis of our hypothesis, we designed other back pocket binders (**42i**–**m**), which could also potentially make direct hydrogen bonding interactions with Thr798 and/or Ser783 without the presence of an amide group (Figure 5).

Because the oral absorption properties of lead compound **42h** were insufficient in mice, replacements of the 1,3-dihydro-2*H*-indol-2-one ring (**42h**) with other bicyclic fused rings (**42i**–**m**) were examined to explore novel back pocket binders with the potential for both potent cellular GI activity and good pharmacokinetic PK properties.

As shown in Table 3, the 1*H*-indole (**42i**) and the 1-benzothiophene (**42j**) derivatives showed good HER2/EGFR and cellular growth inhibitory activities based on their strong PI profiles, similar to **42h**. It was found that removal of the amide carbonyl group of **42h** did not reduce GI activity nor did it affect the PI profiles. In addition, the 1-benzothiophene derivative **42j** enhanced oral absorption in mice. The 1*H*-indazole derivative **42k**, which was formed by the introduction of a nitrogen

Table 4. Biological Profiles for *N*-5 Modified Benzoisothiazole Derivatives



Comp.	R^2	Enzyme IC_{50} (nM) [b]		Cell growth GI_{50} (nM) [b]	Metabolic Stability μL /min/mg		PK $AUC_{0\text{-}8h}$ μg·h/ml [a]	CYP Inhib. % at 10μM
		HER2	EGFR	BT-474	Mouse	Human	Mouse	CYP 3A4
42m	-OH	2.1 (1.9-2.4)	4.1 (3.6-4.6)	3.6 (2.5-4.9)	142	129	1.860	47.2
45m	$-NHCOCH_3$	1.6 (1.5-1.7)	2.9 (2.5-3.4)	4.0 (3.0-5.0)	100	133	0.276	57.0
46m	$-NHCOC(CH_3)_2OH$	2.2 (2.0-2.4)	7.0 (5.4-8.9)	9.4 (7.5-12)	145	117	0.432	62.6
47m	$-NHCOC(CH_3)_2CH_2OH$	3.3 (3.1-3.5)	7.0 (5.9-8.4)	8.6 (7.2-10)	88	113	1.126	-
48m	$-NHCOC(CH_3)_2SO_2CH_3$	2.0 (1.8-2.3)	5.5 (4.2-7.3)	8.0 (6.7-9.6)	76	113	0.198	49.9
49m	$-NHCOC(CH_3)_3$	6.1 (5.3-7.0)	21 (16-29)	9.2 (5.3-13)	108	94	2.268	33.5
50m		0.92 (0.83-1.0)	3.0 (2.5-3.5)	5.8 (1.2-12)	74	61	0.690	35.0
51m	$-NHCOC(CH_3)_2NH_2$	0.98 (0.73-1.3)	2.6 (2.3-3.0)	2.0 (1.6-2.5)	77	88	0.903	21.2

[a]See corresponding footnotes in Table 2. [b]95% confidence interval.

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Table 5. Kinase Selectivity of 51m, IC_{50}(nM)

HER2	0.98	LCK	1,700	LYNB	3,000	LYNA	7,100
EGFR	2.6	c-met	1,800	TIE2	3,200	Src	8,400
HER4	79	MEK1	2,100	CSK	3,900	other 27 kinases[a]	> 10,000

[a]Other kinases: c-kit, AuroraB, FGFR1, FGFR3, ASK1, FAK, MEK5, MEKK, VEGFR1, VEGFR2, PDGFRa, PDGFRb, TTK, TAK1, PLK, PKCtheta, PKA, BMX, IGF1-R, InsR, ZAP70, B-rafw, ERK1, GSK3b, IKK, JNK, and P38a.

atom into the 1*H*-indole (**42i**) and could potentially interact with both Thr798 and Ser783, exhibited potent HER2/EGFR kinase inhibitory activities as well as cellular GI activity; however, **42k** exhibited a poor oral PK profile. Introduction of a methyl group at the *N*-1 position on the 1*H*-indazole ring of **42k**, yielding **42l**, provided improvement of the PK profile but also caused a drop in cellular GI activity based on a reduction of its PI profile. We confirmed the importance of the PI profile for cellular GI activity based on the interaction with Ser783. On the basis of these results, we next designed and synthesized a 1,2-benzisothiazole derivative **42m**, which possesses a hybrid structure of both 1-benzothiophene (**42j**) and 1*H*-indazole (**42k**). We envisioned that the good oral PK profile of **42j**, and the potent GI activity due to the PI profile of **42k**, could be combined in **42m**. As we expected, **42m** showed not only potent HER2/EGFR inhibition, PI behavior, and GI activities but also good oral absorption. These results suggested that the sulfur atom of the 1,2-benzisothiazole ring can interact with the Ser783 residue in the lipophilic back pocket as predicted by our design and that the PI profile correlates well with the GI activity (Figure 6). Since the target profile was achieved, we selected the 1,2-benzisothiazole ring (**42m**) as a suitable back pocket binder for further optimization.

Next, we carried out optimization of the *N*-5 substituents on the pyrrolo[3,2-*d*]pyrimidine core to improve *in vitro* oxidative metabolic stability (MS) in human hepatic microsomes, as well as the CYP3A4 inhibitory activity of 1,2-benzisothiazole derivative **42m**. On the basis of our previous research[3] on compound **1**, we believed that replacement of the hydroxy group with a carboxamide linkage at the *N*-5 position could provide favorable physicochemical properties without a loss of potency. The *N*-5 substituent was thought to be directed toward the solvent contact region based on the cocrystal structural analysis of **1**.[3,4] These results are shown in Table 4. All of the various synthesized *N*-5 carboxamide derivatives (**45m**–**51m**) showed potent HER2/EGFR kinase inhibitory and BT-474 cellular GI activities comparable to those of **42m**. However, compounds **45m**–**48m** did not exhibit improved MS. In addition, these compounds retained strong CYP3A4 inhibitory properties, despite the introduction of polar substituents such as hydroxy (**46m** and **47m**) and sulfonyl (**48m**) moieties into the side chains. However, when the end of the side chain was replaced by a *tert*-butylcarbonyl group (**49m**) or a prolinyl group (**50m**), their MS and CYP3A4 inhibitory profiles were changed significantly. We found that a bulky substituent in **49m** could hinder interaction with the heme protein CYP3A4 and that the basic group in **50m** provided suitable polarity to enhance MS. On the basis of these results, we designed and synthesized the 2-aminoisobutyl (AIB) amide derivative **51m**, which shared the structural features of both the bulky *tert*-butylcarbonyl (**49m**) and basic prolinyl (**50m**) groups. As we expected, compound **51m** achieved acceptable MS and CYP3A4 profiles without loss of potency. On the basis of its *in vitro* potency and good absorption, distribution, metabolism, and excretion (ADME) profiles, compound **51m** was selected as a candidate for further investigation.

The inhibitory activity of **51m** against various kinases is summarized in Table 5. Besides HER2 and EGFR, compound **51m** exhibited HER4 inhibitory activity with an IC_{50} value of 79 nM. As these results show, we confirmed that **51m** is a HER family selective inhibitor.

As shown in Figure 7, compound **51m** was evaluated *in vivo* using a 4–1ST HER2-overexpressing human gastric cancer tumor xenograft model in mice. Reflecting its potent HER2 inhibitory activity, compound **51m** exhibited potent efficacy with complete regression (CR, tumor/control ratio [T/C]: −23%) at an oral dose of 50 mg/kg without significant body weight loss in mice. Furthermore, in accordance with its potent EGFR inhibitory activity, compound **51m** also showed regressive efficacy (T/C: −30%) against a CAL-27 EGFR-overexpressing

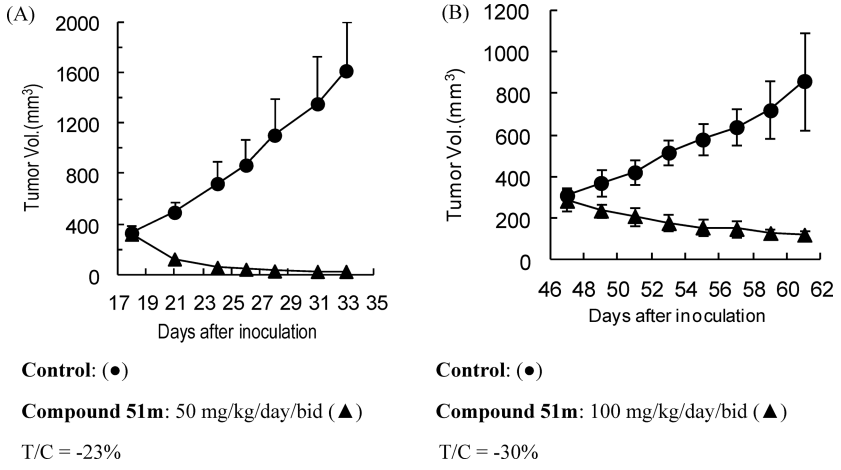

Figure 7. Efficacy studies of **51m** in the 4–1ST xenograft model (A) and the CAL27 xenograft model (B) in mice. (A) Dose level 50 mg/kg; $P \leq 0.0025$ vs control at day 14 (one-tailed Shirley–Williams test). (B) Dose level 100 mg/kg; $P \leq 0.0025$ vs control at day 14 (one-tailed Shirley–Williams test).

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human head and neck cancer xenograft model in mice at an oral dose of 100 mg/kg (Figure 7).

CONCLUSIONS

To develop novel HER2/EGFR kinase inhibitors, we explored pyrrolo[3,2-*d*]pyrimidine derivatives bearing novel back pocket binders. Among the prepared derivatives, the 1,2-benzisothiazole (**42m**) ring was selected as a suitable back pocket binder for further optimization because of the strong cellular GI activity that arose from its PI profile. Ultimately, we reached our preclinical candidate **51m** by optimization of the *N*-5 side chain to reduce CYP inhibition and improve its MS properties. Compound **51m** showed both potent HER2/EGFR inhibitory activities (IC_{50}, 0.98/2.5 nM), as well as breast cancer cell BT-474 GI (GI_{50}, 2.0 nM) activity. Reflecting these strong *in vitro* activities, compound **51m** exhibited potent tumor regressive efficacy against both HER2- and EGFR-overexpressing tumor (4−1ST and CAL27) xenograft models in mice at doses of 50 mg/kg and 100 mg/kg.

