Bioorganic & Medicinal Chemistry 18 (2010) 5157–5171

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

2-Acylamino-4,6-diphenylpyridine derivatives as novel GPR54 antagonists with good brain exposure and in vivo efficacy for plasma LH level in male rats

Toshitake Kobayashi [a,*], Satoshi Sasaki [a], Naoki Tomita [a], Seiji Fukui [b], Masaharu Nakayama [b], Atsushi Kiba [a], Masami Kusaka [a], Shin-ichi Matsumoto [b], Masashi Yamaguchi [b], Fumio Itoh [b], Atsuo Baba [a]

[a] Pharmaceutical Research Division, Takeda Pharmaceutical Company, Ltd, 10 Wadai, Tsukuba-shi, Ibaraki 300-4293, Japan
[b] Pharmaceutical Research Division, Takeda Pharmaceutical Company, Ltd, 17-85, Jusohonmachi 2-Chome, Yodogawa-ku, Osaka 532-8686, Japan

ARTICLE INFO

Article history:
Received 26 April 2010
Revised 21 May 2010
Accepted 22 May 2010
Available online 1 June 2010

Keywords:
GPR54 antagonist
2-Acylamino-4,6-diphenylpyridine
Metastin
Kisspeptin

ABSTRACT

GPR54 is a G protein-coupled receptor (GPCR) which was formerly an orphan receptor. Recent functional study of GPR54 revealed that the receptor plays an essential role to modulate sex-hormones including GnRH. Thus, antagonists of GPR54 are expected to be novel drugs for sex-hormone dependent diseases such as prostate cancer or endometriosis. We recently reported 2-acylamino-4,6-diphenylpyridines as the first small molecule GPR54 antagonists with high potency. However, the representative compound **1** showed low brain exposure, where GPR54 acts as a modulator of gonadotropins by binding with its endogenous ligand, metastin. In order to discover compounds that have not only potent GPR54 antagonistic activity but also good brain permeability, we focused on converting the primary amine on the side chain to a secondary or tertiary amine, and finally we identified **15a** containing a piperazine group. This compound exhibited high affinity to human and rat GPR54, apparent antagonistic activity, and high brain exposure. In addition, intravenous administration of **15a** to castrated male rat suppressed plasma LH level, which indicates the possibility of a small molecule GPR54 antagonist as a novel drug for sex-hormone dependent diseases.

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 gonadotropic hormones and such compounds would be novel drugs for sex-hormone dependent diseases, including prostate cancer and endometriosis.

We have previously reported 2-acylamino-4,6-diphenylpyridine derivatives as the first small molecule GPR54 antagonists.[13] By a structure–activity relationship (SAR) study, we revealed that the 2-acylamino-4,6-diphenylpyridine derivatives bearing a primary amine on their side chain showed high antagonistic activities for GPR54. We then conducted in vivo evaluation using compounds

* Corresponding author. Tel.: +81 29 864 6465; fax: +81 29 864 6308.
E-mail address: Kobayashi_Toshitake@takeda.co.jp (T. Kobayashi).

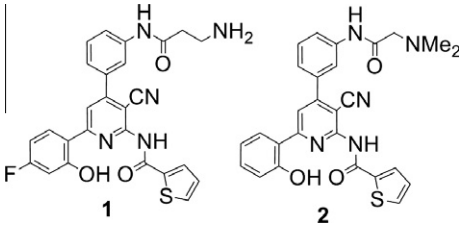
0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.05.061

including **1**, which has potent binding activity (Table 1, hGPR54 IC_{50} = 7.4 nM), however, those compounds did not show in vivo efficacy. After detailed investigation, exposure of these compounds in brain was revealed to be unsatisfactory, and compounds with a primary amine moiety appeared to be the substrate of P-glycoprotein (P-gp) transporters by Caco-2 permeability test. For example, the ratio of basal-to-apical (B-to-A) versus apical-to-basal (A-to-B) flux of compound **1** was 49, and these compounds were supposed to have low blood–brain-barrier (BBB) penetration. As mentioned above, metastin acts on GPR54 in the brain to modulate gonadotropins, therefore these compounds were thought to be unsuitable for in vivo evaluation. We also found that modification of the primary amine at the side chain to secondary or tertiary amines improves Caco-2 permeability as well as enhances lipophilicity, though in vitro GPR54 antagonistic potency declined. For example, compound **2**, which possesses a dimethylamino moiety, showed good Caco-2 permeability and high concentration in rat brain in a rat cassette dosing test, while the affinity for GPR54 was deteriorated (Table 1).

Based on this information, we focused on modifying the primary amine moiety to secondary or tertiary amines to identify compounds with good brain permeability as well as potent GPR54 antagonistic activity (Fig. 1). Other parts of the compounds were fixed to the substituents of compound **3**,[13] which has the most potent affinity for GPR54 (hGPR54 IC_{50} = 3.7 nM) in our previous study; the 2-position of the pyridine ring is a 2-furoylamine and the 6-position is a 4-fluoro-2-hydroxybenzene.

Herein, we report the SAR studies of 2-acylamino-4,6-diphenylpyridine derivatives with secondary or tertiary amines on the side chains, their brain exposure, and the efficacy of in vivo examination.

Table 1
Receptor binding assay for human GPR54, Caco-2 permeability, log *D*, and plasma and brain exposure data of compounds **1** and **2**



Compound		1	2
hGPR54 RBA	IC_{50} (nM)	7.4	180
Caco-2 permeability	B-to-A/A-to-B	49	1
Log *D* at pH 7.4		2.14	3.07
iv rat cassette dosing test (0.2 mpk, 30 min)	Brain (ng/g)	2.2	39.2
	Plasma (ng/ml)	13.4	29.6
	Kp	0.16	1.32

RBA = receptor binding assay.
Kp = the brain-to-plasma concentration ratio.

2. Chemistry

The synthesis of pyridine derivatives **6a–i** and **7a–j**, which possess a secondary or tertiary amine on their side chain is shown in Scheme 1. Compounds **5a–j** were synthesized by condensation of the known aniline **4**[13] and *tert*-butoxycarbonyl(Boc)-protected amino acids. Deprotection of Boc and methoxymethyl (MOM) groups of **5a–j** with HCl gave **3** and **6a–i**. Amines **3** and **6d–g** were converted to **7a–j** under the conditions of reductive alkylation using $NaBH_3(CN)$. In the case of **6f**, a large amount of acetaldehyde (20 equiv) was needed to afford the diethylated compound **7i**. Alkylation of amine **6e** with a large excess of acetone gave only the monoalkylated compound **7e**.

Compounds **10a–c** having *N*,*N*-dialkylated glycine moiety on their side chain were prepared as Scheme 2. Aniline **4** was treated with chloroacetyl chloride to give compound **8**. Amination of compound **8** afforded **9a–c**, followed by deprotection of MOM group with HCl to produce **10a–c**.

The synthesis of pyridine derivatives **15a–d** bearing a piperazine or homopiperazine moiety on the 4-phenyl group is shown in Scheme 3. The four component condensation reaction of ketone **11**,[13] 3-bromobenzaldehyde, malononitrile, and ammonium acetate constructed the pyridine ring to give compound **12**. Acylation of the amine at the 2-position of the pyridine ring with furoyl chloride gave the diacylated compound, which was converted to the monoacylated compound **13** by treatment with basic silica gel. The coupling reaction of **13** with piperazine or homopiperazine derivatives, catalyzed by Pd complex, afforded compounds **14a–d**. Treatment with HCl gave deprotected compounds **15a–d**.

Compounds **21a** and **21b**, with a methylene linker inserted between a piperazine or homopiperazine ring and the phenyl ring, were synthesized as described in Scheme 4. The reduction of the methyl ester **16**[13] afforded alcohol **17**. After mesylation of the hydroxy group of **17**, displacement with a Boc-protected piperazine or homopiperazine was conducted to give compounds **19a** and **19b**. Treatment with TFA or HCl to remove the protecting groups afforded compounds **20a** and **20b**. For compound **20a**, reductive alkylation yielded *N*-methyl (**21a**) and *N*-ethyl (**21b**) derivatives.

The synthesis of the inverse-amide type compound **24** is shown in Scheme 5. Carboxylic acid **22**[13] was condensed with *N*,*N*-dimethylethylenediamine to give amide **23**, followed by deprotection of MOM group with HCl to afford amine **24**.

Conversion of the amide linker between the phenyl ring and amino group to ethylene is shown in Scheme 6. Reductive alkylation of **4** with *N*-Boc-2-aminoacetaldehyde gave **25**, followed by treatment with HCl to afford amine **26**. Subsequent methylation of the amino group provided **27**.

3. Results and discussion

All compounds synthesized were initially evaluated by their binding affinities for human GPR54 against ^{125}I-labeled human metastin (40–54) in a receptor binding assay.

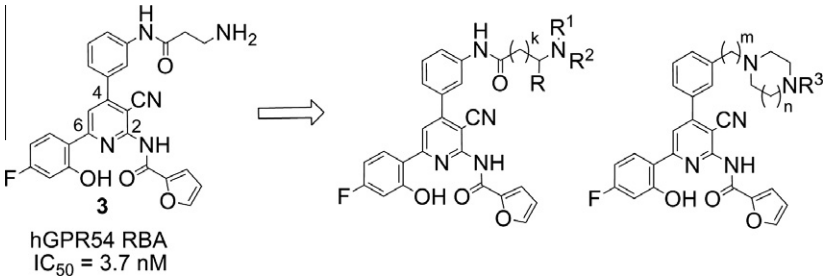
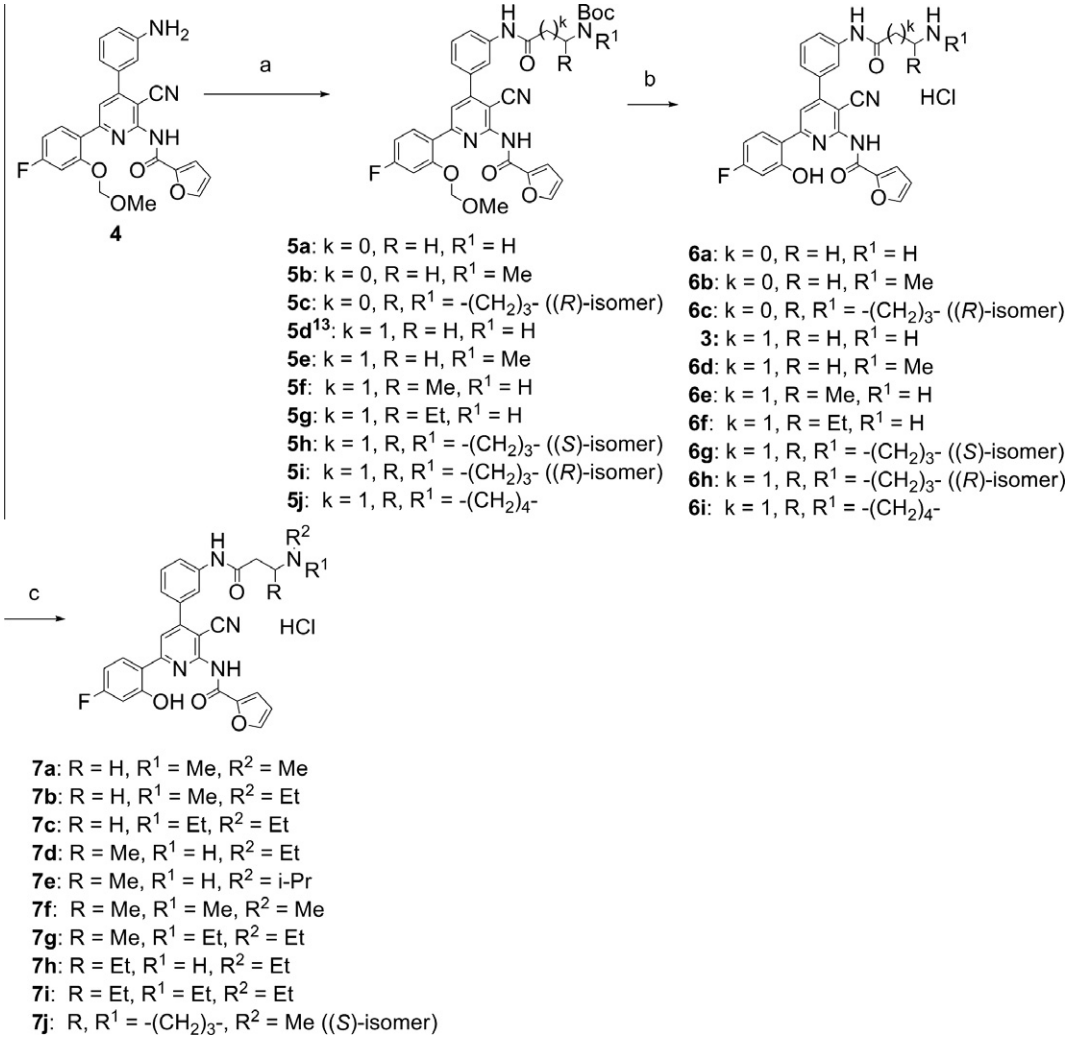
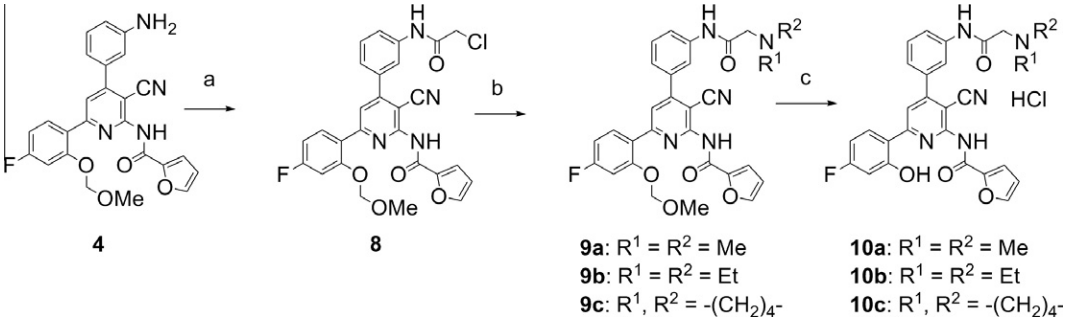


Figure 1. The strategy of identification of compounds with good brain permeability as well as potent GPR54 binding activity.



