Discovery of Novel Hedgehog Signaling Inhibitor

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Abbreviations

AUC	area under the blood concentration time curve
AcOEt	ethyl acetate
AcOH	acetic acid
AcONa	sodium acetate
BCC	basal cell carcinoma
BID	bis in die
$BnEt_3NCl$	benzyltriethylammonium chloride
Boc	*tert*-butoxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
CL	clearance
C_{max}	maximum concentration
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DMA	*N*,*N*-dimethylacetamide
DME	1,2-dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
Dhh	Desert hedgehog
EDC	1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride
Et_2O	diethyl ether
Et_3N	trimethylamine
hERG	human ether-a-go-go related gene
HOBt	1-hydroxybenzotriazole
HTS	high-throughput screening
Hh	hedgehog
HhL	hedgehog ligand
IPE	diisopropyl ether
Ihh	Indian hedgehog
LC-MS	liquid chromatography-mass spectrometry
*m*CPBA	*m*-chloroperbenzoic acid
MePhos	2-(dicyclohexylphosphino)-2'-methylbiphenyl
MOE	molecular operating environment
*m*RNA	messenger ribonucleic acid
MRT	mean residence time
MeOH	methanol
NBS	*N*-bromosuccinimide
NH_4OAc	ammonium acetate
NMR	nuclear magnetic resonance
OTf	triflate
PD	pharmacodynamic
PK	pharmacokinetic
PMB	*p*-methoxybenzyl
$Pd(PPh_3)_4$	tetrakis(triphenylphosphine)-palladium(0)
Pd/C	palladium/carbon
$Pd_2(dba)_3$	tris(dibenzylideneacetone)-dipalladium(0)
$Pd(OAc)_2$	palladium(II) acetate
Ph	phenyl
Ptch	patched
QD	quaque die
rt	room temperature
SAR	structure-activity relationship
± SD	standard deviation
Shh	Sonic hedgehog
Smo	smoothened
T/C	treatment/control ratio
tert-BuONa	sodium *tert*-butoxide
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
Tf_2O	trifluoromethanesulfonic anhydride
TfOH	trifluoromethanesulfonic acid
Vss	steady state volume of distribution
xantphos	4,5-bis(diphenylphosphino)-9,9-dimethylxanthene

INDEX

1. Introduction

1.1. Current status of cancer therapy

Recently, cancer becomes a curable disease owing to discovery of many therapeutic options. The major therapeutic options consist of operation therapy, radiation therapy and chemotherapy, and the combination of these options is used for the treatment in many cases. The recent development of these therapeutic options contributes to cure some kinds of cancer, for example advanced cancer, micro cancer, and metastatic cancer. But some problems were still left in each options. The operation therapy is not suitable in case of metastatic cancer, and stressed physically or mentally to patients. In radiation therapy, the radiation hazards is concerned. In chemotherapy, generally used anticancer drug has a problem of adverse effect which triggered to interact with normal cells as well as cancer cells.

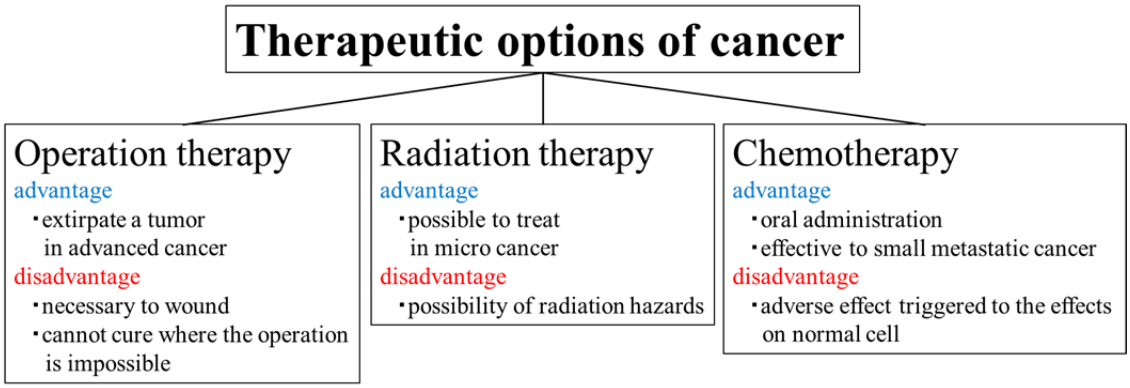


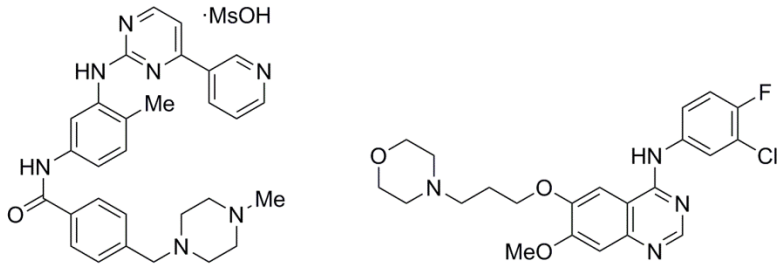
Figure 1. Therapeutic options of cancer.

To dissolve these problems, technical or chemical approaches are developed. Laparoscopic or endoscopic operation lowers the patients' stress in operation therapy. A technique of pinpoint radiation to the tumor can decrease the radiation hazards in radiation therapy. In chemotherapy, a molecular targeted drug is developed to decrease the adverse effect. The molecular targeted drug has been developed over the past decades, and enhances the growth inhibition or malfunction to interact with protein specifically expressed in cancer cell. For this selective interaction with cancer cell, the molecular targeted drug is expected to have less adverse effect.

1.2. Molecular targeted drug and signal transduction

A molecular targeted drug consists of small molecular drug and antibody drug. An antibody drug has been developed since the technology development of monoclonal antibody in 1970's.[1] The antibody drug binds to an antigen specifically exists in the surface of cancer cell, and inhibits the function. The molecular weight of antibody drug is too big, thus mainly developed as injections. On the other hands, a small molecular drug has been developed from the late of 1990's. In contrast to antibody drug, the small molecule drug has advantages to pass through the cell membrane, and develop as oral drug owing to low molecular weight.

Many small molecular drugs have already approved by Food and Drug Administration (FDA).[2] Bcr-Abl protein is specifically overexpressed in chronic myelogenous leukemia (CML) cells and leads to abnormal signal transduction for cell proliferation. Imatinib[3] is the Bcr-Abl protein targeted drug for the inhibition of this signal transduction. Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase, its activation occurred by stimulation with EGF. It induces the signal transduction to Ras/RAF/MAPK pathway and PI3K/Akt pathway which play an important role for cell proliferation and cell growth. Gefitinib[4] inhibits these signaling pathways in non-small cell lung cancer (NSCLC) (Figure 2).



Imatinib
(Bcr/Abl tyrosine kinase inhibitor)

Gefitinib
(EGFR tyrosine kinase inhibitor)

Figure 2. Chemical structures of major small molecular drugs.

1.3. Hedgehog signal and cancer

Pancreatic cancer is difficult to discover in early stage because any symptoms are not appeared. Thus the cancer cannot be found until terminal stage in many cases, 5-year survival rate of pancreatic cancer is quite low compared to other cancers. In addition to few drugs for pancreatic cancer, it is hard to cure a pancreatic cancer completely by only operation therapy. Therefore, the development of novel therapeutic method is expected.

In 2003, Thayer *et al.* reported that hedgehog (Hh) signaling is expressed abnormally in pancreatic cancer cells, and may also be an important mediator in pancreatic carcinoma.[5]

Hh signaling plays an essential role for in cell proliferation and differentiation during embryonic development. In mammal, three ligands of hedgehog signaling are identified, i.e. Sonic hedgehog (Shh), Desert hedgehog (Dhh), and Indian hedgehog (Ihh). Among these ligands, Shh expressed across the whole body is most developed. In the normal state, twelve-transmembrane receptor protein Patched (Ptch) suppresses the activation of seven-transmembrane signal component protein Smoothened (Smo)[6] resulted to the inhibition of signal transduction to downstream. When the Hh ligand (HhL) exists, the activation of Smo is triggered by suppression of Ptch with Shh binding. It causes signal transduction, and upregulates Gli transcription activity. Subsequently, genes under the control of Gli activate downstream signals that contribute to cellular proliferation and differentiation.