These preclinical studies demonstrated that **51m** has the potential for use as a HER2/EGFR dual kinase inhibitor to cure HER2- and EGFR-overexpressing cancer.

EXPERIMENTAL SECTION

General Chemistry Information. Melting points were determined on a Yanagimoto micro melting point apparatus or an 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), and 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. The purity of all biologically evaluated compounds was confirmed to be ≥95% by a Waters LC-MS system (ZMD-1). The column used was an L-column 2 ODS (3.0 × 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 and 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). Yields were not optimized.

Methyl 3-(2-Chloro-4-nitrophenoxy)benzoate (6). A mixture of 2-chloro-1-fluoro-4-nitrobenzene (**4**, 5.27 g, 30.0 mmol), methyl 3-hydroxybenzoate (**5**, 4.79 g, 31.5 mmol), and potassium carbonate (6.22 g, 45.0 mmol) in *N*,*N*-dimethylformamide (DMF) (30 mL) was stirred at room temperature for 4 h. The reaction mixture was concentrated *in vacuo*, and then, to the residue was added water (100 mL). The mixture was extracted with ethyl acetate (EtOAc, 500 mL). The separated organic layer was washed with brine (100 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent, EtOAc/hexane = 1:9 to 1:4) to give **6** (9.18 g, 95%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 3.93 (3H, s), 6.92 (1H, d, *J* = 9.1 Hz), 7.28−7.34 (1H, m), 7.53 (1H, t, *J* = 8.0 Hz), 7.70−7.75 (1H, m), 7.92−7.98 (1H, m), 8.08 (1H, dd, *J* = 9.1, 2.7 Hz), 8.41 (1H, d, *J* = 2.5 Hz).

Methyl 3-(4-Amino-2-chlorophenoxy)benzoate (7). To a solution of **6** (3.08 g, 10.0 mmol) in EtOAc (30 mL) was added 5% platinum−carbon (Pt/C) (90 mg), and the reaction mixture was stirred at room temperature under hydrogen atmosphere for 4 h. The insoluble 5% Pt/C was filtered off, and the filtrate was concentrated *in vacuo*. The residual solid was purified by silica gel column chromatography (eluent, EtOAc/hexane = 1:4 to 1:1) to give **7** (2.79 g, quant.) as gray oil. ^{1}H NMR ($CDCl_3$) δ 3.70 (2H, br s), 3.88 (3H, s), 6.58 (1H, dd, *J* = 8.5, 2.7 Hz), 6.79 (1H, d, *J* = 2.7 Hz), 6.91 (1H, d, *J* = 8.8 Hz), 7.09 (1H, ddd, *J* = 8.2, 2.7, 1.1 Hz), 7.31−7.39 (1H, m), 7.48−7.51 (1H, m), 7.70 (1H, dt, *J* = 7.7, 1.2 Hz).

3-(2-Chloro-4-{[5-(2-hydroxyethyl)-5*H*-pyrrolo[3,2-*d*]-pyrimidin-4-yl]amino}phenoxy)benzoic Acid (9). A solution of 2-(4-chloro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl benzoate[3] (**8**, 1.51 g, 5.00 mmol) and **7** (1.39 g, 5.01 mmol) in 2-propanol (20 mL) was stirred at 80 °C for 12 h. After cooling at room temperature, the reaction mixture was quenched with saturated sodium hydrogen carbonate (200 mL) and extracted with EtOAc (400 mL). The separated organic layer was washed with saturated brine (100 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was dissolved in a mixture of MeOH (30 mL) and tetrahydrofuran (THF) (6.0 mL). To the solution was added 1 N NaOH (13.5 mL), and the mixture was stirred at room temperature for 72 h. The mixture was concentrated *in vacuo*, and then, to the residue were added water (100 mL) and 1 N HCl (13.5 mL). The mixture was extracted with a mixed solvent of EtOAc (200 mL) and THF (100 mL). The separated organic layer was washed with water (100 mL) and dried over $MgSO_4$. Insoluble $MgSO_4$ was filtered off, and the filtrate was concentrated *in vacuo*. The residue was recrystallized from acetonitrile/diethyl ether (Et_2O) (1/1, 50 mL) to give **9** (1.88 g, 88%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.88 (2H, m), 4.50−4.60 (2H, m), 6.31 (1H, br s), 6.52 (1H, d, *J* = 3.0 Hz), 7.23−7.34 (3H, m), 7.51 (1H, t, *J* = 8.0 Hz), 7.59−7.71 (3H, m), 7.99 (1H, d, *J* = 2.7 Hz), 8.35 (1H, s), 9.90 (1H, br s).

***N*-*tert*-Butyl-3-(2-chloro-4-{[5-(2-hydroxyethyl)-5*H*-pyrrolo-[3,2-*d*]pyrimidin-4-yl]amino}phenoxy)benzamide (3).** A mixture of **9** (850 mg, 2.00 mmol), 2-methylpropan-2-amine (0.420 mL, 4.00 mmol), 1-hydroxybenzotriazole monohydrate (HOBt) (368 mg, 2.40 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (466 mg, 3.00 mmol) in DMF (10 mL) was stirred at room temperature for 12 h. The mixture was concentrated *in vacuo*, and then, to the residue was added water (100 mL). The mixture was extracted with EtOAc (200 mL). The organic layer was washed successively with brine (40 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure, and the residue was subjected to silica gel column chromatography (eluent, MeOH/EtOAc = 0:100 to 20:80). The objective fractions were collected and concentrated under reduced pressure, and the residue was subjected to basic silica gel column chromatography (eluent, MeOH/EtOAc = 0:100 to 20:80). The objective fractions were collected and concentrated under reduced pressure. The obtained residue was crystallized from EtOAc/diisopropyl ether (1/1, 25 mL) to give **3** (630 mg, 66%) as colorless crystals. mp 175−177 °C. ^{1}H NMR (DMSO-d_6) δ 1.45 (9H, s), 1.66 (1H, br s), 4.08−4.16 (2H, m), 4.35−4.42 (2H, m), 5.99 (1H, br s), 6.16 (1H, d, *J* = 3.3 Hz), 6.98−7.03 (2H, m), 7.04−7.12 (1H, m), 7.30−7.37 (3H, m), 7.41 (1H, dd, *J* = 2.6, 8.8 Hz), 7.80 (1H, d, *J* = 2.6 Hz), 8.23 (1H, s), 9.68 (1H, br s). Anal. Calcd for $C_{25}H_{26}ClN_5O_3$: C, 62.56; H, 5.46; N, 14.59. Found: C, 62.47; H, 5.47; N, 14.57.

4-(2-Chloro-4-nitrophenoxy)-1*H*-indole (11h). A mixture of **4** (12.1 g, 68.7 mmol), 4-hydroxyindole (**10h**, 10.0 g, 75.3 mmol), and potassium carbonate (15.9 g, 115 mmol) in DMF (140 mL) was stirred at room temperature for 16 h. The mixture was quenched with aqueous ammonium chloride (NH_4Cl) solution (200 mL), and the mixture was extracted with EtOAc (500 mL). The separated organic layer was washed with water (500 mL) and brine (100 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent, EtOAc/hexane = 1:19 to 1:1) to give **11h** (12.7 g, 64%) as yellow powder. ^{1}H NMR ($CDCl_3$) δ 6.29−6.35 (1H, m), 6.79 (1H, d, *J* = 9.2 Hz), 6.87 (1H, d, *J* = 7.5 Hz), 7.17−7.25 (2H, m), 7.35 (1H, d, *J* = 8.4 Hz), 7.97 (1H, dd, *J* = 2.7, 9.2 Hz), 8.34 (1H, br s), 8.41 (1H, d, *J* = 2.7 Hz).

The following compounds (**11c**, **11d**, and **11g**) were prepared from **4** and the corresponding phenols (**10c**, **10d**, and **10g**) by a method similar to that described for **11h**.

5-(2-Chloro-4-nitrophenoxy)-1*H*-indole (11c). Yield 87%, yellow solid. ^{1}H NMR ($CDCl_3$) δ 6.53−6.61 (1H, m), 6.79 (1H, d, *J* = 9.1 Hz), 6.96 (1H, dd, *J* = 2.3, 8.7 Hz), 7.32 (1H, t, *J* = 2.8 Hz),

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7.37 (1H, d, J = 2.3 Hz), 7.45 (1H, d, J = 8.7 Hz), 7.98 (1H, dd, J = 9.1, 2.7 Hz), 8.30 (1H, br s), 8.38 (1H, d, J = 2.7 Hz).

6-(2-Chloro-4-nitrophenoxy)-1*H*-indole (11d). Yield 54%, yellow solid. ^{1}H NMR ($CDCl_3$) δ 6.57−6.62 (1H, m), 6.82 (1H, d, J = 9.1 Hz), 6.68 (1H, dd, J = 2.1, 8.7 Hz), 7.13−7.17 (1H, m), 7.27 (1H, dd, J = 2.1 Hz, 3.2 Hz), 7.67 (1H, d, J = 8.7 Hz), 7.98 (1H, dd, J = 2.8, 9.1 Hz), 8.25 (1H, br s), 8.37 (1H, d, J = 2.8 Hz).