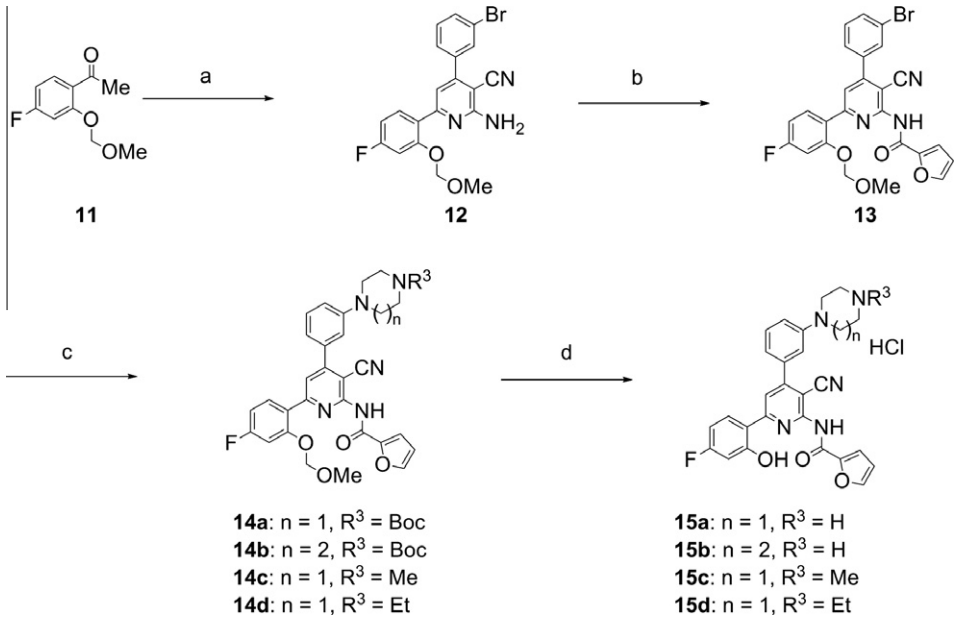
Scheme 1. Synthesis of compounds with a secondary or tertiary amine on their side chain. Reagents and conditions: (a) carboxylic acid, WSCD, HOBt, DMF, rt; (b) 4 M HCl EtOAc solution, EtOAc, rt; (c) (i) aldehyde or ketone, $NaBH_3(CN)$, AcOH, NEt_3, MeOH, rt; (ii) 4 M HCl EtOAc solution, EtOAc, rt.



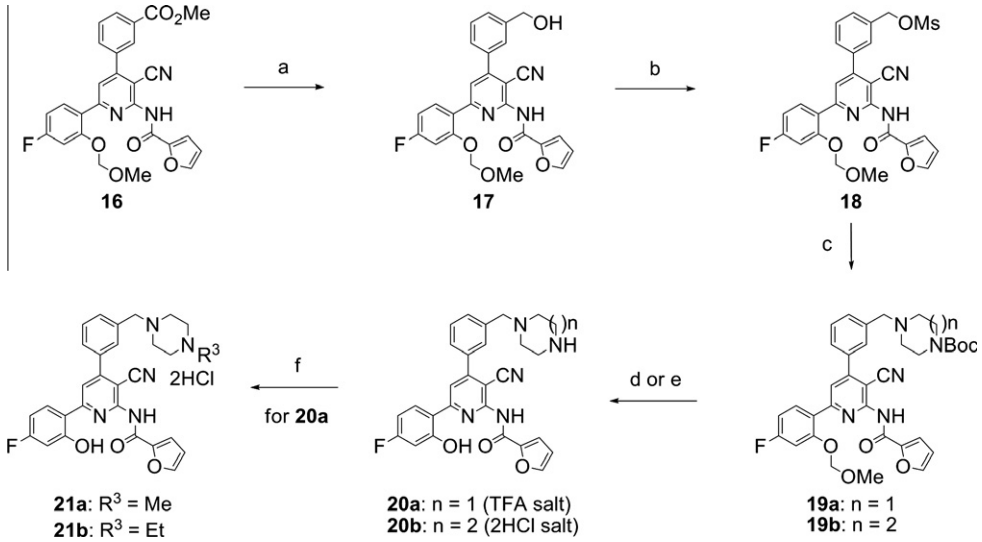
Scheme 2. Synthesis of compounds with a glycine amide as their side chain. Reagents and conditions: (a) chloroacetyl chloride, NEt^iPr_2, THF, 0 °C; (b) amine, K_2CO_3, DMF, rt; (c) 4 M HCl EtOAc solution, MeOH, rt.

The result for compounds possessing an alkylated β-alanine amide moiety is shown in Table 2. Monomethylamine derivative **6d** showed comparable activity to primary amine **3**, however, the affinities of dimethylamine derivative **7a** and other tertiary amine derivatives (**7b**, **7c**) were weakened. These results reconfirmed that the amine group at the end of the side chain is a critical component for activity. Next, we introduced a methyl or ethyl group onto the α-position of the amino group to investigate the sterical tolerance around the amine moiety. Comparing the potency of compounds **7c**, **7g**, and **7i**, introduction of small alkyl groups as R is allowed. Furthermore, the affinity relationship between secondary amines and tertiary amines was retained. When R was methyl, secondary amines **7d** and **7e** were more potent than the tertiary amines **7f** and **7g**, and when R was ethyl, the tendency appeared more clearly (**7h** vs **7i**).

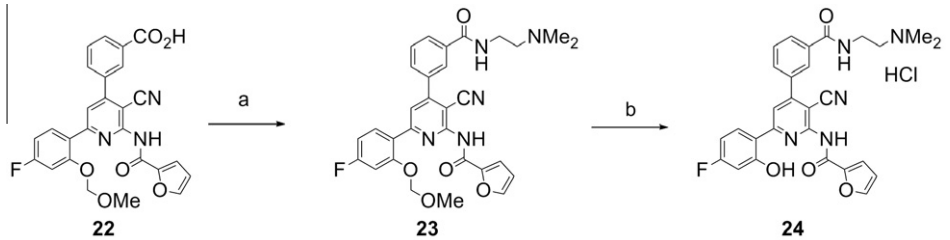
As introduction of an alkyl group at the α-position of the amino group was allowed, cyclic amine derivatives **6g–i** and **7j** were synthesized to evaluate the effect of fixing the amine moiety.



Scheme 3. Synthesis of compounds bearing a piperazine or homopiperazine ring. Reagents and conditions: (a) 3-bromobenzaldehyde, NH_4OAc, malononitrile, toluene, reflux; (b) 2-furoyl chloride, pyridine, 0 °C then purification by basic silica gel; (c) amine, $Pd_2(dba)_3$, XPhos, NaO^tBu, toluene, reflux; (d) 4 M HCl EtOAc solution, MeOH, rt.

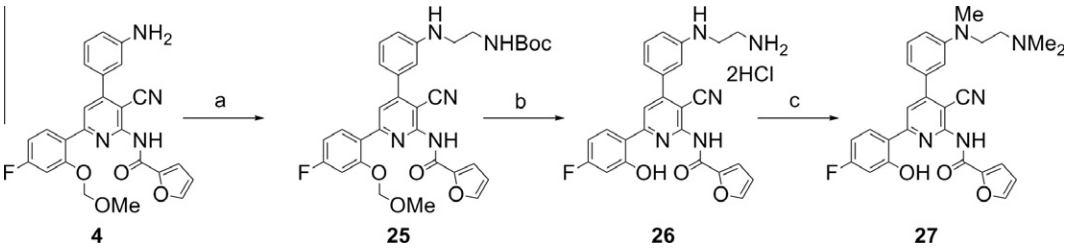


Scheme 4. Synthesis of compounds possessing a methylene linker between a piperazine or piperazine ring and the phenyl ring. Reagents and conditions: (a) $LiBH_4$, THF, 45 °C; (b) MsCl, NEt_3, CH_2Cl_2, rt; (c) amine, NEt_3, THF, rt; (d) TFA, CH_2Cl_2, rt for **19a**; (e) 4 M HCl EtOAc solution, MeOH, rt for **19b**; (f) (i) aldehyde, $NaBH_3(CN)$, AcOH, Et_3N, MeOH, rt; (ii) 4 M HCl EtOAc solution, EtOAc, rt.



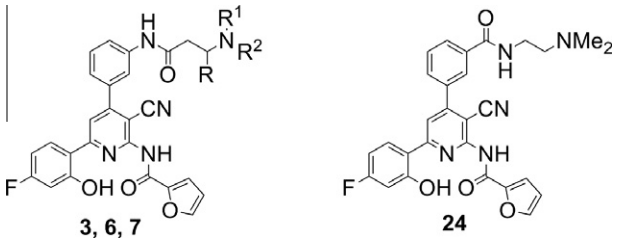
Scheme 5. Synthesis of inverse-amide type compound **24**. Reagents and conditions: (a) *N*,*N*-dimethylethylenediamine, WSCD, HOBt, DMF, rt; (b) 4 M HCl EtOAc solution, MeOH, rt.

Compound **7j** showed similar affinity to the linear analogue **7f**. Secondary amines **6g** and **6h** increased activity compared to tertiary amine **7j**, and the (*S*)-isomer **6g** was more potent than the (*R*)-isomer **6h**. Compound **6i**, which has a piperidine instead of the pyrrol-



Scheme 6. Synthesis of compound **27** having an ethylene linker between the phenyl ring and amino group. Reagents and conditions: (a) *N*-Boc-2-aminoacetaldehyde, $NaBH(OAc)_3$, AcOH, THF, rt; (b) 4 M HCl EtOAc solution, EtOAc, rt; (c) formaldehyde, $NaBH_3(CN)$, AcOH, MeOH, rt.

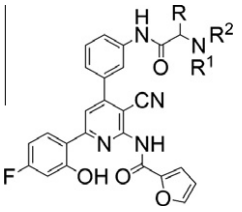
Table 2
The binding affinities of β-alanine amide derivatives for human type GPR54



Compound	R	R^1	R^2		hGPR54 RBA IC_{50} (nM)
3	H	H	H		3.7
6d	H	Me	H		4.6
7a	H	Me	Me		10
7b	H	Me	Et		10
7c	H	Et	Et		16
7d	Me	H	Et	Racemate	10
7e	Me	H	*i*-Pr	Racemate	9.7
7f	Me	Me	Me	Racemate	18
7g	Me	Et	Et	Racemate	17
7h	Et	H	Et	Racemate	8.0
7i	Et	Et	Et	Racemate	22
6g	$-(CH_2)_3-$	H		(*S*)-Isomer	7.9
6h	$-(CH_2)_3-$	H		(*R*)-Isomer	10
6i	$-(CH_2)_4-$	H		Racemate	14
7j	$-(CH_2)_3-$	Me		(*S*)-Isomer	18
24					20

RBA = receptor binding assay.

Table 3
The binding affinities of glycine amide derivatives for human type GPR54



Compound	R^1	R^2	R		hGPR54 RBA IC_{50} (nM)
6a	H	H	H		4.1
6b	H	Me	H		8.4
6c	H	$-(CH_2)_3-$		(*R*)-Isomer	16
10a	Me	Me	H		12
10b	Et	Et	H		30
10c	$-(CH_2)^4-$		H		15

RBA = receptor binding assay.

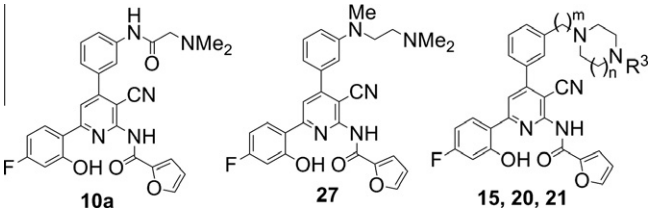
idine of β-proline, also showed potent activity, though it was racemic.

Compound **24**, which is an inverse-amide analogue of **7a**, reduced the activity, indicating that the anilide derivatives are preferable.

The affinities of compounds possessing an alkylated glycinyl group, for human type GPR54, are described in Table 3. The tendency of the affinity was similar to that of compounds with an alkylated β-alaninyl moiety. The primary amine **6a** was the most potent, secondary amine **6b** slightly reduced the affinity, and tertiary amine **10a** was the weakest. The diethylamine **10b** had reduced affinity compared with the dimethyl amine **10a**, while the cyclic amine derivative **10c** retained affinity. As the proline derivative **6c** resulted in a twofold decrease of affinity compared to **6b**, fixing the linker was not preferable, unlike for the β-alaninyl analogue.