This signaling pathway is inactive in most adult tissues; however, aberrant activation driven by Ptch-defective mutations or Hh-ligand overexpression have been detected in certain types of cancers.[7] In the mutation-driven mechanism, tumor growth is enhanced by autonomous activation of Gli caused by constitutive Smo activation due to loss of Ptch function. This mechanism has been observed in medulloblastoma[8] and basal cell carcinoma (BCC).[9] In the Hh-ligand driven mechanism, the overexpression of Hh ligand in cancer cells leads to abnormal activation of Gli in neighboring stromal cells, and induces the enhancement of tumor growth by a paracrine mechanism.[10] This mechanism has been detected in a wide range of solid tumors including colon,[11] prostate,[12] and pancreatic cancers (Figure 3).[13]

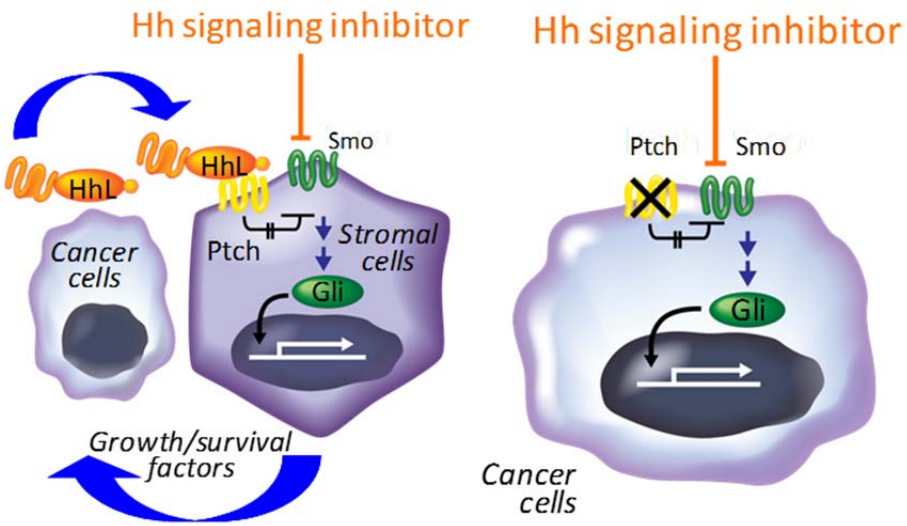


Figure 3. Aberrant activation of Hh signaling pathway in cancer.

1.4. Small molecule Hh signaling inhibitors

To date, several small molecule Hh signaling inhibitors have been identified (Figure 4). Cyclopamine, a steroid jerveratum alkaloid derived from corn lily, showed significant activity in Hh signaling pathway inhibition[14] and directly interacts with Smo protein, thereby inhibiting tumor growth in several cancer cell types. The synthetic small molecule Hh signaling inhibitor, vismodegib[15] has been approved by FDA in locally advanced or metastatic BCC. Sonidegib[16] was discovered by Novartis which has also approved in locally advanced or metastatic BCC. Other compounds in development include a cyclopamine-derivative, saridegib (IPI-926),[17] a synthetic compound, NVP-LEQ506,[18] PF-04449913,[19] and LY-2940680.[20]

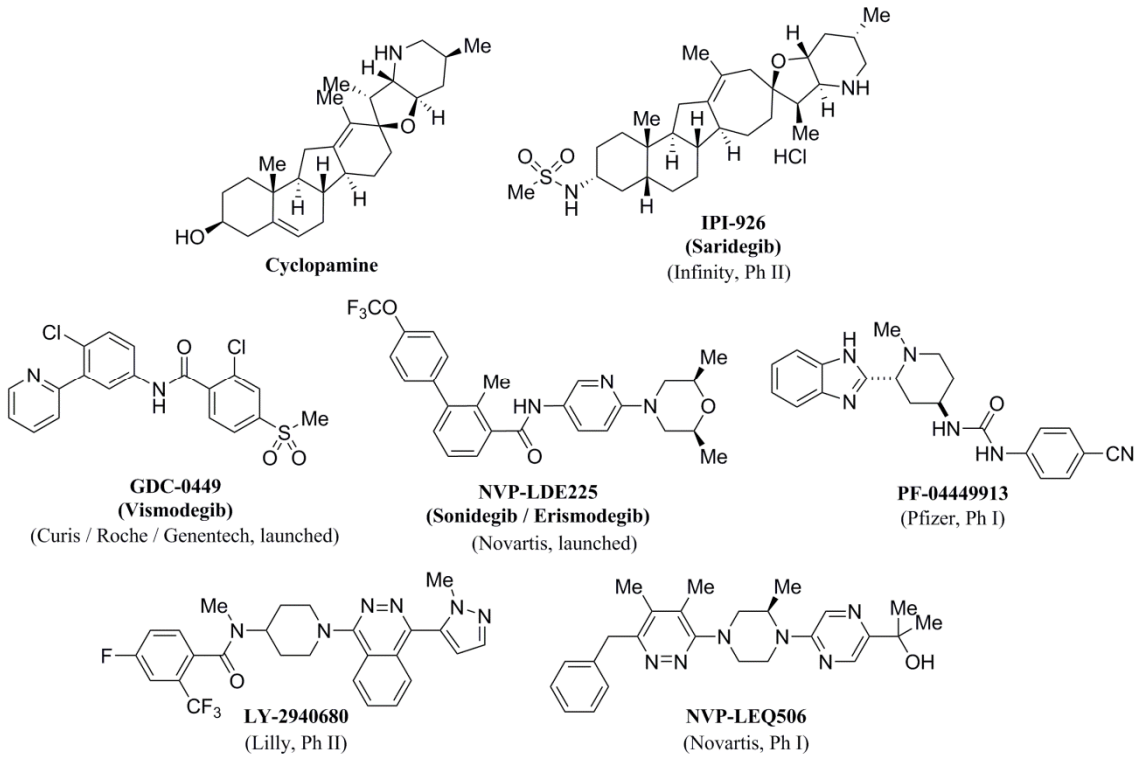


Figure 4. Various small molecule Hh signaling inhibitors.

1.5. The strategy for drug discovery

Based on these results, it has been suggested that an Hh signaling inhibitor may be useful as a therapeutic agent for many kinds of cancers. Therefore, the discovery of novel and potent Hh signaling inhibitor which is structurally different from known compounds was started (Figure 5).

In chapter I, the discovery of pyrrolo[3,2-*c*]quinoline-4-one derivative **12b** and its result of *in vivo* study are included. The modification of thieno[3,2-*c*]quinoline-4-one derivative **1a** identified by high-throughput screening (HTS) was conducted at the upper part to improve stability in mouse and human microsomes while considering to metabolite analysis. The results of Smo binding assay and *in vivo* study are also included.

In chapter II, the discovery of pyrrolo[3,2-*d*]pyridine-4-one derivative **22d** (TAK-441) and the result of *in vivo* study are described. Because of plateaued pharmacokinetic data in high-dose of **12b**, the modification at core ring was conducted to improve solubility. The pharmacokinetic study in rats and dogs also conducted.

In chapter III, some novel Hh signaling inhibitors **25c-f** are discovered by further modification of **22d** at lower part by using X-ray single crystal structure analysis. These compounds showed potent Gli reporter inhibitory activity comparable to **22d**. From the

comparison of pharmacokinetics among **22d** and novel inhibitors **25c-f**, **22d** showed the best pharmacokinetic profile, and was selected as a clinical candidate.

