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Synthesis and structure–activity relationships of 2-acylamino-4,6-diphenylpyridine derivatives as novel antagonists of GPR54

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ABSTRACT

GPR54 is a G protein-coupled receptor (GPCR) which was formerly an orphan receptor. Recent functional study of GPR54 revealed that the receptor has an essential role to modulate sex-hormones including GnRH. Though antagonists of GPR54 are expected to be novel drugs for sex-hormone dependent diseases such as prostate cancer or endometriosis, small molecule GPR54 antagonists have not been reported. We have synthesized a series of 2-acylamino-4,6-diphenylpyridines to identify potent GPR54 antagonists. Detailed structure–activity relationship studies led to compound **9l** with an IC_{50} value of 3.7 nM in a GPR54 binding assay, and apparent antagonistic activity in a cellular functional assay.

1. Introduction

GPR54 (OT7T175, AXOR12) is a G protein-coupled receptor (GPCR) that is highly expressed in brain, including hypothalamus and pituitary as well as peripheral regions.[1] GPR54 was identified as an orphan receptor in rat in 1999,[2] and later Ohtaki et al. discovered that a 54-amino-acid product of a gene called *Kiss-1* was its endogenous ligand.[3] As the *Kiss-1* gene was originally isolated as a tumor metastasis gene, the peptide product was named 'metastin'. Later others also isolated the same peptide and named it 'kisspeptin'.[4]

After much study to understand the function of GPR54, several findings were reported that suggest GPR54 plays an important role in reproduction and pubertal development. The phenotype of GPR54-null mice was consistent with lack of steroid sex hormone production.[5a] In the mutant male mice, the serum testosterone level was similar to that found in normal females. In the case of the mutant females, they did not show the rise in estradiol normally found during estrus. Moreover, mutations of GPR54 were found in patients with idiopathic hypogonadoropic hypogonadism (IHH).[5a,6] Individuals with IHH fail to undergo puberty and are infertile because of failure to secrete the gonadotropic hormones, such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary.

In a recent study, GPR54-metastin signaling was revealed to be essential to initiate gonadotropin secretion.[7] Central or peripheral administration of metastin stimulates a dose-dependent rise in serum levels of LH and FSH in adult rat,[7,8] mice,[9] sheep, macaques, and humans,[10] while metastin stimulation causes no effect on gonadotropin secretion in mice lacking a functional GPR54 gene.[5] Moreover, the effect is blocked by pretreatment with a GnRH antagonist,[7,9,10b,11] therefore, GPR54-metastin signaling is supposed to be up stream of GnRH-GnRH receptor signaling. Furthermore, GPR54 mRNA expression was observed in GnRH neurons of rats and mice, suggesting GPR54 may act directly on GnRH neurons.[10a,11,12] Thus, GPR54 is expected to play a key role on hypothalamus–pituitary–gonadal (HPG) axis.

These facts suggest that small molecule GPR54 antagonists may suppress the release of gonadtropic hormones and such compounds would be novel orally available drugs for sex hormone-dependant diseases, including prostate cancer and endometriosis, however, small molecule GPR54 antagonists have yet to be reported.[13]

High-throughput screening of our proprietary compound collection identified hit compound **1** (Fig. 1) with an IC_{50} value of 1.2 μM in a receptor binding assay (RBA). Since this compound also showed antagonistic activity against stimulation of metastin (40–54) in a cellular Ca^{2+} mobilization assay, we anticipated that this class of compound would be appropriate as leads for a GPR54 antagonist. In this report we describe the structure–activity relationship study that was performed to modify the 4-phenyl ring,

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doi:10.1016/j.bmc.2010.04.036

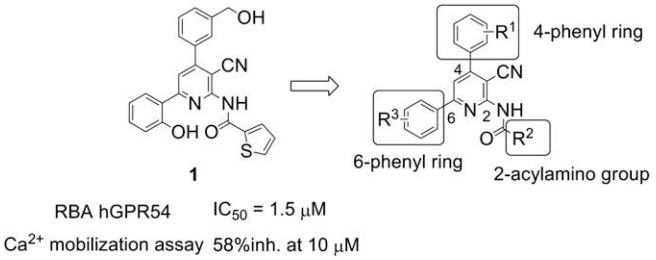


Figure 1. Structure of the hit compound **1** and synthetic strategy.

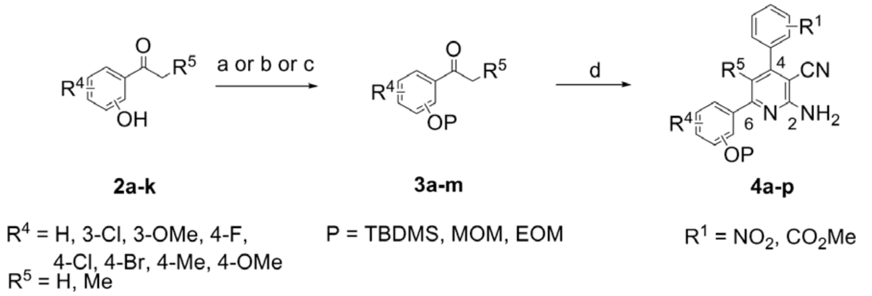
6-phenyl ring, and 2-acylamino group, as shown in Figure 1, and led to the discovery of compound **9l** as a potent GPR54 antagonist.

2. Chemistry

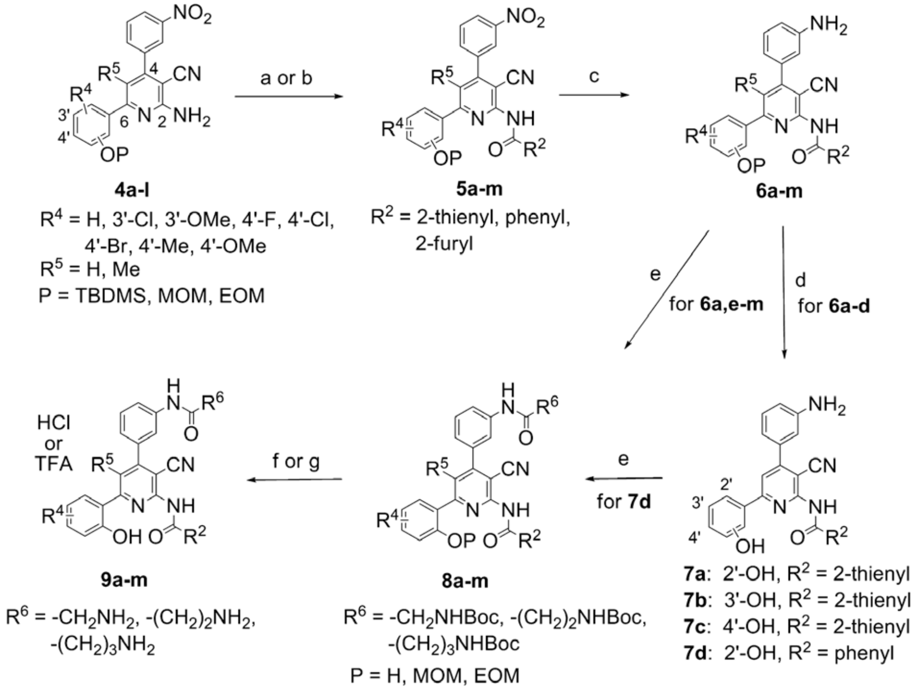
The 2-amino-4,6-diphenylpyridines **4a**–**p**, the key intermediates of 2-acylamino derivatives, were prepared as shown in Scheme 1. The hydroxy groups of **2a**–**k** were protected by *tert*-butyldimethylsilyl (TBDMS), methoxymethyl (MOM), or ethoxymethyl (EOM) groups to give **3a**–**m**. These ketones were treated with the corresponding benzaldehydes, malononitrile, and ammonium acetate to afford the 2-amino-4,6-diphenylpyridines **4a**–**p**.

Compounds with an amino group on the 4-phenyl ring and their amide derivatives were synthesized as shown in Scheme 2. Acylation of the 2-amino group of compounds **4a–l** with an excess amount of acid chloride in pyridine gave the diacylated compounds, which were easily converted to the monoacylated compounds in basic conditions, followed by reduction of the nitro group to yield the anilines **6a–m**. Cleavage of the protective group of the phenolic hydroxy group gave **7a**–**d**, which possess amino groups on the 4-phenyl ring and phenol at the 6-position of the pyridine ring.

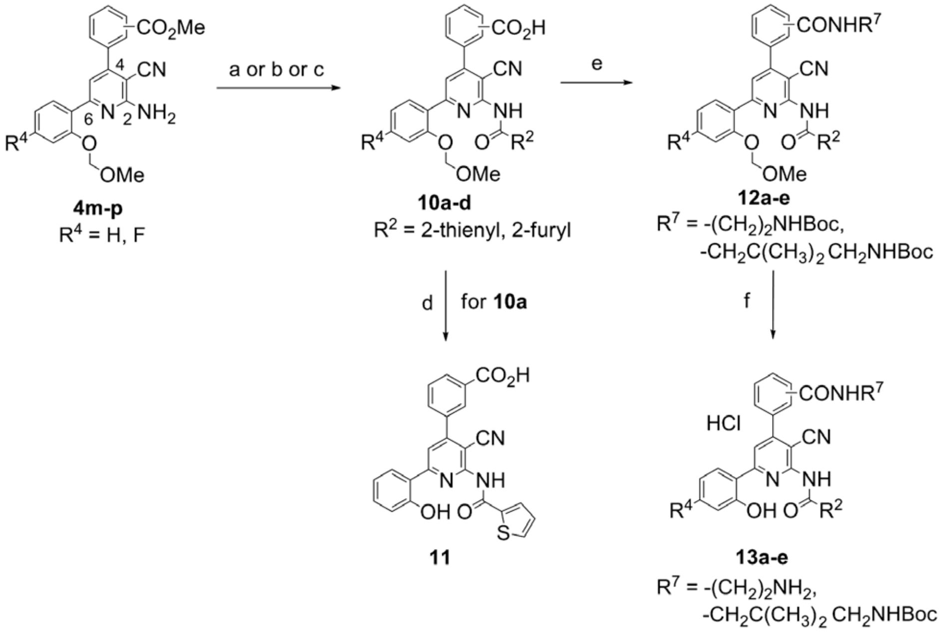
Condensation of anilines **6** with the corresponding carboxylic acids afforded amides **8a**,**b**,**d–m**. For compounds having TBDMS as the protecting group of the hydroxy group, partial or complete deprotection was observed after condensation. Compound **8c** was synthesized from **7d**, which was obtained by deprotection of a



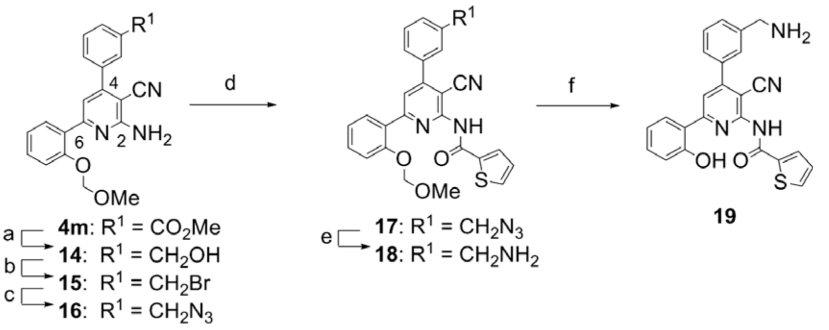
Scheme 1. Synthesis of 2-aminopyridine derivatives. Reagents and conditions: (a) TBDMSCl, imidazole, DMF, rt; (b) MOMCl, NaH, THF, rt; (c) EOMCl, NaH, THF, rt; (d) benzaldehyde, malononitrile, NH_4OAc, toluene, reflux.



Scheme 2. Synthesis of 2-acylamino-4,6-diphenylpyridines with an amino group on the 4-phenyl ring and their amide derivatives. Reagents and conditions: (a) R^2COCl, pyridine, rt, then purification by basic silica gel; (b) R^2COCl, pyridine, rt, then ammonia solution, rt; (c) iron powder, NH_4Cl, EtOH, H_2O, reflux; (d) TBAF, THF, 0 °C; (e) R^6CO_2H, WSCD, HOBt, DMF, rt; (f) 4 M HCl EtOAc solution, rt or 10% HCl MeOH solution, rt; (g) TFA, rt.



Scheme 3. Synthesis of 2-acylamino-4,6-diphenylpyridines with carboxylic acid groups on the 4-phenyl ring and their amide derivatives. Reagents and conditions: (a) (1) R^2COCl, pyridine, rt., then 2 M Na_2CO_3, THF, MeOH, rt; (2) 4 M NaOH, EtOH, THF, rt; (b) (1) R^2COCl, pyridine, rt, then purification by basic silica gel; (2) 4 M NaOH, EtOH, THF, rt; (c) R^2COCl, pyridine, rt, then 4 M NaOH, rt; (d) concd HCl, THF, rt; (e) H_2NR^7, WSCD, HOBt, DMF, rt; (f) 4 M HCl EtOAc solution, rt.



Scheme 4. Synthesis of 2-acylamino-4,6-diphenylpyridine **19** with an aminomethyl group on the 4-phenyl ring. Reagents and conditions: (a) $LiBH_4$, THF, 50 °C; (b) CBr_4, PPh_3, CH_2Cl_2, rt; (c) NaN_3, DMF, rt; (d) 2-thenoyl chloride, pyridine, rt, then purification by basic silica gel; (e) H_2 (3 atm), 10% Pd/C, EtOH, EtOAc, rt; (f) concd HCl, THF, rt.

TBDMS group in advance by TBAF. Removal of the protecting groups of **8a–m**, both on the amide side chain and the phenolic hydroxy group, afforded the amines **9a–m**.

The synthesis of compounds bearing a carboxy group on the 4-phenyl ring and their amide derivatives is outlined in Scheme 3. Acylation of 2-aminopyridines **4m–p** by the method described earlier and hydrolysis of the esters gave **10a–d**. Cleavage of the MOM group of **10a** in acidic condition yielded **11** having a carboxy group on the 4-phenyl ring and a phenol at the 6-position of the pyridine ring.

Condensation of carboxylic acids **10a–d** with amines provided **12a–e**, which were treated with HCl to remove the MOM group and afford **13a–e**.

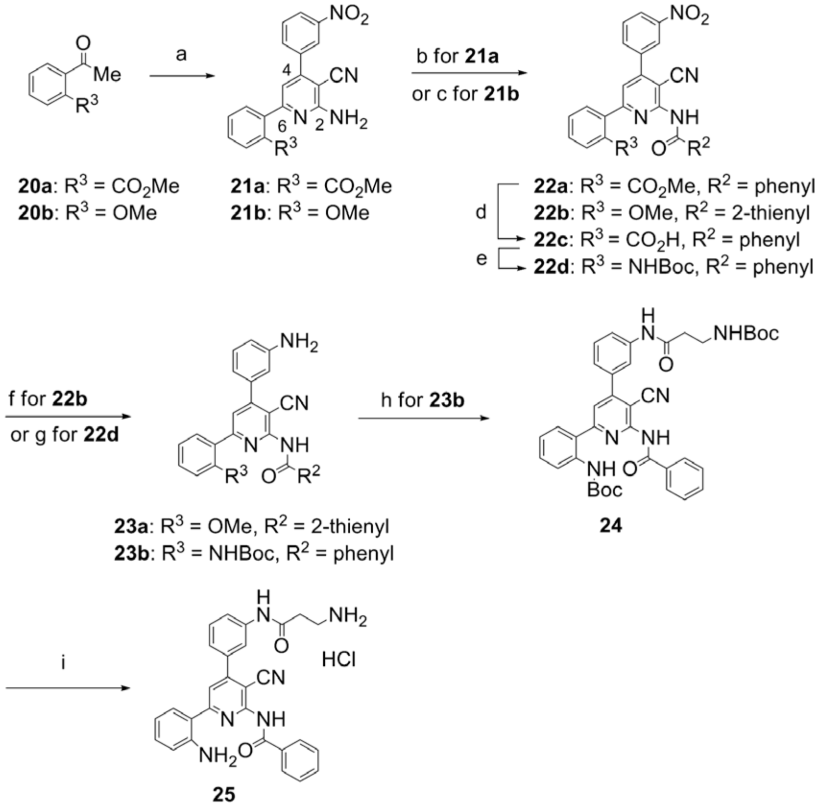
Compound **19** having an aminomethyl moiety on the 4-phenyl ring was prepared as described in Scheme 4. Reduction of the ester of compound **4m** afforded the alcohol **14**, followed by bromination and introduction of an azide to give compound **16**. After acylation of the 2-amino group with 2-thenoyl chloride, the azide was hydrogenated under three atm hydrogen atmosphere to yield **18**, which was converted to **19** by treatment with HCl.

The synthesis of compounds without a phenolic hydroxy group on the 6-phenyl ring is summarized in Scheme 5. The four components condensation reaction described above was applied to ketones **20a**,**b** to yield the 2-amino-4,6-diphenylpyridines **21a**,**b**, followed by acylation of the amino group to provide **22a**,**b**. After hydrolysis of ester **22a**, carboxylic acid **22c** was converted to the *tert*-butoxycarbonyl(Boc)-protected aniline **22d** by Curtius rearrangement reaction. Reduction of the nitro group of compounds **22b**,**d** gave anilines **23a**,**b**, and for **23b**, subsequent condensation with Boc-protected β-alanine yielded **24**. Treatment of **24** with HCl to remove the Boc group provided **25**.

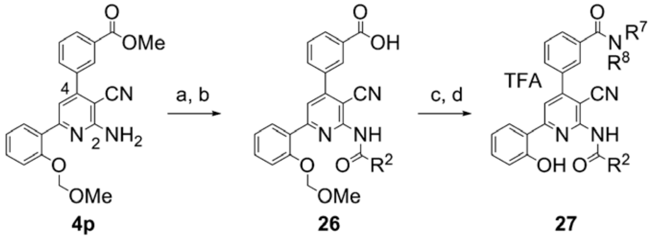
In order to optimize the amine side chain on the 4-phenyl ring and the 2-acylamino group, a combinatorial protocol was applied as summarized in Scheme 6. Acylation of the amino group of **4p** and hydrolysis of the ester gave carboxylic acid **26**, followed by condensation with amines, cleavage of MOM group, and purification with HPLC to afford compound **27**. We planned to synthesize 168 compounds constructed by combining 14 acyl groups with each of 12 amines and actually obtained 148 compounds discarding samples that had insufficient quality or quantity.

3. Results and discussion

All compounds synthesized were initially evaluated by their binding affinities for human GPR54 against ^{125}I-labeled human



Scheme 5. Synthesis of 2-acylamino-4,6-diphenylpyridines without a phenolic hydroxy group on the 6-phenyl ring. Reagents and conditions: (a) benzaldehyde, malononitrile, NH_4OAc, toluene, reflux; (b) benzoyl chloride, pyridine, rt, then NaOMe, MeOH, rt; (c) 2-thenoyl chloride, pyridine, rt, then purification by basic silica gel; (d) 4 M NaOH, MeOH, THF, rt; (e) DPPA, Et_3N, toluene, reflux, then *t*-BuOH, reflux; (f) H_2 (3 atm), 10% Pd/C, THF, 2-propanol, rt; (g) iron powder, NH_4Cl, EtOH, H_2O, reflux; (h) *N*-Boc-β-alanine, WSCD, HOBt, DMF, rt; (i) 4 M HCl EtOAc solution, CH_2Cl_2, rt.



Scheme 6. Synthesis of 2-acylamino-4,6-diphenylpyridines by a combinatorial protocol. Reagents and conditions: (a) R^2COCl, pyridine, rt; (b) 4 M NaOH, THF, MeOH, rt; (c) HNR^7R^8, WSCD, HOBt, triethylamine, DMAP, DMF, CH_2Cl_2, 50 °C then purification by HPLC; (d) TFA, rt, then purification by HPLC.

metastin (40–54) in a receptor binding assay. For compounds having potent affinities for GPR54, antagonistic activities were evaluated in a cellular Ca^{2+} mobilization assay against stimulation of metastin (40–54). The results are shown in Tables 1–4.