Reducing hydrogen bonds, especially proton donors, and enhancing lipophilicity appropriately are common strategies to improve brain penetration.[14] We prepared compound **27**, bearing an amine linker instead of the polar amide linker in expectation of increasing brain permeation, however, the potency declined compared to that of the corresponding amide analogue **10a** (Table 4). We speculated that converting the linker amide to methylene increased the flexibility of the terminal amino group, which is essential for the affinity, and therefore it could not be located at an appropriate position. With the hypothesis above, we planned to fix the conformation of the linker and designed compound **15c**, having a piperazine ring instead of the amide linker. In the overlay of stable conformations of **10a** and **15c**, calculated by MOE,[15] the nitrogen atoms of **10a** and **15c** were located at similar positions (Fig. 2). Encouraged by this result, we prepared **15c** and its analogues and evaluated their affinities for human type GPR54 in a binding assay (Table 4). As expected, **15c** showed almost the same affinity as **10a**. Removal of the methyl group of

Table 4
The binding affinities of compounds without amide linker for human GPR54



Compound	*m*	*n*	R^3	hGPR54 RBA IC_{50} (nM)
10a				12
27				30
15a	0	1	H	3.6
15b	0	2	H	7.3
15c	0	1	Me	15
15d	0	1	Et	11
20a	1	1	H	9.7
20b	1	2	H	25
21a	1	1	Me	18
21b	1	1	Et	20

RBA = receptor binding assay.

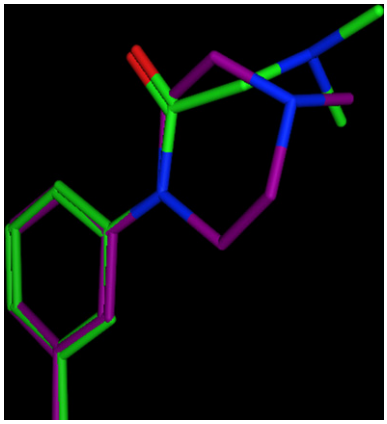


Figure 2. Overlay of the 4-phenyl moiety of **10a** (green) and **15c** (purple) in stable conformations calculated by MOE.[15]

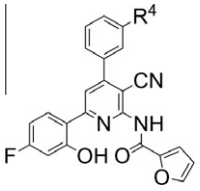
15c increased potency (**15a**) and converting the methyl group to an ethyl group retained the affinity (**15c** vs **15d**). A compound bearing a homopiperazine ring instead of the piperazine ring also showed potent activity (**15a** vs **15b**). Insertion of a methylene linker between the phenyl group and piperazine ring resulted in decreased activity, regardless of it being a secondary amine (**15a** vs **20a**) or tertiary amine (**15c** vs **21a**, **15d** vs **21b**).

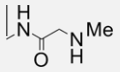
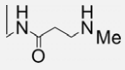
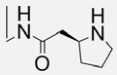
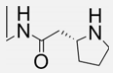
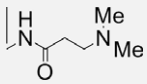
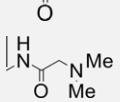
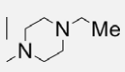
Selected compounds with potent affinities for human type GPR54 in a receptor binding assay were then evaluated by their ability to penetrate the brain. The brain and plasma levels of compounds in rats after 30 min of 0.2 mg/kg intravenous cassette administration are shown in Table 5. Generally, tertiary amine derivatives were superior to secondary amines regarding brain exposure (**6b** vs **10a**, **6d** vs **7a**, **15a** vs **15d**). Especially, tertiary amines **10a** and **15d** showed high concentration in brain and good brain-to-plasma concentration ratio (Kp). In the case of secondary amines, although almost all compounds showed low concentration in brain, compound **15a**, with amide linker converted to a piperazine ring, showed relatively high brain exposure, as was expected.

Caco-2 permeability test for **7a**, **10a**, **15a**, and **15d**, as representative compounds, was conducted to evaluate their P-gp susceptibility (Table 5). A value of B-to-A/A-to-B above 3.0 indicates that a compound is a P-gp substrate. Compound **7a**, which was revealed to be a substrate of P-gp, exhibited low concentration in brain and low Kp value compared to compound **15a**, without efflux effect by P-gp. From the brain exposure data of compounds **10a**, **15a**, and **15d**, lipophilicity can be seen to be essential for permeability to brain, when the effect of P-gp mediated efflux is negligible. Especially, a log *D* value above three looks preferable for the brain penetration considering the high brain concentration of **10a** (log *D* = 3.31) and **15d** (log *D* = 4.10).

Next we investigated the affinity of compounds for rat type GPR54 (Table 5). The result of a binding assay using rat type GPR54 was approximately parallel to that of human type GPR54, although the affinities were attenuated. Particularly, the rat receptor binding affinity of compound **10a**, having high brain permeabil-

Table 5
Brain and plasma exposure data in rats, Caco-2 permeability, and rat GPR54 binding affinities



Compound	R^4	iv rat cassette dosing test (0.2 mpk, 30 min) Concentration (plasma; ng/ml, brain; ng/g)		Caco-2 permeability B-to-A/A-to-B	Log *D* at pH 7.4	RBA IC_{50} (nM) Rat GPR54
6b		Plasma Brain Kp	11.7 <5 —	NT	2.66	26
6d		Plasma Brain Kp	5.2 <5 —	NT	2.13	20
6g		Plasma Brain Kp	11.6 3.7 0.32	NT	2.29	26
6h		Plasma Brain Kp	5.5 2.0 0.36	NT	2.13	38
7a		Plasma Brain Kp	15.9 8.1 0.51	4.3	2.95	47
10a		Plasma Brain Kp	19.3 80.3 4.16	1.4	3.31	130
15a		Plasma Brain Kp	14.0 13.2 0.94	1.2	2.72	15
15d		Plasma Brain Kp	23.8 93.1 3.91	1.0	4.10	47

Kp = the brain-to-plasma concentration ratio. NT = not tested. RBA = receptor binding assay.

ity, was reduced remarkably. On the other hand, compound **15a**, which showed moderate brain penetration in spite of a secondary amine, exhibited high affinity for rat GPR54. Considering both brain permeability and affinity for rat GPR54, **15a** and **15d** were selected for further examinations.

In order to evaluate the antagonistic activities of **15a** and **15d**, a cellular Ca^{2+} mobilization assay was carried out using metastin (40–54) as a stimulator. The result is shown in Figure 3. Compound **15a** exhibited fully potent antagonist activity (IC_{50} = 0.93 μM), however, **15d** did not suppress the Ca^{2+} influx completely at an adequately high concentration. We concluded that **15d** was not suitable for further investigation, and **15a** was selected for in vivo study.

Finally we tested the effect of **15a** on the plasma LH level in castrated male rats. Administration of **15a** intravenously at 0.22 mg/kg was repeated five times at 10 min intervals, ending up with a total dosage at 1.1 mg/kg. The result of plasma LH level measurement is shown in Figure 4. The first administration was performed at 0 min, which is indicated by an arrow, and the following four administrations are indicated with arrowheads. The vehicle administration did not show any effects on plasma LH level during 180 min of blood collection. On the contrary, 60 min after the administration of compound **15a** the plasma LH level was reduced by 32%. This result is the first example of a small molecule GPR54 antagonist suppressing the plasma LH level and suggests the possibility of GPR54 antagonists as drugs for sex-hormone dependent diseases such as prostate cancer and endometriosis.

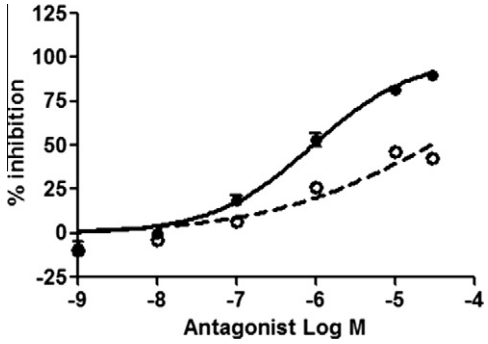


Figure 3. The antagonistic activities of **15a** (solid line) and **15d** (broken line) against metastin (40–54) in CHO cell.

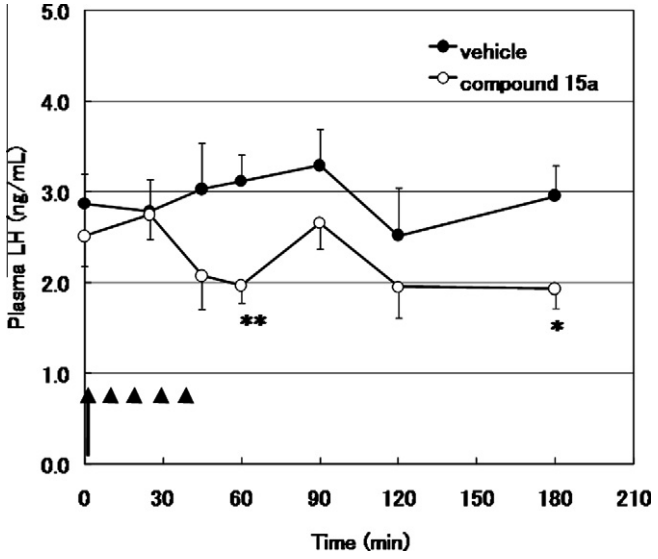


Figure 4. Effect of compound **15a** on plasma LH level in castrated male rats. Values are means ± SE, determined from eight experiments. $^{*}P$ <0.05 and $^{**}P$ <0.01 compared with the vehicle control.

4. Conclusion

To identify compounds having potent GPR54 antagonistic activity and brain permeability, chemical modification focused on the side chain on the 4-phenyl ring was performed. As a result, secondary or tertiary amine derivatives **10a**, **15a**, and **15d** were discovered, which had high affinity for human GPR54 and good brain permeability. Excluding **10a**, because of low potency in a rat receptor binding assay, the cellular Ca^{2+} mobilization assay was conducted for **15a** and **15d**. Unfortunately **15d** did not suppress the Ca^{2+} influx completely at adequately high concentration, however, **15a** showed fully potent antagonistic activity. Finally, compound **15a** was selected for an in vivo examination, and the intravenous injection of **15a** for castrated male rats apparently suppressed the plasma LH level. The result indicates the possibility of small molecule GPR54 antagonists for treatment of sex-hormone dependent diseases such as prostate cancer and endometriosis.

5. Experimental section

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). Element 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. *tert*-Butyl {2-[(3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)amino]-2-oxoethyl}carbamate (5a)

To an ice-cooled mixture of *N*-{4-(3-aminophenyl)-3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]pyridin-2-yl}furan-2-carboxamide[13] (**4**, 0.20 g, 0.44 mmol), *N*-(*tert*-butoxycarbonyl)glycine (0.15 g, 0.88 mmol), and HOBt (0.12 g, 0.88 mmol) in DMF (5 ml) was added WSCD (0.17 g, 0.88 mmol). After stirring at room temperature for 40 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 = 3/2). Recrystallization from EtOAc/hexane gave **5a** (0.21 g, 78%) as colorless crystals: mp 119–121 °C; ^{1}H NMR (DMSO-d_6) δ 1.49 (9H, s), 3.47 (3H, s), 3.95 (2H, d, *J* = 6.0 Hz), 5.10–5.25 (1H, m), 5.30 (2H, s), 6.61 (1H, q, *J* = 1.8 Hz), 6.84–6.91 (1H, m), 7.02 (1H, dd, *J* = 10.5, 2.4 Hz), 7.39 (1H, dd, *J* = 3.3, 1.5 Hz), 7.48–7.61 (4H, m), 7.97 (1H, s), 8.00–8.03 (1H, m), 8.20–8.35 (1H, br s), 8.81 (1H, s).

5.2. *tert*-Butyl {2-[(3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)amino]-2-oxoethyl}methylcarbamate (5b)

Compound **5b** was prepared from **4** (500 mg, 1.1 mmol) and *N*-(*tert*-butoxycarbonyl)-*N*-methylglycine (410 mg, 2.2 mmol) in a manner similar to that described for **5a**. Compound **5b** (540 mg, 78%) was obtained as a colorless solid: mp 162–164 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 1.51 (9H, s), 3.03 (3H, s), 3.48 (3H, s), 4.00 (2H, s), 5.31 (2H, s), 6.61 (1H, dd, J = 3.6, 1.8 Hz), 6.84–6.90 (1H, m), 7.03 (1H, dd, J = 10.8, 2.4 Hz), 7.39 (1H, dd, J = 3.3, 0.6 Hz), 7.45 (3H, br s), 7.59–7.61 (1H, m), 7.97–8.05 (3H, m), 8.60 (1H, br s), 8.82 (1H, br s).

5.3. *tert*-Butyl (*2R*)-2-[(3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)carbamoyl]pyrrolidine-1-carboxylate (5c)

Compound **5c** was prepared from **4** (40 mg, 0.087 mmol) and *N*-(*tert*-butoxycarbonyl)-D-proline (40 mg, 0.19 mmol) in a manner similar to that described for **5a**. Compound **5c** (39 mg, 68%) was obtained as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.51 (10H, s), 1.85–2.00 (2H, m), 2.45–2.65 (1H, br), 3.30–3.50 (5H, m), 4.40–4.55 (1H, m), 5.32 (2H, s), 6.61 (1H, q, J = 1.8 Hz), 6.84–6.91 (1H, m), 7.03 (1H, dd, J = 10.8, 2.4 Hz), 7.39 (1H, dd, J = 6.6, 0.6 Hz), 7.48 (3H, br s), 7.59–7.61 (1H, m), 7.98 (1H, s), 8.03 (1H, dd, J = 8.7, 6.9 Hz), 8.12 (1H, s), 8.81 (1H, s), 9.65–9.85 (1H, m).