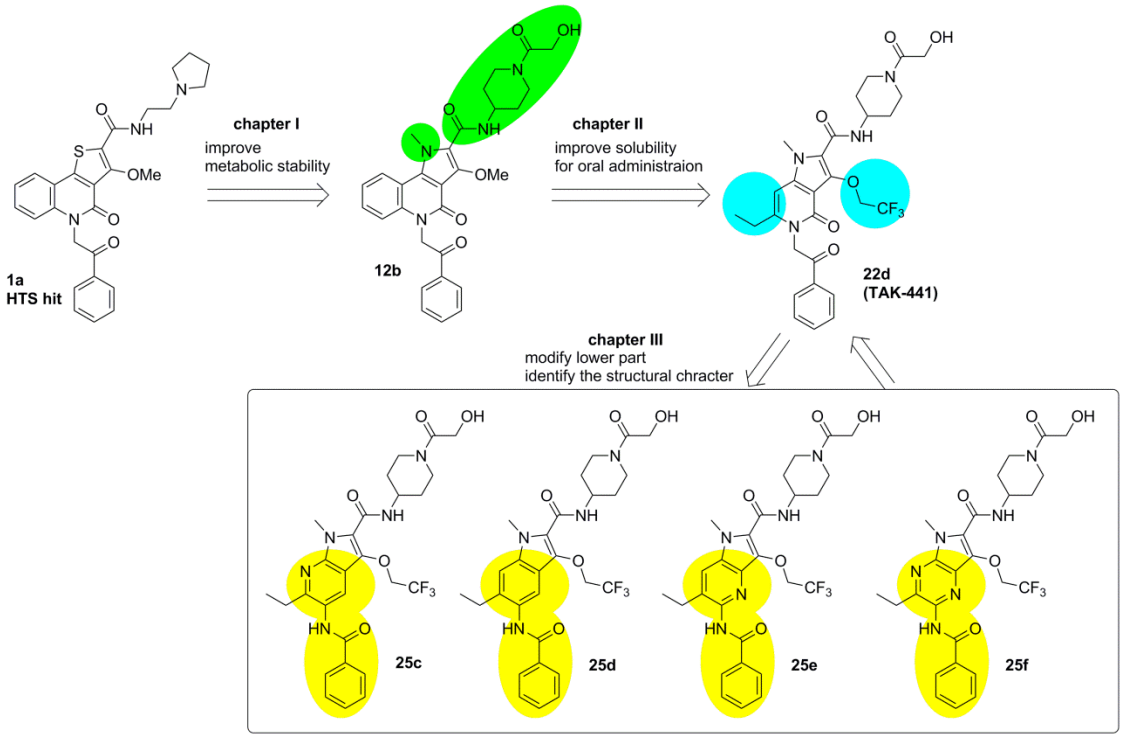


Figure 5. Synthetic strategy and representative compound in each chapter.

Chapter I

Discovery of pyrrolo[3,2-*c*]quinoline-4-one derivatives

2.1. Introduction

Thieno[3,2-*c*]quinoline-4-one derivative **1a** was identified as a Hh signaling inhibitor by HTS of the compound library in Takeda Pharmaceutical Company. The chemical structure of **1a** is distinct from those of known Hh signaling inhibitors currently in clinical trials. Compound **1a** demonstrated potent inhibition of Hh activity with an IC_{50} of 5.1 nM in the Gli-luc reporter assay; however **1a** was unstable in mouse hepatic microsome. In the metabolite analysis, the oxidation of the amide moiety was observed in mouse hepatic microsome, thus the chemical modifications of **1a** focused on amide moiety in an effort to enhance metabolic stability while maintaining *in vitro* activity were conducted (**Figure 6**). In this chapter, the synthesis and structure-activity relationship (SAR) of this new class of Hh signaling inhibitors are described.

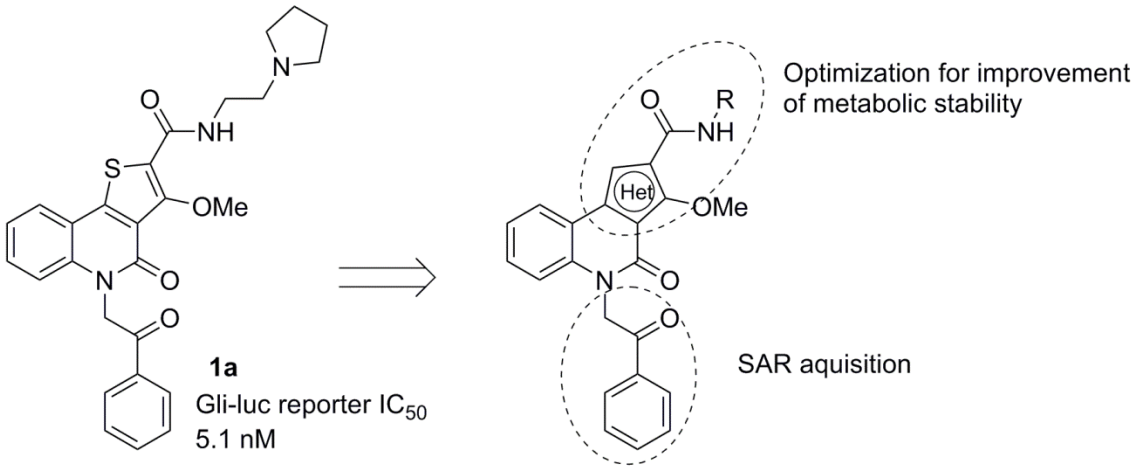


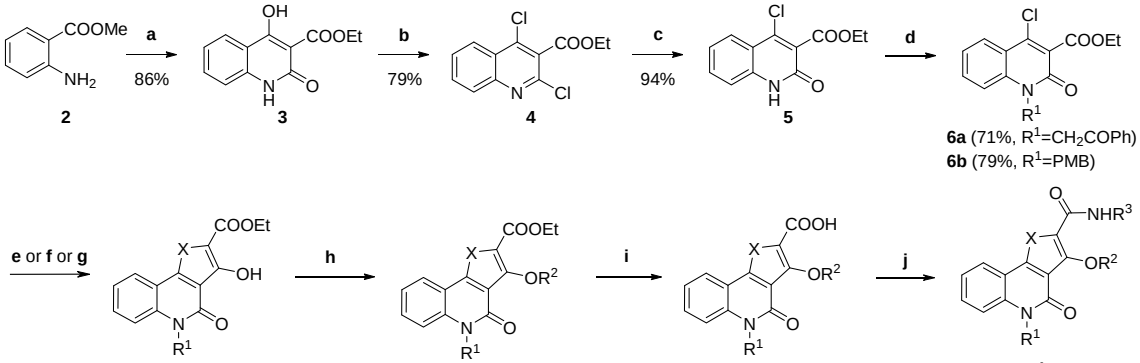
Figure 6. Structure of a hit compound **1a** and synthetic strategy.

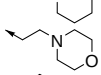
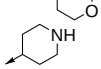
2.2. Synthesis

Thieno-, pyrrolo-, furo-[3,2-*c*]quinoline-4-one derivatives were prepared as shown in Scheme 1 and Scheme 2. Treatment of methyl anthranilate **2** with diethyl malonate under basic conditions gave quinoline compound **2**. Chlorination of **3** using phosphoryl chloride was achieved in 79% yield. Hydrolysis of **4** with sodium acetate led to 4-chloroquinoline **5** selectively, which was *N*-alkylated with phenacyl bromide or *p*-methoxybenzyl chloride to afford **6a**, **b**. The tricyclic core ring was constructed by treatment of **6** with corresponding ester reagent under basic conditions. Alkylation of

hydroxyl group of **7** afforded alkoxy derivative **8**, and following hydrolysis gave derivative **9**. Carboxylic acid **9** was converted to various amides (**1a-h**) by condensation with corresponding amines, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt) in 18-93% yield. Piperidine derivative **1i** was afforded by removal of the Boc group of **1h** with HCl (Scheme 1).

Scheme 1. Synthesis of thieno-, pyrrolo-, furo-[3,2-*c*]quinoline-4-one derivatives.



cmpds.	X	R^1	R^2	R^3	yield (%)
1a	S	CH_2COPh	Me		62
1b	O	CH_2COPh	Me		66
1c	NMe	CH_2COPh	Me		78
1d	NMe	CH_2COPh	Et		18
1e	NMe	CH_2COPh	Me		90
1f	NMe	CH_2COPh	Me		82
1g	NMe	CH_2COPh	Me		85
1h	NMe	PMB	Me		93
1i	NMe	PMB	Me		99[1)]