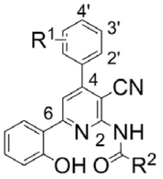
We initially modified the hydroxymethyl group on the 4-phenyl ring of hit compound **1** (Table 1). Replacement of the hydroxymethyl group by a carboxylic acid (**11**) or amine (**7a**) retained activity, while introduction of an aminomethyl group (**19**) resulted in threefold enhancement of activity compared to **1**. As we speculated that introduction of an amino group at this position is preferable, detailed modification of the aminomethyl group was conducted. Introduction of a glycine amide at the 3′-position of the phenyl ring significantly improved activity with an IC_{50} value of 51 nM (**9a**) with apparent antagonistic activity. Next, the length of the methylene linker was examined. Introduction of a β-alanine amide (**9b**) improved potency further compared with the glycine amide **9a**, though the γ-amino butyryl amide **9d** slightly decreased activity. We concluded that the β-alanine amide is the optimum linker between the amino group and the phenyl ring. Compound **13b**, which is an inverse amide derivative of **9b**, also showed potent activity, while **9b** was twofold more active. Shifting the amine side chain to the 2′- or 4′-position of the phenyl ring (**13a**,**c**) resulted in decreased potency.

Regarding modification of the acyl group at the 2-position of the pyridine ring, replacement of the 2-thienyl group with a phenyl group retained activity, though the 2-thienyl group was preferable (**9b** vs **9c**).

Next, we conducted modification of the 6-phenyl ring (Table 2). Initially, investigation of the effect of a hydroxy group at the 2′-position of the phenyl ring was conducted. Methylation of the hydroxy group attenuated activity (**7a** vs **23a**), suggesting that it may be important as a proton donor. Then we replaced the hydroxy group to an amino group as an alternative proton donor, however, it resulted in moderate activity (**9c** vs **25**). Thus a proton donor at the 2′-position of the 6-phenyl ring was considered essential for the affinity to GPR54 and the phenolic hydroxy group was appropriate as the proton donor.

With the information above, we introduced a second substituent on the 6-phenyl ring to optimize the proton donor ability of the phenol. Introduction of a chloro (**9e**) or methoxy group (**9f**) at the 5′-position of the phenyl ring reduced activity compared to the unsubstituted compound **9b**, suggesting substitution at this position is sterically limited. Compounds having fluoro (**9g**) or chloro (**9h**) groups at 4′-position of the phenyl ring improved potency compared to **9b**, though the methoxy group (**9k**) showed decreased activity. These results indicate that an electron-withdrawing group, especially a fluoro group, is preferable at 4′-position of the phenyl ring.

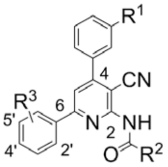
Table 1
Human GPR54 binding affinities and antagonistic activities (**1**)



Compound	R^1	R^2	RBA hGPR54 IC_{50} (μM)	Ca^{2+} mobilization assay hGPR54 IC_{50} (μM)
1	3′-CH_2OH	2-Thienyl	1.5	58% inhibition at 10 μM
11	3′-CO_2H	2-Thienyl	9.9	NT
7a	3′-NH_2	2-Thienyl	2.1	NT
19	3′-CH_2NH_2	2-Thienyl	0.57	NT
9a	3′-$NHCOCH_2NH_2$	2-Thienyl	0.051	2.8
9b	3′-$NHCO(CH_2)_2NH_2$	2-Thienyl	0.021	1.1
9c	3′-$NHCO(CH_2)_2NH_2$	Phenyl	0.051	24
9d	3′-$NHCO(CH_2)_3NH_2$	2-Thienyl	0.034	1.4
13a	2′-$CONH(CH_2)_2NH_2$	2-Thienyl	33	NT
13b	3′-$CONH(CH_2)_2NH_2$	2-Thienyl	0.045	5.1
13c	4′-$CONH(CH_2)_2NH_2$	2-Thienyl	0.12	NT

RBA = receptor binding assay. NT = not tested.

Table 2
Human GPR54 binding affinities and antagonistic activities (**2**)



Compound	R^1	R^2	R^3	RBA hGPR54 IC_{50} (μM)	Ca^{2+} mobilization hGPR54 IC_{50} (μM)
7a	NH_2	2-Thienyl	2′-OH	2.1	NT
23a	NH_2	2-Thienyl	2′-OMe	>30	NT
9b	$NHCO(CH_2)_2NH_2$	2-Thienyl	2′-OH	0.021	1.1
9c	$NHCO(CH_2)_2NH_2$	Phenyl	2′-OH	0.051	24
25	$NHCO(CH_2)_2NH_2$	Phenyl	2′-NH_2	0.89	NT
9e	$NHCO(CH_2)_2NH_2$	2-Thienyl	2′-OH-5′-Cl	0.49	NT
9f	$NHCO(CH_2)_2NH_2$	2-Thienyl	2′-OH-5′-OMe	0.48	NT
9g	$NHCO(CH_2)_2NH_2$	2-Thienyl	2′-OH-4′-F	0.0074	3.6
9h	$NHCO(CH_2)_2NH_2$	2-Thienyl	2′-OH-4′-Cl	0.0098	4.1
9i	$NHCO(CH_2)_2NH_2$	2-Thienyl	2′-OH-4′-Br	0.019	6.3
9j	$NHCO(CH_2)_2NH_2$	2-Thienyl	2′-OH-4′-Me	0.012	0.91
9k	$NHCO(CH_2)_2NH_2$	Phenyl	2′-OH-4′-OMe	0.12	NT

RBA = receptor binding assay. NT = not tested.

In order to optimize the amine moiety on the 4-phenyl ring and 2-acylamino group, we applied a combinatorial protocol to synthesize 148 compounds that satisfied both quality and quantity criteria. After rough RBA screening, compounds with high activity were evaluated for their IC_{50} value, as shown in Table 3. The results show that primary amines connected with a linear linker were suitable as the 3′-substituent of the 4-phenyl ring. For the acyl group, a 2-furyl group was most appropriate and a phenyl group bearing a methoxy group at the 2′- or 4′-position was comparable to a 2-thienyl group.

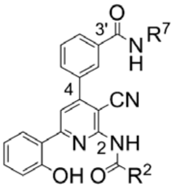
On the basis of the earlier results, we synthesized compounds with combinations of substituents that showed high potency (Table 4). As expected, these compounds exhibited markedly high potency, especially, compound **9l** showed the most potent affinity with an IC_{50} value of 3.7 nM, almost 1000-fold more active compared to hit compound **1**, with appropriate antagonistic activity of GPR54 in a cellular functional assay.

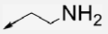
We succeeded to obtain an X-ray crystal structure of compound **13b** from methanol as shown in Figure 2. In the crystal structure, the 4-phenyl ring is twisted perpendicular to the pyridine ring, which may be caused by the steric repulsion of the cyano group. In contrast, the pyridine ring and 6-phenyl ring exist on same plane because of an intramolecular hydrogen bond between the pyridine nitrogen atom and the phenolic hydroxy group.

The intramolecular hydrogen bond is also indicated by the ^1H NMR spectrum in DMSO-d_6 using tetramethylsilane as internal standard (0 ppm).[14] As shown in Table 5, the chemical shift of the phenolic hydroxy group at the 2′-position, which can form an intramolecular hydrogen bond, was around 12 ppm (**7a**). On the other hand, the chemical shifts of the hydroxy groups at 3′- or 4′-position, which cannot make hydrogen bonds with the pyridine nitrogen atom, were approximately 10 ppm (**7b**,**c**).

In order to confirm the importance of the stereo disposition mentioned above towards the affinity for GPR54, we prepared

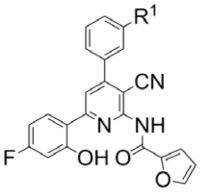
Table 3
Human GPR54 binding affinities for compounds synthesized by a combinatorial protocol

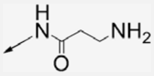
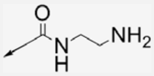
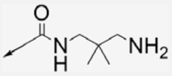


Compound	R^7	R^2	RBA hGPR54 IC_{50} (μM)
27a		2-Thienyl	0.030
27b		2-Furyl	0.014
27c		2′-MeOPh	0.045
27d		4′-MeOPh	0.031
27e		2-Furyl	0.015
27f		2′-MeOPh	0.032
27g		4′-MeOPh	0.032

RBA = receptor binding assay.

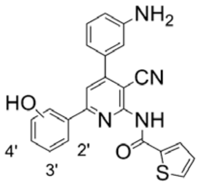
Table 4
Human GPR54 binding affinities and antagonistic activities (3)



Compound	R^1	RBA hGPR54 IC_{50} (μM)	Ca^{2+} mobilization hGPR54 IC_{50} (μM)
9l		0.0037	0.46
13d		0.0080	0.66
13e		0.011	2.5

RBA = receptor binding assay.

Table 5
The position of the phenolic hydroxy group on the 6-phenyl ring and the ^1H NMR chemical shifts in DMSO-d_6



Compound	**7a**	**7b**	**7c**
Position of OH	2′	3′	4′
Chemical shift of OH in ^1H NMR	11.87 ppm	9.69 ppm	10.07 ppm

DMSO-d_6 was used as solvent.

compound **9m** bearing a methyl group at the 5-position of the pyridine ring, which sterically hinders the intramolecular hydrogen bond (Fig. 3). As we expected, the chemical shift of the phenolic hydroxy group of **9m** was 9.89 ppm, which indicates cleavage of the intramolecular hydrogen bond. Moreover, the receptor binding affinity of **9m** was 100-fold less than that of **9b**, which may form the hydrogen bond as estimated by the chemical shift of the 2′-OH at 11.79 ppm.

These experimental results suggest the active conformation of the 4,6-diphenyl pyridines is almost the same as the crystal structure of compound **13b** and, particularly, the intramolecular hydrogen bond between the phenol and pyridine nitrogen atom appears to be essential for binding affinity.

4. Conclusion

In an attempt to find novel GPR54 antagonists, we synthesized 2-acylamino-4,6-diphenylpyridine derivatives and evaluated their activities. Since compound **19**, with a methylamino group at the 3′-position of the 4-phenyl ring showed moderate activity, detailed investigation of the amine side chain was conducted and led to identification of the β-alanine amide derivative **9b** having potent GPR54 binding affinity and antagonistic activity. Further investigation to optimize the 6-phenyl ring revealed that the hydroxy group at the 2′-position is essential for activity and introduction of an electron-withdrawing group at the 4′-position improved potency. With the use of combinatorial chemistry technology, a 2-furoyl

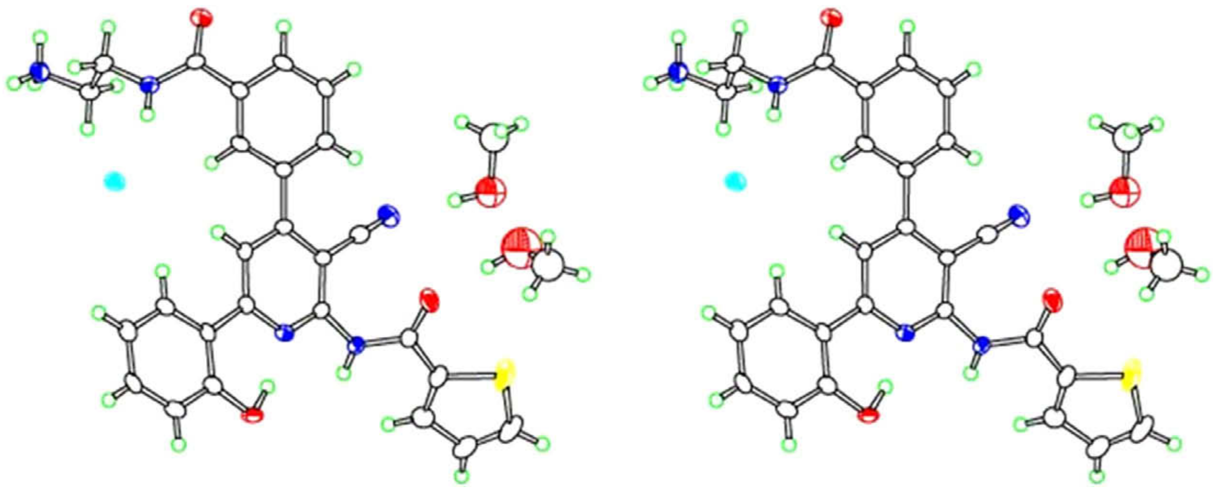
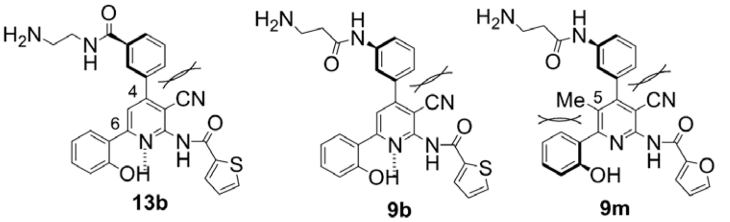


Figure 2. X-ray crystal structure of compound **13b**.



	13b	9b	9m
hGPR54 RBA (IC_{50})	0.045 μM	0.021 μM	1.2 μM
Chemical shift of 2'-OH in ^{1}H NMR	11.90 ppm	11.79 ppm	9.89 ppm

Figure 3. Human GPR54 binding affinities and ^{1}H NMR chemical shifts of the 2′-OH in DMSO-d_6.

group was found to be the most suitable 2-acyl group, and finally these structure–activity relationship studies led to compound **9l** with an IC_{50} value of 3.7 nM in a GPR54 binding assay. Moreover, these compounds showed apparent antagonistic activity in a cellular functional assay. In vivo examination, using these compounds for a proof-of-concept study as a drug for sex-hormone dependent disease, is now ongoing.

5. Experimental

Melting points were determined on a Büchi melting point apparatus and were not corrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker DPX300 (300 MHz) instruments. Chemical shifts are reported as δ values (ppm) downfield from internal tetramethylsilane of the indicated solution. Peak multiplicities are expressed as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; ddd, doublet of doublet of doublets; dt, doublet of triplet; br s, broad singlet; m, multiplet. Coupling constants (*J* values) are given in hertz (Hz). Elemental analyses were carried out by Takeda Analytical Laboratories. Reaction progress was determined by thin layer chromatography (TLC) analysis on Silica Gel 60 F_{254} plate (Merck) or NH TLC plates (Fuji Silysia chemical Ltd). Chromatographic purification was carried on silica gel columns 60 (0.063–0.200 mm or 0.040–0.063 mm, Merck), basic silica gel (ChromatorexNH, 100–200 mesh, Fuji silysia chemical Ltd) or Purif-Pack (SI 60 μM or NH 60 μM, Fuji Silysia, Ltd). Commercial reagents and solvents were used without additional purification.

5.1. 1-[5-Chloro-2-(methoxymethoxy)phenyl]ethanone (3d)

To an ice-cooled solution of 1-(5-chloro-2-hydroxyphenyl)ethanone (**2d**, 5.0 g, 29.3 mmol) in THF (50 ml) was added a 60% suspension of NaH in mineral oil (1.2 g, 29.3 mmol). After stirring at 0 °C for 30 min, to the mixture was added chloromethyl methyl ether (4.7 g, 58.6 mmol) and the whole mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/4) to give **3d** (5.6 g, 89%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.63 (3H, s), 3.51 (3H, s), 5.26 (2H, s), 7.15 (1H, d, *J* = 9.0 Hz), 7.37 (1H, dd, *J* = 9.0, 2.7 Hz), 7.67 (1H, d, *J* = 2.7 Hz).

5.2. 1-[5-Methoxy-2-(methoxymethoxy)phenyl]ethanone (3e)

Compound **3e** was prepared from 1-(2-hydroxy-5-methoxyphenyl)ethanone (**2e**) and chloromethyl methyl ether in a manner similar to that described for **3d** as a colorless oil in 79% yield: ^{1}H NMR ($CDCl_3$) δ 2.64 (3H, s), 3.51 (3H, s), 3.80 (3H, s), 5.21 (2H, s), 7.00 (1H, dd, *J* = 9.0, 3.3 Hz), 7.14 (1H, d, *J* = 9.0 Hz), 7.25 (1H, d, *J* = 3.3 Hz).

5.3. 1-[2-(Ethoxymethoxy)-4-fluorophenyl]ethanone (3f)

Compound **3f** was prepared from 1-(4-fluoro-2-hydroxyphenyl)ethanone (**2f**) and chloromethyl ethyl ether in a manner similar to that described for **3d** as a colorless oil in 90% yield: ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, *J* = 7.2 Hz), 2.61 (3H, s), 3.76 (2H, q, *J* = 7.2 Hz), 5.32 (2H, s), 6.74 (1H, tt, *J* = 8.1, 2.4 Hz), 6.96 (1H, dd, *J* = 10.8, 2.4 Hz), 7.78 (1H, t, *J* = 8.1 Hz).

5.4. 1-[4-Chloro-2-(methoxymethoxy)phenyl]ethanone (3g)

Compound **3g** was prepared from 1-(4-chloro-2-hydroxyphenyl)ethanone (**2g**) and chloromethyl methyl ether in a manner similar to that described for **3d** as a colorless oil in 68% yield: ^{1}H NMR ($CDCl_3$) δ 2.62 (3H, s), 3.53 (3H, s), 5.28 (2H, s), 7.03 (1H, dd, *J* = 8.4, 2.1 Hz), 7.22 (1H, d, *J* = 2.1 Hz), 7.68 (1H, d, *J* = 8.7 Hz).

5.5. 1-[4-Bromo-2-(methoxymethoxy)phenyl]ethanone (3h)

Compound **3h** was prepared from 1-(4-bromo-2-hydroxyphenyl)ethanone (**2h**) and chloromethyl methyl ether in a manner similar to that described for **3d** as a colorless oil in 79% yield: ^{1}H NMR ($CDCl_3$) δ 2.61 (3H, s), 3.52 (3H, s), 5.28 (2H, s), 7.19 (1H, dd, *J* = 8.4, 1.8 Hz), 7.37 (1H, d, *J* = 1.8 Hz), 7.60 (1H, d, *J* = 8.4 Hz).

5.6. 1-[2-(Ethoxymethoxy)-4-methylphenyl]ethanone (3i)

Compound **3i** was prepared from 1-(2-hydroxy-4-methylphenyl)ethanone (**2i**) and chloromethyl ethyl ether in a manner similar to that described for **3d** as a colorless oil in 75% yield: ^{1}H NMR ($CDCl_3$) δ 1.26 (3H, t, *J* = 7.2 Hz), 2.37 (3H, s), 2.61 (3H, s), 3.76 (2H, q, *J* = 7.2 Hz), 5.32 (2H, s), 6.84 (1H, d, *J* = 7.8 Hz), 7.01 (1H, s), 7.65 (1H, d, *J* = 7.8 Hz).

5.7. 1-[4-Methoxy-2-(methoxymethoxy)phenyl]ethanone (3j)

Compound **3j** was prepared from 1-(2-hydroxy-4-methoxyphenyl)ethanone (**2j**) and chloromethyl methyl ether in a manner similar to that described for **3d** as a colorless oil in 70% yield: ^{1}H NMR ($CDCl_3$) δ 2.60 (3H, s), 3.52 (3H, s), 3.84 (3H, s), 5.28 (2H, s), 6.58 (1H, dd, *J* = 8.7, 1.8 Hz), 6.70 (1H, d, *J* = 1.8 Hz), 7.81 (1H, d, *J* = 8.7 Hz).