5.4. *tert*-Butyl {3-[(3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)amino]-3-oxopropyl}methylcarbamate (5e)

Compound **5e** was prepared from **4** (500 mg, 1.1 mmol) and *N*-(*tert*-butoxycarbonyl)-*N*-methyl-β-alanine (440 mg, 2.2 mmol) in a manner similar to that described for **5a**. Compound **5e** (530 mg, 75%) was obtained as a colorless solid: mp 172–174 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 2.69 (2H, br s), 2.91 (3H, s), 3.47 (3H, s), 3.64 (2H, t, J = 6.3 Hz), 5.31 (2H, s), 6.61 (1H, dd, J = 3.3, 1.5 Hz), 6.84–6.91 (1H, m), 7.03 (1H, dd, J = 10.8, 2.4 Hz), 7.38 (1H, dd, J = 3.6, 0.6 Hz), 7.44–7.51 (2H, m), 7.59–7.60 (2H, m), 7.96 (1H, s), 8.02 (1H, dd, J = 8.4, 6.9 Hz), 8.14 (1H, br s), 8.82 (1H, br s), 9.07 (1H, br s).

5.5. *tert*-Butyl 2-{2-[(3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)amino]-2-oxoethyl}piperidine-1-carboxylate (5j)

Compound **5j** was prepared from **4** (190 mg, 0.41 mmol) and [1-(*tert*-butoxycarbonyl)piperidine-2-yl]acetic acid (130 mg, 0.53 mmol) in a manner similar to that described for **5a**. Compound **5j** (230 mg, 82%) was obtained as a pale yellow solid: mp 154–156 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 1.43 (9H, s), 1.52–1.76 (6H, m), 2.57 (1H, dd, J = 5.4 Hz, 16.2 Hz), 2.83–2.96 (2H, m), 3.47 (3H, s), 3.98 (1H, d, J = 14.7 Hz), 4.89 (1H, br s), 5.31 (2H, s), 6.61 (1H, dd, J = 3.3, 1.8 Hz), 6.84–6.91 (1H, m), 7.03 (1H, dd, J = 10.8, 2.4 Hz), 7.38 (1H, dd, J = 3. 6, 0.9 Hz), 7.43–7.50 (2H, m), 7.53–7.61 (2H, m), 7.97 (1H, s), 8.02 (1H, dd, J = 9.0, 6.9 Hz), 8.16 (1H, br s), 8.81 (1H, br s), 9.01 (1H, br s).

5.6. *N*-{3-Cyano-6-(4-fluoro-2-hydroxyphenyl)-4-[3-(glycylamino)phenyl]pyridin-2-yl}furan-2-carboxamide hydrochloride (6a)

A mixture of **5a** (0.20 g, 0.32 mmol) and 4 M HCl EtOAc solution (4 ml) in EtOAc (16 ml) was stirred at room temperature for 40 h. The precipitate was collected by filtration and recrystallization from MeOH/EtOAc gave **6a** (0.16 g, 100%) as a pale yellow solid: mp 220–236 °C; ^{1}H NMR (DMSO-d_6) δ 3.85 (2H, d, J = 5.7 Hz), 6.79–6.87 (3H, m), 7.47 (1H, d, J = 7.8 Hz), 7.54 (1H, d, J = 3.3 Hz), 7.62 (1H, t, J = 7.8 Hz), 7.82 (1H, d, J = 8.1 Hz), 8.00 (1H, s), 8.02 (1H, t, J = 0.9 Hz), 8.16–8.26 (5H, m), 10.87 (1H, s), 11.34 (1H, s), 12.33 (1H, s); Anal. Calcd for $C_{25}H_{18}FN_5O_4{\cdot}HCl{\cdot}H_2O$: C, 57.09; H, 4.02; N, 13.32. Found: C, 56.84; H, 3.83; N, 13.09.

5.7. *N*-[3-Cyano-6-(4-fluoro-2-hydroxyphenyl)-4-{3-[(*N*-methylglycyl)amino]phenyl}pyridin-2-yl]furan-2-carboxamide hydrochloride (6b)

Compound **6b** was prepared from **5b** (500 mg, 0.79 mmol) in a manner similar to that described for **6a**. Compound **6b** (380 mg, 92%) was obtained as a pale yellow solid: mp 294–296 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 2.64 (3H, s), 4.00 (2H, s), 6.79–6.89 (3H, m), 7.47 (1H, d, J = 8.1 Hz), 7.55 (1H, dd, J = 3.6, 0.6 Hz), 7.62 (1H, t, J = 7.8 Hz), 7.83 (1H, d, J = 8.1 Hz), 8.02 (1H, s), 8.08 (1H, dd, J = 1.8, 0.6 Hz), 8.16 (1H, s), 8.24 (1H, dd, J = 8.7, 6.9 Hz), 9.03 (1H, br s), 11.04 (1H, s), 11.33 (1H, s), 12.34 (1H, s); Anal. Calcd for $C_{26}H_{20}FN_5O_4{\cdot}HCl$: C, 59.72; H, 3.86; N, 13.34. Found: C, 59.83; H, 4.06; N, 13.42.

5.8. *N*-(3-{3-Cyano-6-(4-fluoro-2-hydroxyphenyl)-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)-D-prolinamide hydrochloride (6c)

Compound **6c** was prepared from **5c** (190 mg, 0.29 mmol) in a manner similar to that described for **6a**. Compound **6c** (122 mg, 77%) was obtained as a pale yellow solid: mp 216–220 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 1.91–2.07 (3H, m), 2.39–2.51 (1H, m), 3.22–3.29 (2H, m), 4.41 (1H, t, J = 7.2 Hz), 6.79–6.87 (3H, m), 7.47–7.55 (2H, m), 7.63 (1H, t, J = 7.8 Hz), 7.84 (1H, d, J = 8.4 Hz), 8.02 (1H, s), 8.08 (1H, d, J = 0.9 Hz), 8.16 (1H, s), 8.23 (1H, t, J = 7.8 Hz), 9.00–9.60 (2H, m), 11.02 (1H, s), 11.30–11.40 (1H, m), 12.33 (1H, s); $[\alpha]_D^{25}$ = +19.1 (c 1.0, DMSO); Anal. Calcd for $C_{28}H_{22}FN_5O_4{\cdot}HCl{\cdot}H_2O$: C, 59.42; H, 4.45; N, 12.37. Found: C, 59.17; H, 4.52; N, 12.29.

5.9. *N*-[3-Cyano-6-(4-fluoro-2-hydroxyphenyl)-4-{3-[(*N*-methyl-β-alanyl)amino]phenyl}pyridin-2-yl]furan-2-carboxamide hydrochloride (6d)

Compound **6d** was prepared from **5e** (500 mg, 0.78 mmol) in a manner similar to that described for **6a**. Compound **6d** (380 mg, 91%) was obtained as a pale yellow solid: mp 253–255 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 2.58 (3H, t, J = 5.4 Hz), 2.86 (2H, t, J = 6.9 Hz), 3.16–3.22 (2H, m), 6.79–6.88 (3H, m), 7.41 (1H, d, J = 7.8 Hz), 7.53–7.60 (2H, m), 7.83 (1H, d, J = 8.1 Hz), 8.01 (1H, s), 8.08 (1H, dd, J = 1.5, 0.6 Hz), 8.14 (1H, s), 8.23 (1H, dd, J = 8.1, 6.6 Hz), 8.74 (2H, br s), 10.61 (1H, s), 11.31 (1H, s), 12.33 (1H, s); Anal. Calcd for $C_{27}H_{22}FN_5O_4{\cdot}HCl{\cdot}H_2O$: C, 58.54; H, 4.55; N, 12.64. Found: C, 58.80; H, 4.71; N, 12.70.

5.10. *N*-[3-Cyano-6-(4-fluoro-2-hydroxyphenyl)-4-{3-[(piperidin-2-ylacetyl)amino]phenyl}pyridin-2-yl]furan-2-carboxamide hydrochloride (6i)

Compound **6i** was prepared from **5j** (150 mg, 0.22 mmol) in a manner similar to that described for **6a**. Compound **6i** (97 mg, 77%) was obtained as a yellow solid: mp 277–278 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 1.51–1.88 (6H, m), 2.73–2.98 (3H, m), 3.26 (1H, d, J = 12.6 Hz), 6.79–6.88 (3H, m), 7.42 (1H, d, J = 7.8 Hz), 7.54–7.61 (2H, m), 7.82 (1H, d, J = 9.0 Hz), 8.02 (1H, br s), 8.08 (1H, d, J = 0.9 Hz), 8.14 (1H, s), 8.23–8.26 (1H, m), 8.78–8.91 (2H, m), 10.65 (1H, s), 11.32 (1H, s), 12.34 (1H, s); Anal. Calcd

for $C_{30}H_{26}FN_5O_4{\cdot}HCl{\cdot}0.5H_2O$: C, 61.59; H, 4.82; N, 11.97. Found: C, 61.28; H, 4.90; N, 12.05.

5.11. *N*-[3-Cyano-6-(4-fluoro-2-hydroxyphenyl)-4-(3-{[(2*S*)-pyrrolidin-2-ylacetyl]amino}phenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (6g)

To a solution of compound **4** (500 mg, 1.1 mmol), [(2*S*)-1-(*tert*-butoxtcabonyl)pyrrolidin-2-yl]acetic acid[16] (300 mg, 1.3 mmol), and HOBt (180 mg, 1.3 mmol) in DMF (5 ml) was added WSCD (250 mg, 1.3 mmol) and the mixture was stirred at room temperature for 5 days. The solution was diluted with EtOAc, washed with water three times and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane to hexane/EtOAc = 1/1) and basic silica gel column chromatography (hexane to EtOAc) to give *tert*-butyl (2*S*)-2-{2-[(3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)amino]-2-oxoethyl}pyrrolidine-1-carboxylate (**5h**) as an amorphous solid. After the solid was dissolved in EtOAc (5 ml) and MeOH (2 ml), 4 M HCl EtOAc solution (5 ml) was added and the mixture was stirred at room temperature overnight. The solvent was evaporated and the residue was recrystallized from MeOH/Et_2O to give **6g** (390 mg, 63%) as a pale yellow solid: mp 268–269 °C; ^{1}H NMR (DMSO-d_6) δ 1.55–1.68 (1H, m), 1.81–1.97 (2H, m), 2.10–2.20 (1H, m), 2.93 (2H, d, J = 6.9 Hz), 3.13–3.21 (2H, m), 3.76–3.85 (1H, m), 6.79–6.89 (3H, m), 7.41 (1H, d, J = 7.8 Hz), 7.54–7.61 (2H, m), 7.81–7.84 (1H, m), 8.04 (1H, br s), 8.08–8.09 (1H, m), 8.15 (1H, s), 8.23 (1H, dd, J = 8.4, 6.6 Hz), 8.94 (1H, br s), 9.18 (1H, br s), 10.64 (1H, s), 11.33 (1H, s), 12.33 (1H, s); $[\alpha]_D^{25}$ = +16.6 (*c* 1.0, DMSO); Anal. Calcd for $C_{29}H_{24}FN_5O_4{\cdot}HCl{\cdot}0.25H_2O$: C, 61.49; H, 4.54; N, 12.36. Found: C, 61.33; H, 4.52; N, 12.41.

5.12. *N*-[4-{3-[(3-Aminobutanoyl)amino]phenyl}-3-cyano-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (6e)

Compound **6e** was prepared from **4** (500 mg, 1.09 mmol) and *N*-(*tert*-butoxycarbonyl)-3-aminobutanoic acid (443 mg, 2.18 mmol) in a manner similar to that described for **6g**. Compound **6e** (411 mg, 70%) was obtained as a yellow solid: mp 213–215 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 1.26 (3H, d, J = 6.6 Hz), 2.60–2.85 (2H, m), 3.50–3.70 (1H, m), 6.75–6.90 (3H, m), 7.41 (1H, d, J = 8.1 Hz), 7.54 (1H, d, J = 3.9 Hz), 7.59 (1H, d, J = 7.8 Hz), 7.83 (1H, d, J = 8.1 Hz), 7.90–8.10 (5H, m), 8.14 (1H, s), 8.23 (1H, t, J = 7.5 Hz), 10.60 (1H, s), 11.31 (1H, s), 12.34 (1H, s); Anal. Calcd for $C_{27}H_{22}FN_5O_4{\cdot}HCl{\cdot}1.5H_2O$: C, 57.60; H, 4.65; N. 12.44. Found: C, 57.49; H, 4.66; N, 12.38.

5.13. *N*-[4-{3-[(3-Aminopentanoyl)amino]phenyl}-3-cyano-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (6f)

Compound **6f** was prepared from **4** (120 mg, 0.26 mmol) and *N*-(*tert*-butoxycarbonyl)-3-aminopentanoic acid (114 mg, 0.52 mmol) in a manner similar to that described for **6g**. Compound **6f** (105 mg, 73%) was obtained as a yellow solid: mp 212–214 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 0.95 (3H, t, J = 7.6 Hz), 1.55–1.75 (2H, m), 2.65–2.90 (2H, m), 3.46 (1H, br s), 6.75–6.90 (3H, m), 7.42 (1H, d, J = 7.6 Hz), 7.54 (1H, d, J = 3.6 Hz), 7.58 (1H, t, J = 8.0 Hz), 7.83 (1H, d, J = 8.8 Hz), 8.01 (4H, br s), 8.08 (1H, s), 8.14 (1H, s), 8.23 (1H, t, J = 7.6 Hz), 10.63 (1H, s), 11.31 (1H, s), 12.33 (1H, s); Anal. Calcd for $C_{28}H_{24}FN_5O_4{\cdot}HCl{\cdot}1.5H_2O$: C, 58.28; H, 4.89; N. 12.14. Found: C, 58.36; H, 4.77; N, 12.13.