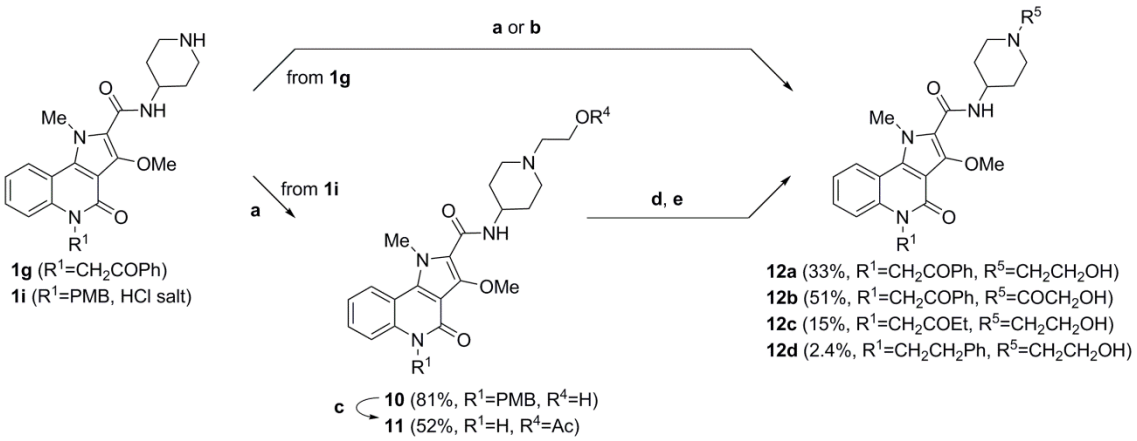
k

1) yield from **1h**

Reagents and conditions: (a) diethyl malonate, NaOEt, EtOH, rt then 140 °C; (b) $POCl_3$, 110°C; (c) AcONa, AcOH, 120 °C; (d) $PhCOCH_2Br$, NaH, DMF, rt for **6a**, or PMBCl, NaH, DMF, rt for **6b**; (e) $EtOOCCH_2SH$ for **7a** or $EtOOCCH_2OH$ for **7b**, NaOEt, EtOH, rt; (f) (1) $MeNHCH_2COOEt$•HCl, Et_3N, EtOH, 85 °C; (2) NaOEt, EtOH, 50 °C for **7c**; (g) $MeNHCH_2COOEt$•HCl, Et_3N, EtOH, 85 °C then NaOEt, EtOH, 60 °C for **7d**; (h) MeI or EtI, DBU, DMF, rt.; (i) NaOH, EtOH, H_2O, rt; (j) R^3NH_2, EDC, HOBt, Et_3N, DMF; (k) HCl, AcOEt, rt.

N-Hydroxyethyl **12a** and *N*-hydroxyacetyl **12b** derivatives were obtained from **1g** by its alkylation or acylation in 33% and 51% yield, respectively. **1i** was alkylated with 2-bromoethanol to afford **10**, and the removal of the *p*-methoxybenzyl group was carried out with trifluoroacetic acid (TFA), anisole, and trifluoromethanesulfonic acid (TfOH) at 80 °C, followed by acetylation to give **11**. *N*-alkylation of **11** using the corresponding alkyl halides in the presence of sodium *tert*-butoxide (*tert*-BuONa) followed by hydrolysis of the acetyl group gave **12c**, **12d** in 15% and 2.4% yield, respectively (Scheme 2).

Scheme 2. Synthesis of **12a-d**.



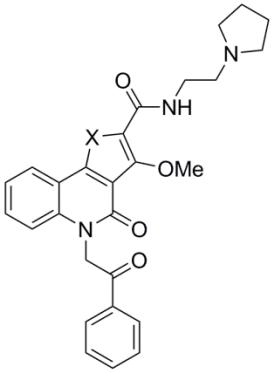
Reagents and conditions: (a) 2-bromoethanol, K_2CO_3, DMF, 80 °C for **10**, 100 °C for **12a**; (b) (1) $ClCOCH_2OAc$, Et_3N, THF, 0 °C, (2) NaOH, EtOH, rt; (c) (1) TFA, anisole, TfOH, 80 °C; (2) AcCl, Et_3N, THF, 0 °C; (d) $EtCOCH_2Br$ or $PhCH_2CH_2Br$, *tert*-BuONa, LiBr, DME/DMF, 0 °C to rt. (e) NaOH, EtOH, H_2O, rt.

2.3. Results and discussion

2.3.1 Biological data for hedgehog, and SAR study

The *in vitro* effects of the tricyclic core on activity (Table 1) were initially assessed by using a luciferase reporter in NIH3T3 cells carrying a stably transfected Gli-reporter construct (Gli-luc reporter cell line).[21] The furo analog **1b** clearly had decreased Hh inhibitory activity compared with **1a**. On the other hand, a significant increase in Hh inhibition was observed with *N*-methylpyrrolo analog **1c** compared to **1b**. *N*-methyl moiety of the 5-membered ring was considered to be essential for potent activity and metabolic stability based on the low lipophilicity. Thus *N*-methylpyrrolo[3,2-*c*]quinoline-4-one was selected as a core ring system for further investigation.

Table 1. Effect of tricyclic ring core against Gli-luc reporter assay.



cmpds	X	Gli-luc reporter IC_{50} (nM)[a]
1a	S	5.1
1b	O	>1000
1c	NMe	2.9

[a] IC_{50} values represent the mean of four measurements.

Although **1c** showed potent *in vitro* activity as mentioned above, the metabolic stability of this compound assessed by incubation in mouse hepatic microsomes was still insufficient to suggest that **1c** would have *in vivo* efficacy in mice (Table 2). Thus, our interest was shifted to improvement of the metabolic stability by optimization of the substituents at the 2, 3, 5-positions of the pyrrolo[3,2-*c*]quinoline-4-one. Substitution of methoxy group at the 3-position reduced activity and was apparently unstable in mouse hepatic microsomes (**1d** vs **1c**). Therefore, the substituent at this position was fixed as methoxy in the remaining studies. Several derivatives were prepared with various cyclic amines at the terminal of the side chain at the 2-position. Ethyl groups with piperidine (**1e**) or morpholine (**1f**) derivatives resulted in decreased Gli-luc reporter inhibitory activity without improvement of metabolic stability. The 4-piperidinyl derivative **1g** showed dramatic improvement in metabolic stability compared to **1c**, indicating that the cyclic amine component attached directly to the amide linker decreased lipophilicity and improved metabolic stability. The side chain of **1g** is shorter than that of **1c** and the author speculated that introduction of the ethyl group on to the nitrogen of the piperidine ring in **1g** was responsible for enhancing activity. The overlays of the stable conformation of **1g** and ethylated **1g** (**Et-1g**) calculated by Molecular Operating Environment (MOE) software were shown in Figure 7. It demonstrated that the ethyl group of **Et-1g** could occupy the same space as the pyrrolidine ring of **1c**. The lipophilicity of **Et-1g** was predicted to be higher than that of **1g** (cLogP value : **Et-1g** = 3.5 vs **1g** = 2.5), suggesting that **Et-1g** may have decreased microsomal stability as a

result. Thus a hydroxyethyl substituent (clogP value: 2.2) onto the piperidine nitrogen of **1g** instead of the ethyl group position was introduced. As expected, **12a** demonstrated potent inhibition of Gli-luc reporter activity while retaining metabolic stability both in mouse and human microsomes. However, further evaluation of **12a** showed strong inhibition of the human ether-a-go-go related gene (hERG; data not shown) which is involved in cardiac repolarization. Since basicity of compounds is often correlated with hERG inhibition,[22] the acyl function was introduced to decrease basicity. Compound **12b**, bearing a *N*-[1-(hydroxyacetyl)piperidinyl] amide moiety, achieved both potent activity and good metabolic stability without hERG inhibition, as expected.

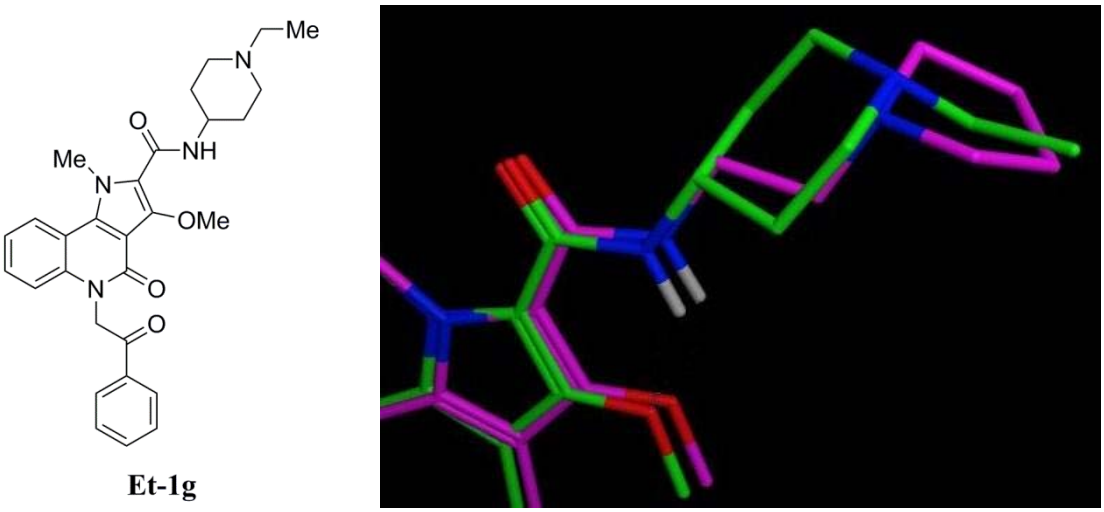
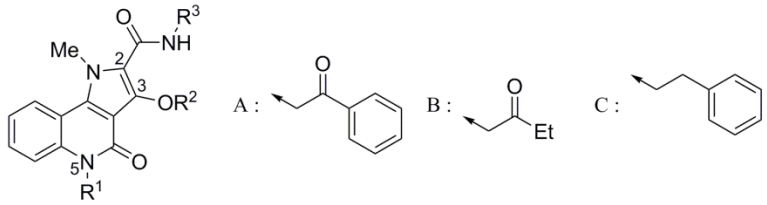