5.8. 1-[4-Fluoro-2-(methoxymethoxy)phenyl]ethanone (3k)

Compound **3k** was prepared from 1-(4-fluoro-2-hydroxyphenyl)ethanone (**2f**) and chloromethyl methyl ether in a manner similar to that described for **3d** as a colorless oil in 92% yield: ^{1}H NMR (CDCl$_3$) δ 2.62 (3H, s), 3.53 (3H, s), 5.28 (2H, s), 6.70–6.80 (1H, m), 6.93 (1H, dd, J = 10.8, 2.4 Hz), 7.79 (1H, dd, J = 8.7, 6.9 Hz).

5.9. 1-[2-(Methoxymethoxy)phenyl]propan-1-one (3l)

Compound **3l** was prepared from 1-(2-hydroxyphenyl)propan-1-one (**2k**) and chloromethyl methyl ether in a manner similar to that described for **3d** as a colorless oil in 82% yield: ^{1}H NMR (CDCl$_3$) δ 1.18 (3H, t, J = 7.2 Hz), 3.01 (2H, q, J = 7.2 Hz), 3.51 (3H, s), 5.27 (2H, s), 7.05 (1H, td, J = 7.5, 0.6 Hz), 7.18 (1H, dd, J = 8.4, 0.6 Hz), 7.42 (1H, td, J = 7.5, 1.8 Hz), 7.65 (1H, dd, J = 7.5, 1.8 Hz).

5.10. 1-[2-(Methoxymethoxy)phenyl]ethanone (3m)

Compound **3m** was prepared from 1-(2-hydroxyphenyl)ethanone (**2a**) and chloromethyl methyl ether in a manner similar to that described for **3d** as a colorless oil in 100% yield: ^{1}H NMR (CDCl$_3$) δ 2.64 (3H, s), 3.50 (3H, s), 5.29 (2H, s), 7.05 (1H, t, J = 7.5 Hz), 7.13 (1H, d, J = 8.4 Hz), 7.44 (1H, t, J = 7.8 Hz), 7.71 (1H, d, J = 7.8 Hz).

5.11. 2-Amino-6-(3-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-4-(3-nitrophenyl)pyridine-3-carbonitrile (4b)

A mixture of 1-(3-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)ethanone[15] (**3b**, 9.86 g, 39.7 mmol), 3-nitrobenzaldehyde (6.00 g, 39.7 mmol), malononitrile (2.62 g, 39.7 mmol), and NH$_4$OAc (4.60 g, 59.6 mmol) in toluene (30 ml) was refluxed for 16 h. After cooling to room temperature, the reaction mixture was concentrated in vacuo and the residue was partitioned between saturated aqueous NaHCO$_3$ and EtOAc. The organic layer was washed with brine, dried over MgSO$_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/4) and triturated with Et$_2$O/hexane to give **4b** (1.88 g, 11%) as a colorless solid: ^{1}H NMR (CDCl$_3$) δ 0.25 (6H, s), 1.01 (9H, s), 5.42 (2H, s), 6.96 (1H, d, J = 8.1 Hz), 7.17 (1H, s), 7.34 (1H, t, J = 8.1 Hz), 7.49 (1H, t, J = 2.1 Hz), 7.57 (1H, d, J = 8.1 Hz), 7.74 (1H, t, J = 8.1 Hz), 7.99 (1H, dt, J = 7.5, 1.2 Hz), 8.38 (1H, dq, J = 8.4, 1.2 Hz), 8.47 (1H, t, J = 1.2 Hz).

5.12. 2-Amino-6-(4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-4-(3-nitrophenyl)pyridine-3-carbonitrile (4c)

Compound **4c** was prepared from 1-(4-{[*tert*-butyl(dimethyl)-silyl]oxy}phenyl)ethanone[16] (**3c**) and 3-nitrobenzaldehyde in a manner similar to that described for **4b** as a colorless amorphous solid in 11% yield: ^{1}H NMR (CDCl$_3$) δ 0.24 (6H, s), 1.00 (9H, s), 5.38 (2H, br s), 6.94 (2H, d, J = 8.7 Hz), 7.16 (1H, s), 7.73 (1H, t, J = 8.0 Hz), 7.91–8.01 (3H, m), 8.36 (1H, d, J = 8.4 Hz), 8.47 (1H, s).

5.13. 2-Amino-6-[5-chloro-2-(methoxymethoxy)phenyl]-4-(3-nitrophenyl)pyridine-3-carbonitrile (4d)

Compound **4d** was prepared from **3d** and 3-nitrobenzaldehyde in a manner similar to that described for **4b** as a light brown solid in 11% yield: mp 192–193 °C: ^{1}H NMR (CDCl$_3$) δ 3.46 (3H, s), 5.22 (2H, s), 5.42 (2H, br s), 7.18 (1H, d, J = 9.0 Hz), 7.35 (1H, dd, J = 9.0, 2.4 Hz), 7.41 (1H, s), 7.74 (1H, t, J = 7.1 Hz), 7.83 (1H, d, J = 2.4 Hz), 8.02 (1H, d, J = 8.7 Hz), 8.36 (1H, d, J = 8.4 Hz), 8.46 (1H, s).

5.14. 2-Amino-6-[5-methoxy-2-(methoxymethoxy)phenyl]-4-(3-nitrophenyl)pyridine-3-carbonitrile (4e)

Compound **4e** was prepared from **3e** and 3-nitrobenzaldehyde in a manner similar to that described for **4b** as a yellow solid in 39% yield: ^{1}H NMR (DMSO-d_6) δ 3.31 (3H, s), 3.77 (3H, s), 5.14 (2H, s), 7.02 (1H, d, J = 9.0, 3.3 Hz), 7.11 (2H, br s), 7.17 (1H, d, J = 9.0 Hz), 7.29 (1H, s), 7.33 (1H, d, J = 3.3 Hz), 7.87 (1H, t, J = 8.1 Hz), 8.12 (1H, d, J = 7.8 Hz), 8.39 (1H, dd, J = 7.8, 1.8 Hz), 8.44 (1H, d, J = 1.8 Hz).

5.15. 2-Amino-6-[2-(ethoxymethoxy)-4-fluorophenyl]-4-(3-nitrophenyl)pyridine-3-carbonitrile (4f)

Compound **4f** was prepared from **3f** and 3-nitrobenzaldehyde in a manner similar to that described for **4b** as a yellow solid in 19% yield: ^{1}H NMR (CDCl$_3$) δ 1.21 (3H, t, J = 7.2 Hz), 3.70 (2H, q, J = 7.2 Hz), 5.28 (2H, s), 5.39 (2H, br s), 6.85 (1H, t, J = 8.4 Hz), 7.01 (1H, dd, J = 10.8, 2.4 Hz), 7.38 (1H, s), 7.73 (1H, t, J = 8.1 Hz), 7.84 (1H, t, J = 7.8 Hz), 8.00 (1H, d, J = 7.8 Hz), 8.37 (1H, dq, J = 8.1, 1.2 Hz), 8.46 (1H, t, J = 2.4 Hz).

5.16. 2-Amino-6-[4-chloro-2-(methoxymethoxy)phenyl]-4-(3-nitrophenyl)pyridine-3-carbonitrile (4g)

Compound **4g** was prepared from **3g** and 3-nitrobenzaldehyde in a manner similar to that described for **4b** as a yellow solid in 25% yield: mp 196–198 °C; ^{1}H NMR (CDCl$_3$) δ 3.48 (3H, s), 5.24 (2H, s), 5.40 (2H, br s), 7.13 (1H, dd, J = 8.4, 2.1 Hz), 7.26 (1H, d, J = 2.1 Hz), 7.39 (1H, s), 7.71–7.81 (2H, m), 8.01 (1H, dt, J = 8.1, 0.9 Hz), 8.37 (1H, dq, J = 8.1, 0.9 Hz), 8.46 (1H, t, J = 1.8 Hz).

5.17. 2-Amino-6-[4-bromo-2-(methoxymethoxy)phenyl]-4-(3-nitrophenyl)pyridine-3-carbonitrile (4h)

Compound **4h** was prepared from **3h** and 3-nitrobenzaldehyde in a manner similar to that described for **4b** as a yellow solid in 27% yield: mp 201–202 °C; ^{1}H NMR (CDCl$_3$) δ 3.48 (3H, s), 5.23 (2H, s), 5.40 (2H, br s), 7.29 (1H, dd, J = 8.4, 1.8 Hz), 7.40 (2H, d, J = 7.5 Hz), 7.73 (2H, t, J = 8.1 Hz), 8.00 (1H, dt, J = 7.5, 1.2 Hz), 8.38 (1H, dt, J = 8.1, 1.2 Hz), 8.46 (1H, t, J = 1.8 Hz).

5.18. 2-Amino-6-[2-(ethoxymethoxy)-4-methylphenyl]-4-(3-nitrophenyl)pyridine-3-carbonitrile (4i)

Compound **4i** was prepared from **3i** and 3-nitrobenzaldehyde in a manner similar to that described for **4b** as a yellow solid in 31% yield: mp 148–149 °C; ^{1}H NMR (CDCl$_3$) δ 1.20 (3H, t, J = 7.2 Hz), 2.40 (3H, s), 3.70 (2H, q, J = 7.2 Hz), 5.27 (2H, s), 5.38 (2H, br s), 6.95 (1H, dd, J = 8.4, 0.9 Hz), 7.05 (1H, s), 7.42 (1H, s), 7.69–7.75 (2H, m), 8.02 (1H, d, J = 7.8 Hz), 8.36 (1H, dq, J = 8.4, 0.9 Hz), 8.46 (1H, t, J = 1.8 Hz).

5.19. 2-Amino-6-[4-methoxy-2-(methoxymethoxy)phenyl]-4-(3-nitrophenyl)pyridine-3-carbonitrile (4j)

Compound **4j** was prepared from **3j** and 3-nitrobenzaldehyde in a manner similar to that described for **4b** as a yellow solid in 22% yield: mp 171–172 °C; ^{1}H NMR (CDCl$_3$) δ 3.47 (3H, s), 3.86 (3H, s), 5.24 (2H, s), 5.37 (2H, br s), 6.69 (1H, dd, J = 8.7, 2.4 Hz), 6.78 (1H, d, J = 2.4 Hz), 7.44 (1H, s), 7.72 (1H, t, J = 8.0 Hz), 7.86 (1H, d, J = 8.7 Hz), 8.01 (1H, dd, J = 6.6, 1.2 Hz), 8.36 (1H, d, J = 8.4 Hz), 8.47 (1H, d, J = 2.1 Hz).

5.20. 2-Amino-6-[4-fluoro-2-(methoxymethoxy)phenyl]-4-(3-nitrophenyl)pyridine-3-carbonitrile (4k)

Compound **4k** was prepared from **3k** and 3-nitrobenzaldehyde in a manner similar to that described for **4b** as a pale yellow solid in 34% yield: mp 202–203 °C; ^{1}H NMR (CDCl$_3$) δ 3.48 (3H, s), 5.23 (2H, s), 5.39 (2H, s), 6.80–6.90 (1H, m), 6.99 (1H, dd, J = 10.8, 2.4 Hz), 7.38 (1H, s), 7.73 (1H, t, *J* = 7.8 Hz), 7.84 (1H, dd, *J* = 8.7, 6.9 Hz), 7.96–8.05 (1H, m), 8.35–8.40 (1H, m), 8.45–8.50 (1H, m).

5.21. 2-Amino-6-[2-(methoxymethoxy)phenyl]-5-methyl-4-(3-nitrophenyl)pyridine-3-carbonitrile (4l)

Compound **4l** was prepared from **3l** and 3-nitrobenzaldehyde in a manner similar to that described for **4b** as a pale yellow amorphous solid in 37% yield: ^{1}H NMR ($CDCl_3$) δ 1.82 (3H, s), 3.42 (3H, s), 5.17 (2H, s), 5.25 (2H, s), 7.13 (1H, t, *J* = 6.6 Hz), 7.23–7.28 (2H, m), 7.37–7.42 (1H, m), 7.72–7.74 (2H, m), 8.25 (1H, br s), 8.34–8.37 (1H, m).

5.22. Methyl 3-{2-amino-3-cyano-6-[2-(methoxymethoxy)-phenyl]pyridin-4-yl}benzoate (4m)

Compound **4m** was prepared from **3m** and methyl 3-formylbenzoate in a manner similar to that described for **4b** as a colorless solid in 28% yield: mp 129–130 °C; ^{1}H NMR ($CDCl_3$) δ 3.45 (3H, s), 3.95 (3H, s), 5.22 (2H, s), 5.35 (2H, br s), 7.14 (1H, t, *J* = 7.5 Hz), 7.22 (1H, d, *J* = 7.5 Hz), 7.37–7.42 (2H, m), 7.61 (1H, t, *J* = 7.8 Hz), 7.77 (1H, dd, *J* = 7.8, 1.8 Hz), 7.85–7.89 (1H, m), 8.15–8.19 (1H, m), 8.28 (1H, t, *J* = 1.7 Hz).

5.23. Methyl 2-{2-amino-3-cyano-6-[2-(methoxymethoxy)-phenyl]pyridin-4-yl}benzoate (4n)

Compound **4n** was prepared from **3m** and methyl 2-formylbenzoate in a manner similar to that described for **4b** as an orange solid in 19% yield: mp 109–110 °C; ^{1}H NMR (DMSO-d_6) δ 3.31 (3H, s), 3.68 (3H, s), 5.14 (2H, s), 6.93 (2H, s), 7.02 (1H, s), 7.13 (1H, t, *J* = 8.0 Hz), 7.20 (1H, d, *J* = 7.8 Hz), 7.37–7.49 (2H, m), 7.63 (1H, t, *J* = 8.2 Hz), 7.71–7.80 (2H, m), 7.95 (1H, dd, *J* = 7.8, 1.8 Hz).

5.24. Methyl 4-{2-amino-3-cyano-6-[2-(methoxymethoxy)-phenyl]pyridin-4-yl}benzoate (4o)

Compound **4o** was prepared from **3m** and methyl 4-formylbenzoate in a manner similar to that described for **4b** as a yellow solid in 35% yield: mp 170–171 °C; ^{1}H NMR ($CDCl_3$) δ 3.43 (3H, s), 3.96 (3H, s), 5.21 (2H, s), 5.36 (2H, br s), 7.14 (1H, td, *J* = 7.5, 0.9 Hz), 7.21 (1H, dd, *J* = 8.4, 0.9 Hz), 7.35–7.42 (2H, m), 7.69 (2H, d, *J* = 8.4 Hz), 7.77 (1H, dd, *J* = 7.2, 1.8 Hz), 8.18 (2H, d, *J* = 8.4 Hz).

5.25. Methyl 3-{2-amino-3-cyano-6-[4-fluoro-2-(methoxy-methoxy)phenyl]pyridin-4-yl}benzoate (4p)

Compound **4p** was prepared from **3k** and methyl 3-formylbenzoate in a manner similar to that described for **4b** as a yellow solid in 35% yield: mp 155–158 °C; ^{1}H NMR ($CDCl_3$) δ 3.46 (3H, s), 3.95 (3H, s), 5.22 (2H, s), 5.36 (2H, br s), 6.85 (1H, td, *J* = 8.4, 2.4 Hz), 6.98 (1H, dd, *J* = 10.8, 2.4 Hz), 7.34 (1H, s), 7.61 (1H, t, *J* = 7.8 Hz), 7.75–7.90 (2H, m), 8.10–8.20 (1H, m), 8.27 (1H, s).

5.26. *N*-[6-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}phenyl)-3-cyano-4-(3-nitrophenyl)pyridin-2-yl]thiophene-2-carboxamide (5a)

To a solution of 2-amino-6-(2-{[*tert*-butyl(dimethyl)silyl]oxy}-phenyl)-4-(3-nitrophenyl)pyridine-3-carbonitrile[17] (**4a**, 1.0 g, 2.2 mmol) in pyridine (10 ml) was added 2-thenoyl chloride (0.6 ml, 5.6 mmol) at room temperature and the whole was stirred at room temperature for 20 h. After the solvent was evaporated in vacuo, the residue was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc/hexane = 5/95 to 50/50) and recrystallization from EtOAc/hexane to give **5a** (0.59 g, 47%) as a colorless solid: mp 106–108 °C; ^{1}H NMR ($CDCl_3$) δ 0.12 (6H, s), 0.83 (9H, s), 6.95 (1H, dd, *J* = 0.9, 9.3 Hz), 7.13 (1H, t, *J* = 7.5 Hz), 7.18–7.21 (1H, m), 7.37 (1H, t, *J* = 7.8 Hz), 7.67 (1H, dd, *J* = 4.8, 0.9 Hz), 7.75 (1H, t, *J* = 8.1 Hz), 7.78–7.81 (1H, m), 7.85 (1H, dd, *J* = 7.5, 1.5 Hz), 7.97 (1H, s), 8.05 (1H, d, *J* = 7.5 Hz), 8.41 (1H, d, *J* = 7.5 Hz), 8.46 (1H, s), 8.49 (1H, s).

5.27. *N*-[6-(3-{[*tert*-Butyl(dimethyl)silyl]oxy}phenyl)-3-cyano-4-(3-nitrophenyl)pyridin-2-yl]thiophene-2-carboxamide (5b)

Compound **5b** was prepared from **4b** in a manner similar to that described for **5a** as a pale yellow amorphous solid in 98% yield: ^{1}H NMR ($CDCl_3$) δ 0.23 (6H, s), 0.99 (9H, s), 6.87 (1H, d, *J* = 9.0 Hz), 7.15 (1H, dd, *J* = 4.8, 3.9 Hz), 7.56 (1H, s), 7.62–7.69 (2H, m), 7.86 (1H, dd, *J* = 3.6, 0.6 Hz), 7.93–7.99 (3H, m), 8.31 (1H, dd, *J* = 6.9, 1.8 Hz), 8.41 (1H, t, *J* = 1.8 Hz), 8.83 (1H, s).

5.28. *N*-[6-(4-{[*tert*-Butyl(dimethyl)silyl]oxy}phenyl)-3-cyano-4-(3-nitrophenyl)pyridin-2-yl]thiophene-2-carboxamide (5c)

Compound **5c** was prepared from **4c** in a manner similar to that described for **5a** as a pale yellow amorphous solid in 78% yield: ^{1}H NMR ($CDCl_3$) δ 0.23 (6H, s), 1.00 (9H, s), 6.97 (1H, d, *J* = 8.1 Hz), 7.17 (1H, dd, *J* = 4.8, 3.9 Hz), 7.35 (1H, t, *J* = 8.1 Hz), 7.52 (1H, t, *J* = 1.8 Hz), 7.62–7.66 (3H, m), 7.74 (1H, t, *J* = 8.1 Hz), 7.83 (1H, dd, *J* = 3.9, 0.9 Hz), 8.06 (1H, d, *J* = 7.8 Hz), 8.38 (1H, d, *J* = 8.1 Hz), 8.66 (1H, s).

5.29. *N*-[6-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}phenyl)-3-cyano-4-(3-nitrophenyl)pyridin-2-yl]benzamide (5d)

Compound **5d** was prepared from **4a** and benzoyl chloride in a manner similar to that described for **5a** as a colorless solid in 77% yield: ^{1}H NMR ($CDCl_3$) δ 0.12 (6H, s), 0.80 (9H, s), 6.94–6.97 (1H, m), 7.09–7.15 (1H, m), 7.33–7.39 (1H, m), 7.52–7.57 (2H, m), 7.61–7.66 (1H, m), 7.76 (1H, t, *J* = 7.8 Hz), 7.83 (1H, dd, *J* = 7.8, 1.8 Hz), 7.98 (1H, s), 8.00–8.09 (3H, m), 8.38–8.42 (1H, m), 8.50 (1H, t, *J* = 1.8 Hz), 8.57 (1H, br s).