5.14. *N*-[3-Cyano-6-(4-fluoro-2-hydroxyphenyl)-4-(3-{[(2*R*)-pyrrolidin-2-ylacetyl]amino}phenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (6h)

Compound **6h** was prepared from **4** (400 mg, 0.87 mmol) and [(2*R*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]acetic acid[16] (300 mg, 1.31 mmol) in a manner similar to that described for **6g**. Compound **6h** (340 mg, 70%) was obtained as a yellow solid: mp 269–270 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 1.55–1.68 (1H, m), 1.81–1.97 (2H, m), 2.10–2.20 (1H, m), 2.93 (2H, d, J = 6.9 Hz), 3.13–3.21 (2H, m), 3.76–3.85 (1H, m), 6.79–6.89 (3H, m), 7.41 (1H, d, J = 7.8 Hz), 7.54–7.61 (2H, m), 7.81–7.84 (1H, m), 8.04 (1H, br s), 8.08–8.09 (1H, m), 8.15 (1H, s), 8.23 (1H, dd, J = 8.4, 6.6 Hz), 8.94 (1H, br s), 9.18 (1H, br s), 10.64 (1H, s), 11.33 (1H, s), 12.33 (1H, s); $[\alpha]_D^{25}$ = −14.7 (*c* 1.0, DMSO); Anal. Calcd for $C_{29}H_{24}FN_5O_4{\cdot}HCl$: C, 61.98; H, 4.48; N, 12.46. Found: C, 61.77; H, 4.53; N, 12.34.

5.15. *N*-[3-Cyano-4-(3-{[3-(ethylamino)pentanoyl]amino}phenyl)-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (7h)

To a solution of **6f** (150 mg, 0.27 mmol), acetaldehyde (97%, 26 mg, 0.60 mmol), and AcOH (0.1 ml) in MeOH (10 ml) was added $NaBH_3(CN)$ (38 mg, 0.60 mmol) and the mixture was stirred at room temperature for 4 h. The mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. A solution of the residue in EtOAc (5 ml) was treated with 4 M HCl EtOAc solution (0.5 ml) at room temperature and then concentrated. The residue was recrystallized from MeOH/Et_2O to give **7h** (137 mg, 88%) as a pale yellow solid: mp 233–238 °C; ^{1}H NMR (DMSO-d_6) δ 0.96 (3H, t, J = 7.5 Hz), 1.23 (3H, t, J = 7.2 Hz), 1.61–1.84 (2H, m), 2.80–2.95 (2H, m), 3.02 (2H, q, J = 7.2 Hz), 3.48 (1H, br s), 6.79–6.88 (3H, m), 7.42 (1H, d, J = 7.8 Hz), 7.53–7.61 (2H, m), 7.83 (1H, d, J = 9.0 Hz), 8.01 (1H, d, J = 1.5 Hz), 8.07 (1H, t, J = 0.9 Hz), 8.21 (1H, s), 8.26 (1H, t, J = 7.8 Hz), 8.50–8.85 (2H, m), 10.70 (1H, s), 11.25–11.40 (1H, m), 12.34 (1H, br s); Anal. Calcd for $C_{30}H_{28}FN_5O_4{\cdot}HCl{\cdot}0.5H_2O$: C, 61.38; H, 5.15; N, 11.93. Found: C, 61.21; H, 5.18; N, 11.92.

5.16. *N*-[3-Cyano-4-{3-[(*N*,*N*-dimethyl-β-alanyl)amino]phenyl}-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (7a)

Compound **7a** was prepared from **3** (120 mg, 0.23 mmol) and formaldehyde (37%, 37 μL, 0.46 mmol) in a manner similar to that described for **7h**. Compound **7a** (65 mg, 51%) was obtained as a pale yellow solid: mp 240–241 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 2.79 (6H, s), 2.93 (2H, t, J = 7.2 Hz), 3.30–3.45 (2H, m), 6.75–6.90 (3H, m), 7.41 (1H, d, J = 7.8 Hz), 7.50–7.65 (2H, m), 7.82 (1H, d, J = 8.4 Hz), 8.01 (1H, s), 8.08 (1H, s), 8.14 (1H, s), 8.20–8.30 (1H, m), 9.99 (1H, br s), 10.65 (1H, s), 11.32 (1H, s), 12.33 (1H, s); Anal. Calcd for $C_{28}H_{24}FN_5O_4{\cdot}HCl{\cdot}0.5H_2O$: C, 60.16; H, 4.69; N. 12.53. Found: C, 60.44; H, 4.66; N, 12.68.

5.17. *N*-[3-Cyano-4-{3-[(*N*-ethyl-*N*-methyl-β-alanyl)amino]phenyl}-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (7b)

Compound **7b** was prepared from **6d** (150 mg, 0.28 mmol) and acetaldehyde (90%, 41 mg, 0.84 mmol) in a manner similar to that described for **7h**. Compound **7b** (92 mg, 58%) was obtained as a pale yellow solid: mp 223–224 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 1.25 (3H, t, J = 7.2 Hz), 2.75 (3H, d, J = 3.9 Hz), 2.95 (2H, t, J = 7.2 Hz), 3.07–3.44 (4H, m), 6.79–6.88 (3H, m), 7.41 (1H, d,

J = 8.1 Hz), 7.53–7.61 (2H, m), 7.81–7.84 (1H, m), 8.01 (1H, s), 8.08 (1H, dd, *J* = 1.8, 0.9 Hz), 8.14 (1H, s), 8.21–8.26 (1H, m), 9.97 (1H, br s), 10.65 (1H, s), 11.32 (1H, s), 12.33 (1H, d, *J* = 1.2 Hz); 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, 60.46; H, 5.06; N, 12.00.

5.18. *N*-[3-Cyano-4-{3-[(*N*,*N*-diethyl-β-alanyl)amino]phenyl}-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (7c)

Compound **7c** was prepared from **3** (200 mg, 0.38 mmol) and acetaldehyde (97%, 0.6 ml) in a manner similar to that described for **7h**. Compound **7c** (194 mg, 88%) was obtained as a pale yellow solid: mp 209–213 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 1.24 (6H, t, *J* = 7.2 Hz), 2.94 (2H, t, *J* = 7.2 Hz), 3.08–3.24 (4H, m), 3.34–3.39 (2H, m), 6.79–6.88 (3H, m), 7.41 (1H, d, *J* = 7.8 Hz), 7.54–7.60 (2H, m), 7.83 (1H, d, *J* = 8.4 Hz), 8.01 (1H, s), 8.08 (1H, s), 8.14 (1H, s), 8.23 (1H, t, *J* = 7.8 Hz), 10.07 (1H, br s), 10.67 (1H, s), 11.32 (1H, s), 12.34 (1H, s); Anal. Calcd for $C_{30}H_{28}FN_5O_4{\cdot}HCl{\cdot}0.5H_2O$: C, 61.38; H, 5.15; N, 11.93. Found: C, 61.23; H, 4.95; N, 11.80.

5.19. *N*-[3-Cyano-4-(3-{[3-(ethylamino)butanoyl]amino}phenyl)-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (7d)

Compound **7d** was prepared from **6e** (100 mg, 0.19 mmol) and acetaldehyde (97%, 14 mg, 0.28 mmol) in a manner similar to that described for **7h**. Compound **7d** (83 mg, 77%) was obtained as a pale yellow solid: mp 249–255 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 1.23 (3H, t, *J* = 7.2 Hz), 1.30 (3H, d, *J* = 6.3 Hz), 2.69–2.77 (1H, m), 2.91–3.02 (3H, m), 3.60 (1H, q, *J* = 5.7 Hz), 6.78–6.86 (3H, m), 7.41 (1H, d, *J* = 7.5 Hz), 7.53–7.60 (2H, m), 7.83 (1H, d, *J* = 8.1 Hz), 8.01 (1H, d, *J* = 1.2 Hz), 8.14 (1H, s), 8.21 (1H, s), 8.23 (1H, t, *J* = 7.8 Hz), 8.60–8.90 (2H, m), 10.65 (1H, s), 11.30–11.50 (1H, m), 12.30–12.50 (1H, m); Anal. Calcd for $C_{29}H_{26}FN_5O_4{\cdot}HCl$: C, 61.76; H, 4.83; N, 12.42. Found: C, 61.39; H, 4.81; N, 12.38.

5.20. *N*-{3-Cyano-6-(4-fluoro-2-hydroxyphenyl)-4-[3-({3-[(1-methylethyl)amino]butanoyl}amino)phenyl]pyridin-2-yl}furan-2-carboxamide hydrochloride (7e)

Compound **7e** was prepared from **6e** (200 mg, 0.37 mmol) and acetone (0.2 ml) in a manner similar to that described for **7h**. Compound **7e** (170 mg, 79%) was obtained as a pale yellow solid: mp 197–199 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 1.26–1.33 (9H, m), 2.75 (1H, dd, *J* = 15.9, 7.8 Hz), 2.94 (1H, dd, *J* = 15.9, 4.8 Hz), 3.44 (1H, br s), 3.68 (1H, br s), 6.79–6.88 (3H, m), 7.42 (1H, d, *J* = 7.8 Hz), 7.53–7.61 (2H, m), 7.84 (1H, d, *J* = 8.1 Hz), 8.01 (1H, s), 8.08 (1H, d, *J* = 1.2 Hz), 8.14 (1H, s), 8.21–8.26 (1H, m), 8.62 (1H, br s), 8.76 (1H, br s), 10.65 (1H, s), 11.31 (1H, s), 12.34 (1H, s); Anal. Calcd for $C_{30}H_{28}FN_5O_4{\cdot}HCl{\cdot}1.5H_2O$: C, 59.55; H, 5.33; N, 11.57. Found: C, 59.51; H, 5.32; N, 11.61.

5.21. *N*-[3-Cyano-4-(3-{[3-(dimethylamino)butanoyl]amino}phenyl)-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (7f)

Compound **7f** was prepared from **6e** (100 mg, 0.19 mmol) and formaldehyde (37%, 30 μL, 0.37 mmol) in a manner similar to that described for **7h**. Compound **7f** (68 mg, 64%) was obtained as a pale yellow solid: mp 233–234 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 1.30 (3H, d, *J* = 6.6 Hz), 2.65–2.85 (7H, m), 2.90–3.10 (1H, m), 3.70–3.90 (1H, m), 6.75–6.90 (3H, m), 7.43 (1H, d, *J* = 7.5 Hz), 7.54 (1H, d, *J* = 3.3 Hz), 7.59 (1H, t, *J* = 7.8 Hz), 7.82 (1H, d, *J* = 9.0 Hz), 8.00 (1H, s), 8.08 (1H, s), 8.15 (1H, s), 8.20–8.30 (1H, m), 10.03 (1H, br s), 10.65 (1H, s), 11.32 (1H, s), 12.34 (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, 60.84; H, 4.85; N, 12.27.

5.22. *N*-[3-Cyano-4-(3-{[3-(diethylamino)butanoyl]amino}phenyl)-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (7g)

Compound **7g** was prepared from **6e** (100 mg, 0.19 mmol) and acetaldehyde (97%, 17 mg, 0.38 mmol) in a manner similar to that described for **7h**. Compound **7g** (60 mg, 54%) was obtained as a pale yellow solid: mp 193–195 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 1.25–1.45 (9H, m), 2.70–2.85 (1H, m), 3.05–3.30 (5H, m), 3.92 (1H, br s), 6.75–6.90 (3H, m), 7.43 (1H, d, *J* = 7.8 Hz), 7.54 (1H, d, *J* = 3.0 Hz), 7.58 (1H, t, *J* = 7.8 Hz), 7.82 (1H, d, *J* = 9.0 Hz), 8.01 (1H, s), 8.08 (1H, s), 8.15 (1H, s), 8.24 (1H, t, *J* = 7.5 Hz), 9.79 (1H, s), 10.68 (1H, s), 11.31 (1H, s), 12.35 (1H, s); Anal. Calcd for $C_{31}H_{30}FN_5O_4{\cdot}HCl{\cdot}1.5H_2O$: C, 60.14; H, 5.54; N. 11.31. Found: C, 60.51; H, 5.89; N, 11.25.

5.23. *N*-[3-Cyano-4-(3-{[3-(diethylamino)pentanoyl]amino}phenyl)-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (7i)

Compound **7i** was prepared from **6f** (150 mg, 0.22 mmol) and acetaldehyde (97%, 268 mg, 5.46 mmol) in a manner similar to that described for **7h**. Compound **7i** (130 mg, 79%) was obtained as a pale yellow solid: mp 171–175 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 0.98 (3H, t, *J* = 7.2 Hz), 1.28–1.33 (6H, m), 1.60–1.78 (1H, m), 1.86–2.06 (1H, m), 2.76 (1H, dd, *J* = 16.2, 6.6 Hz), 3.04–3.39 (5H, m), 3.80 (1H, br s), 6.79–6.88 (3H, m), 7.42 (1H, d, *J* = 8.1 Hz), 7.54–7.61 (2H, m), 7.83 (1H, d, *J* = 8.7 Hz), 8.02 (1H, s), 8.08 (1H, d, *J* = 0.6 Hz), 8.15 (1H, s), 8.23 (1H, t, *J* = 7.8 Hz), 9.61 (1H, br s), 10.78 (1H, s), 11.31 (1H, s), 12.34 (1H, s); Anal. Calcd for $C_{32}H_{32}FN_5O_4{\cdot}HCl{\cdot}H_2O$: C, 61.58; H, 5.65; N, 11.22. Found: C, 61.43; H, 5.69; N, 11.20.