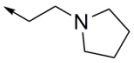
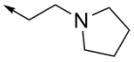
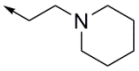
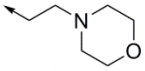
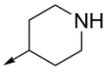
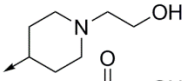
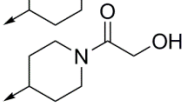
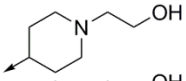
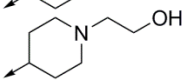
Figure 7. Overlay of ethylated **1g** (Et-**1g**, green) and **1c** (purple) in stable conformation calculated by MOE software.[a]

[a] MOE version 2010.10; Chemical Computing Group, Inc., Montreal, Quebec, Canada. ClogP values, which indicate the index of lipophilicity, were calculated by Daylight Software ClogP, version 4.82, Daylight Chemical Information Systems, Inc., Aliso Viejo, CA.

Finally, the SAR around the 5-position was investigated. The 2-butanoyl derivative **12c**, which had an ethyl group in place of the benzene ring in **12b**, exhibited significantly decreased potency. The phenethyl derivative **12d** without the carbonyl group showed 30-fold drop in activity compared with **12a**. These results suggested that an aromatic substituent at the 5-position would be necessary for tight binding to the Smo protein. Therefore, the phenacyl group in **12b** was selected as the best substituent at the 5-position for further evaluation.

Table 2. Effect of substituents on pyrrolo[3,2-*c*]quinoline-4-one at 2, 3, and 5-position.



cmpds	R^1	R^2	R^3	Gli-luc reporter IC_{50} (nM)[a]	metabolic stability[b] (μL / min / mg)	
					Mouse	Human
1c	A	Me		2.9	95	14
1d	A	Et		7.0	155	26
1e	A	Me		9.6	192	30
1f	A	Me		22	237	73
1g	A	Me		21	10	2
12a	A	Me		6.6	9	6
12b	A	Me		4.6	11	0
12c	B	Me		>1000	0	3
12d	C	Me		150	48	72

[a] IC_{50} values are the mean of four measurements. [b] Hepatic microsome.

2.3.2 Pharmacodynamic, pharmacokinetic data and *in vivo* study of 12b in mouse

The *in vivo* pharmacodynamic (PD) and pharmacokinetic (PK) profiles of **12b** are shown in Table 3. PAN-04 is a human pancreatic xenograft tumor line derived from a clinical specimen established by the Central Institute for Experimental Animals (Kanagawa, Japan). This xenograft tumor significantly expressed stroma-derived Gli1 and cancer-derived Shh activity. The reduction of Gli1 *m*RNA expression levels was measured as a PD marker in this model. Compound **12b** showed potent activity in a Smo binding assay (IC_{50}: 41 nM), favorable *in vitro* metabolic stability in both mouse and human microsomes, and favorable mouse PK profile. Consistent with the results of *in vitro* activities and ADME profiles, **12b** strongly suppressed Gli1 *m*RNA expression after a single cycle of dosing at 25 mg/kg, twice daily (BID).

Table 3. Activity in *in vivo* PD assay, properties and pharmacokinetic data of **12b**.

cmpd	Smo binding (nM)	*in vivo* PD Gli1 *m*RNA (% of ctrl)[a]	metabolic stability[b] (μL / min / mg)		Mouse PK[c]				
			Mouse	Human	Cmax(μg/mL)	Tmax (h)	MRT (h)	AUC (μgh/mL)	C_{8h} (h)
12b	41	5	11	ND[d]	2.65	1.00	2.99	12.1	0.604

[a] Gli1 *m*RNA expression at 25 mg/kg bid. The value is indicated the Gli1 *m*RNA expression level compare to control. The compound **12b** was dosed at 25 mg/kg bid.

[b] Hepatic microsome.

[c] Cassette dosing at 10 mg/kg po.

[d] Not detected. The compound did not reduce under metabolism test.

Compound **12b** demonstrated marked suppression of Gli1 *m*RNA in the PAN-04 model. This supported *in vivo* efficacy studies with compound **12b** in a medulloblastoma allograft model generated from Ptc+/- p53-/- mice in which the Hh signaling pathway was constitutively activated. Oral administration of compound **12b** for two weeks at 6.25 mg/kg BID resulted in virtually complete suppression of tumor growth (*T*/*C* ratio = 3%; Figure 8, left) without notable body weight loss (Figure 8, right). It was suggested that compound **12b** might be effective in the treatment of cancers that are activated by Hh signaling in mice.

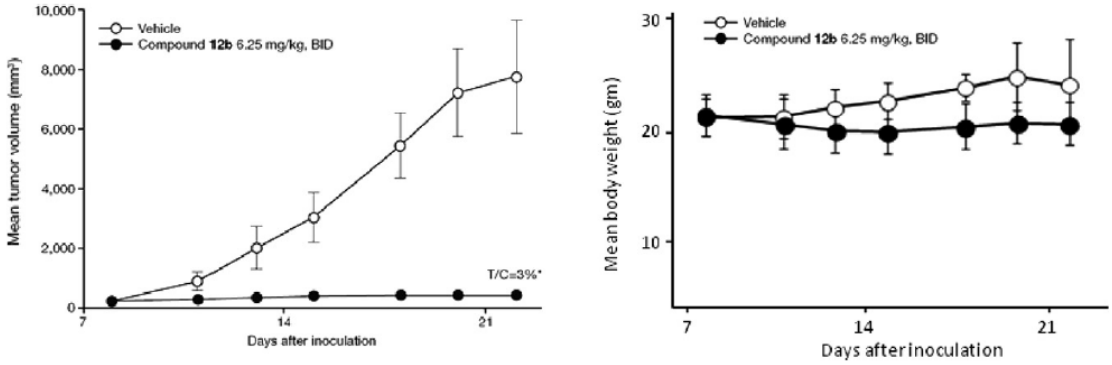


Figure 8. Antitumor activity (left) and body weight change (right) upon treatment with **12b** in nude mice carrying Ptc+/- p53-/- medulloblastoma allograft tumors.[a]

[a] Compound **12b** was administrated orally at a dose of 6.25 mg/kg BID for 14 days (●); vehicle controls (○). Each point represents the mean ± SD of duplicate values. *P < 0.025 by a 1-tailed Dunnett's test compared to controls.

2.4. Conclusion

In these studies, HTS to identify Hh signaling inhibitors resulted in a novel lead compound **1a**, which possesses a thieno[3,2-*c*]quinoline-4-one core structure. The

medicinal chemistry efforts, focusing on maintaining *in vitro* activity while significantly improving metabolic stability, led to pyrrolo[3,2-*c*]quinoline-4-one **12b** with a characteristic side chain at the 2-position. Compound **12b** showed suppression of Gli1 *m*RNA expression and virtually complete growth suppression in the medulloblastoma allograft model. This *in vivo* efficacy encouraged to seek Hh signaling inhibitors that might be considered as candidates for clinical research in patients with cancers that are characterized by aberrant Hh signaling activity.