5.30. *N*-{6-[5-Chloro-2-(methoxymethoxy)phenyl]-3-cyano-4-(3-nitrophenyl)pyridin-2-yl}thiophene-2-carboxamide (5e)

To a solution of **4d** (0.50 g, 1.2 mmol) in pyridine (30 ml) was added 2-theonyl chloride (0.45 g, 3.0 mmol) and the whole was stirred at room temperature for 16 h. To the solution was added 2-theonyl chloride (0.98 g, 6.7 mmol) and the mixture was stirred at room temperature for 72 h. To the solution was added 28% aqueous ammonia solution (3 ml) and the mixture was stirred at room temperature for 1 h. After the solvent was evaporated in vacuo, the residue was diluted with water and extracted with EtOAc twice. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/2 to 1/1) and recrystallization from EtOAc/Et_2O to give **5e** (0.36 g, 57%) as a colorless solid: mp 161–162 °C; ^{1}H NMR (DMSO-d_6) δ 3.48 (3H, s), 5.27 (2H, s), 7.19–7.26 (3H, m), 7.39–7.42 (1H, m), 7.68 (1H, d, *J* = 4.8 Hz), 7.75–7.83 (2H, m), 7.95 (1H, s), 7.99 (1H, s), 8.13–8.52 (3H, m).

5.31. *N*-{3-Cyano-6-[5-methoxy-2-(methoxymethoxy)phenyl]-4-(3-nitrophenyl)pyridin-2-yl}thiophene-2-carboxamide (5f)

Compound **5f** was prepared from **4e** in a manner similar to that described for **5e** as a pale yellow amorphous solid in 24% yield: ^{1}H NMR ($CDCl_3$) δ 3.47 (3H, s), 3.86 (3H, s), 5.20 (2H, s), 7.00 (1H, dd, *J* = 9.0, 3.3 Hz), 7.19–7.26 (3H, m), 7.47 (1H, d, *J* = 3.3 Hz), 7.67 (1H, d, *J* = 4.5 Hz), 7.45–7.83 (2H, m), 8.00 (1H, s), 8.14 (1H, d, *J* = 7.8 Hz), 8.40 (1H, d, *J* = 7.8 Hz), 8.53 (1H, t, *J* = 2.1 Hz).

5.32. *N*-{3-Cyano-6-[2-(ethoxymethoxy)-4-fluorophenyl]-4-(3-nitrophenyl)pyridin-2-yl}thiophene-2-carboxamide (5g)

Compound **5g** was prepared from **4f** in a manner similar to that described for **5e** as a yellow powder in 70% yield: mp 166–167 °C; ^{1}H NMR (CDCl$_3$) δ 1.22 (3H, t, *J* = 6.9 Hz), 3.73 (2H, q, *J* = 6.9 Hz), 5.33 (2H, s), 6.88 (1H, t, *J* = 7.2 Hz), 7.05 (1H, d, *J* = 10.8 Hz), 7.20 (1H, t, *J* = 4.4 Hz), 7.63–7.84 (3H, m), 7.97–8.02 (2H, m), 8.13 (1H, d, *J* = 7.8 Hz), 8.39–8.51 (3H, m).

5.33. *N*-{6-[4-Chloro-2-(methoxymethoxy)phenyl]-3-cyano-4-(3-nitrophenyl)pyridin-2-yl}thiophene-2-carboxamide (5h)

Compound **5h** was prepared from **4g** in a manner similar to that described for **5e** as a pale yellow amorphous solid in 74% yield: ^{1}H NMR (CDCl$_3$) δ 3.51 (3H, s), 5.29 (2H, s), 7.14–7.21 (2H, m), 7.30 (1H, d, *J* = 1.8 Hz), 7.67 (1H, d, *J* = 5.1 Hz), 7.75–7.82 (2H, m), 7.92–7.98 (2H, m), 8.13 (1H, d, *J* = 7.8 Hz), 8.41 (1H, d, *J* = 7.5 Hz), 8.43–8.52 (2H, m).

5.34. *N*-{6-[4-Bromo-2-(methoxymethoxy)phenyl]-3-cyano-4-(3-nitrophenyl)pyridin-2-yl}thiophene-2-carboxamide (5i)

Compound **5i** was prepared from **4h** in a manner similar to that described for **5e** as a yellow solid in 87% yield: mp 166–167 °C; ^{1}H NMR (CDCl$_3$) δ 3.51 (3H, s), 5.29 (2H, s), 7.20 (1H, t, *J* = 4.4 Hz), 7.32 (1H, dd, *J* = 8.1, 1.5 Hz), 7.45 (1H, s), 7.67 (1H, d, *J* = 4.4 Hz), 7.75–7.87 (3H, m), 7.98 (1H, s), 8.13 (1H, d, *J* = 7.5 Hz), 8.41 (1H, dd, *J* = 8.1, 1.5 Hz), 8.47 (1H, s), 8.51 (1H, t, *J* = 1.5 Hz).

5.35. *N*-{3-Cyano-6-[2-(ethoxymethoxy)-4-methylphenyl]-4-(3-nitrophenyl)pyridin-2-yl}thiophene-2-carboxamide (5j)

Compound **5j** was prepared from **4i** in a manner similar to that described for **5e** as a yellow solid in 50% yield: mp 121–123 °C; ^{1}H NMR (CDCl$_3$) δ 1.21 (3H, t, *J* = 6.8 Hz), 2.42 (3H, s), 3.73 (2H, q, *J* = 6.8 Hz), 5.33 (2H, s), 6.98 (1H, d, *J* = 7.8 Hz), 7.10 (1H, s), 7.19 (1H, t, *J* = 4.5 Hz), 7.65–7.87 (4H, m), 8.01 (1H, br s), 8.14 (1H, d, *J* = 7.8 Hz), 8.40 (1H, dt, *J* = 7.5, 0.9 Hz), 8.49–8.52 (2H, m).

5.36. *N*-{3-Cyano-6-[4-methoxy-2-(methoxymethoxy)phenyl]-4-(3-nitrophenyl)pyridin-2-yl}benzamide (5k)

Compound **5k** was prepared from **4j** and benzoyl chloride in a manner similar to that described for **5e** as a yellow amorphous solid in 95% yield: ^{1}H NMR (CDCl$_3$) δ 3.49 (3H, s), 3.83 (3H, s), 5.27 (2H, s), 6.64 (1H, dd, *J* = 8.7, 2.1 Hz), 6.77 (1H, d, *J* = 2.1 Hz), 7.48–7.62 (3H, m), 7.73 (1H, t, *J* = 8.1 Hz), 7.94 (1H, d, *J* = 8.7 Hz), 8.00–8.02 (3H, m), 8.11 (1H, d, *J* = 7.2 Hz), 8.36 (1H, d, *J* = 7.2 Hz), 8.49 (1H, d, *J* = 1.8 Hz), 8.86 (1H, s).

5.37. *N*-{3-Cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-4-(3-nitrophenyl)pyridin-2-yl}furan-2-carboxamide (5l)

Compound **5l** was prepared from **4k** and 2-furoyl chloride in a manner similar to that described for **5a** as a colorless solid in 90% yield: mp 177–179 °C; ^{1}H NMR (DMSO-d_6) δ 3.37 (3H, s), 5.36 (2H, s), 6.77 (1H, dd, *J* = 3.4, 1.9 Hz), 7.01–7.10 (1H, m), 7.18 (1H, dd, *J* = 11.3, 2.3 Hz), 7.55 (1H, d, *J* = 3.8 Hz), 7.90–8.06 (3H, m), 8.11 (1H, s), 8.17–8.23 (1H, m, *J* = 8.3 Hz), 8.43–8.48 (1H, m), 8.55 (1H, t, *J* = 1.9 Hz), 11.37 (1H, br s). Anal. Calcd for $C_{25}H_{17}FN_4O_6$: C, 61.48; H, 3.51; N, 11.47. Found: C, 61.58; H, 3.69; N, 11.43.

5.38. *N*-{3-Cyano-6-[2-(methoxymethoxy)phenyl]-5-methyl-4-(3-nitrophenyl)pyridin-2-yl}furan-2-carboxamide (5m)

Compound **5m** was prepared from **4l** and 2-furoyl chloride in a manner similar to that described for **5a** as a colorless amorphous solid in 100% yield: ^{1}H NMR (DMSO-d_6) δ 2.00 (3H, s), 3.42 (3H, s), 5.17 (2H, s), 6.59 (1H, q, *J* = 1.8 Hz), 7.16 (1H, td, *J* = 7.5, 0.9 Hz), 7.26–7.37 (3H, m), 7.41–7.47 (1H, m), 7.55 (1H, q, *J* = 0.9 Hz), 7.74–7.82 (2H, m), 8.32 (1H, s), 8.37–8.41 (1H, m), 8.71 (1H, s).

5.39. *N*-[4-(3-Aminophenyl)-6-(2-{[*tert*-butyl(dimethyl)silyl]-oxy}phenyl)-3-cyanopyridin-2-yl]thiophene-2-carboxamide (6a)

A mixture of **5a** (0.20 g, 0.36 mmol), iron powder (0.12 g, 2.16 mmol), and NH$_4$Cl (0.13 g, 2.3 mmol) in 50% aqueous EtOH (20 ml) was stirred under reflux for 20 min. After cooling to room temperature, the solution was diluted with saturated aqueous NaHCO$_3$ and extracted with EtOAc. The extract was washed with brine, dried over MgSO$_4$, and concentrated in vacuo to give **6a** (0.18 g, 95%) as an amorphous solid: ^{1}H NMR (DMSO-d_6) δ 0.18 (6H, s), 0.76 (9H, s), 5.39–5.43 (2H, m), 6.71–6.81 (2H, m), 6.88 (1H,s), 7.03 (1H, d, *J* = 8.1 Hz), 7.12–7.31(3H, m), 7.44 (1H, t, *J* = 7.7 Hz), 7.79–7.92 (2H, m), 7.96 (1H, d, *J* = 4.8 Hz), 8.14 (1H, d, *J* = 3.0 Hz), 11.46 (1H, s).

5.40. *N*-[4-(3-Aminophenyl)-6-(3-{[*tert*-butyl(dimethyl)-silyl]oxy}phenyl)-3-cyanopyridin-2-yl]thiophene-2-carboxamide (6b)

Compound **6b** was prepared from **5b** in a manner similar to that described for **6a** as a yellow amorphous solid in 89% yield: ^{1}H NMR (CDCl$_3$) δ 0.21 (6H, s), 0.99 (9H, s), 3.84–3.99 (2H, br s), 6.74 (1H, dd, *J* = 8.1, 1.8 Hz), 6.90–6.97 (3H, m), 7.06 (1H, t, *J* = 4.4 Hz), 7.18–7.31 (2H, m), 7.49–7.61 (4H, m), 7.76 (1H, d, *J* = 3.6 Hz), 8.91(1H, s).

5.41. *N*-[4-(3-Aminophenyl)-6-(4-{[*tert*-butyl(dimethyl)silyl]-oxy}phenyl)-3-cyanopyridin-2-yl]thiophene-2-carboxamide (6c)

Compound **6c** was prepared from **5c** in a manner similar to that described for **6a** as a yellow amorphous solid in 91% yield: ^{1}H NMR (CDCl$_3$) δ 0.23 (6H, s), 1.00 (9H, s), 3.80–4.00 (2H, br s), 6.74 (1H, dd, *J* = 7.8, 1.8 Hz), 6.84–6.96 (4H, m), 7.11 (1H, dd, *J* = 4.8, 3.9 Hz), 7.21 (1H, t, *J* = 7.8 Hz), 7.52 (1H, s), 7.58 (1H, d, *J* = 3.9 Hz), 7.81 (1H, d, *J* = 2.7 Hz), 7.93 (2H, d, *J* = 8.7 Hz), 8.82(1H, s).

5.42. *N*-{4-(3-Aminophenyl)-6-[5-chloro-2-(methoxymethoxy)-phenyl]-3-cyanopyridin-2-yl}thiophene-2-carboxamide (6e)

Compound **6e** was prepared from **5e** in a manner similar to that described for **6a** as a light brown solid in 74% yield: mp 180–182 °C; ^{1}H NMR (CDCl$_3$) δ 3.45 (3H, s), 5.23 (2H, s), 6.69–6.91 (1H, m), 7.04–7.08 (2H, m), 7.19–7.38 (4H, m), 7.64 (1H, d, *J* = 7.5 Hz), 7.81–7.88 (3H, m), 8.30–8.60 (1H, m).

5.43. *N*-{4-(3-Aminophenyl)-3-cyano-6-[5-methoxy-2-(methoxymethoxy)phenyl]pyridin-2-yl}thiophene-2-carboxamide (6f)

Compound **6f** was prepared from **5f** in a manner similar to that described for **6a** as a yellow amorphous solid in 99% yield: ^{1}H NMR (CDCl$_3$) δ 3.42 (3H, s), 3.85 (3H, s), 5.16 (2H, s), 6.84 (1H, d, *J* = 7.8 Hz), 6.97 (1H, dd, *J* = 9.0, 3.3 Hz), 7.03–7.06 (2H, m), 7.16–7.34 (4H, m), 7.40 (1H, br s), 7.63 (1H, d, *J* = 4.8 Hz), 7.80 (1H, d, *J* = 3.6 Hz), 7.85–8.00 (1H, m).

5.44. *N*-{4-(3-Aminophenyl)-6-[4-chloro-2-(methoxymethoxy)-phenyl]-3-cyanopyridin-2-yl}thiophene-2-carboxamide (6h)

Compound **6h** was prepared from **5h** in a manner similar to that described for **6a** as a green amorphous solid in 100% yield: ^{1}H NMR ($CDCl_3$) δ 3.39 (3H, s), 3.70–4.00 (2H, m), 5.15 (2H, s), 6.72 (1H, d, *J* = 7.2 Hz), 6.90–7.20 (5H, m), 7.54 (1H, br s), 7.65–7.80 (4H, m), 8.54 (1H, br s).

5.45. *N*-{4-(3-Aminophenyl)-3-cyano-6-[4-methoxy-2-(methoxymethoxy)phenyl]pyridin-2-yl}benzamide (6k)

Compound **6k** was prepared from **5k** in a manner similar to that described for **6a** as a yellow amorphous solid in 100% yield: ^{1}H NMR ($CDCl_3$) δ 3.42 (3H, s), 3.80 (3H, s), 5.20 (2H, s), 6.62 (1H, dd, *J* = 9.0, 2.1 Hz), 6.75–6.77 (2H, m), 6.96–7.00 (2H, m), 7.25 (1H, t, *J* = 7.8 Hz), 7.46 (2H, t, *J* = 7.4 Hz), 7.53 (1H, d, *J* = 7.2 Hz), 7.85–7.98 (4H, m), 8.85 (1H, s).

5.46. *N*-{4-(3-Aminophenyl)-3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]pyridin-2-yl}furan-2-carboxamide (6l)

Compound **6l** was prepared from **5l** in a manner similar to that described for **6a** as a colorless solid in 90% yield: mp 204–206 °C; ^{1}H NMR (DMSO-d_6) δ 3.36 (3H, s), 5.38 (2H, s), 5.45 (2H, s), 6.71–6.81 (3H, m), 6.85 (1H, d, *J* = 1.9 Hz), 6.99–7.09 (1H, m), 7.13–7.27 (2H, m), 7.52 (1H, d, *J* = 3.4 Hz), 7.92–7.99 (2H, m), 8.02 (1H, s), 11.09 (1H, br s). Anal. Calcd for $C_{25}H_{19}FN_4O_4 \cdot H_2O$: C, 63.02; H, 4.44; N, 11.75. Found: C, 63.28; H, 4.07; N, 11.59.

5.47. *N*-{4-(3-Aminophenyl)-3-cyano-6-[2-(methoxymethoxy)-phenyl]-5-methylpyridin-2-yl}furan-2-carboxamide (6m)

Compound **6m** was prepared from **5m** in a manner similar to that described for **6a** as a colorless amorphous solid in 92% yield: ^{1}H NMR ($CDCl_3$) δ 2.00 (3H, s), 3.38 (3H, s), 3.89 (2H, br s), 5.13 (2H, s), 6.53 (1H, q, *J* = 1.8 Hz), 6.69–6.77 (3H, m), 7.12 (1H, td, *J* = 7.8, 0.9 Hz), 7.22–7.42 (5H, m), 7.50 (1H, s), 8.82 (1H, s).

5.48. *N*-[4-(3-Aminophenyl)-3-cyano-6-(2-hydroxyphenyl)-pyridin-2-yl]thiophene-2-carboxamide (7a)

To an ice-cooled solution of **6a** (0.33 g, 0.63 mmol) in THF (6 ml) was added TBAF (1 M in THF, 2 ml). After stirring at 0 °C for 40 min, the reaction mixture was diluted with water and extracted with EtOAc. The extract was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was treated with $CHCl_3$ and the precipitated solid was collected by filtration. The mother liquid was purified by silica gel column chromatography (EtOAc/hexane = 2/98 to 70/30) to give a yellow solid. The solids were combined and recrystallized from DMF/Et_2O to give **7a** (45 mg, 17%) as a yellow solid: mp 283–284 °C; ^{1}H NMR (DMSO-d_6) δ 5.44 (2H, s), 6.72–6.85 (2H, m), 6.88 (1H, s), 7.18–7.46 (3H, m), 8.00 (1H, d, *J* = 4.8 Hz), 8.08–8.19 (3H, m), 11.39 (1H, s), 11.87 (1H, s). Anal. Calcd for $C_{23}H_{16}N_4O_2S$: C, 66.97; H, 3.91; N, 13.58. Found: C, 66.77; H, 4.02; N, 13.60.

5.49. *N*-[4-(3-Aminophenyl)-3-cyano-6-(3-hydroxyphenyl)-pyridin-2-yl]thiophene-2-carboxamide (7b)

Compound **7b** was prepared from **6b** in a manner similar to that described for **7a** as light brown solid in 57% yield: mp 246–247 °C; ^{1}H NMR (DMSO-d_6) δ 5.42 (2H, s), 6.75 (1H, d, *J* = 7.8 Hz), 6.84 (1H, d, *J* = 7.5 Hz), 6.89–6.95 (2H, m), 7.21 (1H, d, *J* = 7.8 Hz), 7.26–7.37 (2H, m), 7.64 (1H, s), 7.66 (1H, d, *J* = 8.1 Hz), 7.90 (1H, s), 7.97 (1H, d, *J* = 0.6 Hz), 8.16 (1H, d, *J* = 3.0 Hz), 9.69 (1H, s), 11.32 (1H, s). Anal. Calcd for $C_{23}H_{16}N_4O_2S$: C, 66.97; H, 3.91; N, 13.58. Found: C, 66.93; H, 3.91; N, 13.69.

5.50. *N*-[4-(3-Aminophenyl)-3-cyano-6-(4-hydroxyphenyl)-pyridin-2-yl]thiophene-2-carboxamide (7c)

Compound **7c** was prepared from **6c** in a manner similar to that described for **7a** as a light brown solid in 51% yield: mp 264–265 °C; ^{1}H NMR (DMSO-d_6) δ 5.40 (2H, s), 6.75 (2H, d, *J* = 7.8 Hz), 6.82 (1H, d, *J* = 7.8 Hz), 6.88–6.92 (3H, m), 7.20–7.30 (2H, m), 7.86 (1H, s), 7.96 (1H, d, *J* = 4.8 Hz), 8.11–8.16 (3H, m), 10.07 (1H, br s), 11.35 (1H, br s). Anal. Calcd for $C_{23}H_{16}N_4O_2S$: C, 66.97; H, 3.91; N, 13.58. Found: C, 66.82; H, 3.90; N, 13.63.

5.51. *N*-[4-(3-Aminophenyl)-3-cyano-6-(2-hydroxyphenyl)-pyridin-2-yl]benzamide (7d)

A mixture of **5d** (0.80 g, 1.5 mmol), iron powder (0.41 g, 7.3 mmol), and NH_4Cl (0.40 g, 7.5 mmol) in EtOH (10 ml), THF (5 ml) and water (5 ml) was stirred under reflux for 2 h. After cooling to room temperature, the solution was diluted with water and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/2) to give *N*-[4-(3-aminophenyl)-6-(2-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-3-cyanopyridin-2-yl]benzamide (**6d**, 0.55 g, 72%) as a yellow amorphous solid.