5.24. *N*-{3-Cyano-6-(4-fluoro-2-hydroxyphenyl)-4-[3-({[(2*S*)-1-methylpyrrolidin-2-yl]acetyl}amino)phenyl]pyridin-2-yl}furan-2-carboxamide hydrochloride (7j)

Compound **7j** was prepared from **6g** (200 mg, 0.36 mmol) and formaldehyde (37%, 60 mg, 0.74 mmol) in a manner similar to that described for **7h**. Compound **7j** (180 mg, 87%) was obtained as a pale yellow solid: mp 257–258 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 1.67–1.79 (1H, m), 1.87–2.06 (2H, m), 2.24–2.35 (1H, m), 2.79–2.93 (4H, m), 3.00–3.24 (2H, m), 3.52–3.73 (2H, m), 6.79–6.88 (3H, m), 7.42 (1H, d, *J* = 8.1 Hz), 7.53–7.60 (2H, m), 7.81–7.84 (1H, m), 8.01 (1H, t, *J* = 1.8 Hz), 8.08 (1H, dd, *J* = 1.5 Hz, 0.6 Hz), 8.15 (1H, s), 8.21–8.26 (1H, m), 10.48 (1H, br s), 10.70 (1H, s), 11.31 (1H, s), 12.35 (1H, s); $[\alpha]_D^{25} = -6.6$ (*c* 1.0, DMSO); Anal. Calcd for $C_{30}H_{26}FN_5O_4{\cdot}HCl{\cdot}0.25H_2O$: C, 62.07; H, 4.77; N, 12.06. Found: C, 61.84; H, 4.65; N, 12.15.

5.25. *N*-(4-{3-[(Chloroacetyl)amino]phenyl}-3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]pyridin-2-yl)furan-2-carboxamide (8)

To an ice-cooling solution of **4** (0.50 g, 1.1 mmol) and *N*,*N*-diisopropylethylamine (0.17 g, 1.3 mmol) in THF (5 ml) was added chloroacetyl chloride (0.10 ml, 1.3 mmol) and the mixture was stirred at the same temperature for 3 h. The solution was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc). Recrystallization from EtOAc/hexane gave **8** (0.55 g, 93%) as a pale yellow solid: mp 174–176 °C; ^{1}H NMR ($CDCl_3$) δ 3.47 (3H, s), 4.23 (2H, d, *J* = 2.1 Hz), 5.30 (2H, s),

6.62 (1H, dd, J = 3.3, 1.5 Hz), 6.84–6.91 (1H, m), 7.03 (1H, dd, J = 10.8, 2.4 Hz), 7.40 (1H, dd, J = 3.6, 0.9 Hz), 7.53–7.66 (4H, m), 7.97 (1H, s), 8.00–8.05 (2H, m), 8.38 (1H, br s), 8.82 (1H, br s).

5.26. *N*-(3-Cyano-4-{3-[(*N*,*N*-dimethylglycyl)amino]phenyl}-6-[4-fluoro-2-(methoxymethoxy)phenyl]pyridin-2-yl)furan-2-carboxamide (9a)

A mixture of compound **8** (200 mg, 0.37 mmol), dimethylamine hydrochloride (60 mg, 0.74 mmol), and K_2CO_3 (150 mg, 0.11 mmol) in DMF (2 ml) was stirred at room temperature for 14 h. The solution was diluted with EtOAc, washed with water twice and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (hexane to EtOAc). Recrystallization from EtOAc/hexane gave **9a** (110 mg, 55%) as a colorless solid: mp 174–176 °C; ^{1}H NMR ($CDCl_3$) δ 2.41 (6H, s), 3.11 (2H, s), 3.47 (3H, s), 5.30 (2H, s), 6.62 (1H, q, J = 1.8 Hz), 6.84–6.91 (1H, m), 7.02 (1H, dd, J = 10.8 Hz, 2.4 Hz), 7.39 (1H, dd, J = 3.6 Hz, 0.9 Hz), 7.45–7.59 (2H, m), 7.60 (1H, s), 7.73–7.77 (1H, m), 7.98–8.04 (3H, m), 8.81 (1H, s), 9.30 (1H, s).

5.27. *N*-(3-Cyano-4-{3-[(*N*,*N*-diethylglycyl)amino]phenyl}-6-[4-fluoro-2-(methoxymethoxy)phenyl]pyridin-2-yl)furan-2-carboxamide (9b)

Compound **9b** was prepared from **8** (300 mg, 0.56 mmol) and diethylamine (0.12 ml, 1.2 mmol) in a manner similar to that described for **9a**. Compound **9b** (230 mg, 72%) was obtained as a colorless solid: mp 126–128 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 1.11 (6H, t, J = 7.2 Hz), 2.68 (4H, q, J = 7.2 Hz), 3.17 (2H, s), 3.47 (3H, s), 5.30 (2H, s), 6.62 (1H, dd, J = 3.6, 1.8 Hz), 6.84–6.91 (1H, m), 7.03 (1H, dd, J = 10.8, 2.4 Hz), 7.39 (1H, dd, J = 3.6, 0.9 Hz), 7.45–7.54 (2H, m), 7.59–7.60 (1H, m), 7.67–7.70 (1H, m), 7.98–8.05 (3H, m), 8.82 (1H, s), 9.58 (1H, br s).

5.28. *N*-(3-Cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-4-{3-[(pyrrolidin-1-ylacetyl)amino]phenyl}pyridin-2-yl)furan-2-carboxamide (9c)

Compound **9c** was prepared from **8** (250 mg, 0.47 mmol) and pyrrolidine (37 mg, 0.52 mmol) in a manner similar to that described for **9a**. Compound **9c** (180 mg, 67%) was obtained as a colorless solid: mp 152–154 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 1.87–1.91 (4H, m), 2.73 (4H, br s), 3.32 (2H, s), 3.47 (3H, s), 5.30 (2H, s), 6.62 (1H, dd, J = 1.8 Hz, 3.6 Hz), 6.84–6.91 (1H, m), 7.02 (1H, dd, J = 2.4 Hz, 10.8 Hz), 7.39–7.40 (1H, m), 7.45–7.54 (2H, m), 7.59–7.60 (1H, m), 7.69–7.73 (1H, m), 7.98–8.04 (3H, m), 8.82 (1H, br s), 9.28 (1H, br s).

5.29. *N*-[3-Cyano-4-{3-[(*N*,*N*-dimethylglycyl)amino]phenyl}-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (10a)

Compound **10a** was prepared from **9a** (110 mg, 0.20 mmol) in a manner similar to that described for **6a**. Compound **10a** (102 mg, 95%) was obtained as a pale yellow solid: mp 217–222 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 2.88 (6H, s), 4.19 (2H, s), 6.79–6.88 (3H, m), 7.48 (1H, d, J = 7.2 Hz), 7.54 (1H, d, J = 3.6 Hz), 7.63 (1H, t, J = 7.8 Hz), 7.83 (1H, d, J = 7.8 Hz), 8.02 (1H, s), 8.08 (1H, s), 8.16 (1H, s), 8.23 (1H, t, J = 7.8 Hz), 9.97 (1H, s), 11.06 (1H, s), 11.34 (1H, s), 12.32 (1H, s); Anal. Calcd for $C_{27}H_{22}FN_5O_4{\cdot}HCl{\cdot}1.5H_2O$: C, 59.29; H, 4.79; N, 12.80. Found: C, 59.28; H, 4.57; N, 12.56.

5.30. *N*-[3-Cyano-4-{3-[(*N*,*N*-diethylglycyl)amino]phenyl}-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (10b)

Compound **10b** was prepared from **9b** (150 mg, 0.26 mmol) in a manner similar to that described for **6a**. Compound **10b** (130 mg, 89%) was obtained as a pale yellow solid: mp 262–263 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 1.25 (6H, t, J = 7.2 Hz), 3.26 (4H, br s), 4.20 (2H, d, J = 3.9 Hz), 6.79–6.89 (3H, m), 7.49 (1H, d, J = 8.1 Hz), 7.54 (1H, dd, J = 3.6, 0.6 Hz), 7.63 (1H, t, J = 8.1 Hz), 7.82–7.86 (1H, m), 8.03–8,04 (1H, m), 8.08 (1H, dd, J = 1.5, 0.6 Hz), 8.16 (1H, s), 8.23 (1H, dd, J = 8.4, 6.9 Hz), 9.75 (1H, br s), 11.18 (1H, s), 11.34 (1H, s), 12.31 (1H, s); Anal. Calcd for $C_{29}H_{26}FN_5O_4{\cdot}HCl{\cdot}0.25H_2O$: C, 61.27; H, 4.88; N, 12.32. Found: C, 61.22; H, 4.85; N, 12.30.

5.31. *N*-[3-Cyano-6-(4-fluoro-2-hydroxyphenyl)-4-{3-[(pyrrolidin-1-ylacetyl)amino]phenyl}pyridin-2-yl]furan-2-carboxamide hydrochloride (10c)

Compound **10c** was prepared from **9c** (150 mg, 0.26 mmol) in a manner similar to that described for **6a**. Compound **10c** (140 mg, 96%) was obtained as a pale yellow solid: mp 203–205 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 1.94–2.00 (4H, m), 3.17 (2H, br s), 3.63 (2H, br s), 4.32 (2H, s), 6.79–6.90 (3H, m), 7.47 (1H, d, J = 8.1 Hz), 7.55 (1H, dd, J = 0.9 Hz, 3.6 Hz), 7.63 (1H, t, J = 8.1 Hz), 7.82–7.85 (1H, m), 8.03 (1H, br s), 8.08 (1H, dd, J = 0.6 Hz, 1.5 Hz), 8.16 (1H, s), 8.24 (1H, dd, J = 6.9 Hz, 8.7 Hz), 10.33 (1H, br s), 11.10 (1H, s), 11.35 (1H, s), 12.33 (1H, s); Anal. Calcd for $C_{29}H_{24}FN_5O_4{\cdot}HCl{\cdot}H_2O$: C, 60.05; H, 4.69; N, 12.07. Found: C, 59.90; H, 4.69; N, 12.02.

5.32. 2-Amino-4-(3-bromophenyl)-6-[4-fluoro-2-(methoxymethoxy)phenyl]pyridine-3-carbonitrile (12)

A mixture of 1-[4-fluoro-2-(methoxymethoxy)phenyl]ethanone[13] (**11**, 5.0 g, 25.3 mmol), 3-bromobenzaldehyde (4.68 g, 25.23 mmol), malononitrile (1.67 g, 25.3 mmol), and NH_4OAc (2.92 g, 37.9 mmol) in toluene (60 ml) was stirred under reflux 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 recrystallized from EtOAc/hexane to give **12** (3.28 g, 30%) as a yellow solid: mp 165–166 °C; ^{1}H NMR ($CDCl_3$) δ 3.46 (3H, s), 5.22 (2H, s), 5.34 (2H, br s), 6.81–6.88 (1H, m), 6.98 (1H, dd, J = 10.8, 2.4 Hz), 7.29 (1H, s), 7.39 (1H, t, J = 7.8 Hz), 7.57–7.65 (2H, m), 7.73 (1H, t, J = 1.8 Hz), 7.79 (1H, dd, J = 8.7, 6.9 Hz).

5.33. *N*-{4-(3-Bromophenyl)-3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]pyridin-2-yl}furan-2-carboxamide (13)

To a solution of **12** (2.2 g, 5.1 mmol) in pyridine (30 ml) was added 2-furoyl chloride (1.7 g, 12.8 mmol) at room temperature and the whole was stirred at room temperature for 18 h. The reaction mixture 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 triturated with diisopropyl ether to give **13** (2.4 g, 90%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 3.49 (3H, s), 5.27 (2H, s), 6.62 (1H, q, J = 1.8 Hz), 6.85–6.91 (1H, m), 7.01 (1H, dd, J = 10.8, 1.8 Hz), 7.39–7.46 (2H, m), 7.60 (1H, d, J = 0.9 Hz), 7.66–7.69 (2H, m), 7.80 (1H, t, J = 1.8 Hz), 7.90 (1H, s), 8.01 (1H, t, J = 7.8 Hz), 8.81 (1H, s).

5.34. *tert*-Butyl 4-(3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)piperazine-1-carboxylate (14a)

A mixture of **13** (300 mg, 0.57 mmol), *tert*-butyl piperazine-1-carboxylate (128 mg, 0.69 mmol), Xphos (55 mg, 0.11 mmol), NaO^tBu (83 mg, 0.86 mmol), and $Pd_2(dba)_3$ (53 mg, 0.057 mmol) in toluene (15 ml) was stirred under reflux for 2 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 give **14a** (300 mg, 84%) as a pale brown amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.49 (9H, s), 3.24 (4H, t, J = 5.1 Hz), 3.45 (3H, s), 3.61 (4H, t, J = 5.1 Hz), 5.20 (2H, s), 6.61 (1H, q, J = 1.8 Hz), 6.84–6.90 (1H, m), 6.98–7.14 (3H, m), 7.27 (1H, s), 7.39–7.46 (2H, m), 7.58 (1H, t, J = 0.9 Hz), 7.93 (1H, s), 8.00 (1H, dd, J = 8.7, 6.9 Hz), 8.84(1H, s).