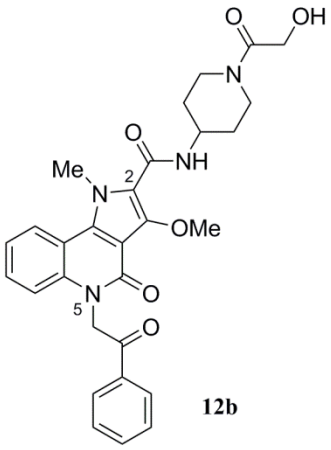
Chapter II

Discovery of TAK-441 as orally available candidate

3.1. Introduction

In a previous chapter, the synthesis and biological activity of *N*-(1-glycoloylpiperidin-4-yl)-3-methoxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxamide **12b** as a potent Hh signaling inhibitor were described. The phenacyl group at the 5-position is important for its potent *in vitro* activity, and the *N*-[1-(hydroxyacetyl)piperidinyl] amide side chain at the 2-position provided high metabolic stability in mouse and human. Moreover, the amide was effective substituent for the reduction of hERG inhibition, which is important in cardiac repolarization, based on the avoidance of tertiary amine structure.

Table 4. Activity, solubility and PK profile of compound **12b**.



12b

Gli-luc reporter [a] (nM)	*in vivo* PD Gli1 *m*RNA[b] (% of ctrl)	solubility[c] (μg/mL)	Mouse PK 10 mg/kg[d]		Mouse PK 100 mg/kg[e]	
			C_{max} (μg/mL)	AUC (μgh/mL)	C_{max} (μg/mL)	AUC (μgh/mL)
4.6	5	8.4	2.65	12.1	3.63	32.3

[a] IC_{50} values are the mean of four measurements.

[b] The value is the Gli1 *m*RNA expression level at 25 mg/kg BID of compound **12b** compared to controls.

[c] Measured at pH 6.8 (Japanese Pharmacopoeia second fluid).

[d] Cassette dosing, $AUC_{0\text{-}8h}$

[e] BALB/c-nu/nu mice, $AUC_{0\text{-}24h}$

Compound **12b** exhibited potent suppression of tumor growth in *in vivo* experiments in a mouse medulloblastoma allograft model. However, the PK profile of **12b** at high dose in mice (100 mg/kg) showed poor oral absorption that was not proportional to dose ($AUC_{0\text{-}24h}$ 32.3 μgh/mL; Table 4). Considering that this poor oral absorption could be attributed to low solubility (8.4 μg/mL at pH 6.8), the author sought to improve the solubility while maintaining Hh pathway inhibitory activity as shown in Figure 9. In general, compounds with a tricyclic core show poor solubility and often low bioavailability.[23] Thus, the benzene ring on pyrrolo[3,2-*c*]quinoline-4-one was removed in order to decrease planarity. In addition, it was speculated that the residual substituent at the 3-position was available for modification of the physicochemical properties. The investigations in chapter I demonstrated that substituents at the 2- and 5-positions were fixed because they played important roles in potency and metabolic stability as mentioned above. Herein, the design and synthesis of a novel series of derivatives leading to the identification of clinical candidate are described.

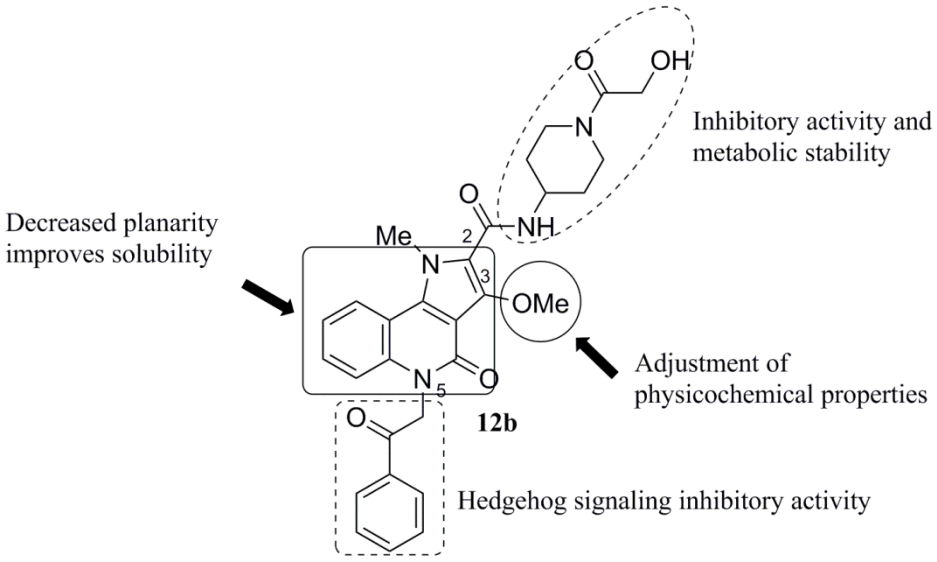


Figure 9. Compound design strategy for improving solubility and maintaining potency.

3.2 Synthesis

The removal of benzene ring from compound **12b** was expected to reduce planarity. Retrosynthetic analysis (Figure 10) demonstrated that ketoesters bearing R^6 and R^7 substituents would allow investigation of replacements for the phenyl ring that was being removed.

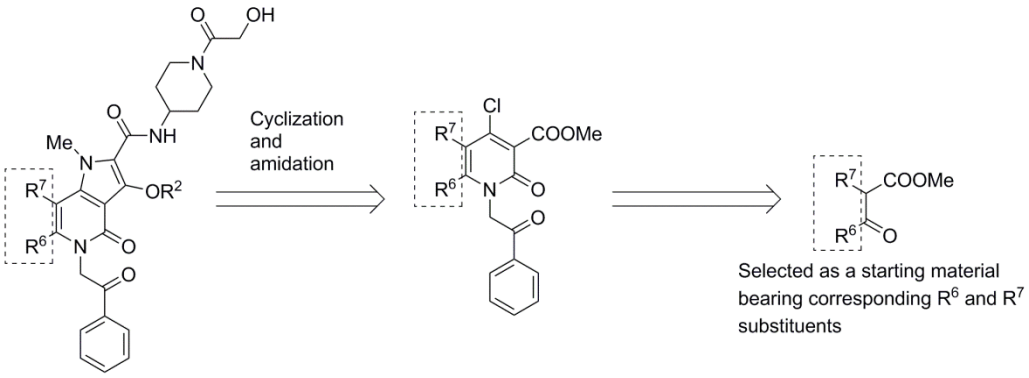
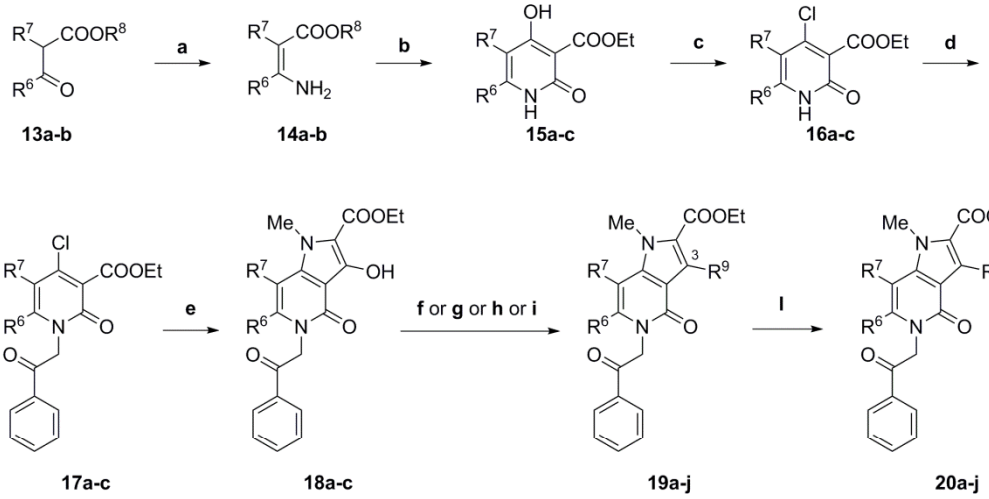


Figure 10. Synthetic strategy.