7-(2-Chloro-4-nitrophenoxy)-1*H*-indole (11g). Yield 72%, yellow solid. ^{1}H NMR ($CDCl_3$) δ 6.62−6.66 (1H, m), 6.88 (1H, d, J = 8.0 Hz), 6.90 (1H, d, J = 9.1 Hz), 7.12 (1H, t, J = 8.0 Hz), 7.21−7.25 (1H, m), 7.56 (1H, d, J = 8.0 Hz), 8.00 (1H, dd, J = 9.1, 2.8 Hz), 8.25 (1H, br s), 8.41 (1H, d, J = 2.8 Hz).

4-(2-Chloro-4-nitrophenoxy)-1,3-dihydro-2*H*-indol-2-one (13h). To a solution of **11h** (2.51 g, 8.69 mmol) in *tert*-butanol (50 mL) was added *N*-bromosuccinimide (NBS) (1.69 g, 9.49 mmol), and the reaction mixture was stirred at room temperature for 2 h. The mixture was quenched with saturated sodium hydrogen carbonate solution (50 mL), and the mixture was extracted with EtOAc (250 mL). The separated organic layer was washed with water (50 mL) and brine (10 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent, EtOAc/hexane = 1:19 to 1:1) to give **12h** (1.63 g) as a crude product. To a solution of **12h** (1.63 g) in THF (25 mL) was added 1 N HCl solution (8 mL), and the reaction mixture was stirred at 70 °C for 70 h. The mixture was diluted with water (100 mL) and extracted with a mixed solvent of EtOAc (75 mL) and THF (75 mL). The organic layer was washed with saturated sodium hydrogen carbonate solution (50 mL) and brine (15 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residual solid was collected by filtration to give **13h** (1.08 g, 80% from **11h**) as pale gray powder. ^{1}H NMR ($CDCl_3$) δ 3.42 (2H, s), 6.65 (1H, d, J = 8.0 Hz), 6.79 (1H, d, J = 8.0 Hz), 6.96 (1H, d, J = 9.0 Hz), 7.28 (1H, t, J = 8.0 Hz), 8.00 (1H, br s), 8.09 (1H, dd, J = 9.0, 2.7 Hz), 8.41 (1H, d, J = 2.7 Hz).

The following compounds (**13c**, **13d**, and **13g**) were prepared from the corresponding 1*H*-indoles (**11c**, **11d** and **11g**) by a method similar to that described for **13h**.

5-(2-Chloro-4-nitrophenoxy)-1,3-dihydro-2*H*-indol-2-one (13c). Yield 61%, yellow solid. ^{1}H NMR ($CDCl_3$) δ 3.53 (2H, s), 6.90 (1H, d, J = 8.5 Hz), 6.94 (1H, d, J = 9.1 Hz), 7.04 (1H, dd, J = 8.5, 2.3 Hz), 7.13 (1H, d, J = 2.3 Hz), 8.16 (1H, dd, J = 9.1, 2.7 Hz), 8.45 (1H, d, J = 2.7 Hz), 10.50 (1H, s).

6-(2-Chloro-4-nitrophenoxy)-1,3-dihydro-2*H*-indol-2-one (13d). Yield 45%, brown solid. ^{1}H NMR ($CDCl_3$) δ 3.56 (2H, s), 6.63 (1H, d, J = 2.2 Hz), 6.72 (1H, dd, J = 8.0, 2.2 Hz), 6.94 (1H, d, J = 9.0 Hz), 7.15−7.37 (1H, m), 7.78 (1H, br s), 8.07 (1H, dd, J = 9.0, 2.8 Hz), 8.39 (1H, d, J = 2.8 Hz).

7-(2-Chloro-4-nitrophenoxy)-1,3-dihydro-2*H*-indol-2-one (13g). Yield 45%, pale pink solid. ^{1}H NMR ($CDCl_3$) δ 3.63 (2H, s), 6.88−6.98 (2H, m), 7.06 (1H, t, J = 7.8 Hz), 7.12−7.20 (1H, m), 7.60 (1H, br s), 8.08 (1H, dd, J = 9.1, 2.8 Hz), 8.41 (1H, d, J = 2.8 Hz).

4-(4-Amino-2-chlorophenoxy)-1,3-dihydro-2*H*-indol-2-one (14h). To a solution of **13h** (1.07 g, 3.51 mmol) in a mixture of MeOH (30 mL)/THF (30 mL) was added 5% Pt/C (111 mg), and the reaction mixture was stirred at room temperature under hydrogen atmosphere for 2 h. The insoluble 5% Pt/C was filtered off, and the filtrate was concentrated *in vacuo*. The residual solid was collected by filtration to give **14h** (926 mg, 96%) as pale gray powder. ^{1}H NMR ($CDCl_3$) δ 3.46 (2H, s), 3.69 (2H, br s), 6.37 (1H, d, J = 8.5 Hz), 6.50−6.64 (2H, m), 6.78 (1H, d, J = 2.6 Hz), 6.91 (1H, d, J = 8.5 Hz), 7.10 (1H, t, J = 8.5 Hz), 7.53 (1H, br s).

The following compounds (**14c**, **14d**, and **14g**) were prepared from the corresponding 1,3-dihydro-2*H*-indol-2-ones (**13c**, **13d**, and **13g**) by a method similar to that described for **14h**.

5-(4-Amino-2-chlorophenoxy)-1,3-dihydro-2*H*-indol-2-one (14c). Yield 96%, white solid. ^{1}H NMR ($CDCl_3$) δ 3.50 (2H, s), 3.66 (2H, br s), 6.56 (1H, dd, J = 8.7, 2.8 Hz), 6.72−6.84 (4H, m), 6.85 (1H, d, J = 8.7 Hz), 7.72 (1H, br s).

6-(4-Amino-2-chlorophenoxy)-1,3-dihydro-2*H*-indol-2-one (14d). Yield 61%, yellow solid. ^{1}H NMR ($CDCl_3$) δ 3.47 (2H, s), 3.69 (2H, s), 6.41 (1H, d, J = 2.3 Hz), 6.52 (1H, dd, J = 8.0, 2.3 Hz), 6.58 (1H, dd, J = 8.7, 2.8 Hz), 6.78 (1H, d, J = 2.8 Hz), 6.91 (1H, d, J = 8.7 Hz), 7.09 (1H, d, J = 8.0 Hz), 7.68 (1H, br s).

7-(4-Amino-2-chlorophenoxy)-1,3-dihydro-2*H*-indol-2-one (14g). Yield 92%, brown solid. ^{1}H NMR ($CDCl_3$) δ 3.58 (2H, s), 3.69 (2H, br s), 6.49−6.64 (2H, m), 6.78 (1H, d, J = 2.8 Hz), 6.83−6.97 (3H, m), 7.61 (1H, br s).

Methyl 3-(2-chloro-4-nitrophenoxy)-2-methylbenzoate (16e). A mixture of **4** (4.4 g, 25.0 mmol), methyl 3-hydroxy-2-methylbenzoate (**15e**, 4.2 g, 27.1 mmol), and potassium carbonate (6.9 g, 50 mmol) in DMF (50 mL) was stirred at room temperature for 16 h. The mixture was quenched with water (200 mL), and the mixture was extracted with EtOAc (400 mL). The organic layer was washed with water (400 mL) and brine (10 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent, EtOAc/hexane = 1:1) to give **16e** (7.7 g, 96%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 2.40 (3H, s), 3.93 (3H, s), 6.63 (1H, d, J = 9.1 Hz), 7.16 (1H, dd, J = 8.0, 1.4 Hz), 7.33 (1H, t, J = 7.8 Hz), 7.82 (1H, dd, J = 8.0, 1.4 Hz), 8.01 (1H, dd, J = 9.1, 2.7 Hz), 8.39 (1H, d, J = 2.7 Hz).

The following compound **16a** was prepared from **15a** by methods similar to that described for **16e**.

Methyl 5-(2-chloro-4-nitrophenoxy)-2-methylbenzoate (16a). Yield 92%, yellow powder. ^{1}H NMR ($CDCl_3$) δ 2.63 (3H, s), 3.89 (3H, s), 6.86 (1H, d, J = 9.1 Hz), 7.15 (1H, dd, J = 8.3, 2.7 Hz), 7.33 (1H, d, J = 8.3 Hz), 7.64 (1H, d, J = 2.7 Hz), 8.06 (1H, dd, J = 9.1, 2.7 Hz), 8.39 (1H, d, J = 2.7 Hz).

Methyl 2-(bromomethyl)-3-(2-chloro-4-nitrophenoxy)-benzoate (17e). A mixture of **16e** (4.8 g, 14.9 mmol), NBS (3.2 g, 10.0 mmol), and 2,2′-azobis(isobutylonitrile) (AIBN) (123 mg, 0.75 mmol) in trifluoromethylbenzene (50 mL) was stirred at 100 °C for 16 h. The mixture was allowed to cool at room temperature, and then insoluble materials were removed by filtration. The filtrate was washed with 1 N NaOH (50 mL) and brine (10 mL) successively, and the separated organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent, EtOAc/hexane = 15:85 to 25:75) to give **17e** (4.2 g, 70%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 3.98 (3H, s), 5.05 (2H, s), 6.92 (1H, d, J = 9.1 Hz), 7.08 (1H, dd, J = 8.1, 1.2 Hz), 7.42 (1H, t, J = 8.0 Hz), 7.86 (1H, dd, J = 7.8, 1.2, Hz), 8.08 (1H, dd, J = 9.1, 2.6 Hz), 8.41 (1H, d, J = 2.6 Hz).

The following compound **17a** was prepared from **16a** by methods similar to that described for **17e**.

Methyl 2-(bromomethyl)-5-(2-chloro-4-nitrophenoxy)-benzoate (17a). Yield 37%, yellow powder. ^{1}H NMR ($CDCl_3$) δ 3.94 (3H, s), 4.97 (2H, s), 6.98 (1H, d, J = 9.0 Hz), 7.21 (1H, dd, J = 8.3, 2.7 Hz), 7.55 (1H, d, J = 8.3 Hz), 7.65 (1H, d, J = 2.7 Hz), 8.11 (1H, dd, J = 9.0, 2.8 Hz), 8.41 (1H, d, J = 2.8 Hz).