5.35. *tert*-Butyl 4-(3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)-1,4-diazepane-1-carboxylate (14b)

Compound **14b** was prepared from **13** (300 mg, 0.57 mmol) and *tert*-butyl homopiperazine-1-carboxylate (138 mg, 0.69 mmol) in a manner similar to that described for **14a**. Compound **14b** (155 mg, 42%) was obtained as a pale yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.39 (4H, s), 1.44 (5H, s), 2.00–2.15 (2H, m), 3.20–3.40 (2H, m), 3.46 (3H, s), 3.58–3.70 (6H, br s), 5.25 (2H, s), 6.61 (1H, q, J = 1.8 Hz), 6.83–6.95 (3H, m), 6.99–7.03 (2H, m), 7.34–7.39 (2H, m), 7.59 (1H, t, J = 0.9 Hz), 7.94 (1H, s), 8.03 (1H, t, J = 7.8 Hz), 8.81 (1H, s).

5.36. *N*-{3-Cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-4-[3-(4-methylpiperazin-1-yl)phenyl]pyridin-2-yl}furan-2-carboxamide (14c)

Compound **14c** was prepared from **13** (300 mg, 0.57 mmol) and 1-methylpiperazine (69 mg, 0.69 mmol) in a manner similar to that described for **14a**. Compound **14c** (103 mg, 53%) was obtained as a pale yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 2.37 (3H, s), 2.60 (4H, t, J = 5.1 Hz), 3.32 (4H, t, J = 5.1 Hz), 3.46 (3H, s), 5.24 (2H, s), 6.61 (1H, q, J = 1.8 Hz), 6.85–6.91 (1H, m), 7.01 (1H, dd, J = 10.8, 2.4 Hz), 7.06–7.12 (2H, m), 7.24–7.26 (1H, m), 7.39–7.45 (2H, m), 7.59 (1H, s), 7.93 (1H, s), 8.00 (1H, dd, J = 8.7, 7.2 Hz), 8.81 (1H, s).

5.37. *N*-{3-Cyano-4-[3-(4-ethylpiperazin-1-yl)phenyl]-6-[4-fluoro-2-(methoxymethoxy)phenyl]pyridin-2-yl}furan-2-carboxamide (14d)

Compound **14d** was prepared from **13** (300 mg, 0.57 mmol) and 1-ethylpiperazine (79 mg, 0.69 mmol) in a manner similar to that described for **14a**. Compound **14d** (94 mg, 30%) was obtained as a pale yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.14 (3H, t, J = 7.2 Hz), 2.49 (2H, q, J = 7.2 Hz), 2.63 (4H, t, J = 5.1 Hz), 3.30 (4H, t, J = 5.1 Hz), 3.46 (3H, s), 5.24 (2H, s), 6.61 (1H, q, J = 1.8 Hz), 6.71–6.91 (1H, m), 7.00 (1H, dd, J = 10.8, 2.4 Hz), 7.06–7.12 (2H, m), 7.23–7.26 (1H, m), 7.38–7.45 (2H, m), 7.59 (1H, q, J = 0.9 Hz), 7.93 (1H, s), 8.00 (1H, dd, J = 8.7, 6.6 Hz), 8.81 (1H, s).

5.38. *N*-[3-Cyano-6-(4-fluoro-2-hydroxyphenyl)-4-(3-piperazin-1-ylphenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (15a)

Compound **15a** was prepared from **14a** (300 mg, 0.48 mmol) in a manner similar to that described for **6a**. Compound **15a** (194 mg, 73%) was obtained as a pale yellow solid: mp 266–273 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 3.26 (4H, s), 3.50 (4H, s), 6.79–6.87 (3H, m), 7.22 (2H, t, J = 9.0 Hz), 7.30 (1H, s), 7.48–7.54 (2H, m), 8.08 (1H, s), 8.16 (1H, s), 8.25 (1H, t, J = 8.1 Hz), 8.80–9.20 (2H, m), 11.10–11.50 (1H, m), 12.43 (1H, br s); Anal. Calcd for $C_{27}H_{22}FN_5O_3\cdot HCl\cdot H_2O$: C, 59.29; H, 4.79; N, 12.80. Found: C, 59.61; H, 4.97; N, 12.76.

5.39. *N*-{3-Cyano-4-[3-(1,4-diazepan-1-yl)phenyl]-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl}furan-2-carboxamide hydrochloride (15b)

Compound **15b** was prepared from **14b** (140 mg, 0.22 mmol) in a manner similar to that described for **6a**. Compound **15b** (108 mg, 86%) was obtained as a pale yellow solid: mp 254–259 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 2.09–2.20 (2H, m), 3.10–3.20 (2H, m), 3.30–3.40 (2H, m), 3.61 (2H, t, J = 5.7 Hz), 3.75–3.95 (2H, m), 6.79–6.86 (3H, m), 6.99–7.07 (3H, m), 7.43 (1H, t, J = 7.8 Hz), 7.53 (1H, d, J = 3.6 Hz), 8.08 (1H, t, J = 1.5 Hz), 8.13 (1H, s), 8.22 (1H, dd, J = 9.9, 6.9 Hz), 8.96 (2H, br s), 11.23 (1H, s), 12.41 (1H, s); Anal. Calcd for $C_{28}H_{24}FN_5O_3\cdot HCl\cdot 0.5H_2O$: C, 61.94; H, 4.83; N, 12.90. Found: C, 61.86; H, 4.82; N, 12.64.

5.40. *N*-{3-Cyano-6-(4-fluoro-2-hydroxyphenyl)-4-[3-(4-methylpiperazin-1-yl)phenyl]pyridin-2-yl}furan-2-carboxamide hydrochloride (15c)

Compound **15c** was prepared from **14c** (95 mg, 0.18 mmol) in a manner similar to that described for **6a**. Compound **15c** (82 mg, 80%) was obtained as a pale yellow solid: mp 254–259 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 2.83 (3H, s), 3.10–3.30 (4H, m), 3.40–3.65 (2H, m), 3.80–4.15 (2H, m), 6.79–6.87 (3H, m), 7.20–7.27 (2H, m), 7.32 (1H, s), 7.48–7.54 (2H, m), 8.08 (1H, s), 8.16 (1H, s), 8.25 (1H, dd, J = 9.9, 6.9 Hz), 10.62 (1H, br s), 11.25 (1H, s), 11.44 (1H, s); Anal. Calcd for $C_{28}H_{24}FN_5O_3\cdot HCl\cdot H_2O$: C, 60.93; H, 4.93; N, 12.68. Found: C, 61.32; H, 4.85; N, 12.69.

5.41. *N*-{3-Cyano-4-[3-(4-ethylpiperazin-1-yl)phenyl]-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl}furan-2-carboxamide hydrochloride (15d)

Compound **15d** was prepared from **14d** (90 mg, 0.16 mmol) in a manner similar to that described for **6a**. Compound **15d** (77 mg, 82%) was obtained as a pale yellow solid: mp 218–222 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 1.29 (3H, t, J = 7.2 Hz), 3.10–3.25 (6H, m), 3.59 (2H, d, J = 11.4 Hz), 3.98 (2H, d, J = 11.4 Hz), 6.79–6.87 (3H, m), 7.20–7.27 (2H, m), 7.33 (1H, s), 7.48–7.54 (2H, m), 8.08 (1H, t, J = 0.9 Hz), 8.16 (1H, s), 8.25 (1H, dd, J = 9.6, 6.9 Hz), 10.53 (1H, br s), 11.25 (1H, s), 12.44 (1H, s); Anal. Calcd for $C_{29}H_{26}FN_5O_3\cdot HCl\cdot H_2O$: C, 61.54; H, 5.16; N, 12.37. Found: C, 61.72; H, 5.30; N, 12.34.

5.42. *N*-{3-Cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-4-[3-(hydroxymethyl)phenyl]pyridin-2-yl}furan-2-carboxamide (17)

To a solution of methyl 3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}benzoate[13] (**16**, 640 mg, 1.28 mmol) in THF (30 ml) was added $LiBH_4$ (56 mg, 2.55 mmol) and the mixture was stirred at 45 °C for 96 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 = 2/1) to give **17** (350 mg, 58%) as a yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 3.45 (3H, s), 4.80 (2H, s), 5.24 (2H, s), 6.60 (1H, q,

J = 1.8 Hz), 6.83–6.90 (1H, m), 7.00 (1H, dd, *J* = 10.5, 2.4 Hz), 7.38 (1H, dd, *J* = 3.3, 0.6 Hz), 7.51–7.62 (4H, m), 7.70 (1H, s), 7.92 (1H, s), 7.96 (1H, dd, *J* = 8.7, 6.9 Hz), 8.84 (1H, br s).

5.43. 3-{3-Cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}benzyl methanesulfonate (18)

To a solution of **17** (90 mg, 0.19 mmol) and triethylamine (38 mg, 0.38 mmol) in CH_2Cl_2 (5 ml) was added methanesulfonyl chloride (44 mg, 0.38 mmol). After stirring 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 = 1/1) to give **18** (60 mg, 57%) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ 3.02 (3H, s), 3.47 (3H, s), 5.27 (2H, s), 5.34 (2H, s), 6.62 (1H, q, *J* = 1.8 Hz), 6.85–6.92 (1H, m), 7.02 (1H, dd, *J* = 10.5, 2.4 Hz), 7.40 (1H, dd, *J* = 3.6, 0.6 Hz), 7.60–7.61 (3H, m), 7.72–7.76 (2H, m), 7.94 (1H, s), 8.02 (1H, dd, *J* = 8.7, 6.9 Hz), 8.81 (1H, s).

5.44. *tert*-Butyl 4-(3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}benzyl)piperazine-1-carboxylate (19a)

A solution of **18** (60 mg, 0.11 mmol), *tert*-butyl piperazine-1-carboxylate (24 mg, 0.13 mmol), and triethylamine (14 mg, 0.13 mmol) in THF (5 ml) was stirred at room temperature for 18 h. The mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with 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 = 1/1 to 2/1) to give **19a** (40 mg, 57%) as a pale yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s), 2.44 (4H, s), 3.46 (7H, s), 3.61 (2H, s), 5.25 (2H, s), 6.62 (1H, q, *J* = 1.8 Hz), 6.89–6.91 (1H, m), 7.01 (1H, dd, *J* = 10.8, 2.4 Hz), 7.40 (1H, q, *J* = 2.4 Hz), 7.49–7.60 (4H, m), 7.72 (1H, s), 7.92 (1H, s), 8.00 (1H, dd, *J* = 8.7, 6.6 Hz), 8.82 (1H, s).

5.45. *tert*-Butyl 4-(3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}benzyl)-1,4-diazepane-1-carboxylate (19b)

Compound **19b** was prepared from **18** (100 mg, 0.18 mmol) and *tert*-butyl homopiperazine-1-carboxylate (54 mg, 0.27 mmol) in a manner similar to that described for **19a**. Compound **19b** (80 mg, 68%) was obtained as a pale yellow amorphous solid: ^{1}H NMR (DMSO-d_6) δ 1.45 (9H, s), 1.75–1.94 (2H, m), 2.62–2.78 (4H, m), 3.43–3.55 (7H, m), 3.72 (2H, s), 5.25 (2H, s), 6.61 (1H, q, *J* = 1.8 Hz), 6.84–6.90 (1H, m), 7.00 (1H, dd, *J* = 10.8, 2.4 Hz), 7.39 (1H, d, *J* = 3.6 Hz), 7.49–7.59 (4H, m), 7.69 (1H, s), 7.92 (1H, s), 8.00 (1H, dd, *J* = 8.7, 6.9 Hz), 8.85 (1H, s).

5.46. *N*-{3-Cyano-6-(4-fluoro-2-hydroxyphenyl)-4-[3-(piperazin-1-ylmethyl)phenyl]pyridin-2-yl}furan-2-carbox- amide trifluoroacetate (20a)

To a solution of **19a** (50 mg, 0.078 mmol) in CH_2Cl_2 (2 ml) was added TFA (1 ml) and the mixture was stirred at room temperature for 1 h. The solution was diluted with Et_2O and the precipitate was collected by filtration. Recrystallization from MeOH/Et_2O gave **20a** (29 mg, 35%) as a colorless solid: mp 144–150 °C; ^{1}H NMR (DMSO-d_6) δ 2.66 (4H, br s), 3.13 (4H, br s), 3.73 (2H, s), 6.80–6.87 (3H, m), 7.52–7.74 (5H, m), 8.08 (1H, d, *J* = 0.9 Hz), 8.18 (1H, s), 8.26 (1H, dd, *J* = 9.6, 6.9 Hz), 8.59 (2H, br s), 11.29 (1H, s), 12.40 (1H, s); Anal. Calcd for $C_{28}H_{24}FN_5O_3\cdot C_2HF_3O_2\cdot 1.5H_2O$: C, 56.43; H, 4.40; N, 10.96. Found: C, 56.33; H, 4.09; N, 10.65.

5.47. *N*-{3-Cyano-4-[3-(1,4-diazepan-1-ylmethyl)phenyl]-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl}furan-2-carboxamide dihydrochloride (20b)

Compound **20b** was prepared from **19b** (75 mg, 0.11 mmol) in a manner similar to that described for **6a**. Compound **20b** (63 mg, 98%) was obtained as a pale yellow solid: mp 199–206 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 2.10–2.30 (2H, m), 3.10–3.80 (8H, m), 4.53 (2H, br s), 6.80–6.87 (3H, m), 7.55 (1H, d, *J* = 3.6 Hz), 7.73 (1H, t, *J* = 7.5 Hz), 7.85 (2H, br s), 8.09 (2H, br s), 8.37–8.43 (2H, m), 9.32 (1H, br s), 9.60 (1H, br s), 11.34 (1H, s), 11.82 (1H, s), 12.54 (1H, s); Anal. Calcd for $C_{29}H_{26}FN_5O_3\cdot 2HCl\cdot 1.5H_2O$: C, 56.96; H, 5.11; N, 11.45. Found: C, 57.05; H, 5.15; N, 11.43.