The general synthesis of the compounds in this study is outlined below. Pyridone **15**, except for commercially available **15c**, was prepared from **13**. Ketoester **13** was converted to **14** by amination. Cyclization of **14** with diethyl malonate afforded **15** in 60-92% yield. Monochlorination of **15** was achieved using phosphoryl chloride in 17-60% yield. Alkylation of **16** with phenacyl bromide and potassium carbonate proceeded in low yield (7-23%) because of the production of *O*-phenacylated compound as a major product. Substitution and cyclization of **17** was conducted with sarcosine ethyl ester hydrochloride under basic conditions[24] to obtain pyrrolo[3,2-*c*]pyridine **18**. The hydroxyl group at the 3-position of **18** was treated with various alkylating reagents to afford corresponding alkoxy derivatives **19a-e, 19i,** and **19j**. Ethyl derivative **19h** was prepared by treatment of **18a** with trifluoromethanesulfonic anhydride and afforded triflate **19f**. Following Stille coupling of the obtained triflate **19f** with vinyltributyltin, ethenyl derivative **19g** was treated with palladium on carbon under hydrogen atmosphere to give the 3-ethyl derivative **19h**. Finally, saponification of **19** afforded the corresponding carboxylic acid **20** in 23-99% yield (Scheme 3).

Scheme 3. Synthesis of carboxylic acid **20**.



cmpds	R^6	R^7	R^8	yield
13a 14a	Et	H	Me	92
13b 14b	-$(CH_2)_4$-		Et	60

cmpds	R^6	R^7	yield **14** to **15**	**15** to **16**	**16** to **17**	**17** to **18**
15-18a	Et	H	46	60	7	74
15-18b	-$(CH_2)_4$-		58	17	16	-
15-18c	Me	H		44	23	68

cmpds	R^6	R^7	R^9	yield
19a	Et	H	OEt	82
19b	Et	H	OCH_2CH_2F	77
19c	Et	H	OCH_2CHF_2	77
19d	Et	H	OCH_2CF_3	86
19e	Et	H	OMe	23
19f	Et	H	OTf	59
19g	Et	H	ethenyl	72[1)]
19h	Et	H	Et	100[2)]
19i	-$(CH_2)_4$-		OMe	11[3)]
19j	Me	H	OMe	26

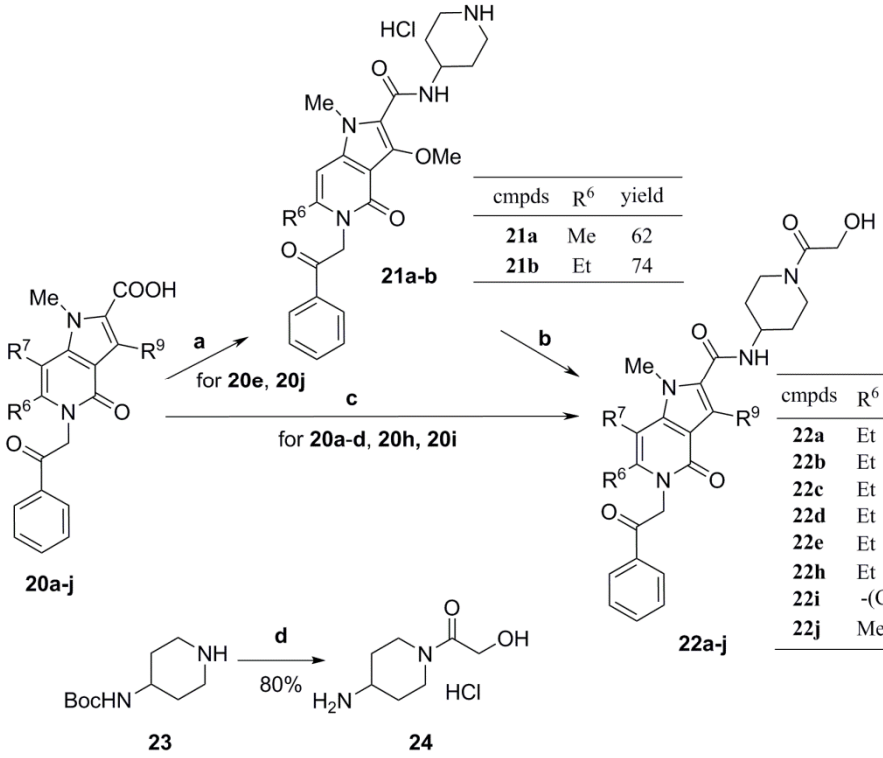
1) yield from **19f**
2) yield from **19g**
3) 2 steps yield from **17b**

cmpds	R^6	R^7	R^9	yield
20a	Et	H	OEt	89
20b	Et	H	OCH_2CH_2F	56
20c	Et	H	OCH_2CHF_2	83
20d	Et	H	OCH_2CF_3	93
20e	Et	H	OMe	79
20h	Et	H	Et	99
20i	-$(CH_2)_4$-		OMe	23
20j	Me	H	OMe	89

Reagents and conditions: (a) NH_4OAc, MeOH, rt; (b) diethyl malonate, NaOEt, EtOH, xylene, 120 °C, then 150 °C; (c) $POCl_3$ for **16a,b** or $POCl_3$, $BnEt_3NCl$, MeCN, 40 °C, then reflux for **16c**; (d) $PhCOCH_2Br$, K_2CO_3, DMF, rt for **17a,c** or $PhCOCH_2Br$, NaH, DMA, rt for **17b**; (e) $MeNHCH_2COOEt$•HCl, Et_3N, EtOH, reflux; (f) R'X, DBU, DMF, rt for **19b** (R'=CH_2CH_2F), **19e, j** (R'=Me); (g) R'_2SO_4, K_2CO_3, acetone, reflux for **19a** (R'=Et), **19i** (R'=Me); (h) R'OTf, Cs_2CO_3, DMF, rt for **19c** (R'=CH_2CHF_2), **19d** (R'=CH_2CF_3); (i) Tf_2O, pyridine, 60 °C for **19f**; (j) vinyltributyltin, $Pd(PPh_3)_4$, DMF, 100 °C; (k) Pd/C, H_2, THF/MeOH, rt; (l) NaOH, EtOH, H_2O, 60 °C.

Amidation of carboxylic acid **20** is shown in Scheme 4. Compounds **22e** and **22j** were synthesized from corresponding carboxylic acids **20e** and **20j** by stepwise acylation. Compounds **22a-d**, **22h,** and **22i** were obtained by condensation of corresponding carboxylic acid **20** with amine **24** directly. The requisite **24** was prepared from 4-Boc-aminopiperidine (**23**) in three steps: acylation with acetoxyacetyl chloride, followed by removal of the acetyl and *tert*-butoxycarbonyl groups in 80% yield .

Scheme 4.



Reagents and conditions : (a) (1) 4-amino-1-Boc-piperidine, EDC, HOBt, DMF, rt, (2) 4M HCl in AcOEt, AcOEt, rt; (b) (1) $ClCOCH_2OAc$, Et_3N, THF, rt, (2) NaOH, EtOH, H_2O, rt for **22e**, **22j**; (c) **24**, EDC, HOBt, DMF, rt for **22a-d**, **22h**, **22i**; (d) (1) $ClCOCH_2OAc$, Et_3N, THF, 0 °C; (2) 8M NaOH, EtOH, H_2O, rt; (3) 4M HCl in AcOEt, AcOEt, rt.

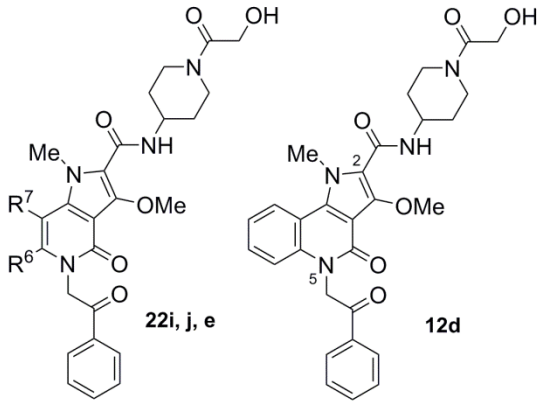
3.3 Results and discussion

3.3.1 Biological data for hedgehog, and SAR study

In vitro activities of compounds **22a-j** were evaluated using luciferase reporter activities in NIH3T3 cells carrying a stably-transfected Gli-reporter construct, designated the Gli-luc reporter cell line. First, modification of the benzene ring to cyclohexane in the core structure was examined. In the Gli-luc reporter assay, cyclohexene **22i** showed an approximately 20-fold decreased activity compared with **12d** (Table 5). This result implied that a substituent with specific size and shape would be required for maintaining the potent inhibition of Gli-luc reporter activity. Downsizing the cyclohexene ring to a monomethyl group (R^6) was effective in maintaining activity (**22j** vs **22i**). In addition, solubility of **22j** was improved compared with that of **12d**, as expected. Introduction of an ethyl group at the 6-position resulted in further enhancement of Hh inhibitory activity without loss of solubility (**22e** vs **22j**). Among the compounds listed in Table 5, the 6-ethyl derivative **22e** which had potent activity in

the Gli-luc reporter assay and good solubility was selected for further investigation.