4-(2-Chloro-4-nitrophenoxy)-2,3-dihydro-1*H*-isoindol-1-one (18e). A mixture of **17e** (601 mg, 1.50 mmol) and 28% aqueous ammonia solution (3 mL) in a mixture of THF (27 mL) and MeOH (1 mL) was stirred at room temperature for 16 h. The reaction mixture was concentrated *in vacuo*, and the residual solid was collected and washed with water (10 mL), EtOAc (10 mL), and Et_2O (10 mL) successively to give **18e** (500 mg, 96%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 4.28 (2H, s), 7.16 (1H, d, J = 9.1 Hz), 7.32 (1H, dd, J = 7.1, 1.4 Hz), 7.54−7.65 (2H, m), 8.17 (1H, dd, J = 9.1, 2.7 Hz), 8.49 (1H, d, J = 2.7 Hz), 8.74 (1H, br s).

The following compound **18a** was prepared from **17a** by methods similar to that described for **18e**.

6-(2-Chloro-4-nitrophenoxy)-2,3-dihydro-1*H*-isoindol-1-one (18a). Yield 96%, white powder. ^{1}H NMR ($CDCl_3$) δ 4.50 (2H, s), 6.90 (1H, br s), 6.97 (1H, d, J = 9.1 Hz), 7.34 (1H, dd, J = 8.3, 2.3 Hz), 7.48 (1H, d, J = 2.3 Hz), 7.56 (1H, d, J = 8.3 Hz), 8.09 (1H, dd, J = 9.1, 2.7 Hz), 8.41 (1H, d, J = 2.7 Hz).

4-(4-Amino-2-chlorophenoxy)-2,3-dihydro-1*H*-isoindol-1-one (14e). To a solution of **18e** (948 mg, 3.21 mmol) in a mixture of MeOH (20 mL)/THF (20 mL)/EtOAc (20 mL) was added 5% Pt/C (25 mg), and the mixture was stirred at room temperature under hydrogen atmosphere for 16 h. The insoluble 5% Pt/C was filtered off, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent, MeOH/EtOAc = 0:100 to

20:80) to give **14e** (390 mg, 50%) as a pale gray powder. ^{1}H NMR ($CDCl_3$) δ 3.73 (2H, br s), 4.45 (2H, s), 6.58 (1H, dd, J = 8.7, 2.7 Hz), 6.75−6.84 (3H, m), 6.94 (1H, d, J = 8.7 Hz), 7.35 (1H, t, J = 7.8 Hz), 7.54 (1H, d, J = 7.7 Hz).

The following compound **14a** was prepared from **18a** by methods similar to that described for **14e**.

6-(4-Amino-2-chlorophenoxy)-2,3-dihydro-1*H*-isoindol-1-one (14a). Yield 70%, yellow powder. ^{1}H NMR ($CDCl_3$) δ 3.70 (2H, br s), 4.40 (2H, s), 6.30 (1H, br s), 6.58 (1H, dd, J = 8.5, 2.8 Hz), 6.78 (1H, d, J = 2.8 Hz), 6.93 (1H, d, J = 8.5 Hz), 7.15−7.30 (2H, m), 7.40 (1H, d, J = 8.3 Hz).

5-Methoxy-2,3-dihydro-1*H*-isoindol-1-one (21). Compound **21** was obtained as a white solid in 39% yield from **19** by a method similar to that described for **18e**. ^{1}H NMR ($CDCl_3$) δ 3.88 (3H, s), 4.41 (2H, s), 6.39 (1H, br s), 6.90−7.05 (2H, m), 7.77 (1H, d, J = 8.4 Hz).

5-Hydroxy-2,3-dihydro-1*H*-isoindol-1-one (22). A mixture of **21** (950 mg, 5.82 mmol) and 48% aqueous hydrogen bromide solution (15 mL) was stirred at 100 °C for 48 h. The mixture was allowed to cool at room temperature. To the mixture was added brine (50 mL), and the mixture was extracted with EtOAc (150 mL). The organic layer was washed with water (50 mL) and brine (10 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residual solid was washed with EtOAc (10 mL) to give **22** (385 mg, 44%) as a pale yellow solid. ^{1}H NMR (95%$CDCl_3$ + 5%DMSO-d_6) δ 4.32 (2H, s), 6.85−6.95 (2H, m), 7.30−7.45 (1H, br s), 7.62 (1H, d, J = 8.7 Hz).

5-(2-Chloro-4-nitrophenoxy)-2,3-dihydro-1*H*-isoindol-1-one (23). Compound **23** was obtained as a white solid in 74% yield from **22** by a method similar to that described for **16e**. ^{1}H NMR ($CDCl_3$) δ 4.46 (2H, s), 6.47 (1H, br s), 7.03 (1H, d, J = 9.0 Hz), 7.10−7.20 (2H, m), 7.91 (1H, d, J = 8.4 Hz), 8.11 (1H, dd, J = 9.0, 2.7 Hz), 8.41 (1H, d, J = 2.7 Hz).

5-(4-Amino-2-chlorophenoxy)-2,3-dihydro-1*H*-isoindol-1-one (14b). Compound **14b** was obtained as a white solid in quantitative yield from **23** by a method similar to that described for **14e**. ^{1}H NMR ($CDCl_3$) δ 3.73 (2H, br s), 4.37 (2H, s), 6.16 (1H, br s), 6.60 (1H, dd, J = 8.4, 2.7 Hz), 6.80 (1H, d, J = 2.7 Hz), 6.85−6.90 (1H, m), 6.94 (1H, d, J = 8.4 Hz), 6.95−7.00 (1H, m), 7.77 (1H, d, J = 8.4 Hz).

4-Bromo-7-methoxy-2,3-dihydro-1*H*-isoindol-1-one (26). Compound **26** was obtained as a white solid in 40% yield from **24** by a method similar to that described for **18e**. ^{1}H NMR ($CDCl_3$) δ 3.97 (3H, s), 4.29 (2H, s), 6.47 (1H, br s), 6.84 (1H, d, J = 8.7 Hz), 7.59 (1H, d, J = 8.7 Hz).

4-Bromo-7-hydroxy-2,3-dihydro-1*H*-isoindol-1-one (27). Compound **27** was obtained as a pale yellow solid in 71% yield from **26** by a method similar to that described for **22**. ^{1}H NMR (95% $CDCl_3$+5%DMSO-d_6) δ 4.28 (2H, s), 6.77 (1H, d, J = 8.7 Hz), 7.46 (1H, d, J = 8.7 Hz), 8.13 (1H, br s).

7-Hydroxy-2,3-dihydro-1*H*-isoindol-1-one (28). To a solution of **27** (228 mg, 1.53 mmol) in MeOH (10 mL) was added 5% Pt/C (38 mg), and the reaction mixture was stirred at room temperature under hydrogen atmosphere for 2 h. The insoluble 5% Pt/C was filtered off, and the filtrate was concentrated *in vacuo*. The residual solid was collected by filtration to give **28** (149 mg, quant.) as a pale yellow powder. ^{1}H NMR ($CDCl_3$+5%DMSO-d_6) δ 4.37 (2H, s), 6.79 (1H, d, J = 8.4 Hz), 6.94 (1H, d, J = 7.5 Hz), 7.30−7.45 (1H, m), 8.20 (1H, br s).

7-(2-Chloro-4-nitrophenoxy)-2,3-dihydro-1*H*-isoindol-1-one (29). Compound **29** was obtained as a pale gray solid in 58% yield from **28** by a method similar to that described for **16e**. ^{1}H NMR ($CDCl_3$ + 5%DMSO-d_6) δ 4.43 (2H, s), 6.82 (1H, d, J = 9.3 Hz), 7.06 (1H, d, J = 7.8 Hz), 7.41 (1H, d, J = 7.8 Hz), 7.55−7.70 (1H, m), 7.95−8.10 (2H, m), 8.34 (1H, d, J = 2.7 Hz).

7-(4-Amino-2-chlorophenoxy)-2,3-dihydro-1*H*-isoindol-1-one (14f). Compound **14f** was obtained as a white solid in quantitative yield from **29** by a method similar to that described for **14e**. ^{1}H NMR ($CDCl_3$) δ 3.70 (2H, br s), 4.43 (2H, s), 6.37 (1H, br s), 6.52 (1H, d, J = 8.7 Hz), 6.55−6.60 (1H, m), 6.78 (1H, d, J = 2.4 Hz), 7.01 (1H, d, J = 8.1 Hz), 7.06 (1H, d, J = 7.5 Hz), 7.36 (1H, t, J = 8.1 Hz).

3-Chloro-4-(1*H*-indol-4-yloxy)aniline (14i). To a solution of **11h** (1.00 g, 3.46 mmol) in EtOH (17 mL) were added calcium chloride (1.50 g, 1.08 mmol), reduced iron (750 mg, 13.4 mmol), and water (3 mL), and the mixture was refluxed at 100 °C for 8 h. The mixture was allowed to cool at room temperature. The mixture was filtered through a Celite pad, and the filtrate was concentrated *in vacuo*. The residue was partitioned between water (50 mL) and EtOAc (100 mL). The organic layer was washed with brine (10 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 **14i** (620 mg, 73%) as a purple oil. ^{1}H NMR ($CDCl_3$) δ 3.63 (2H, br s), 6.40 (1H, dd, J = 7.4, 1.0 Hz), 6.50−6.64 (2H, m), 6.82 (1H, d, J = 2.8 Hz), 6.92 (1H, d, J = 8.5 Hz), 7.00−7.19 (3H, m), 8.20 (1H, br s).