5.48. *N*-[3-Cyano-6-(4-fluoro-2-hydroxyphenyl)-4-{3-[(4-methylpiperazin-1-yl)methyl]phenyl}pyridin-2-yl]furan-2-carboxamide dihydrochloride (21a)

Compound **21a** was prepared from **20a** (60 mg, 0.083 mmol) and formaldehyde (37%, 0.5 ml, 3.20 mmol) in a manner similar to that described for **7h**. Compound **21a** (40 mg, 82%) was obtained as a pale yellow solid: mp 205–210 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 2.73 (3H, s), 3.30–4.50 (10H, m), 6.80–6.87 (3H, m), 7.54 (1H, d, *J* = 3.6 Hz), 7.62–7.81 (3H, m), 7.90–8.05 (1H, m), 8.08 (1H, s), 8.32–8.39 (2H, m), 11.32 (1H, s), 12.51 (1H, br s); Anal. Calcd for $C_{29}H_{26}FN_5O_3\cdot 2HCl\cdot 1.5H_2O$: C, 56.96; H, 5.11; N, 11.45. Found: C, 56.93; H, 5.16; N, 11.45.

5.49. *N*-[3-Cyano-4-{3-[(4-ethylpiperazin-1-yl)methyl]phenyl}-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide dihydrochloride (21b)

Compound **21b** was prepared from **20a** (60 mg, 0.083 mmol) and acetaldehyde (97%, 0.5 ml, 9.53 mmol) in a manner similar to that described for **7h**. Compound **21b** (41 mg, 83%) was obtained as a pale yellow solid: mp 215–222 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 1.25 (3H, t, *J* = 7.2 Hz), 3.10–4.50 (12H, m), 6.80–6.87 (3H, m), 7.54 (1H, d, *J* = 3.6 Hz), 7.68–7.82 (3H, m), 7.95–8.05 (1H, m), 8.09 (1H, s), 8.33–8.39 (2H, m), 11.33 (1H, s), 12.52 (1H, br s); Anal. Calcd for $C_{30}H_{28}FN_5O_3\cdot 2HCl\cdot 1.5H_2O$: C, 57.60; H, 5.32; N, 11.20. Found: C, 57.88; H, 5.23; N, 11.14.

5.50. *N*-{3-Cyano-4-(3-{[2-(dimethylamino)ethyl]carbamoyl}phenyl)-6-[4-fluoro-2-(methoxymethoxy)phenyl]pyridin-2-yl}furan-2-carboxamide (23)

Compound **23** was prepared from 3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}benzoic acid[13] (**22**, 200 mg, 0.41 mmol) and *N*,*N*-dimethylethylenediamine (43 mg, 0.49 mmol) in a manner similar to that described for **5a**. Compound **23** (90 mg, 39%) was obtained as a pale brown amorphous solid: ^{1}H NMR ($CDCl_3$) δ 2.29 (6H, s), 2.56 (2H, t, *J* = 5.7 Hz), 3.47 (3H, s), 3.56 (2H, q, *J* = 5.5 Hz), 5.27 (2H, s), 6.61 (1H, q, *J* = 1.8 Hz), 6.84–6.90 (1H, m), 7.01 (1H, dd, *J* = 10.8, 2.4 Hz), 7.13 (1H, br s), 7.39 (1H, dd, *J* = 3.6, 0.6 Hz), 7.59–7.64 (2H, m), 7.83–7.86 (1H, m), 7.95–8.01 (3H, m), 8.15 (1H, d, *J* = 1.5 Hz), 8.70–9.10 (1H, m).

5.51. *N*-[3-Cyano-4-(3-{[2-(dimethylamino)ethyl]carbamoyl}phenyl)-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide hydrochloride (24)

Compound **24** was prepared from **23** (80 mg, 0.14 mmol) in a manner similar to that described for **6a**. Compound **24** (60 mg,

76%) was obtained as a colorless solid: mp 200–204 °C (MeOH/Et_2O); ^1H NMR (DMSO-d_6) δ 2.84 (6H, s), 3.26–3.33 (2H, m), 3.65–3.69 (2H, m), 6.80–6.87 (3H, m), 7.54 (1H, d, J = 3.6 Hz), 7.76 (1H, t, J = 7.8 Hz), 7.93 (1H, d, J = 7.8 Hz), 8.08–8.14 (2H, m), 8.26–8.35 (3H, m), 9.03 (1H, t, J = 5.4 Hz), 9.89 (1H, br s), 11.32 (1H, s), 12.43 (1H, s); Anal. Calcd for $C_{28}H_{24}FN_5O_4{\cdot}HCl{\cdot}1.5H_2O$: C, 58.28; H, 4.89; N, 12.14. Found: C, 58.49; H, 4.69; N, 12.12.

5.52. *tert*-Butyl {2-[(3-{3-cyano-6-[4-fluoro-2-(methoxymethoxy)phenyl]-2-[(furan-2-ylcarbonyl)amino]pyridin-4-yl}phenyl)amino]ethyl}carbamate (25)

To a solution of **4** (500 mg, 1.09 mmol), *N*-Boc-2-aminoacetaldehyde (347 mg, 2.18 mmol), and AcOH (1.0 ml) in THF (10 ml) was added $NaBH(OAc)_3$ (462 mg, 2.18 mmol) and the whole was stirred at room temperature for 18 h. The mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with 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 = 1/1) and recrystallization from Et_2O to give **25** (395 mg, 60%) as a yellow solid: mp 145–146 °C; ^1H NMR ($CDCl_3$) δ 1.45 (9H, s), 3.33–3.52 (7H, m), 4.11 (1H, br s), 4.34 (1H, br s), 5.25 (2H, s), 6.61 (1H, q, J = 1.8 Hz), 6.75 (1H, dd, J = 8.1, 1.8 Hz), 6.85–7.03 (4H, m), 7.26 (1H, s), 7.32 (1H, t, J = 7.8 Hz), 7.38 (1H, dd, J = 3.0, 0.6 Hz), 7.92 (1H, s), 8.01 (1H, dd, J = 8.4, 6.6 Hz), 8.80 (1H, s).

5.53. *N*-[4-{3-[(2-Aminoethyl)amino]phenyl}-3-cyano-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide dihydrochloride (26)

Compound **26** was prepared from **25** (200 mg, 0.33 mmol) in a manner similar to that described for **6a**. Compound **26** (120 mg, 69%) was obtained as a yellow solid: mp 203–205 °C (MeOH); ^1H NMR (DMSO-d_6) δ 2.91–3.11 (2H, m), 3.31–3.46 (2H, m), 6.74–6.98 (6H, m), 7.29–7.41 (1H, m), 7.53 (1H, d, J = 3.6 Hz), 7.90–8.15 (5H, m), 8.18–8.29 (1H, m), 11.24 (1H, s), 12.39 (1H, br s); Anal. Calcd for $C_{25}H_{20}FN_5O_3{\cdot}2HCl{\cdot}H_2O$: C, 54.75; H, 4.41; N, 12.77. Found: C, 54.81; H, 4.32; N, 12.77.

5.54. *N*-[3-Cyano-4-(3-{[2-(dimethylamino)ethyl](methyl)amino}phenyl)-6-(4-fluoro-2-hydroxyphenyl)pyridin-2-yl]furan-2-carboxamide (27)

To a solution of **26** (50 mg, 0.094 mmol), formaldehyde (37%, 46 mg, 0.57 mmol), and AcOH (0.10 ml) in MeOH (5 ml) was added $NaBH_3(CN)$ (35 mg, 0.56 mmol) and the whole was stirred at room temperature for 3 days. The mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by recrystallization from EtOAc/hexane to give **27** (36 mg, 77%) as a yellow solid: mp 195–197 °C; ^1H NMR ($CDCl_3$) δ 2.33 (6H, s), 2.57 (2H, t, J = 7.5 Hz), 3.06 (3H, s), 3.55 (2H, t, J = 7.5 Hz), 6.59–6.68 (2H, m), 6.79 (1H, dd, J = 10.6, 2.6 Hz), 6.84–6.93 (3H, m), 7.36–7.47 (2H, m), 7.59–7.67 (2H, m), 7.80 (1H, dd, J = 9.1, 6.4 Hz), 9.21 (1H, s), 13.60 (1H, d, J = 1.3 Hz); Anal. Calcd for $C_{28}H_{26}FN_5O_3{\cdot}0.25H_2O$: C, 66.72; H, 5.30; N, 13.89. Found: C, 66.45; H, 5.12; N, 13.78.

5.55. 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% dialyzed fetal bovine serum (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.

5.56. Receptor binding assay

The procedure of this binding assay was based on the method of Ohtaki et al.[17] 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, pH 7.4). 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).

5.57. 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% dFBS, 100 U/ml penicillin, and 100 μg/ml streptomycin in 5% CO_2/95% air atmosphere.

5.58. 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.).

5.59. Brain and plasma concentration in rats

Test compounds were administrated intravenously as a cassette dosing to male Crj/CD(SD)(IGS) rats. After dosing, blood samples were collected into heparinized tubes, centrifuged to obtain plasma. The brain was immediately harvested, weighted, homoge-

nized with saline to 20% (w/v). The plasma and brain homogenate samples were deproteinized with acetonitrile, followed by centrifugation to obtain supernatants. The supernatants were diluted with 0.01 M ammonium formate (0.2% formic acid) in water/acetonitrile (9:1, v/v) containing internal standard. The supernatants were injected into a LC/MS/MS system to measure the compound concentrations.

5.60. Permeability of test compounds across Caco-2 monolayers

Caco-2 monolayers were grown to confluence on collagen-coated, microporous, polycarbonate membranes in 12-well Costar Transwell® plates. The permeability assay buffer was Hanks' Balanced Salt Solution containing 10 mmol/L HEPES, 15 mmol/L glucose, and 1% bovine serum albumin at a pH of 7.3–7.5. The test compound dosing concentrations were 10 μmol/L in the assay buffer. The cells were dosed on the apical side (A-to-B) or basolateral side (B-to-A) and incubated at 37 °C with 5% CO_2 in a humidified chamber. At each time point, 1 and 2 h, 200 μL were taken from the A-to-B receivers, and 50 μL were taken from the B-to-A receivers. Fresh assay buffer was added to the receivers after the 1-hour sampling. Also, at 2 h, 50 μL of the donors were taken. Each determination was performed in duplicate. The permeability through a cell-free (blank) membrane was studied to determine non-specific binding and free diffusion of the test compounds through the device. The lucifer yellow flux was also measured for each monolayer after being subjected to the test compounds to ensure no damage was inflicted to the cell monolayers during the flux period. All samples were assayed by LC/MS/MS using electrospray ionization. The apparent permeability, P_{app}, and percent recovery were calculated as follows:

$$P_{app} = (dC_r/dt) \times V_r/A \times C_0 \quad (1)$$

$$\text{Percent Recovery} = 100 \times ((V_r \times C_r^{final}) + (V_d \times C_d^{final}))/(V_d \times C_0) \quad (2)$$

where, dC_r/dt is the slope of the cumulative concentration in the receiver compartment versus time in μmol/L s^{-1}. V_r is the volume of the receiver compartment in cm^3. V_d is the volume of the donor compartment in cm^3. A is the area of the cell monolayer (1.1 cm^2 for 12-well Transwell®). C_0 is the nominal concentration of the dosing solution in μmol/L. C_r^{final} is the cumulative receiver concentration in μmol/L at the end of the incubation period. C_d^{final} is the concentration of the donor in μmol/L at the end of the incubation period.

5.61. In vivo evaluation in castrated rat model

Male Wister rats were purchased from SLC Japan. These animals were 11 weeks old when subjected for the test, and castration was performed 7 days prior to the test. The compound **15a** was suspended in 20% DMSO-40% PEG400 to the final concentration at 1.5 mmol/L. Administration of the compound **15a** at 0.22 mg/kg (0.8 ml/kg, $n = 8$) was repeated five times with 10 min intervals, through the jugular catheter, ending up with a total dosage at 1.1 mg/kg. The vehicle without compound was used as a negative control ($n = 8$). The blood sample was collected through the jugular catheter immediately before the administration and 25, 45, 60, 90, 120 and 180 min after the administration. The plasma was prepared by the treatment with aprotinin–EDTA and the centrifugation at 15,000 rpm for 10 min. The supernatant was stored at −20 °C until used for Radioimmunoassay (RIA) to measure the plasma LH levels.

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., Jr.; Acierno, J. S.; 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., Jr.; Crowley, W. F.; 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.
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.-I. *Endocrinology* **2005**, *146*, 4431.
13. Kobayashi, T.; Sasaki, S.; Tomita, N.; Fukui, S.; Kuroda, N.; Nakayama, M.; Kiba, A.; Takatsu, Y.; Ohtaki, T.; Itoh, F.; Baba, A. *Bioorg. Med. Chem.* **2010**, *18*, 3841.
14. Kerns, E. H.; Di, L. *Drug-like Properties: Concepts, Structure Design and Methods: From ADME to Toxicity Optimization*; Academic press: Burlington, Massachusetts, 2008. Chapter 10.
15. Molecular Operating Environment (MOE 2010.03); Chemical Computing Group Inc.: Montreal, Quebec, Canada; http://www.chemcomp.com.
16. Giuliana, C.; Luca, G.; Ahmed, R. Q.; Fabio, S.; Santi, S. *J. Med. Chem.* **2002**, *45*, 2571 (*S*)-isomer was prepared in the same manner.
17. 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.