Compound **7d** was prepared from **6d** in a manner similar to that described for **7a** as a yellow solid in 65% yield: mp 259–261 °C; ^{1}H NMR (DMSO-d_6) δ 5.45 (2H, br s), 6.76–6.79 (1H, m), 6.82–6.85 (1H, m), 6.90 (1H, t, *J* = 2.1 Hz), 6.94–7.01 (2H, m), 7.25 (1H, t, *J* = 7.8 Hz), 7.36–7.42 (1H, m), 7.59–7.64 (2H, m), 7.67–7.72 (1H, m), 8.05–8.08 (2H, m), 8.12 (1H, s), 8.15 (1H, dd, *J* = 8.1, 1.5 Hz), 11.41(1H, s), 11.99 (1H, s). Anal. Calcd for $C_{25}H_{18}N_4O_2$: C, 73.88; H, 4.46; N, 13.78. Found: C, 73.60; H, 4.42; N, 13.73.

5.52. *tert*-Butyl {2-[(3-{3-cyano-6-(2-hydroxyphenyl)-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)amino]-2-oxoethyl}carbamate (8a)

To an ice-cooled solution of **6a** (0.13 g, 0.25 mmol), [(*tert*-butoxycarbonyl)amino]acetic acid (86 mg, 0.50 mmol), and HOBt (66 mg, 0.50 mmol) in DMF (4 ml) was added WSCD (94 mg, 0.50 mmol). After stirring at room temperature for 17 h, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc twice. The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/1 to 3/2) to give a mixture of *tert*-butyl {2-[(3-{6-(2-{[*tert*-butyl(dimethyl)silyl]-oxy}phenyl)-3-cyano-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)amino]-2-oxoethyl}carbamate and **8a** (0.10 g) as a pale yellow solid.

The mixture was treated with TBAF in a manner similar to that described for **7a** to give **8a** as a yellow solid in 40% yield from **6a**: mp 206–215 °C; ^{1}H NMR ($CDCl_3$) δ 1.56 (9H, s), 3.98 (2H, d, *J* = 6.0 Hz), 5.20–5.30 (1H, m), 6.90 (1H, t, *J* = 7.5 Hz), 7.10 (1H, d, *J* = 7.5 Hz), 7.19 (1H, dd, *J* = 4.8, 3.9 Hz), 7.35–7.40 (2H, m), 7.50 (1H, t, *J* = 7.8 Hz), 7.60 (1H, d, *J* = 8.1 Hz), 7.67–7.69 (2H, m), 7.77–7.81 (2H, m), 7.95 (1H, s), 8.45–8.50 (1H, m), 8.80 (1H, s), 13.15–13.25 (1H, m).

5.53. *tert*-Butyl {3-[(3-{3-cyano-6-(2-hydroxyphenyl)-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)amino]-3-oxopropyl}carbamate (8b)

To an ice-cooled solution of **6a** (0.30 g, 0.57 mmol), 3-[(*tert*-butoxycarbonyl)amino]propanoic acid (0.11 g, 0.57 mmol), and HOBt (77 mg, 0.57 mmol) in DMF (8 ml) was added WSCD (0.11 g, 0.57 mmol). After stirring at room temperature for 12 h, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/1 to 2/1) and recrystallization from $CHCl_3/Et_2O$ to give **8b** (0.13 g, 40%) as a pale yellow solid: mp 230–231 °C; ^{1}H NMR (DMSO-d_6) δ 1.38 (9H, s), 2.40–2.54 (2H, m), 3.22–3.27 (2H, m), 6.89–7.01 (3H, m), 7.28–7.42 (3H, m), 7.54 (1H, t, J = 8.0 Hz), 7.80 (1H, d, J = 8.1 Hz), 7.99 (2H, s), 8.12–8.14 (3H, m), 10.24 (1H, s), 11.44 (1H, s), 11.75–11.85 (1H, m).

5.54. *tert*-Butyl {3-[(3-{3-cyano-6-(2-hydroxyphenyl)-2-[(phenylcarbonyl)amino]pyridin-4-yl}phenyl)amino]-3-oxopropyl}-carbamate (8c)

Compound **8c** was prepared from **7d** and 3-[(*tert*-butoxycarbonyl)amino]propanoic acid in a manner similar to that described for **8b** as a pale yellow solid in 74% yield: mp 220–221 °C; ^{1}H NMR (DMSO-d_6) δ 1.38 (9 H, s), 2.53–2.55 (2H, m), 3.21–3.27 (2H, m), 6.88 (1H, br s), 6.95–7.01 (2H, m), 7.37–7.43 (2H, m), 7.52–7.73 (4H, m), 7.82 (1H, brd, J = 8.4 Hz), 8.00 (1H, br s), 8.06–8.08 (2H, m), 8.13–8.17 (2H, m), 10.24 (1H, br s), 11.45 (1H, br s), 11.92 (1H, br s). Anal. Calcd for $C_{33}H_{31}N_5O_5$: C, 68.62; H, 5.41; N, 12.12. Found: C, 68.37; H, 5.36; N, 12.11.

5.55. *tert*-Butyl {4-[(3-{3-cyano-6-(2-hydroxyphenyl)-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)amino]-4-oxobutyl}carbamate (8d)

Compound **8d** was prepared from **6a** and 4-[(*tert*-butoxycarbonyl)amino]butanoic acid in a manner similar to that described for **8b** as a colorless amorphous solid in 91% yield: ^{1}H NMR (DMSO-d_6) δ 1.56 (9H, s), 1.66–1.75 (2H, m), 2.35 (2H, t, J = 7.4 Hz), 2.89–3.01 (2H, m), 6.80–6.90 (1H, m), 6.95–7.01 (2H, m), 7.31–7.59 (4H, m), 7.70–7.80 (1H, m), 7.90–8.05 (3H, m), 8.14 (2H, d, J = 7.8 Hz), 10.21 (1H, s), 11.45 (1H, s), 11.80–11.90 (1H, m).

5.56. *tert*-Butyl {3-[(3-{6-[5-chloro-2-(methoxymethoxy)-phenyl]-3-cyano-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)amino]-3-oxopropyl}carbamate (8e)

Compound **8e** was prepared from **6e** and 3-[(*tert*-butoxycarbonyl)amino]propanoic acid in a manner similar to that described for **8b** as a colorless solid in 69% yield: mp 230–231 °C; ^{1}H NMR (DMSO-d_6) δ 1.38 (9H, s), 3.21–3.28 (2H, m), 3.78 (3H, s), 5.37 (2H, s), 6.80–6.90 (1H, m), 7.27–7.40 (3H, m), 7.52–7.58 (2H, m), 7.73 (1H, d, J = 7.8 Hz), 7.98 (2H, d, J = 3.0 Hz), 8.06–8.16 (3H, m), 10.24 (1H, s), 11.41–11.50 (1H, m).

5.57. *tert*-Butyl {3-[(3-{3-cyano-6-[5-methoxy-2-(methoxymethoxy)phenyl]-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)amino]-3-oxopropyl}carbamate (8f)

Compound **8f** was prepared from **6f** and 3-[(*tert*-butoxycarbonyl)amino]propanoic acid in a manner similar to that described for **8b** as a yellow solid in 63% yield: mp 167–168 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (9H, s), 2.62 (2H, t, J = 5.7 Hz), 3.43 (3H, s), 3.49 (2H, q, J = 5.9 Hz), 3.84 (3H, s), 5.12 (2H, s), 6.97 (1H, dd, J = 9.0, 3.3 Hz), 7.15–7.22 (2H, m), 7.43–7.50 (3H, m), 7.62–7.68 (2H, m), 7.81 (1H, d, J = 3.3 Hz), 7.95 (2H, br s), 7.98–8.10 (1H, m), 8.45–8.64 (1H, m).

5.58. *tert*-Butyl {3-[(3-{3-cyano-6-[2-(ethoxymethoxy)-4-fluorophenyl]-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)amino]-3-oxopropyl}carbamate (8g)

A mixture of **5g** (0.20 g, 0.39 mmol), iron powder (0.13 g, 2.31 mmol), and NH_4Cl (0.13 g, 2.50 mmol) in 50% aqueous EtOH (20 ml) was stirred under reflux for 2 h. After cooling to room temperature, the solution was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give *N*-{4-(3-aminophenyl)-3-cyano-6-[2-(ethoxymethoxy)-4-fluorophenyl]pyridin-2-yl}-thiophene-2-carboxamide (**6g**, 0.15 g, 79%) as an amorphous solid.

Compound **8g** was prepared from **6g** and 3-[(*tert*-butoxycarbonyl)amino]propanoic acid in a manner similar to that described for **8b** as a yellow solid in 63% yield: ^{1}H NMR ($CDCl_3$) δ 1.21 (3H, t, J = 7.2 Hz), 1.44 (9H, s), 2.63 (2H, t, J = 6.0 Hz), 3.49 (2H, q, J = 6.0 Hz), 3.71 (2H, q, J = 7.2 Hz), 5.12–5.20 (1H, m), 5.34 (2H, s), 6.85 (1H, td, J = 8.4, 2.4 Hz), 7.05 (1H, d, J = 10.5 Hz), 7.17 (1H, t, J = 4.5 Hz), 7.42–7.50 (2H, m), 7.60–7.65 (2H, m), 7.75–7.80 (1H, m), 7.93–7.98 (3H, m), 8.08–8.20 (1H, m), 8.52 (1H, br s).

5.59. *tert*-Butyl {3-[(3-{6-[4-chloro-2-(methoxymethoxy)-phenyl]-3-cyano-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)amino]-3-oxopropyl}carbamate (8h)

Compound **8h** was prepared from **6h** and 3-[(*tert*-butoxycarbonyl)amino]propanoic acid in a manner similar to that described for **8b** as a colorless solid in 54% yield: mp 194–195 °C; ^{1}H NMR (DMSO-d_6) δ 1.37 (9H, s), 2.50–2.55 (2H, m), 3.22–3.30 (2H, m), 3.36 (3H, s), 5.41 (2H, s), 6.80–6.95 (1H, m), 7.25–7.29 (2H, m), 7.37–7.39 (2H, m), 7.54 (1H, td, J = 7.8, 3.0 Hz), 7.72 (1H, d, J = 7.5 Hz), 7.94–7.98 (2H, m), 8.02 (1H, s), 8.15 (1H, s), 8.16 (1H, s), 10.24 (1H, s), 11.46 (1H, s).

5.60. *tert*-Butyl {3-[(3-{6-[4-bromo-2-(methoxymethoxy)-phenyl]-3-cyano-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)amino]-3-oxopropyl}carbamate (8i)

A mixture of **5i** (0.15 g, 0.27 mmol), iron powder (88 mg, 1.59 mmol), and NH_4Cl (92 mg, 1.72 mmol) in 50% aqueous EtOH (10 ml) was stirred under reflux for 2 h. After cooling to room temperature, the solution was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give *N*-{4-(3-aminophenyl)-6-[4-bromo-2-(methoxymethoxy)phenyl]-3-cyanopyridin-2-yl}thiophene-2-carboxamide (**6i**, 0.14 g, 100%) as an amorphous solid.

Compound **8i** was prepared from **6i** and 3-[(*tert*-butoxycarbonyl)amino]propanoic acid in a manner similar to that described for **8b** as a yellow solid in 63% yield: mp 144–145 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (9H, t, J = 7.2 Hz), 2.64 (2H, t, J = 6.0 Hz), 3.45–3.54 (5H, m), 5.10–5.18 (1H, m), 5.30 (2H, s), 7.18 (1H, t, J = 4.4 Hz), 7.26–7.32 (2H, m), 7.45–7.58 (3H, m), 7.61–7.66 (2H, m), 7.79–7.81 (2H, m), 7.91–8.02 (2H, m), 8.40–8.50 (1H, m).

5.61. *tert*-Butyl {3-[(3-{3-cyano-6-[4-methoxy-2-(methoxymethoxy)phenyl]-2-[(phenylcarbonyl)amino]pyridin-4-yl}phenyl)-amino]-3-oxopropyl}carbamate (8k)

Compound **8k** was prepared from **6k** and 3-[(*tert*-butoxycarbonyl)amino]propanoic acid in a manner similar to that described for **8b** as a colorless amorphous solid in 32% yield: ^{1}H NMR ($CDCl_3$) δ 1.44 (9H, s), 2.64 (2H, t, J = 6.0 Hz), 3.48–3.55 (5H, m), 3.86 (3H, s), 5.10–5.20 (1H, m), 5.31 (2H, s), 6.69 (1H, dd, J = 8.7, 2.1 Hz), 6.82 (1H, s), 7.48–7.63 (6H, m), 7.91–8.04 (6H, m).

5.62. *tert*-Butyl {3-[(3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)-phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)-amino]-3-oxopropyl}carbamate (8l)

Compound **8l** was prepared from **6l** and 3-[(*tert*-butoxycarbonyl)amino]propanoic acid in a manner similar to that described for **8b** as a colorless solid in 19% yield: mp 198–200 °C; ^{1}H NMR (DMSO-d_6) δ 1.38 (9H, s), 2.48–2.55 (2H, m), 3.20–3.28 (2H, m), 3.37 (3H, s), 5.40 (2H, s), 6.77 (1H, dd, J = 3.4, 1.9 Hz), 6.88 (1H, t, J = 5.7 Hz), 7.01–7.10 (1H, m), 7.18 (1H, dd, J = 11.3, 2.6 Hz), 7.38 (1H, d, J = 7.5 Hz), 7.50–7.59 (2H, m), 7.71 (1H, d, J = 8.3 Hz), 7.95–8.06 (3H, m), 8.11 (1H, br s), 10.24 (1H, br s), 11.28 (1H, br s). Anal. Calcd for $C_{33}H_{32}FN_5O_7$: C, 62.95; H, 5.12; N, 11.12. Found: C, 62.76; H, 5.10; N, 11.10.

5.63. *tert*-Butyl {3-[(3-{3-cyano-2-[(furan-2-ylcarbonyl)amino]-6-[2-(methoxymethoxy)phenyl]-5-methylpyridin-4-yl}phenyl)-amino]-3-oxopropyl}carbamate (8m)

Compound **8m** was prepared from **6m** and 3-[(*tert*-butoxycarbonyl)amino]propanoic acid in a manner similar to that described for **8b** as a colorless amorphous solid in 73% yield: ^{1}H NMR (CDCl$_3$) δ 1.44 (9H, s), 2.01 (3H, s), 2.61 (2H, t, J = 5.7 Hz), 3.41 (3H, s), 3.49 (2H, q, J = 5.9 Hz), 5.19 (2H, s), 5.20–5.30 (1H, m), 6.57 (1H, q, J = 1.8 Hz), 7.12–7.16 (2H, m), 7.24–7.35 (3H, m), 7.38–7.53 (4H, m), 7.65–7.85 (1H, m), 7.97 (1H, br s), 8.70 (1H, s).

5.64. *N*-{3-Cyano-4-[3-(glycylamino)phenyl]-6-(2-hydroxyphenyl)pyridin-2-yl}thiophene-2-carboxamide dihydrochloride (9a)

To a solution of **8a** (60 mg, 0.11 mmol) in EtOAc (5 ml) was added 4 M HCl EtOAc solution (2 ml) and the whole was stirred at room temperature for 2 h. The precipitated solid was collected by filtration and washed with EtOAc to give **9a** (53 mg, 95%) as a yellow solid: mp 210–217 °C; ^{1}H NMR (DMSO-d_6) δ 3.85 (2H, t, J = 5.7 Hz), 6.96–7.05 (2H, m), 7.31 (1H, t, J = 2.6 Hz), 7.37–7.49 (2H, m), 7.62 (1H, t, J = 8.0 Hz), 7.83 (1H, d, J = 9.0 Hz), 8.00–8.04 (2H, m), 8.13–8.25 (6H, m), 10.97 (1H, s), 11.09 (1H, s). Anal. Calcd for $C_{25}H_{19}N_5O_3S\cdot 2HCl\cdot 0.5H_2O$: C, 54.45; H, 4.02; N, 12.70. Found: C, 54.65; H, 4.01; N, 12.61.

5.65. *N*-{4-[3-(β-Alanylamino)phenyl]-3-cyano-6-(2-hydroxyphenyl)pyridin-2-yl}thiophene-2-carboxamide hydrochloride (9b)

Compound **9b** was prepared from **8b** in a manner similar to that described for **9a** as a yellow solid in 88% yield: mp 223–228 °C; ^{1}H NMR (DMSO-d_6) δ 2.78 (2H, t, J = 6.8 Hz), 3.10–3.17 (2H, m), 6.95–7.04 (2H, m), 7.30–7.33 (1H, m), 7.40 (1H, t, J = 7.8 Hz), 7.58 (1H, t, J = 7.8 Hz), 7.80–8.00 (4H, m), 8.00–8.02 (1H, m), 8.12–8.18 (3H, m), 10.54 (1H, s), 11.50 (1H, s), 11.79 (1H, s). Anal. Calcd for $C_{26}H_{21}N_5O_3S\cdot HCl\cdot 1.5H_2O$: C, 57.19; H, 4.43; N, 12.83. Found: C, 57.36; H, 4.58; N, 12.85.

5.66. *N*-{4-[3-(β-Alanylamino)phenyl]-3-cyano-6-(2-hydroxyphenyl)pyridin-2-yl}benzamide trifluoroacetate (9c)

A solution of **8c** (0.15 g, 0.26 mmol) in TFA (5 ml) was stirred at room temperature for 90 min. The solvent was evaporated in vacuo and the residual solid was recrystallized from EtOH/Et$_2$O to give **9c** (0.12 g, 78%) as a pale yellow solid: mp 225–226 °C; ^{1}H NMR (DMSO-d_6) δ 2.76 (2H, t, J = 6.9 Hz), 3.12 (2H, br s), 6.95–7.02 (2H, m), 7.37–7.45 (2H, m), 7.56–7.64 (3H, m), 7.68–7.85 (5H, m), 8.00 (1H, br s), 8.05–8.08 (2H, m), 8.14 (1H, dd, J = 8.1, 1.5 Hz), 8.17 (1H, s), 10.46 (1H, br s), 11.49 (1H, br s), 11.87 (1H, br s). Anal. Calcd for $C_{28}H_{23}N_5O_3\cdot C_2HF_3O_2$: C, 60.91; H, 4.09; N, 11.84. Found: C, 60.75; H, 4.17; N, 11.90.

5.67. *N*-[4-{3-[(4-Aminobutanoyl)amino]phenyl}-3-cyano-6-(2-hydroxyphenyl)pyridin-2-yl]thiophene-2-carboxamide hydrochloride (9d)

Compound **9d** was prepared from **8d** in a manner similar to that described for **9a** as a pale yellow solid in 41% yield: mp 236–238 °C; ^{1}H NMR (DMSO-d_6) δ 1.83–1.93 (2H, m), 2.47–2.51 (2H, m), 2.87 (2H, t, J = 7.8 Hz), 6.95–7.03 (2H, m), 7.30 (1H, t, J = 4.4 Hz), 7.38–7.41 (2H, m), 7.56 (1H, t, J = 8.0 Hz), 7.60–7.70 (3H, m), 7.99–8.02 (2H, m), 8.13–8.15 (3H, m), 10.35 (1H, s), 11.40–11.90 (2H, m). Anal. Calcd for $C_{27}H_{23}N_5O_3S\cdot HCl\cdot 0.5H_2O$: C, 59.71; H, 4.64; N, 12.90. Found: C, 60.02; H, 4.50; N, 13.02.