1-Benzothiophene-4-ol (31). To a solution of 6,7-dihydro-4(5*H*)-benzothiophene (**30**, 10.0 g, 65.7 mmol) in THF (160 mL) was added phenyltrimethylammonium tribromide (27.1 g, 72.3 mmol) at 0 °C, and the mixture was stirred at 0 °C for 1.5 h and at room temperature for 2.5 h. The mixture was quenched with water (300 mL), and the mixture was extracted with EtOAc (500 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was dissolved in DMF (400 mL), and to the solution were added lithium carbonate (21.7 g, 293 mmol) and lithium bromide (25.7 g, 295 mmol). The mixture was stirred at 150 °C for 4 h, and quenched with water (400 mL). The mixture was extracted with EtOAc (800 mL). The organic layer was washed with water (800 mL) and brine (80 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent, EtOAc/hexane = 0:1 to 1:9) to give **31** (8.98 g, 91%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 5.16 (1H, s), 6.72 (1H, d, J = 7.6 Hz), 7.20 (1H, t, J = 8.0 Hz), 7.33−7.40 (1H, m), 7.42−7.54 (2H, m).

4-(2-Chloro-4-nitrophenoxy)-1-benzothiophene (32). A mixture of **31** (5.00 g, 33.3 mmol), **4** (5.80 g, 33.0 mmol), and potassium carbonate (5.10 mmol, 36.9 mmol) in DMF (50 mL) was stirred at room temperature for 48 h. The mixture was quenched with water (150 mL) and extracted with EtOAc (300 mL). The organic layer was washed with water (300 mL) and brine (30 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent, EtOAc/hexane = 1:19 to 1:4) to give **32** (7.80 g, 66%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 6.94 (1H, d, J = 9.1 Hz), 7.18 (1H, d, J = 8.1 Hz), 7.25 (1H, dd, J = 5.7, 0.8 Hz), 7.48 (1H, t, J = 8.1 Hz), 7.85 (1H, d, J = 5.7 Hz), 8.01 (1H, d, J = 8.1 Hz), 8.14 (1H, dd, J = 9.1, 2.7 Hz), 8.52 (1H, d, J = 2.7 Hz).

4-(1-Benzothiophen-4-yloxy)-3-chloroaniline (14j). Compound **14j** was obtained as a pale orange solid in 80% yield from **32** by a method similar to that described for **14i**. ^{1}H NMR (DMSO-d_6) δ 3.67 (2H, br s), 6.49−6.61 (2H, m), 6.81 (1H, d, J = 2.8 Hz), 6.91 (1H, d, J = 8.5 Hz), 7.14−7.22 (1H, m), 7.39 (1H, dd, J = 5.5, 0.6 Hz), 7.51−7.56 (1H, m), 7.58 (1H, dd, J = 5.5, 0.8 Hz).

1*H*-Indazol-4-ol (34k). 1*H*-Indazol-4-amine (**33**, 800 mg, 6.01 mmol) was dissolved in a mixture of concentrated sulfuric acid (25 mL) and water (20 mL). To the solution was added a solution of sodium nitrite (415 mg, 6.01 mmol) in water (5 mL) at 0 °C and refluxed at 100 °C for 15 h. The mixture was allowed to cool at 0 °C and neutralized with 1 N NaOH (470 mL). The mixture was partitioned between water (50 mL) and EtOAc (100 mL). The organic layer was washed with brine (10 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent, EtOAc/hexane = 1:2 to 1:0) to give **34k** (266 mg, 32%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 6.36−6.39 (1H, m), 6.92−7.12 (2H, m), 8.02 (1H, s), 9.81 (1H, s), 12.79 (1H, s).

3-Chloro-4-(1*H*-indazol-4-yloxy)aniline (14k). Compound **14k** was obtained as a yellow solid in 10% yield from **34k** by a method similar to that described for **6**. ^{1}H NMR ($CDCl_3$) δ 5.39 (2H, s), 6.19 (1H, dd, J = 6.3, 2.0 Hz), 6.60 (1H, dd, J = 8.7, 2.6 Hz), 6.77 (1H, d, J = 2.6 Hz), 7.01 (1H, d, J = 8.7 Hz), 7.13−7.28 (2H, m), 7.86 (1H, s), 13.16 (1H, br s).

3-Chloro-4-[(1-methyl-1*H*-indazol-4-yl)oxy]aniline (14l). Compound **14l** was obtained as a yellow solid in 59% yield from commercially available **34l** by a method similar to that described for **6**. ^{1}H NMR ($CDCl_3$) δ 3.64 (2H, br s), 3.96 (3H, s), 6.41 (1H, d, J = 6.8

Hz), 6.60 (1H, dd, J = 8.6, 2.0 Hz), 6.81 (1H, d, J = 2.0 Hz), 6.98 (1H, d, J = 8.5 Hz), 7.16 (1H, dd, J = 8.5, 6.8 Hz), 7.45−7.55 (1H, m), 7.99 (1H, s).

2-(Benzylsulfanyl)-6-methoxybenzaldehyde (37). To a suspension of sodium *tert*-butoxide (3.47 g, 36.1 mmol) in THF (50 mL) was added benzylmercaptan (4.24 mL, 32.2 mmol) at 0 °C, and the mixture was stirred at 0 °C for 30 min. To the reaction mixture was added dropwise a solution of 2-fluoro-6-methoxybenzaldehyde (**36**, 4.64 g, 30.1 mmol) in THF (10 mL), and the mixture was stirred at room temperature for 1 h. To the reaction mixture was added water (150 mL), and the resulting precipitate was collected by filtration. The collected solid was washed with water (20 mL) and Et_2O (50 mL) successively and dried *in vacuo* to give **37** (7.08 g, 91%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 3.91 (3H, s), 4.15 (2H, s), 6.72 (1H, d, J = 8.3 Hz), 6.95 (1H, J = 8.3 Hz), 7.25 - 7.43 (6H, m), 10.59 (1H, s).

4-Methoxy-1,2-benzisothiazole (38). Compound **37** (18.8 g, 72.8 mmol) and thioanisole (18.0 g, 145 mmol) were dissolved in a mixed solvent of acetonitrile (175 mL) and water (175 mL), and the solution was stirred at room temperature for 30 min. To the reaction mixture was added hydroxylamine-*O*-sulfonic acid (12.4 g, 109 mmol), and the mixture was stirred at room temperature for 2 h. To the reaction mixture was added saturated sodium hydrogen carbonate (400 mL), and the mixture was extracted with EtOAc (500 mL). The organic layer was washed with water (250 mL) and dried over $MgSO_4$. The solvent was evaporated *in vacuo*, and the residue was purified by silica gel column chromatography (eluent, EtOAc/hexane = 0:10 to 7:3) to give **38** (10.2 g, 85%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 4.00 (3H, s), 6.75 (1H, dd, J = 7.4, 0.9 Hz), 7.35 - 7.62 (2H, m), 9.02 (1H, d, J = 0.8 Hz).

1,2-Benzisothiazol-4-ol (39). A mixture of **38** (22.0 g, 133 mmol) and pyridine hydrochloride (140 g, 1.21 mol) was stirred at 195 °C for 2 h. After cooling at room temperature, water (1.0 L), 1 N HCl (12 mL), and EtOAc (1 L) were added to the mixture. After stirring at room temperature for 2 h, the separated organic layer was washed with water (500 mL) and brine (200 mL) successively, and the organic layer was dried over $MgSO_4$. Insoluble $MgSO_4$ was filtered off, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent, EtOAc/hexane = 0:10 to 10:0) to give **39** (15.7 g, 78%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 6.08 (1H, s), 6.74 (1H, dd, J = 8.0, 0.6 Hz), 7.38 (1H, t, J = 8.0 Hz), 7.51 (1H, dt, J = 8.0, 0.9 Hz), 9.06 (1H, d, J = 0.9 Hz).

4-(2-Chloro-4-nitrophenoxy)-1,2-benzisothiazole (40). To a solution of **4** (5.70 g, 32.5 mmol) and **39** (5.40 g, 35.7 mmol) in DMF (50 mL) was added potassium carbonate (9.40 g, 68.0 mmol), and the mixture was stirred at room temperature for 24 h. Water (100 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (150 mL). The organic layer was washed with water (250 mL) and brine (100 mL), and dried over $MgSO_4$. Insoluble $MgSO_4$ was filtered off, and the filtrate was concentrated under reduced pressure to give **40** (9.60 g, 96%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 6.89 (1H, dd, J = 8.0, 0.6 Hz), 7.04 (1H, d, J = 9.1 Hz), 7.53 (1H, t, J = 8.0 Hz), 7.83 (1H, dd, J = 8.0, 0.8 Hz), 8.11 (1H, dd, J = 9.1, 2.6 Hz), 8.46 (1H, d, J = 2.6 Hz), 8.95 (1H, d, J = 0.9 Hz).

4-(1,2-Benzisothiazol-4-yloxy)-3-chloroaniline (14m). Compound **40** (9.60 g, 31.3 mmol) and reduced iron (20.0 g, 358 mmol) were suspended in EtOH (150 mL), and the suspension was heated at 80 °C. To the mixture was added 1 N HCl (5 mL), and the mixture was refluxed at 80 °C for 5 h. The reaction mixture was allowed to cool at room temperature. To the mixture was added 1 N NaOH (15 mL), and the reaction mixture was filtered through a Celite pad. The filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc (400 mL), and the mixture was washed with water (200 mL), saturated sodium hydrogen carbonate (200 mL), 1 N NaOH (100 mL), and brine (100 mL) successively. The separated organic layer was dried over $MgSO_4$. The insoluble $MgSO_4$ was filtered off, and the filtrate was concentrated under reduced pressure to give **14m** (8.50 g, 98%) as a pale purple solid. ^{1}H NMR ($CDCl_3$) δ 3.74 (2H, br s), 6.49 (1H, dd, J = 8.0, 0.6 Hz), 6.62 (1H, dd, J = 8.6, 2.6 Hz), 6.83 (1H, d, J = 2.6 Hz), 7.01 (1H, d, J = 8.6 Hz), 7.35 (1H, t, J = 8.0 Hz), 7.57 (1H, dt, J = 8.0, 0.8 Hz), 9.15 (1H, d, J = 0.9 Hz).