5.68. *N*-{4-[3-(β-Alanylamino)phenyl]-6-(5-chloro-2-hydroxyphenyl)-3-cyanopyridin-2-yl}thiophene-2-carboxamide trifluoroacetate (9e)

Compound **9e** was prepared from **8e** in a manner similar to that described for **9c** as a yellow solid in 95% yield: mp 197–204 °C; ^{1}H NMR (DMSO-d_6) δ 2.76 (2H, t, J = 6.6 Hz), 3.10–3.13 (2H, m), 7.05 (1H, d, J = 8.7 Hz), 7.31 (1H, t, J = 4.4 Hz), 7.41–7.45 (2H, m), 7.58 (1H, t, J = 8.0 Hz), 7.60–7.85 (4H, m), 8.01 (2H, d, J = 5.1 Hz), 8.14 (1H, d, J = 3.6 Hz), 8.21 (1H, d, J = 2.7 Hz), 8.26 (1H, s), 10.46 (1H, s), 11.44 (1H, s), 11.26(1H, s). Anal. Calcd for $C_{26}H_{20}ClN_5O_3S\cdot C_2HF_3O_2\cdot H_2O$: C, 51.74; H, 3.56; N, 10.77. Found: C, 51.74; H, 3.57; N, 10.77.

5.69. *N*-{4-[3-(β-Alanylamino)phenyl]-3-cyano-6-(2-hydroxy-5-methoxyphenyl)pyridin-2-yl}thiophene-2-carboxamide hydrochloride (9f)

Compound **9f** was prepared from **8f** in a manner similar to that described for **9a** as an orange solid in 100% yield: mp 197–201 °C; ^{1}H NMR (DMSO-d_6) δ 2.79 (2H, t, J = 6.6 Hz), 3.10 (2H, q, J = 6.0 Hz), 3.78 (3H, s), 6.95–7.05 (2H, m), 7.31 (1H, t, J = 4.4 Hz), 7.40 (1H, d, J = 7.5 Hz), 7.55–7.63 (2H, m), 7.82–8.00 (4H, m), 8.01 (2H, d, J = 4.0 Hz), 8.15 (1H, d, J = 4.0 Hz), 8.22 (1H, s), 10.55 (1H, s), 11.20–11.45 (1H, m), 11.49 (1H, s). Anal. Calcd for $C_{27}H_{23}N_5O_4S\cdot HCl\cdot 2.5H_2O$: C, 54.50; H, 4.91; N, 11.77. Found: C, 54.46; H, 4.78; N, 11.75.

5.70. *N*-{4-[3-(β-Alanylamino)phenyl]-3-cyano-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl}thiophene-2-carboxamide hydrochloride (9g)

Compound **9g** was prepared from **8g** in a manner similar to that described for **9a** as a yellow solid in 64% yield: mp 217–220 °C; ^{1}H NMR (DMSO-d_6) δ 2.80 (2H, t, J = 6.8 Hz), 3.05–3.15 (2H, m), 6.81–6.90 (2H, m), 7.31 (1H, t, J = 4.4 Hz), 7.41 (1H, t, J = 7.8 Hz), 7.57 (1H, t, J = 7.8 Hz), 7.83 (1H, d, J = 8.4 Hz), 7.85–8.02 (5H, m), 8.15–8.25 (3H, m), 10.58 (1H, s), 11.47 (1H, s), 12.25 (1H, s). Anal. Calcd for $C_{26}H_{20}FN_5O_3S\cdot HCl\cdot 1.5H_2O$: C, 55.27; H, 4.28; N, 12.40. Found: C, 55.20; H, 4.31; N, 12.39.

5.71. *N*-{4-[3-(β-Alanylamino)phenyl]-6-(4-chloro-2-hydroxyphenyl)-3-cyanopyridin-2-yl}thiophene-2-carboxamide trifluoroacetate (9h)

Compound **9h** was prepared from **8h** in a manner similar to that described for **9c** as a pale green solid in 58% yield: mp 206–209 °C;

^{1}H NMR (DMSO-d_6) δ 2.76 (2H, q, *J* = 6.6 Hz), 3.09–3.15 (2H, m), 7.03–7.10 (2H, m), 7.31 (1H, t, *J* = 4.4 Hz), 7.41 (1H, t, *J* = 7.5 Hz), 7.58 (1H, t, *J* = 8.0 Hz), 7.65–7.80 (4H, m), 8.01 (2H, d, *J* = 4.4 Hz), 8.13–8.19 (3H, m), 10.47 (1H, s), 11.48 (1H, s), 12.09 (1H, s). Anal. Calcd for $C_{26}H_{20}ClN_5O_3S\cdot C_2HF_3O_2\cdot 2H_2O$: C, 50.34; H, 3.77; N, 10.48. Found: C, 50.19; H, 3.76; N, 10.51.

5.72. *N*-{4-[3-(β-Alanylamino)phenyl]-6-(4-bromo-2-hydroxyphenyl)-3-cyanopyridin-2-yl}thiophene-2-carboxamide hydrochloride (9i)

Compound **9i** was prepared from **8i** in a manner similar to that described for **9a** as a yellow solid in 100% yield: mp 203–211 °C; ^{1}H NMR (DMSO-d_6) δ 2.78 (2H, t, *J* = 6.6 Hz), 3.05–3.16 (2H, m), 7.17 (1H, t, *J* = 8.7 Hz), 7.26–7.33 (2H, m), 7.40 (1H, d, *J* = 7.2 Hz), 7.57 (1H, t, *J* = 8.1 Hz), 7.80–7.98 (4H, m), 8.00–8.18 (5H, m), 10.53 (1H, s), 11.44 (1H, s), 11.90–12.05 (1H, m). Anal. Calcd for $C_{26}H_{20}BrN_5O_3S\cdot HCl\cdot H_2O$: C, 48.50; H, 4.07; N, 10.88. Found: C, 48.40; H, 4.35; N, 11.06.

5.73. *N*-{4-[3-(β-Alanylamino)phenyl]-3-cyano-6-(2-hydroxy-4-methylphenyl)pyridin-2-yl}thiophene-2-carboxamide hydrochloride (9j)

A mixture of **5j** (0.20 g, 0.39 mmol), iron powder (0.13 g, 2.33 mmol), and NH_4Cl (0.14 g, 2.53 mmol) in 50% aqueous EtOH (20 ml) was stirred under reflux for 20 min. After cooling to room temperature, the solution was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give *N*-{4-(3-aminophenyl)-3-cyano-6-[2-(ethoxymethoxy)-4-methylphenyl]-pyridin-2-yl}thiophene-2-carboxamide (**6j**, 0.14 g, 74%) as an amorphous solid.

To an ice-cooled solution of **6j** (0.14 g, 0.29 mmol), 3-[(*tert*-butoxycarbonyl)amino]propanoic acid (0.11 g, 0.58 mmol), and HOBt (78 mg, 0.58 mmol) in DMF (8 ml) was added WSCD (0.11 g, 0.57 mmol). After stirring at room temperature for 18 h, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/1 to 3/2) and recrystallization from EtOAc/Et_2O to give *tert*-butyl {3-[(3-{3-cyano-6-[2-(ethoxymethoxy)-4-methylphenyl]-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)amino]-3-oxopropyl}-carbamate (**8j**, 55 mg, 29%) as a yellow solid.

Compound **9j** was prepared from **8j** in a manner similar to that described for **9a** as a yellow solid in 100% yield: mp 204–210 °C; ^{1}H NMR (DMSO-d_6) δ 2.31 (3H, s), 2.79 (2H, t, *J* = 6.8 Hz), 3.10 (2H, t, *J* = 6.0 Hz), 6.79–6.84 (2H, m), 7.31 (1H, t, *J* = 4.4 Hz), 7.41 (1H, t, *J* = 7.8 Hz), 7.57 (1H, t, *J* = 7.8 Hz), 7.82–7.96 (4H, m), 8.00–8.07 (3H, m), 8.14 (2H, d, *J* = 3.9 Hz), 10.54 (1H, s), 11.43 (1H, s), 11.91 (1H, s). Anal. Calcd for $C_{27}H_{23}N_5O_3S\cdot HCl\cdot H_2O$: C, 58.74; H, 4.75; N, 12.69. Found: C, 58.42; H, 4.90; N, 12.30.

5.74. *N*-{4-[3-(β-Alanylamino)phenyl]-3-cyano-6-(2-hydroxy-4-methoxyphenyl)pyridin-2-yl}benzamide trifluoroacetate (9k)

Compound **9k** was prepared from **8k** in a manner similar to that described for **9c** as a yellow solid in 86% yield: mp 164–170 °C; ^{1}H NMR (DMSO-d_6) δ 2.76 (2H, t, *J* = 7.1 Hz), 3.12 (2H, q, *J* = 5.9 Hz), 3.81 (3H, s), 6.53–6.58 (2H, m), 7.42 (1H, d, *J* = 7.5 Hz), 7.55–7.85 (8H, m), 7.97 (1H, s), 8.05–8.14 (4H, m), 10.45 (1H, s), 11.45 (1H, s), 12.40(1H, s). Anal. Calcd for $C_{29}H_{25}N_5O_4\cdot C_2HF_3O_2\cdot H_2O$: C, 58.22; H, 4.41; N, 10.95. Found: C, 58.45; H, 4.48; N, 10.92.

5.75. *N*-{4-[3-(β-Alanylamino)phenyl]-3-cyano-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl}furan-2-carboxamide hydrochloride (9l)

A solution of **8l** (97 mg, 0.15 mmol) in 10% HCl MeOH solution (6 ml) was stirred at room temperature for 3.5 h. The solvent was evaporated in vacuo and the residual solid was washed with hot EtOAc. Recrystallization from EtOH/Et_2O gave **9l** (26 mg, 32%) as a yellow solid: mp 269–270 °C; ^{1}H NMR (DMSO-d_6) δ 2.70–2.80 (2H, m), 3.11 (2H, t, *J* = 6.8 Hz), 6.76–6.89 (3H, m), 7.41 (1H, d, *J* = 7.5 Hz), 7.50 (1H, br s), 7.58 (1H, t, *J* = 8.1 Hz), 7.60–7.95 (4H, m), 7.99 (1H, s), 8.03–8.14 (2H, m), 8.22 (1H, dd, *J* = 9.8, 6.8 Hz), 10.47 (1H, br s), 12.40 (2H, br s). Anal. Calcd for $C_{26}H_{20}FN_5O_4\cdot HCl\cdot 2H_2O$: C, 55.97; H, 4.52; N, 12.55. Found: C, 55.85; H, 4.42; N, 12.31.

5.76. *N*-{4-[3-(β-Alanylamino)phenyl]-3-cyano-6-(2-hydroxyphenyl)-5-methylpyridin-2-yl}furan-2-carboxamide hydrochloride (9m)

Compound **9m** was prepared from **8m** in a manner similar to that described for **9a** as a yellow amorphous solid in 77% yield: ^{1}H NMR (DMSO-d_6) δ 1.95 (3H, s), 2.78 (2H, t, *J* = 3.3 Hz), 3.09 (2H, q, *J* = 3.3 Hz), 6.73 (1H, q, *J* = 1.8 Hz), 6.90–7.02 (2H, m), 7.10 (1H, d, *J* = 7.5 Hz), 7.26–7.32 (2H, m), 7.51–7.56 (2H, m), 7.71 (1H, d, *J* = 8.4 Hz), 7.78 (1H, s), 7.80–8.00 (4H, m), 9.89 (1H, br s), 10.56 (1H, s), 11.22 (1H, s). Anal. Calcd for $C_{27}H_{23}N_5O_4\cdot HCl\cdot 1.5H_2O$: C, 59.50; H, 4.99; N, 12.85. Found: C, 59.86; H, 5.10; N, 12.55.

5.77. 3-{3-Cyano-6-[2-(methoxymethoxy)phenyl]-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}benzoic acid (10a)

To a solution of **4m** (1.5 g, 3.9 mmol) in pyridine (15 ml) was added 2-thenoyl chloride (1.0 ml, 9.6 mmol) at room temperature and the whole was stirred at room temperature for 17 h. After the solvent was evaporated in vacuo, the residue was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved into MeOH (15 ml) and to the solution was added 2 M Na_2CO_3 (20 ml). After the whole was stirred at room temperature for 20 h, the mixture was diluted with water and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/1) and recrystallization from EtOAc/hexane to give methyl 3-{3-cyano-6-[2-(methoxymethoxy)phenyl]-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}benzoate (0.94 g, 49%) as a colorless solid: mp 159–160 °C; ^{1}H NMR ($CDCl_3$) δ 3.32 (3H, s), 3.92 (3H, s), 5.32 (2H, s), 7.19 (1H, t, *J* = 7.5 Hz), 7.27–7.31 (2H, m), 7.50 (1H, t, *J* = 7.8 Hz), 7.80 (1H, t, *J* = 7.8 Hz), 7.91 (1H, d, *J* = 7.8 Hz), 7.97–8.08 (3H, m), 8.16–8.19 (2H, m), 8.26 (1H, s), 11.49 (1H, s).

To a solution of methyl 3-{3-cyano-6-[2-(methoxymethoxy)-phenyl]-2-[(thiophen-2-ylcarbonyl)amino]pyridine-4-yl}benzoate (1.0 g, 2.1 mmol) in THF (15 ml) and EtOH (8 ml) was added 4 M NaOH (1.5 ml) and the whole was stirred at room temperature for 16 h. The reaction mixture was neutralized with 1 M HCl and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give **10a** (1.01 g, 100%) as a colorless solid: mp 179–180 °C; ^{1}H NMR (DMSO-d_6) δ 3.36 (3H, s), 5.32 (2H, s), 7.17 (1H, t, *J* = 7.5 Hz), 7.28–7.31 (2H, m), 7.47–7.53 (1H, m), 7.78 (1H, t, *J* = 7.8 Hz), 7.92 (1H, dd, *J* = 7.5, 1.5 Hz), 7.98–8.01 (2H, m), 8.09 (1H, s), 8.14–8.20 (2H, m), 8.29 (1H, s), 11.47 (1H, s), 13.15–13.35 (1H, m).

5.78. 2-{3-Cyano-6-[2-(methoxymethoxy)phenyl]-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}benzoic acid (10b)

To a solution of **4n** (0.60 g, 1.5 mmol) in pyridine (10 ml) was added 2-thenoyl chloride (0.57 g, 3.9 mmol) at room temperature and the whole was stirred at room temperature for 48 h. After the solvent was evaporated in vacuo, the residue was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc/hexane = 1/1 to 2/1) and recrystallization from Et_2O to give methyl 2-{3-cyano-6-[2-(methoxymethoxy)-phenyl]-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}benzoate (0.69 g, 90%) as a colorless solid: mp 190–191 °C; ^{1}H NMR ($CDCl_3$) δ 3.41 (3H, s), 3.77 (3H, s), 5.22 (2H, s), 7.14–7.26 (4H, m), 7.38–7.46 (2H, m), 7.57–7.70 (3H, m), 7.76 (1H, s), 7.95 (1H, dd, *J* = 8.1, 1.8 Hz), 8.12 (1H, d, *J* = 8.0 Hz), 8.38 (1H, s).

Compound **10b** was prepared from methyl 2-{3-cyano-6-[2-(methoxymethoxy)phenyl]-2-[(thiophen-2-ylcarbonyl)amino]-pyridin-4-yl}benzoate in a manner similar to that described for **10a** as a colorless solid in 89% yield: mp 182–183 °C; ^{1}H NMR (DMSO-d_6) δ 3.33 (3H, s), 5.26 (2H, s), 7.19 (1H, t, *J* = 7.7 Hz), 7.26–7.29 (2H, m), 7.45–7.51 (2H, m), 7.67 (1H, t, *J* = 7.7 Hz), 7.77 (1H, t, *J* = 7.5 Hz), 7.87 (1H, s), 7.91–7.97 (2H, m), 8.08 (1H, dd, *J* = 7.8, 1.2 Hz), 8.14–8.15 (1H, m), 11.35 (1H, s), 13.10 (1H, s).

5.79. 4-{3-Cyano-6-[2-(methoxymethoxy)phenyl]-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}benzoic acid (10c)

To a solution of **4o** (0.30 g, 0.77 mmol) in pyridine (10 ml) was added 2-thenoyl chloride (0.28 g, 1.9 mmol) at room temperature and the whole was stirred at room temperature for 18 h. To the reaction mixture was added 4 M NaOH (2.5 ml) and stirred at room temperature for 1 h. The solution was diluted with water, neutralized with 5 M HCl, and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by recrystallization from EtOAc to give **10c** (0.30 g, 81%) as a yellow solid: mp 205–206 °C; ^{1}H NMR (DMSO-d_6) δ 3.34 (3H, s), 5.32 (2H, s), 7.19 (1H, t, *J* = 8.4 Hz), 7.27–7.31 (2H, m), 7.50 (1H, t, *J* = 7.8 Hz), 7.84–8.00 (4H, m), 8.06 (1H, d, *J* = 2.4 Hz), 8.14–8.18 (3H, m), 11.47 (1H, s), 13.10–13.40 (1H, m).

5.80. 3-{3-Cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}benzoic acid (10d)

To a solution of **4p** (4.0 g, 9.8 mmol) in pyridine (30 ml) was added 2-furoyl chloride (3.2 g, 24.5 mmol) at room temperature and the whole was stirred at 60 °C for 3 h. The mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved into THF (50 ml) and to the solution was added MeOH (50 ml) and 2 M Na_2CO_3 (30 ml). After the whole was stirred at room temperature overnight, the mixture was concentrated in vacuo, diluted with water, and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/2) and recrystallization from EtOAc/hexane to give methyl 3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}benzoate (3.1 g, 64%) as a colorless solid: mp 160–162 °C; ^{1}H NMR ($CDCl_3$) δ 3.48 (3H, s), 3.97 (3H, s), 5.27 (2H, s), 6.60–6.65 (1H, m), 6.85–6.95 (1H, m), 7.01 (1H, dd, *J* = 10.8, 2.4 Hz), 7.05 (1H, d, *J* = 3.3 Hz), 7.60 (1H, s), 7.66 (1H, t, *J* = 7.5 Hz), 7.90–8.00 (2H, m), 8.02 (1H, dd, *J* = 8.7, 6.9 Hz), 8.18–8.25 (1H, m), 8.30–8.35 (1H, m), 8.83 (1H, s).

To a solution of methyl 3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}benzoate (3.0 g, 5.9 mmol) in THF (40 ml) and EtOH (20 ml) was added 4 M NaOH (4.5 ml) and the whole was stirred at room temperature for 6 h. The reaction mixture was neutralized with 1 M HCl and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give **10d** (2.6 g, 92%) as a pale yellow solid: mp 254–255 °C; ^{1}H NMR (DMSO-d_6) δ 3.38 (3H, s), 5.36 (2H, s), 6.77 (1H, dd, *J* = 3.3, 1.8 Hz), 7.05 (1H, td, *J* = 8.4, 2.4 Hz), 7.17 (1H, dd, *J* = 11.1, 2.4 Hz), 7.55 (1H, d, *J* = 3.3 Hz), 7.78 (1H, t, *J* = 7.8 Hz), 7.95–8.05 (3H, m), 8.08 (1H, s), 8.12–8.20 (1H, m), 8.27 (1H, s), 11.35 (1H, s).

5.81. 3-{3-Cyano-6-(2-hydroxyphenyl)-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}benzoic acid (11)

To a solution of **10a** (0.15 g, 0.31 mmol) in THF (10 ml) was added concd HCl (0.5 ml) with ice-cooling and the whole was stirred at room temperature for 1 h. The reaction mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was washed with EtOAc to give **11** (0.12 g, 84%) as a colorless solid: mp 308–309 °C; ^{1}H NMR (DMSO-d_6) δ 6.97–7.02 (2H, m), 7.31 (1H, dd, *J* = 4.8, 3.9 Hz), 7.41 (1H, t, *J* = 7.8 Hz), 7.78 (1H, t, *J* = 7.8 Hz), 8.00–8.03 (2H, m), 8.14–8.18 (3H, m), 8.27–8.30 (2H, m), 11.49 (1H, br s), 11.95 (1H, s), 13.33 (1H, br s). Anal. Calcd for $C_{24}H_{15}N_3O_4S$: C, 65.30; H, 3.42; N, 9.52. Found: C, 65.05; H, 3.41; N, 9.41.

5.82. *tert*-Butyl (2-{[(3-{3-cyano-6-[2-(methoxymethoxy)-phenyl]-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}-phenyl)carbonyl]amino}ethyl)carbamate (12b)

Compound **12b** was prepared from **10a** and *tert*-butyl (2-aminoethyl)carbamate in a manner similar to that described for **8b** as a colorless solid in 89% yield: mp 200–201 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (9H, s), 3.09–3.16 (2H, m), 3.30–3.33 (5H, m), 5.31 (2H, s), 6.93 (1H, t, *J* = 5.7 Hz), 7.20 (1H, t, *J* = 7.5 Hz), 7.27–7.31 (2H, m), 7.47–7.53 (1H, m), 7.72 (1H, t, *J* = 7.8 Hz), 7.88 (2H, dd, *J* = 7.8, 1.8 Hz), 7.97–8.06 (3H, m), 8.15–8.17 (2H, m), 8.65 (1H, t, *J* = 5.7 Hz), 11.48 (1H, s).

5.83. *tert*-Butyl (2-{[(4-{3-cyano-6-[2-(methoxymethoxy)-phenyl]-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}-phenyl)carbonyl]amino}ethyl)carbamate (12c)

Compound **12c** was prepared from **10c** and *tert*-butyl (2-aminoethyl)carbamate in a manner similar to that described for **8b** as a colorless solid in 64% yield: ^{1}H NMR (DMSO-d_6) δ 1.38 (9H, s), 2.49–2.51 (2H, m), 3.10–3.17 (2H, m), 3.34 (3H, s), 5.32 (2H, s), 6.93 (1H, t, *J* = 5.4 Hz), 7.19 (1H, t, *J* = 7.5 Hz), 7.27–7.31 (2H, m), 7.49 (1H, td, *J* = 7.8, 1.8 Hz), 7.81 (2H, d, *J* = 8.1 Hz), 7.91 (1H, dd, *J* = 7.5, 1.8 Hz), 7.97 (1H, d, *J* = 5.1 Hz), 8.04 (3H, d, *J* = 6.3 Hz), 8.16 (1H, d, *J* = 3.6 Hz), 8.62–8.65 (1H, m), 11.47 (1H, s).

5.84. *tert*-Butyl (2-{[(3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)-phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)-carbonyl]amino}ethyl)carbamate (12d)

Compound **12d** was prepared from **10d** and *tert*-butyl (2-aminoethyl)carbamate in a manner similar to that described for **8b** as a colorless solid in 80% yield: mp 172–173 °C; ^{1}H NMR ($CDCl_3$) δ 1.38 (9H, s), 3.30–3.50 (2H, m), 3.47 (3H, s), 3.50–3.65 (2H, m), 5.04 (1H, br s), 5.27 (2H, s), 6.60–6.65 (1H, m), 6.80–6.95 (1H, m), 7.01 (1H, dd, *J* = 10.8, 2.4 Hz), 7.35–7.45 (2H, m), 7.60 (1H, s), 7.62 (1H, t, *J* = 7.8 Hz), 7.88 (1H, d, *J* = 7.8 Hz), 7.95–8.05 (3H, m), 8.13 (1H, s), 8.83 (1H, s).

5.85. *tert*-Butyl (3-{[(3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)carbonyl]amino}-2,2-dimethylpropyl)carbamate (12e)

Compound **12e** was prepared from **10d** and *tert*-butyl (3-amino-2,2-dimethylpropyl)carbamate in a manner similar to that described for **8b** as a yellow oil in 83% yield: ^{1}H NMR ($CDCl_3$) δ 0.92 (6H, s), 1.40 (9H, s), 2.96 (2H, d, *J* = 5.9 Hz), 3.26 (2H, d, *J* = 6.9 Hz), 3.45 (3H, s), 5.20–5.40 (3H, m), 6.55–6.65 (1H, m), 6.80–6.90 (1H, m), 6.95–7.05 (1H, m), 7.38 (1H, dd, *J* = 3.6, 0.6 Hz), 7.55–7.70 (2H, m), 7.85–8.10 (5H, m), 8.18 (1H, s), 8.92 (1H, br s).

5.86. *N*-[4-{2-[(2-Aminoethyl)carbamoyl]phenyl}-3-cyano-6-(2-hydroxyphenyl)pyridin-2-yl]thiophene-2-carboxamide dihydrochloride (13a)

To an ice-cooled solution of **10b** (0.12 g, 0.25 mmol), *tert*-butyl (2-aminoethyl)carbamate (50 mg, 0.30 mmol), and HOBt (40 mg, 0.30 mmol) in DMF (4 ml) was added WSCD (57 mg, 0.30 mmol). After stirring at room temperature for 19 h, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/1 to 2/1) to give *tert*-butyl (2-{[(2-{3-cyano-6-[2-(methoxymethoxy)phenyl]-2-[(thiophen-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)carbonyl]amino}ethyl)carbamate (**12a**, 0.13 g, 83%) as an amorphous solid.

Compound **13a** was prepared from **12a** in a manner similar to that described for **9a** as a yellow solid in 80% yield: mp 196–201 °C; ^{1}H NMR ($CDCl_3$) δ 2.87–2.93 (2H, m), 3.37–3.43 (2H, m), 5.70–6.50 (3H, m), 6.93–7.03 (2H, m), 7.30 (1H, t, *J* = 4.4 Hz), 7.39 (1H, t, *J* = 7.7 Hz), 7.52 (1H, dd, *J* = 7.5, 2.1 Hz), 7.65–7.74 (2H, m), 7.90–8.01 (3H, m), 8.07–8.10 (2H, m), 8.15 (1H, d, *J* = 3.0 Hz), 8.84 (1H, t, *J* = 5.6 Hz), 11.40 (1H, s). Anal. Calcd for $C_{26}H_{21}N_5O_3S\cdot 2HCl\cdot H_2O$: C, 54.36; H, 4.39; N, 12.19. Found: C, 54.63; H, 4.49; N, 12.38.

5.87. *N*-[4-{3-[(2-Aminoethyl)carbamoyl]phenyl}-3-cyano-6-(2-hydroxyphenyl)pyridin-2-yl]thiophene-2-carboxamide hydrochloride (13b)

Compound **13b** was prepared from **12b** in a manner similar to that described for **9a** as a pale green solid in 88% yield: mp 203–210 °C; ^{1}H NMR (DMSO-d_6) δ 3.03 (2H, t, *J* = 6.0 Hz), 3.54–3.60 (2H, m), 6.96–7.04 (2H, m), 7.30–7.33 (1H, m), 7.40 (1H, t, *J* = 7.7 Hz), 7.75 (1H, t, *J* = 7.7 Hz), 7.91–8.03 (5H, m), 8.11–8.28 (5H, m), 8.96 (1H, t, *J* = 5.4 Hz), 11.40–11.60 (1H, m), 11.90 (1H, br s). Anal. Calcd for $C_{26}H_{21}N_5O_3S\cdot HCl\cdot 1.5H_2O$: C, 57.09; H, 4.61; N. 12.80. Found: C, 57.49; H, 4.81; N, 12.89.

5.88. *N*-[4-{4-[(2-Aminoethyl)carbamoyl]phenyl}-3-cyano-6-(2-hydroxyphenyl)pyridin-2-yl]thiophene-2-carboxamide hydrochloride (13c)

Compound **13c** was prepared from **12c** in a manner similar to that described for **9a** as a yellow solid in 100% yield: mp 202–208 °C; ^{1}H NMR (DMSO-d_6) δ 3.03 (2H, q, *J* = 5.8 Hz), 3.56–3.65 (2H, m), 6.96–7.04 (2H, m), 7.32 (1H, t, *J* = 4.4 Hz), 7.41 (1H, t, *J* = 7.8 Hz), 7.86 (2H, d, *J* = 8.1 Hz), 8.01–8.18 (8H, m), 8.25 (1H, s), 8.93 (1H, t, *J* = 5.4 Hz), 11.50 (1H, s), 11.55–11.90 (1H, m). Anal. Calcd for $C_{26}H_{21}N_5O_3S\cdot HCl\cdot 1.5H_2O$: C, 57.09; H, 4.61; N, 12.80. Found: C, 57.15; H, 4.64; N, 12.57.

5.89. *N*-[4-{3-[(2-Aminoethyl)carbamoyl]phenyl}-3-cyano-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (13d)

Compound **13d** was prepared from **12d** in a manner similar to that described for **9a** as a yellow solid in 55% yield: mp 205–207 °C; ^{1}H NMR (DMSO-d_6) δ 2.95–3.05 (2H, m), 3.55–3.65 (2H, m), 6.80–6.90 (3H, m), 7.54 (1H, d, *J* = 3.6 Hz), 7.75 (1H, t, *J* = 7.5 Hz), 7.92 (1H, d, *J* = 7.8 Hz), 8.01 (3H, br s), 8.05–8.15 (2H, m), 8.26 (2H, s), 8.32 (1H, t, *J* = 7.5 Hz), 8.98 (1H, t, *J* = 5.4 Hz), 11.31 (1H, s), 12.45 (1H, s). Anal. Calcd for $C_{26}H_{20}FN_5O_4\cdot HCl\cdot 3.1H_2O$: C, 54.05; H, 4.75; N, 12.12. Found: C, 53.81; H, 4.48; N, 12.09.

5.90. *N*-[4-{3-[(3-Amino-2,2-dimethylpropyl)carbamoyl]phenyl}-3-cyano-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (13e)

Compound **13e** was prepared from **12e** in a manner similar to that described for **9a** as a yellow solid in 58% yield: mp 256–257 °C; ^{1}H NMR (DMSO-d_6) δ 0.99 (6H, s), 2.60–2.75 (2H, m), 3.20–3.35 (2H, m), 6.75–6.90 (3H, m), 7.54 (1H, d, *J* = 3.6 Hz), 7.70–8.00 (5H, m), 8.05–8.15 (2H, m), 8.20–8.35 (3H, m), 9.02 (1H, t, *J* = 3.3 Hz), 11.31 (1H, s), 12.40 (1H, s). Anal. Calcd for $C_{29}H_{26}FN_5O_4\cdot HCl\cdot 0.5H_2O$: C, 60.79; H, 4.93; N. 12.22. Found: C, 60.53; H, 5.04; N, 12.22.

5.91. 2-Amino-4-[3-(hydroxymethyl)phenyl]-6-[2-(methoxymethoxy)phenyl]pyridine-3-carbonitrile (14)

To a solution of **4m** (1.0 g, 2.6 mmol) in THF (20 ml) was added $LiBH_4$ (56 mg, 2.6 mmol) and the whole was stirred at 50 °C for 24 h. The reaction mixture was diluted with water and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/2 to 1/1) and treated with EtOAc/hexane to give **14** (0.83 g, 89%) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.87 (1H, t, *J* = 5.7 Hz), 3.43 (3H, s), 4.79 (2H, d, *J* = 5.7 Hz), 5.20 (2H, s), 5.37 (2H, s), 7.13 (1H, t, *J* = 7.2 Hz), 7.21 (1H, d, *J* = 8.4 Hz), 7.34–7.41 (2H, m), 7.47–7.58 (3H, m), 7.64 (1H, s), 7.75 (1H, d, *J* = 7.8 Hz).

5.92. 2-Amino-4-[3-(bromomethyl)phenyl]-6-[2-(methoxymethoxy)phenyl]pyridine-3-carbonitrile (15)

To a solution of **14** (0.63 g, 1.7 mmol) in CH_2Cl_2 (60 ml) was added PPh_3 (0.55 g, 2.1 mmol) and then CBr_4 (0.87 g, 2.6 mmol) successively and the whole was stirred at room temperature for 1 h. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/2 to 3/2) to give **15** (0.47 g, 64%) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ 3.45 (3H, s), 4.56 (2H, s), 5.22 (2H, s), 5.32 (2H, s), 7.15 (1H, t, *J* = 7.5 Hz), 7.22 (1H, d, *J* = 7.5 Hz), 7.36–7.40 (2H, m), 7.49–7.58 (2H, m), 7.59–7.60 (1H, m), 7.66 (1H, s), 7.76 (1H, dd, *J* = 7.8, 1.8 Hz).

5.93. 2-Amino-4-[3-(azidomethyl)phenyl]-6-[2-(methoxymethoxy)phenyl]pyridine-3-carbonitrile (16)

To a solution of **15** (0.20 g, 0.47 mmol) in DMF (10 ml) was added NaN_3 (0.15 g, 2.36 mmol) and the whole was stirred at room temperature for 16 h. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/2) to give **16** (0.17 g, 94%) as a colorless solid: mp 99–100 °C; ^{1}H NMR ($CDCl_3$) δ 3.44 (3H, s), 4.45 (2H, s), 5.21 (2H, s), 5.32 (2H, s), 7.14 (1H, t, *J* = 7.5 Hz), 7.21–7.26 (1H, m), 7.36–7.46 (3H, m), 7.52–7.64 (3H, m), 7.77 (1H, dd, *J* = 7.5, 1.8 Hz).

5.94. *N*-{4-[3-(Azidomethyl)phenyl]-3-cyano-6-[2-(methoxymethoxy)phenyl]pyridin-2-yl}thiophene-2-carboxamide (17)

Compound **17** was prepared from **16** in a manner similar to that described for **5a** as a colorless solid in 79% yield: mp 139–140 °C; ^{1}H NMR ($CDCl_3$) δ 3.34 (3H, s), 4.62 (2H, s), 5.32 (2H, s), 7.19(1H, t, *J* = 7.5 Hz), 7.27–7.31 (2H, m), 7.47–7.52 (1H, m), 7.59–7.74 (4H, m), 7.89–7.92 (1H, m), 7.97–7.98 (1H, m), 8.04 (1H, s), 8.15 (1H, d, *J* = 3.6 Hz), 11.47 (1H, s).

5.95. *N*-{4-[3-(Aminomethyl)phenyl]-3-cyano-6-[2-(methoxymethoxy)phenyl]pyridin-2-yl}thiophene-2-carboxamide (18)

A mixture of **17** (0.15 g, 0.30 mmol) and 10% Pd/C (50%wet, 50 mg) in EtOAc (10 ml) and EtOH (10 ml) was stirred under 3 atm hydrogen atmosphere at room temperature for 4 h. The solid was filtered off and the mother liquid was concentrated to give **18** (0.14 g, 100%) as a pale green amorphous solid: ^{1}H NMR ($CDCl_3$) δ 3.44 (3H, s), 3.98 (2H, s), 5.25 (2H, s), 7.12–7.17 (2H, m), 7.25 (1H, d, *J* = 7.5 Hz), 7.38–7.63 (5H, m), 7.68 (1H, s), 7.79–7.98 (3H, m).

5.96. *N*-{4-[3-(Aminomethyl)phenyl]-3-cyano-6-(2-hydroxyphenyl)pyridin-2-yl}thiophene-2-carboxamide (19)

To a solution of **18** (0.14 g, 0.30 mmol) in THF (10 ml) was added concd HCl (0.5 ml) and the whole was stirred at room temperature for 1 h. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc twice. The extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc/MeOH = 50/1 to 20/1) and recrystallization from EtOAc to give **19** (26 mg, 20%) as a pale yellow solid: mp 203–205 °C; ^{1}H NMR (DMSO-d_6) δ 4.04 (2H, s), 6.89 (2H, t, *J* = 8.9 Hz), 7.15 (1H, t, *J* = 4.4 Hz), 7.33 (1H, t, *J* = 7.1 Hz), 7.49–7.86 (7H, m), 7.52–7.55 (1H, m), 8.00 (1H, d, *J* = 6.6 Hz). Anal. Calcd for $C_{24}H_{18}N_4O_2S$: C, 67.59; H, 4.25; N. 13.14. Found: C, 67.23; H, 4.21; N, 12.90.

5.97. Methyl 2-[6-amino-5-cyano-4-(3-nitrophenyl)pyridin-2-yl]benzoate (21a)

Compound **21a** was prepared from methyl 2-acetylbenzoate (**20a**) and 3-nitrobenzaldehyde in a manner similar to that described for **4b** as a pale yellow solid in 10% yield: mp 202–203 °C; ^{1}H NMR ($CDCl_3$) δ 3.79(3H, s), 5.43 (2H, s), 6.97 (1H, s), 7.51–7.61 (3H, m), 7.73 (1H, t, *J* = 8.0 Hz), 7.85 (1H, d, *J* = 7.5 Hz), 8.01 (1H, d, *J* = 6.6 Hz), 8.37 (1H, d, *J* = 8.4 Hz), 8.47 (1H, t, *J* = 1.8 Hz).

5.98. 2-Amino-6-(2-methoxyphenyl)-4-(3-nitrophenyl)pyridine-3-carbonitrile (21b)

Compound **21b** was prepared from 1-(2-methoxyphenyl)ethanone (**20b**) and 3-nitrobenzaldehyde in a manner similar to that described for **4b** as a pale yellow solid in 10% yield: ^{1}H NMR ($CDCl_3$) δ 3.88 (3H, s), 5.37 (2H, br s), 7.02 (1H, d, *J* = 8.4 Hz), 7.10 (1H, td, *J* = 7.5, 1.8 Hz), 7.38–7.48 (2H, m), 7.72 (1H, t, *J* = 7.8 Hz), 7.82 (1H, dd, *J* = 7.5, 1.8 Hz), 7.96–8.03 (1H, m), 8.32–8.39 (1H, m), 8.43–8.46 (1H, m).

5.99. Methyl 2-{5-cyano-4-(3-nitrophenyl)-6-[(phenylcarbonyl)amino]pyridin-2-yl}benzoate (22a)

To a solution of **21a** (0.29 g, 0.77 mmol) in pyridine (15 ml) was added benzoyl chloride (0.27 g, 1.9 mmol). After stirring at room temperature for 19 h, the reaction mixture was diluted with water and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved into THF (4 ml) and MeOH (3 ml) and to the mixture was added 2 M Na_2CO_3 (4 ml). After stirring at room temperature for 16 h, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc twice. The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was diluted with MeOH (10 ml) and to the solution was added 28% NaOMe solution in MeOH (150 mg) and the whole was stirred at room temperature for 1 h. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc twice. The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/1) to give **22a** (0.24 g, 65%) as a pale brown foam: ^{1}H NMR ($CDCl_3$) δ 3.77(3H, s), 7.46–7.61 (6H, m), 7.70 (1H, t, *J* = 7.8 Hz), 7.82 (1H, d, *J* = 7.8 Hz), 7.90 (2H, d, *J* = 8.1 Hz), 8.11 (1H, d, *J* = 7.8 Hz), 8.36–8.50 (3H, m), 8.99 (1H, s).

5.100. *N*-[3-Cyano-6-(2-methoxyphenyl)-4-(3-nitrophenyl)-pyridin-2-yl]thiophene-2-carboxamide (22b)

Compound **22b** was prepared from **21b** in a manner similar to that described for **5a** as a colorless solid in 72% yield: mp 218–219 °C; ^{1}H NMR ($CDCl_3$) δ 3.88 (3H, s), 7.05 (1H, d, *J* = 8.4 Hz), 7.08–7.21 (2H, m), 7.43–7.52 (1H, m), 7.66 (1H, d, *J* = 4.8 Hz), 7.74–7.82 (2H, m), 7.97 (1H, d, *J* = 7.5 Hz), 8.01 (1H, s), 8.12 (1H, d, *J* = 7.8 Hz), 8.40 (1H, ddd, *J* = 8.1, 2.1, 0.9 Hz), 8.46 (1H, s), 8.52 (1H, s). Anal. Calcd for $C_{24}H_{16}N_4O_4S$: C, 63.15; H, 3.53; N, 12.27. Found: C, 63.10; H, 3.50; N, 12.11.