The following compounds (**42a**−**42m**) were prepared from **8** and the corresponding anilines (**14a**−**14m**) by a method similar to that described for **9**.

6-(2-Chloro-4-{[5-(2-hydroxyethyl)-5*H*-pyrrolo[3,2-*d*]-pyrimidin-4-yl]amino}phenoxy)-2,3-dihydro-1*H*-isoindol-1-one (42a). Yield 71%, colorless crystals, mp 209 °C. ^{1}H NMR (DMSO-d_6) δ 3.82−3.95 (2H, m), 4.34 (2H, s), 4.55 (2H, t, J = 4.4 Hz), 6.30 (1H, br s), 6.52 (1H, d, J = 3.0 Hz), 6.93 (1H, d, J = 2.4 Hz), 7.23−7.35 (2H, m), 7.53−7.71 (3H, m), 7.98 (1H, d, J = 2.4 Hz), 8.35 (1H, s), 8.64 (1H, br s), 9.89 (1H, br s). HRMS (m/z): $[M + H]^+$ calcd for $C_{22}H_{18}ClN_5O_3$, 436.117; found, 436.118.

5-(2-Chloro-4-{[5-(2-hydroxyethyl)-5*H*-pyrrolo[3,2-*d*]-pyrimidin-4-yl]amino}phenoxy)-2,3-dihydro-1*H*-isoindol-1-one (42b). Yield 54%, colorless crystals, mp 210−212 °C. ^{1}H NMR (DMSO-d_6) δ 3.85−3.90 (2H, m), 4.31 (2H, s), 4.50−4.60 (2H, m), 6.32 (1H, br s), 6.51 (1H, d, J = 3.3 Hz), 7.00−7.10 (2H, m), 7.30 (1H, d, J = 9.0 Hz), 7.60−7.70 (3H, m), 7.95−8.00 (1H, m), 8.34 (1H, s), 8.41 (1H, s), 9.89 (1H, br s). HRMS (m/z): $[M + H]^+$ calcd for $C_{22}H_{18}ClN_5O_3$, 436.117; found, 436.117.

5-(2-Chloro-4-{[5-(2-hydroxyethyl)-5*H*-pyrrolo[3,2-*d*]-pyrimidin-4-yl]amino}phenoxy)-1,3-dihydro-2*H*-indol-2-one (42c). Yield 45%, pale orange crystals, mp 253−256 °C. ^{1}H NMR (DMSO-d_6) δ 3.47 (2H, s), 3.86 (2H, t, J = 4.5 Hz), 4.52 (2H, t, J = 4.5 Hz), 6.27 (1H, s), 6.50 (1H, d, J = 3.0 Hz), 6.75−6.80 (2H, m), 6.88 (1H, s), 7.08 (1H, d, J = 8.7 Hz), 7.52 (1H, dd, J = 9.1, 2.7 Hz), 7.65 (1H, d, J = 3.0 Hz), 7.91 (1H, d, J = 2.7 Hz), 8.31 (1H, s), 9.77 (1H, br s), 10.32 (1H, s). Anal. Calcd for $C_{22}H_{18}ClN_5O_3$: C, 60.62; H, 4.16; N, 16.07. Found: C, 60.36; H, 4.18; N, 15.94.

6-(2-Chloro-4-{[5-(2-hydroxyethyl)-5*H*-pyrrolo[3,2-*d*]-pyrimidin-4-yl]amino}phenoxy)-1,3-dihydro-2*H*-indol-2-one (42d). Yield 52%, pale orange crystals, mp 256−257 °C. ^{1}H NMR (DMSO-d_6) δ 3.42 (2H, s), 3.87 (2H, t, J = 4.5 Hz), 4.53 (2H, t, J = 4.5 Hz), 6.29 (1H, br s), 6.31 (1H, d, J = 2.3 Hz), 6.46−6.56 (2H, m), 7.16 (1H, d, J = 8.3 Hz), 7.22 (1H, d, J = 8.7 Hz), 7.58 (1H, dd, J = 8.7, 2.7 Hz), 7.66 (1H, d, J = 3.0 Hz), 7.95 (1H, d, J = 2.7 Hz), 8.34 (1H, s), 9.85 (1H, br s), 10.29 (1H, br s). Anal. Calcd for $C_{22}H_{18}ClN_5O_3$: C, 60.62; H, 4.16; N, 16.07. Found: C, 60.40; H, 4.21; N, 15.97.

4-(2-Chloro-4-{[5-(2-hydroxyethyl)-5*H*-pyrrolo[3,2-*d*]-pyrimidin-4-yl]amino}phenoxy)-2,3-dihydro-1*H*-isoindol-1-one (42e). Yield 98%, colorless crystals, mp 280 °C. ^{1}H NMR (DMSO-d_6) δ 3.88 (2H, t, J = 4.5 Hz), 4.35 (2H, s), 4.53 (2H, t, J = 4.5 Hz), 6.32 (1H, br s), 6.51 (1H, d, J = 3.0 Hz), 6.89 (1H, dd, J = 7.0, 1.8 Hz), 7.30 (1H, d, J = 8.7 Hz), 7.39−7.48 (2H, m), 7.60 (1H, dd, J = 8.7, 2.5 Hz), 7.66 (1H, d, J = 3.0 Hz), 7.99 (1H, d, J = 2.5 Hz), 8.34 (1H, s), 8.68 (1H, br s), 9.88 (1H, br s). Anal. Calcd for $C_{22}H_{18}ClN_5O_3$ 1.2 H_2O: C, 57.76; H, 4.49; N, 15.31. Found: C, 57.73; H, 4.55; N, 15.30.

7-(2-Chloro-4-{[5-(2-hydroxyethyl)-5*H*-pyrrolo[3,2-*d*]-pyrimidin-4-yl]amino}phenoxy)-2,3-dihydro-1*H*-isoindol-1-one (42f). Yield 36%, pale yellow crystals, mp 264−266 °C. ^{1}H NMR (DMSO-d_6) δ 3.80−3.90 (2H, m), 4.37 (2H, s), 4.50−4.60 (2H, m), 6.30 (1H, br s), 6.50 (1H, d, J = 3.0 Hz), 6.59 (1H, d, J = 9.0 Hz), 7.14 (1H, d, J = 9.0 Hz), 7.20−7.30 (1H, m), 7.48 (1H, t, J = 7.2 Hz), 7.55−7.60 (1H, m), 7.65 (1H, d, J = 3.0 Hz), 7.95−8.00 (1H, m), 8.33 (1H, s), 8.42 (1H, s), 9.84 (1H, br s). HRMS (m/z): $[M + H]^+$ calcd for $C_{22}H_{18}ClN_5O_3$, 436.117. Found, 436.113.

7-(2-Chloro-4-{[5-(2-hydroxyethyl)-5*H*-pyrrolo[3,2-*d*]-pyrimidin-4-yl]amino}phenoxy)-1,3-dihydro-2*H*-indol-2-one (42g). Yield 50%, pale pink crystals, mp 244−246 °C. ^{1}H NMR (DMSO-d_6) δ 3.57 (2H, s), 3.87 (2H, t, J = 4.5 Hz), 4.53 (2H, t, J = 4.5 Hz), 6.29 (1H, br s), 6.50 (1H, d, J = 3.2 Hz), 6.54 (1H, dd, J = 8.0, 0.9 Hz), 6.82−6.93 (1H, m), 6.99 (1H, dd, J = 8.0, 0.9 Hz), 7.12 (1H, d, J = 8.9 Hz), 7.54 (1H, dd, J = 8.9, 2.6 Hz), 7.66 (1H, d, J = 3.2 Hz), 7.94 (1H, d, J = 2.6 Hz), 8.32 (1H, s), 9.82 (1H, br s), 10.77 (1H, s). HRMS (m/z): $[M + H]^+$ calcd for $C_{22}H_{18}ClN_5O_3$, 436.117. Found, 436.116.

4-(2-Chloro-4-{[5-(2-hydroxyethyl)-5*H*-pyrrolo[3,2-*d*]-pyrimidin-4-yl]amino}phenoxy)-1,3-dihydro-2*H*-indol-2-one (42h). Yield 27%, colorless crystals, mp 259−261 °C. ^{1}H NMR (DMSO-d_6) δ 3.37 (2H, s), 3.87 (2H, q, J = 4.4 Hz), 4.53 (2H, t, J = 4.3 Hz), 6.26−6.34 (2H, m), 6.50 (1H, d, J = 3.0 Hz), 6.59 (1H, d, J = 7.4 Hz), 7.13 (1H, t, J = 8.1 Hz), 7.20 (1H, d, J = 8.8 Hz), 7.56 (1H, dd, J = 8.8, 2.6 Hz),

7.65 (1H, d, J = 3.0 Hz), 7.95 (1H, d, J = 2.6 Hz), 8.32 (1H, s), 9.84 (1H, br s), 10.51 (1H, br s). Anal. Calcd for $C_{22}H_{18}ClN_5O_3$ 0.2 H_2O: C, 60.13; H, 4.22; N, 15.94. Found: C, 60.01; H, 4.29; N, 15.77.

2-(4-{[3-Chloro-4-(1*H*-indol-4-yloxy)phenyl]amino}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethanol (35j). Yield 86%, colorless crystals, mp 233−235 °C. ^{1}H NMR (DMSO-d_6) δ 3.86 (2H, t, J = 4.5 Hz), 4.54 (2H, t, J = 4.5 Hz), 6.31 (1H, br s), 6.34−6.49 (2H, m), 6.92−7.08 (2H, m), 7.19 (1H, d, J = 7.9 Hz), 7.31 (1H, t, J = 2.7 Hz), 7.42−7.64 (2H, m), 7.94 (1H, d, J = 2.3 Hz), 8.29 (1H, s), 9.83 (1H, br s), 11.28 (1H, br s). Anal. Calcd for $C_{22}H_{18}ClN_5O_2$: C, 62.93; H, 4.32; N, 16.68. Found: C, 62.78; H, 4.47; N, 16.50.