5.101. 2-{5-Cyano-4-(3-nitrophenyl)-6-[(phenylcarbonyl)amino]pyridin-2-yl}benzoic acid (22c)

To a solution of **22a** (0.60 g, 1.0 mmol) in THF (15 ml) and MeOH (10 ml) was added 4 M NaOH (5 ml) and the whole was stirred at room temperature for 1 h. The reaction mixture was neutralized with 1 M HCl and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give **22c** (0.55 g, 100%) as a pale yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 7.37–7.80 (6H, m), 7.93–7.99 (3H, m), 8.09–8.17 (3H, m), 8.42 (1H, d, *J* = 8.1 Hz), 8.53 (1H, t, *J* = 1.8 Hz), 9.61 (1H, s).

5.102. *N*-[4-(3-Aminophenyl)-3-cyano-6-(2-methoxyphenyl)-pyridin-2-yl]thiophene-2-carboxamide (23a)

Compound **23a** was prepared from **22b** in a manner similar to that described for **18** as a colorless solid in 64% yield: mp 260–261 °C; ^{1}H NMR ($CDCl_3$) δ 3.85 (2H, br s), 3.90 (3H, s), 6.82 (1H, dd, *J* = 7.8, 2.1 Hz), 6.98–7.20 (5H, m), 7.25–7.37 (1H, m), 7.40–7.48 (1H, m), 7.63 (1H, d, *J* = 5.1 Hz), 7.77 (1H, d, *J* = 3.9 Hz), 7.88–8.00 (2H, m), 8.41 (1H, s). Anal. Calcd for $C_{24}H_{18}N_4O_2S$: C, 67.59; H, 4.25; N, 13.14. Found: C, 67.50; H, 3.99; N, 13.41.

5.103. *tert*-Butyl (2-{4-(3-aminophenyl)-5-cyano-6-[(phenylcarbonyl)amino]pyridin-2-yl}phenyl)carbamate (23b)

A mixture of **22c** (2.0 g, 4.3 mmol), DPPA (1.4 g, 5.2 mmol), and triethylamine (1.1 g, 10.8 mmol) in toluene (50 ml) was stirred at room temperature for 2 h. To the mixture was added *tert*-butanol

(10 ml) and the whole was stirred at 80 °C for 8 h. After cooling to room temperature, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/2 to 2/3) to give *tert*-butyl (2-{5-cyano-4-(3-nitrophenyl)-6-[(phenylcarbonyl)amino]pyridin-2-yl}phenyl)carbamate (**22d**, 0.27 g, 11%) as a yellow solid.

Compound **23b** was prepared from **22d** in a manner similar to that described for **6a** as a yellow amorphous solid in 51% yield: ^{1}H NMR ($CDCl_3$) δ 1.52 (9H, s), 6.87 (1H, d, J = 7.8 Hz), 6.95 (1H, s), 7.04 (1H, d, J = 7.2 Hz), 7.13 (1H, t, J = 7.8 Hz), 7.36 (1H, t, J = 8.1 Hz), 7.47 (1H, t, J = 7.8 Hz), 7.55–7.67 (5H, m), 8.03 (2H, d, J = 7.5 Hz), 8.29 (1H, d, J = 8.4 Hz), 8.83 (1H, s), 10.80 (1H, s).

5.104. *tert*-Butyl (3-{[3-(6-{2-[(*tert*-butoxycarbonyl)amino]phenyl}-3-cyano-2-[(phenylcarbonyl)amino]pyridin-4-yl)phenyl]amino}-3-oxopropyl)carbamate (24)

Compound **24** was prepared from **23b** and 3-[(*tert*-butoxycarbonyl)amino]propanoic acid in a manner similar to that described for **8b** as a yellow amorphous solid in 100% yield: ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s), 1.55 (9H, s), 2.66 (2H, t, J = 5.7 Hz), 3.53 (2H, q, J = 5.8 Hz), 5.20 (1H, br s), 7.11 (1H, t, J = 7.5 Hz), 7.28–7.71 (9H, m), 7.97–8.04 (3H, m), 8.25–8.32 (2H, m), 8.90 (1H, s), 10.77 (1H, s).

5.105. *N*-{4-[3-(β-Alanylamino)phenyl]-6-(2-aminophenyl)-3-cyanopyridin-2-yl}benzamide dihydrochloride (25)

Compound **25** was prepared from **24** in a manner similar to that described for **9a** as a yellow solid in 78% yield: mp 186–190 °C; ^{1}H NMR (DMSO-d_6) δ 2.79 (2H, t, J = 6.6 Hz), 3.09 (2H, q, J = 6.0 Hz), 6.81 (1H, t, J = 6.3 Hz), 6.99 (1H, d, J = 8.4 Hz), 7.27 (1H, t, J = 7.8 Hz), 7.42 (1H, t, J = 7.8 Hz), 7.54–7.72 (4H, m), 7.83–8.01 (6H, m), 8.09 (2H, d, J = 8.4 Hz), 10.56 (1H, s), 11.42 (1H, s). Anal. Calcd for $C_{28}H_{24}N_6O_2\cdot 2HCl\cdot 1.3H_2O$: C, 58.70; H, 5.03; N, 14.67. Found: C, 58.91; H, 5.34; N, 14.33.

5.106. Synthesis by combinatorial chemistry method

General: To a solution of **4p** (2.0 mmol) in pyridine (8 ml) was added acid chloride (6.0 mmol) at room temperature and the whole was stirred at room temperature for 20 h. The reaction mixture was diluted with EtOAc and washed with water. The organic layer was concentrated in vacuo. The residue was dissolved into MeOH (5 ml) and THF (5 ml) and to the solution was added 4 M NaOH (5 ml). After the whole was stirred at room temperature for 20 h, the mixture was neutralized by 1 M HCl (20 ml). The precipitated solid was collected by filtration and washed with water to give crude **26**. This compound was used next reaction without further purification.

To a 0.12 M solution of **26** in DMF (830 μl, 100 μmol) was added triethylamine (14.1 μl, 1.04 mmol), 0.24 M solution of amine in CH_2Cl_2 (600 μl, 144 μmol), 0.20 M solution of HOBt in CH_2Cl_2 (720 μl, 144 μmol), 0.20 M solution of WSCD in CH_2Cl_2 (720 μl, 144 μmol), and 2.0 M solution of DMAP in CH_2Cl_2 (7.2 μl, 14.4 μmol) successively. The solution was stirred at 50 °C for 24 h and cooled to room temperature. The reaction mixture was diluted with CH_2Cl_2 (3 ml), washed with 5% aqueous $NaHCO_3$ (2 ml), and the organic layer was separated with Presep (dehydration, Wako Pure Chemical Industries, Ltd.). The solvent was evaporated in vacuo and the residue was purified by HPLC (Gilson high-throughput HPLC system using a YMC CombiPrep ODS–A column (S–5 μM 50 mm x 20 mm) with 5–95% gradient water–acetonitril containing 0.1% TFA). The compound obtained was dissolved into TFA (3 ml) and the whole was stirred at room temperature for 20 h. The solvent was evaporated in vacuo and the residue was purified by HPLC (same condition) to give **27**. Purity of the compounds was evaluated by LC/MS analysis (Walters ZMD; column, CAPCELL PAK C18 UG120 S-3 3 mm, 35x1.5 mm; solvent, water/0.05% TFA (A), MeCN/0.04% TFA (B); gradient condition: 0.00 min (A/B = 90/10), 2.00 min (A/B = 5/95), 2.75 min (A/B = 5/95), 2.76 min (A/B = 90/10), 3.60 min (A/B = 90/10); flow rate of 0.5 ml/min). The percentage of the peak area detected at UV: 220 nM of the resultant product peak was taken as the purity of the compound.

Compounds **27a–g** were synthesized as mentioned above (purity >93%).

5.107. Preparation of CHO membranes for GPR54 binding assay

CHO cell lines stably expressing human GPR54 (h175-KB34) or rat GPR54 (r175-KB29) were used for receptor binding assays. Cells were cultured in Dulbecco's Modified Eagle Medium (D-MEM) supplemented with 10% dFBS, 1× Non-Essential Amino Acid (Invitrogen), 50 μg/ml gentamycin (Invitrogen) in 5% CO_2/95% air atmosphere. Cells were harvested within 50% confluency in Ca^{2+}–Mg^{2+}-free phosphate buffered saline containing 1% EDTA and centrifuged (2500 rpm, 4 °C, 10 min, HIMAC rotor #RP24A-132). After twice washed with the same buffer, cells were homogenized in ice-cold homogenization buffer (10 mM $NaHCO_3$, 2 mM EGTA, 0.2 mM Magnesium acetate, 10 μg/ml Leupeptin, 10 μg/ml E-64, 100 μg/ml o-phenanthroline, 90 μg/ml phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml Pepstatin A, pH 7.3 at 25 °C) and collected by centrifugation (30,000 rpm, 1 h, 4 °C, HIMAC rotor #RP42-767). Pellets were resuspended in ice-cold stock buffer (20 mM Tris, 250 mM sucrose, 2 mM EGTA, 10 μg/ml Leupeptin, 10 μg/ml E-64, 100 μg/ml o-phenanthroline, 90 μg/ml PMSF, 10 μg/ml Pepstatin A, pH 7.4 at 25 °C) and stored at −80 °C.

5.108. Receptor binding assay

The procedure of this binding assay was based on the method of Ohtaki et al.[18] The frozen membranes were suspended in binding buffer (20 mM Tris, 2.5 mM magnesium acetate, 2 mM EGTA, 100 μg/ml o-phenanthroline, 90 μg/ml PMSF, 10 μg/ml Leupeptin, 10 μg/ml Pepstatin A, 10 μg/ml E-64, and 1 mg/ml BSA, pH 7.4). Membranes were incubated with 135 pM ^{125}I-metatsin (40–54) (the Peptide Institute products labeled with ^{125}I by lactoperoxidase method) and test compounds at 25 °C for 60 min. The reaction mixtures were filtrated using GF/C filter plates pretreated with 0.3% polyethyleneimine by a cell harvester (PerkinElmer). After filtration, the filter plates were washed with three times 300 μl buffer (50 mM Tris, 5 mM $MgCl_2$, 1 mM EDTA, 0.05% CHAPS, 0.05% NaN_3, 0.1% BSA, pH7.4). The filter plates were dried and the radioactivity was determined after addition of 30 μl Microscint-0 using TopCount (PerkinElmer). Nonspecific binding was defined in the presence of 1.8 μM metastin (45–54).

5.109. Preparation of CHO cell lines

CHO cell lines stably expressing human GPR54 (cell line: h175-KB33, h175-16) were used for Ca^{2+} mobilization assays. Cells were cultured in Eagle's Minimum Essential Medium (MEM) supplemented with 10% dialyzed fetal bovine serum (dFBS), 100 U/ml penicillin, and 100 μg/ml streptomycin in 5% CO_2/95% air atmosphere.

5.110. Ca^{2+} mobilization assay

Cells were plated at 30,000 cells/well in 96-well plate (type 3904, Corning) and cultured overnight. The cells were incubated in Ca^{2+} assay kit solution (115 mM NaCl, 5.4 mM KCl, 0.8 mM

$MgCl_2$, 1.8 mM $CaCl_2$, 20 mM Hepes, 13.8 mM D-glucose; pH 7.4, Dojin) containing 0.1% BSA, 2.5 μg/ml Fluo-3AM, 1.25 mM probenecid and 0.04% Pluronic F-127 for 60 min at 37 °C. They were washed with Ca^{2+} assay kit solution containing 0.1% BSA and 1.25 mM probenecid at 37 °C. After washing cells, the Ca^{2+} mobilization was detected in a fluorometric imaging plate reader (FLIPR, Molecular Devices) as described in the FLIPR system manual. Antagonist activities of test compounds in h175-KB33 and h175-16 were determined as inhibitory response to 30 nM and 100 pM metastin (40–54), respectively. The 50% inhibitory concentration (IC_{50}) was used to calculate by non-linear regression analysis in GraphPad Prism software (GraphPad Software Inc.).

5.111. X-ray crystallographic data

X-ray crystallographic data for compound **13b** has been deposited with the Cambridge Crystallographic Data Center as CCDC771900. The crystallographic data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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References and notes

1. Reviews: (a) Smith, J. T.; Clifton, D. K.; Steiner, R. A. *Reproduction* **2006**, *131*, 623; (b) Dungan, H. M.; Clifton, D. K.; Steiner, R. A. *Endocrinology* **2006**, *147*, 1154.
2. (a) Lee, D. K.; Nguyen, T.; O'Neill, G. P.; Cheng, R.; Liu, Y.; Howard, A. D.; Coulombe, N.; Tan, C. P.; Tang-Nguyen, A.-T.; George, S. R.; O'Dowd, B. F. *FEBS Lett.* **1999**, *446*, 103; (b) Muir, A. I.; Chamberlain, L.; Elshourbagy, N. A.; Michalovich, D.; Moore, D. J.; Calamari, A.; Szekeres, P. G.; Sarau, H. M.; Chambers, J. K.; Murdock, P.; Steplewski, K.; Shabon, U.; Miller, J. E.; Middleton, S. E.; Darker, J. G.; Larminie, C. G. C.; Wilson, S.; Bergsma, D. J.; Emson, P.; Faull, R.; Philpott, K. L.; Harrison, D. C. *J. Biol. Chem.* **2001**, *276*, 28969.
3. Ohtaki, T.; Shintani, Y.; Honda, S.; Matsumoto, H.; Hori, A.; Kanehashi, K.; Terao, Y.; Kumano, S.; Takatsu, Y.; Masuda, Y.; Ishibashi, Y.; Watanabe, T.; Asada, M.; Yamada, T.; Suenaga, M.; Kitada, C.; Usuki, S.; Kurokawa, T.; Onda, H.; Nishimura, O.; Fujino, M. *Nature* **2001**, *411*, 613.
4. Kotani, M.; Detheux, M.; Vandenbogaerde, A.; Communi, D.; Vanderwinden, J.-M.; Poul, E. L.; Brezillon, S.; Tyldesley, R.; Suarez-Huerta, N.; Vandeput, F.; Blanpain, C.; Schiffmann, S. N.; Vassart, G.; Parmentier, M. *J. Biol. Chem.* **2001**, *276*, 34631.
5. (a) Seminara, S. B.; Messager, S.; Chatzidaki, E. E.; Thresher, R. R.; Acierno, J. S., Jr.; Shagoury, J. K.; Bo-Abbas, Y.; Kuohung, W.; Schwinof, K. M.; Hendrick, A. G.; Zahn, D.; Dixon, J.; Kaiser, U. B.; Slaugenhaupt, S. A.; Gusella, J. F.; O'Rahilly, S.; Carlton, M. B. L.; Crowley, W. F., Jr.; Aparicio, S. A. J. R.; Colledge, W. H. *N. Eng. J. Med.* **2003**, *349*, 1614; (b) Funes, S.; Hedrick, J. A.; Vassileva, G.; Markowitz, L.; Abbondanzo, S.; Golovko, A.; Yang, S.; Monsma, F. J.; Gustafson, E. L. *Biochem. Biophys. Res. Commun.* **2003**, *312*, 1357.
6. de Roux, N.; Genin, E.; Carel, J. C.; Matsuda, F.; Chaussain, J. L.; Milgrom, E. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 10972–10976.
7. Matsui, H.; Takatsu, Y.; Kumano, S.; Matsumoto, H.; Ohtaki, T. *Biochem. Biophys. Res. Commun.* **2004**, *320*, 383.
8. (a) Thompson, E. L.; Patterson, M.; Murphy, K. G.; Smith, K. L.; Dhillo, W. S.; Todd, J. F.; Ghatei, M. A.; Bloom, S. R. *J. Neuroendocrinol.* **2004**, *16*, 850; (b) Navarro, V. M.; Castellano, J. M.; Fernandez-Fernandez, R.; Barreiro, M. L.; Roa, J.; Sanchez-Criado, J. E.; Aguilar, E.; Dieguez, C.; Pinilla, L.; Tena-Sempere, M. *Endocrinology* **2004**, *145*, 4565; (c) Navarro, V. M.; Castellano, J. M.; Fernandez-Fernandez, R.; Tovar, S.; Roa, J.; Mayen, A.; Nogueiras, R.; Vazquez, M. J.; Barreiro, M. L.; Magni, P.; Aguilar, E.; Dieguez, C.; Pinilla, L.; Tena-Sempere, M. *Endocrinology* **2005**, *146*, 156; (d) Navarro, V. M.; Castellano, J. M.; Fernandez-Fernandez, R.; Tovar, S.; Roa, J.; Mayen, A.; Barreiro, M. L.; Casanueva, F. F.; Aguilar, E.; Dieguez, C.; Pinilla, L.; Tena-Sempere, M. *Endocrinology* **2005**, *146*, 1689.
9. Gottsch, M. L.; Cunningham, M. J.; Smith, J. T.; Popa, S. M.; Acohido, B. V.; Crowley, W. F.; Seminara, S.; Clifton, D. K.; Steiner, R. A. *Endocrinology* **2004**, *145*, 4073.
10. (a) Messager, S.; Chatzidaki, E. E.; Ma, D.; Hendrick, A. G.; Zahn, D.; Dixon, J.; Thresher, R. R.; Malinge, I.; Lomet, D.; Carlton, M. B. L.; Colledge, W. H.; Caraty, A.; Aparicio, S. A. J. R. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 1761; (b) Sahahab, M. M.; Seminara, S. B.; Crowley, W. F.; Ojeda, S. R.; Plant, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 2129; (c) Dhillo, W. S.; Chaudhri, O. B.; Patterson, M.; Thompson, E. L.; Murphy, K. G.; Badman, M. K.; McGowan, B. M.; Amber, V.; Patel, S.; Ghatei, M. A.; Bloom, S. R. *J. Clin. Endocrinol. Metab.* **2005**, *12*, 6609.
11. Irwig, M. S.; Fraley, G. S.; Smith, J. T.; Acohido, B. V.; Popa, S. M.; Cunningham, M. J.; Gottsch, M. L.; Clifton, D. K.; Steiner, R. A. *Neuroendocrinology* **2004**, *80*, 264.
12. Kinoshita, M.; Tsukamura, H.; Adachi, S.; Matsui, H.; Uenoyama, Y.; Iwata, K.; Yamada, S.; Inoue, K.; Ohtaki, T.; Matsumoto, H.; Maeda, K. *Endocrinology* **2005**, *146*, 4431.
13. Peptide GPR54 antagonists were recently reported: Roseweir, A. K.; Kauffman, A. S.; Smith, J. T.; Guerriero, K. A.; Morgan, K.; Pielecka-Fortuna, J.; Pienda, R.; Gottsch, M. L.; Tena-Sempere, M.; Moenter, S. M.; Terasawa, E.; Clarke, I. J.; Steiner, R. A.; Millar, R. P. *J. Neurosci.* **2009**, *29*, 3920.
14. Murata et al. also estimate the intramolecular hydorogen bond between the phenol and pyridine nitrogen atom by the chemical shift of the phenolic hydroxide in the ^1H NMR spectrum: Murata, T.; Shimada, M.; Sakakibara, S.; Yoshino, T.; Kadono, H.; Masuda, T.; Shimazaki, M.; Shintani, T.; Fuchikami, K.; Sakai, K.; Inbe, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*(5), 913.
15. Aggarwal, R.; Birkbeck, A. A.; Giles, R. G.; Green, I. R.; Gruchlik, Y.; Oosthuizen, F. J. *Aust. J. Chem.* **2003**, *56*, 489.
16. Xing, X.; Ho, P.; Bourquin, G.; Yen, L. A.; Cuny, G. D. *Tetrahedron* **2003**, *59*, 9961.
17. Murata, T.; Sakakibara, S.; Yoshino, T.; Ikegami, Y.; Masuda, T.; Shimada, M.; Shintani, T.; Shimazaki, M.; Lowinger, T. B.; Ziegelbauer, K. B.; Fuchikami, K.; Umeda, M.; Komura, H.; Yoshida, N. WO 0244153, 2002.
18. Ohtaki, T.; Kumano, S.; Ishibashi, Y.; Ogi, K.; Matsui, H.; Harada, M.; Kitada, C.; Kurokawa, T.; Onda, H.; Fujino, M. *J. Biol. Chem.* **1999**, *274*, 37041.