2-(4-{[4-(1-Benzothiophen-4-yloxy)-3-chlorophenyl]amino}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethanol (42j). Yield 62%, colorless crystals, mp 245−246 °C. ^{1}H NMR (DMSO-d_6) δ 3.88 (2H, br s), 4.54 (2H, br s), 6.51 (1H, d, J = 3.0 Hz), 6.62 (1H, d, J = 7.1 Hz), 7.22 (1H, d, J = 8.8 Hz), 7.31 (1H, t, J = 7.9 Hz), 7.48−7.84 (5H, m), 8.00 (1H, d, J = 2.4 Hz), 8.34 (1H, s), 9.85 (1H, br s). Anal. Calcd for $C_{22}H_{17}ClN_4O_2S$ 0.1 H_2O: C, 60.23; H, 3.95; N, 12.77. Found: C, 60.11; H, 3.98; N, 12.73.

2-(4-{[3-Chloro-4-(1*H*-indazol-4-yloxy)phenyl]amino}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethanol (42k). Yield 39%, colorless crystals, mp 195−198 °C. ^{1}H NMR (DMSO-d_6) δ 3.89 (2H, br s), 4.55 (2H, br s), 6.25−6.40 (1H, m), 6.71 (1H, s), 7.15−7.35 (3H, m), 7.50−7.74 (1H, m), 7.92 (2H, s), 8.33 (1H, s), 8.79 (1H, s), 13.26 (1H, s). Anal. Calcd for $C_{21}H_{17}ClN_6O_2$ 0.3 H_2O: C, 59.17; H, 4.16; N, 19.72. Found: C, 59.45; H, 4.45; N, 19.65.

2-[4-({3-chloro-4-[(1-methyl-1*H*-indazol-4-yl)oxy]phenyl}-amino)-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl]ethanol (42l). Yield 57%, colorless crystals, mp 228−229 °C. ^{1}H NMR (DMSO-d_6) δ 3.89 (2H, br s), 4.06 (3H, s), 4.55 (2H, t, J = 4.6 Hz), 6.33 (2H, dd, J = 7.0, 0.7 Hz), 6.52 (1H, d, J = 3.0 Hz), 7.24−7.38 (3H, m), 7.57−7.70 (2H, m), 7.92 (1H, d, J = 0.7 Hz), 8.01 (1H, d, J = 2.4 Hz), 8.36 (1H, s), 9.92 (1H, s). HRMS (m/z): $[M + H]^+$ calcd for $C_{22}H_{19}ClN_6O_2$, 435.133. Found, 435.132.

2-(4-{[4-(1,2-Benzisothiazol-4-yloxy)-3-chlorophenyl]-amino}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethanol (42m). Yield 67%, colorless crystals, mp 218−220 °C. ^{1}H NMR (DMSO-d_6) δ 3.89 (2H, br s), 4.56 (2H, t, J = 4.5 Hz), 6.34 (1H, br s), 6.48−6.63 (2H, m), 7.42 (1H, d, J = 8.8 Hz), 7.53 (1H, t, J = 8.0 Hz), 7.60−7.76 (2H, m), 7.92 (1H, d, J = 8.0 Hz), 8.05 (1H, d, J = 2.4 Hz), 8.38 (1H, s), 9.25 (1H, s), 9.99 (1H, s). Anal. Calcd for $C_{21}H_{16}ClN_5O_2S$: C, 57.60; H, 3.68; N, 15.99. Found: C, 57.37; H, 3.95; N, 15.69.

5-(2-Aminoethyl)-*N*-[4-(1,2-benzisothiazol-4-yloxy)-3-chlorophenyl]-5*H*-pyrrolo[3,2-*d*]pyrimidin-4-amine Dihydrochloride (44). A mixture of *tert*-butyl [2-(4-chloro-5*H*-pyrrolo[3,2-*d*]-pyrimidin-5-yl)ethyl]carbamate[3] (**43**, 2.90 g, 9.77 mmol) and **14m** (2.70 g, 9.77 mmol) in 2-propanol (50 mL) was stirred at 80 °C for 12 h. To the mixture was added saturated sodium hydrogen carbonate (150 mL), and the mixture was extracted with EtOAc (300 mL). The organic layer was washed with brine (30 mL) and dried over $MgSO_4$. Insoluble $MgSO_4$ was filtered off, and the filtrate was concentrated *in vacuo*. The obtained residue was subjected to silica gel column chromatography (eluent, MeOH/EtOAc = 0:100 to 10:90). The objective fractions were collected and concentrated under reduced pressure. To the residue were added 4 N HCl in EtOAc (15 mL) and EtOH (15 mL), and the mixture was stirred at 80 °C for 4 h. Ethyl acetate (100 mL) was added to the reaction mixture, and the resulting solid was collected by filtration to give **44** (4.00 g, 80%) as brown powder. ^{1}H NMR (DMSO-d_6) δ 3.27−3.37 (2H, m), 4.97−5.10 (2H, m), 6.67 (1H, d, J = 7.7 Hz), 6.75 (1H, d, J = 3.0 Hz), 7.44 (1H, d, J = 8.9 Hz), 7.58 (1H, t, J = 7.7 Hz), 7.63−7.74 (1H, m), 7.88−8.03 (2H, m), 8.06 (1H, d, J = 3.0 Hz), 8.29 (3H, br s), 8.74 (1H, s), 9.22 (1H, d, J = 0.8 Hz), 10.10 (1H, br s).

***N*-[2-(4-{[4-(1,2-Benzisothiazol-4-yloxy)-3-chlorophenyl]-amino}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl]acetamide (45m).** A mixture of **44** (560 mg, 1.10 mmol), acetic acid (94 mg, 1.57 mmol), triethylamine (1.5 mL), HOBt (200 mg, 1.48 mmol), and EDC(1.0 g, 4.39 mmol) in DMF (50 mL) was stirred at room temperature for 12 h. Water (50 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (100 mL). The organic layer was washed successively with water (100 mL) and brine (10 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure, and the residue was subjected to silica gel column chromatography (eluent, MeOH/EtOAc = 0:100 to 10:90). The objective fractions were collected and concentrated under reduced pressure, and the residue was crystallized from EtOAc/diisopropyl ether (1/1, 15 mL) to give **45m** (450 mg, 80%) as white crystals. mp 222−224 °C.^{1}H NMR (DMSO-d_6) δ 1.80 (3H, s), 3.35−3.43 (2H, m), 4.52 (2H, br s), 6.52 (1H, d, J = 2.3 Hz), 6.60 (1H, d, J = 8.0 Hz), 7.40 (1H, d, J = 8.7 Hz), 7.54 (1H, t, J = 8.0 Hz), 7.66 (1H, d, J = 2.3 Hz), 7.82 (1H, dd, J = 8.7, 2.3 Hz), 7.92 (1H, d, J = 8.0 Hz), 8.10 (1H, d, J = 2.3 Hz), 8.27 (1H, br s), 8.36 (1H, s), 8.84 (1H, s), 9.25 (1H, s). Anal. Calcd for $C_{23}H_{19}ClN_6O_2S$: C, 57.68; H, 4.00; N, 17.55. Found: C, 57.56; H, 3.98; N, 17.38.

***N*-[2-(4-{[4-(1,2-Benzisothiazol-4-yloxy)-3-chlorophenyl]-amino}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl]-2-hydroxy-2-methylpropanamide Methanesulfonate (46m).** A solution of **44** (100 mg, 0.20 mmol), 2-hydroxy-2-methylpropanoic acid (50.0 mg, 0.48 mmol), EDC (300 mg, 1.32 mmol), HOBt (10.0 mg 0.074 mmol), and triethylamine (0.5 mL) in DMF (10 mL) was stirred at room temperature for 12 h. Water (100 mL) was added to the reaction mixture, and then, the mixture was extracted with EtOAc (100 mL). The organic layer was washed with water (100 mL) and brine (10 mL) successively, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent, MeOH/EtOAc = 0:100 to 10:90) to give a white solid. To a solution of the white solid in EtOAc (5 mL) was added methanesulfonic acid (20 mg, 0.21 mmol) and diethyl ether (1 mL), and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure. The residual crystals were collected by filtration and washed with EtOAc to give **46m** (55.0 mg, 45%) as white crystals. mp 174−176 °C. ^{1}H NMR (DMSO-d_6) δ 2.12 (6H, s), 2.81 (2H, s), 3.47 (2H, t, J = 6.2 Hz), 4.56 (2H, t, J = 6.2 Hz), 6.48 (1H, d, J = 2.8 Hz), 6.66 (1H, d, J = 7.2 Hz), 7.18 (1H, d, J = 8.9 Hz), 7.33 (1H, t, J = 7.9 Hz), 7.53 (1H, dd, J = 5.7, 0.8, Hz), 7.57−7.85 (4H, m), 7.95−8.16 (2H, m), 8.32 (1H, s), 8.72 (1H, s). Anal. Calcd for $C_{26}H_{27}ClN_6O_6S_2$ 1.0 H_2O: C, 49.01; H, 4.59; N, 13.19. Found: C, 48.79; H, 4.42; N, 13.32.

***N*-[2-(4-{[4-(1,2-Benzisothiazol-4-yloxy)-3-chlorophenyl]-amino}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl]-3-hydroxy-2,2-dimethylpropanamide (47m).** Compound **47m** was obtained as pale yellow crystals in 52% yield from **44** by a method similar to that described for **45m**. mp 219−221 °C. ^{1}H NMR (DMSO-d_6) δ 0.98 (6H, s), 3.33 (2H, s), 3.39−3.53 (2H, m), 4.53 (2H, br s), 4.85 (1H, br s), 6.50 (1H, br s), 6.61 (1H, d, J = 7.7 Hz), 7.39 (1H, d, J = 8.9 Hz), 7.44−7.70 (2H, m), 7.81−8.02 (3H, m), 8.16 (1H, br s), 8.36 (1H, s), 8.96 (1H, br s), 9.25 (1H, d, J = 0.9 Hz). Anal. Calcd for $C_{26}H_{25}ClN_6O_3S$ 2.5 H_2O: C, 53.65; H, 5.19; N, 14.44. Found: C, 53.94; H, 5.27; N, 14.75.

***N*-[2-(4-{[4-(1,2-Benzisothiazol-4-yloxy)-3-chlorophenyl]-amino}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl]-2-methyl-2-(methylsulfonyl)propanamide (48m).** Compound **48m** was obtained as white crystals in 75% yield from **44** by a method similar to that described for **45m**. mp 247−248 °C. ^{1}H NMR (DMSO-d_6) δ 1.41 (6H, s), 2.96 (3H, s), 3.41−3.56 (2H, m), 4.59 (2H, t, J = 6.6 Hz), 6.50 (1H, d, J = 2.6 Hz), 6.60 (1H, d, J = 8.0 Hz), 7.40 (1H, d, J = 9.0 Hz), 7.54 (1H, t, J = 8.0 Hz), 7.58 (1H, d, J = 2.6 Hz), 7.81 (1H, dd, J = 9.0, 2.0 Hz), 7.92 (1H, d, J = 8.0 Hz), 8.06 (1H, d, J = 2.0 Hz), 8.22 (1H, t, J = 5.9 Hz), 8.36 (1H, s), 8.74 (1H, s), 9.25 (1H, d, J = 0.8 Hz). Anal. Calcd for $C_{26}H_{25}ClN_6O_4S_2$: C, 53.37; H, 4.31; N, 14.36. Found: C, 53.49; H, 4.45; N, 14.12.

***N*-[2-(4-{[4-(1,2-Benzisothiazol-4-yloxy)-3-chlorophenyl]-amino}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl]-2,2-dimethylpropanamide Methanesulfonate (49m).** A solution of **44** (100 mg, 0.20 mmol), 2,2-dimethylpropanoic acid anhydride (55.0 mg, 0.30 mmol), and triethylamine (0.3 mL) in DMF (10 mL) was stirred at room temperature for 2 h. Water (50 mL) was added to the reaction mixture, and then, the mixture was extracted with EtOAc (100 mL). The organic layer was washed successively with water (100 mL) and brine (10 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure, and the residue was subjected to silica gel column chromatography (eluent, MeOH/EtOAc = 0:100 to 10:90), and the objective fractions were collected and concentrated under reduced pressure. The residue was crystallized from EtOAc/diisopropyl

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ether (1/1, 5 mL) to give *N*-[2-(4-{[4-(1,2-benzoisothiazol-4-yloxy)-3-chlorophenyl]amino}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl]-2,2-dimethylpropanamide (87.0 mg, 1.67 mmol) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.01 (9H, s), 3.64−3.74 (2H, m), 4.51 (2H, t, J = 7.6 Hz), 6.49 (1H, d, J = 3.2 Hz), 6.60 (1H, d, J = 7.6 Hz), 7.37 (1H, d, J = 8.9 Hz), 7.46−7.63 (2H, m), 7.75−7.94 (3H, m), 8.10 (1H, d, J = 2.5 Hz), 8.33 (1H, s), 8.90 (1H, s), 9.22 (1H, d, J = 0.9 Hz). The white solid (1.50 g, 2.88 mmol) was dissolved in EtOAc (100 mL), and methanesulfonic acid (300 mg, 3.12 mmol) was added to the mixture. Diethyl ether (5 mL) was added, and the precipitate was collected by filtration to give **49m** (1.30 g, 63%) as white crystals. mp 216−218 °C. ^{1}H NMR (DMSO-d_6) δ 1.02 (9H, s), 2.32 (3H, s), 3.43−3.52 (2H, m), 4.62−4.69 (2H, m), 6.68 (2H, dd, J = 2.2, 5.4 Hz), 7.47 (1H, d, J = 8.9 Hz), 7.58 (1H, t, J = 7.9 Hz), 7.73−8.10 (5H, m), 8.77 (1H, s), 9.22 (1H, d, J = 0.8 Hz), 10.13 (1H, br s). Anal. Calcd for $C_{26}H_{25}ClN_6O_4S_2$: C, 52.55; H, 4.74; N, 13.62. Found: C, 52.57; H, 4.73; N, 13.62.

***N*-[2-(4-{[4-(1,2-Benzisothiazol-4-yloxy)-3-chlorophenyl]-amino}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl]prolinamide hydrochloride (50m).** A mixture of **44** (150 mg, 0.29 mmol), 1-(*tert*-butoxycarbonyl)proline (72.0 mg, 0.33 mmol), triethylamine (1.2 mL), HOBt (20 mg, 0.15 mmol), and EDC (320 mg, 1.40 mmol) in DMF (15 mL) was stirred at room temperature for 12 h. Water (50 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (100 mL). The organic layer was washed successively with water (100 mL) and brine (10 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure, and the residue was subjected to silica gel column chromatography (eluent, MeOH/EtOAc = 0:100 to 10:90). The objective fractions were collected and concentrated under reduced pressure, and the residue was dissolved in EtOH (5 mL). To the solution was added 4 N HCl in EtOAc (5 mL), and the mixture was stirred at 80 °C for 2 h. After cooling at room temperature, the precipitate was collected by filtration to give **50m** (72.0 mg, 41%) as white crystals. mp 143−145 °C. ^{1}H NMR (DMSO-d_6) δ 1.54−1.93 (3H, m), 2.06−2.25 (1H, m), 2.68−3.74 (4H, m), 3.96−4.10 (1H, m), 4.58−4.90 (2H, m), 6.57−6.74 (2H, m), 7.43 (1H, d, J = 8.9 Hz), 7.51−7.75 (2H, m), 7.84−8.01 (3H, m), 8.43 (1H, br s), 8.69 (1H, s), 8.80 (1H, t, J = 5.8 Hz), 9.19 (1H, d, J = 0.8 Hz), 9.54 (1H, br s), 9.95 (1H, br s). Anal. Calcd for $C_{26}H_{25}Cl_2N_7O_2S$ 2.5 H_2O: C, 50.73; H, 4.91; N, 15.93. Found: C, 50.73; H, 4.96; N, 15.77.

***N*-[2-(4-{[4-(1,2-Benzisothiazol-4-yloxy)-3-chlorophenyl]-amino}-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)ethyl]-2-methylalaninamide (51m).** A mixture of **44** (2.0 g, 3.92 mmol), *N*-(*tert*-butoxycarbonyl)-2-methylalanine (1.00 g, 4.92 mmol), triethylamine (4.5 mL), HOBt (400 mg, 2.96 mmol), and EDC (3.20 g, 14.0 mmol) in DMF (200 mL) was stirred at room temperature for 12 h. Water (200 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (400 mL). The organic layer was washed successively with water (400 mL) and brine (40 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure, and the residue was subjected to silica gel column chromatography (eluent, MeOH/EtOAc = 0:100 to 10:90). The objective fractions were collected and concentrated under reduced pressure, and the residue was dissolved in EtOH (100 mL). To the solution was added 1 N HCl (50 mL), and the mixture was stirred at 70 °C for 10 h. After cooling at room temperature, 1 N NaOH (400 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (400 mL). The organic layer was washed successively with water (200 mL) and brine (40 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure, and the residue was subjected to basic silica gel column chromatography (eluent, MeOH/EtOAc = 0:100 to 10:90), and the objective fractions were collected and concentrated under reduced pressure. The obtained residue was crystallized from EtOAc/diisopropyl ether (1/1, 25 mL) to give **51m** (1.30 g, 64%) as colorless crystals. mp 198 °C. ^{1}H NMR (DMSO-d_6) δ 1.12 (6H, s), 1.85 (2H, br s), 3.44 (2H, t, J = 6.6 Hz), 4.55 (2H, t, J = 6.6 Hz), 6.50 (1H, d, J = 3.0 Hz), 6.61 (1H, d, J = 7.6 Hz), 7.39 (1H, d, J = 8.0 Hz), 7.54 (1H, t, J = 8.0 Hz), 7.61 (1H, d, J = 3.0 Hz), 7.84−7.91 (1H, m), 7.92 (1H, d, J = 8.0 Hz), 8.12 (1H, br s), 8.22 (1H, br s), 8.35 (1H, s), 8.90 (1H, br s), 9.26 (1H, d, J = 0.8 Hz). Anal. Calcd for $C_{25}H_{24}ClN_7O_2S$: C, 57.52; H, 4.63; N, 18.78. Found: C, 57.44; H, 4.49; N, 18.72.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

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

■ AUTHOR INFORMATION

Corresponding Author

*(Y.K.) Tel: +81-466-32-1249. Fax: +81-466-29-4450. E-mail: Kawakita_Youichi@takeda.co.jp:. (T.I.) Tel: +81-466-32-1155. Fax: +81-466-29-4449. E-mail: Ishikawa_Tomoyasu@takeda.co.jp.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

^{1}H NMR, proton nuclear magnetic resonance; AUC, area under the blood concentration time curve; BA, bioavailability; DMF, *N*,*N*-dimethylformamide; EGFR, epidermal growth factor receptor; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; EtOAc, ethyl acetate; EtOH, ethanol; GI, growth inhibitory; HCl, hydrochloric acid; HER2, human epidermal growth factor receptor 2; HOBt, 1-hydroxybenzotriazole monohydrate; HPLC, high-performance liquid chromatography; K_2CO_3, potassium carbonate; mCPBA, 3-chloroperbenzoic acid; $MgSO_4$, magnesium sulfate; MS, mass spectroscopy; NaOH, sodium hydroxide; NBS, *N*-bromosuccinimide; NMP, 1-methyl-2-pyrrolidone; PI, pseudoirreversibility; PK, pharmacokinetic; ppm, parts per million; Pt/C, platinum/carbon; SAR, structure−activity relationships; THF, tetrahydrofuran; TKIs, tyrosine kinase inhibitors

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