Table 5. Modification of the core ring.



cmpds	R^6	R^7	Gli-luc reporter IC_{50}[a] (nM)	solubility[b] (μg / mL)
22i	-$(CH_2)_4$-		96	80
22j	Me	H	14	77
22e	Et	H	5.7	63
12d			4.6	8.4

[a] IC_{50} values are the mean of four measurements.

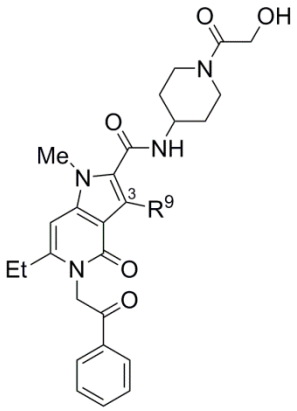
[b] Measured at pH 6.8 (Japanese Pharmacopoeia second fluid).

Compound **22e** was conducted *in vivo* PD study at 25 mg/kg, BID, but the efficacy was weak. It was considered the solubility of the compound **22e** was insufficient to show good efficacy at higher dose, thus the chemical modification for further improvement of solubility was continued. Thus, the synthetic efforts next focused on modification of substituents at the 3-position (Table 6). Exchange of the methoxy group in **22e** to an ethyl group significantly decreased activity (**22h** vs **22e**). This result suggested that the oxygen atom in the 3-methoxy group in **22e** plays an important role in activity. In general, fluorination of alkyl substituents is known to impact the physicochemical profile by changing lipophilic and electronic factors.[25] Therefore, the effect of fluorination on solubility and potency was investigated by using the 3-ethoxy derivative **22a** as a standard compound with good activity and solubility.

Both 2-fluoroethoxy (**22b**) and 2,2-difluoroethoxy (**22c**) groups effectively increased solubility, but exposure (AUC) of these compounds in mice following oral dosing was decreased compared with that of **22a**. On the other hand, 2,2,2-trifluoroethoxy derivative **22d** exhibited favorable exposure compared to **22a** and **22e**. Furthermore, the oral absorption of **22d** was better than that of **12b**, suggesting

that the improvement of solubility was contributed to good PK profile at higher dose (Table 7). Thus, **22d** was selected as a compound for further investigation.

Table 6. Optimization of pyrroro[3,2-*c*]pyridine at the 3-position.



cmpds	R^9	Gli-luc reporter IC_{50}[a] (nM)	solubility[b] (μg / mL)	AUC[c] (μgh/mL)
22e	OMe	5.7	63	31.647
22h	Et	95		
22a	OEt	6.9	58	20.678
22b	OCH_2CH_2F	4.9	69	5.149
22c	OCH_2CHF_2	4.4	84	5.404
22d	OCH_2CF_3	4.4	81	28.346

[a] IC_{50} values are the mean of four measurements.

[b] Measured at pH 6.8 (Japanese Pharmacopoeia second fluid).

[c] Cassette dosing at 10 mg/kg, po in mice. AUC means area under the plasma concentration versus time curve from 0 to 8 hours.

Table 7. Pharmacokinetic data of **12b** and **22d**.

cmpd	Mouse PK 10 mg/kg[a]		Mouse PK 100 mg/kg[b]	
	C_{max} (μg/mL)	AUC (μgh/mL)	C_{max} (μg/mL)	AUC (μgh/mL)
12b	2.65	12.1	3.63	32.3
22d	5.63	28.3	21.5	206

[a] Cassette dosing, $AUC_{0\text{-}8h}$

[b] BALB/c-nu/nu mice, $AUC_{0\text{-}24h}$

3.3.2 X-ray single crystal structure analysis of 22d

As mentioned above, the oxygen atom in the alkoxy group at the 3-position was important for inhibitory activity. An X-ray structural analysis of **22d** suggested an explanation for this finding (Figure 11). An intramolecular hydrogen bond interaction was observed between the oxygen atom in the 2,2,2-trifluoroethoxy group and the amide hydrogen atom at the C2 side chain. This conformation would not be favored in the C3-ethyl derivative. It was speculated that the oxygen atom would be necessary for hydrogen bond formation with the amide, thereby stabilizing the position of the side chain.

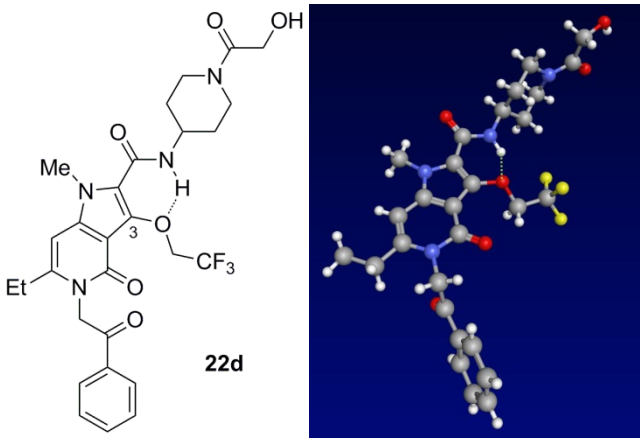
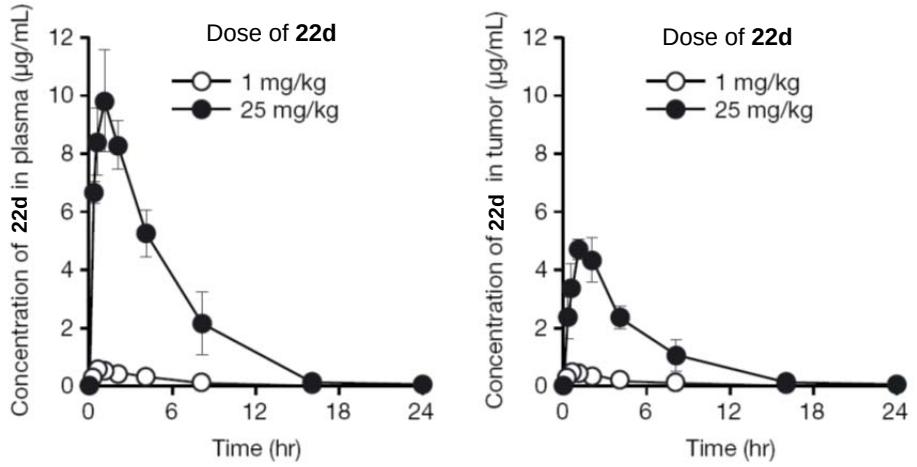


Figure 11. X-ray single crystal structure of compound **22d**.[a]

[a] The figure was described based on X-ray structural analysis data using MOE Software. Molecular Operating Environment (MOE version 2010.10); Chemical Computing Group, Inc.: Montreal, Quebec, Canada; http://www.chemcomp.com.

3.3.3 *In vivo* study of 22d

The *in vivo* efficacy of **22d** was evaluated by using a Ptc1+/- p53-/- medulloblastoma allograft model in mice in which the Hh pathway was activated. Plasma and tumor concentrations of **11d** after a single oral dose are shown in Figure 12 indicating good tumor permeability and dose linearity. Repeated oral administration of compound **22d** (QD, 14 days) resulted in moderate antitumor activity with a *T/C* value of 46% at a dose of 1 mg/kg (Figure 13); complete growth inhibition was observed at 25 mg/kg (*T*/*C* = 1%) without excessive accumulation. These data suggest that improved solubility resulted in a dose-dependent PK profile for compound **22d**.



	Plasma		Tumor	
	C_{max} (μg/mL)	$AUC_{0\text{-}24h}$ (μgh/mL)	C_{max} (μg/mL)	$AUC_{0\text{-}24h}$ (μgh/mL)
1 mg/kg	0.659	3.36	0.244	1.30
25 mg/kg	9.87	54.3	4.65	25.3

Figure 12. Concentrations of compound **22d** in the plasma and tumor after oral administration at doses of 1 and 25 mg/kg in medulloblastoma allografted mice.[a]

[a] Mean ± standard deviation, SD (n=3)

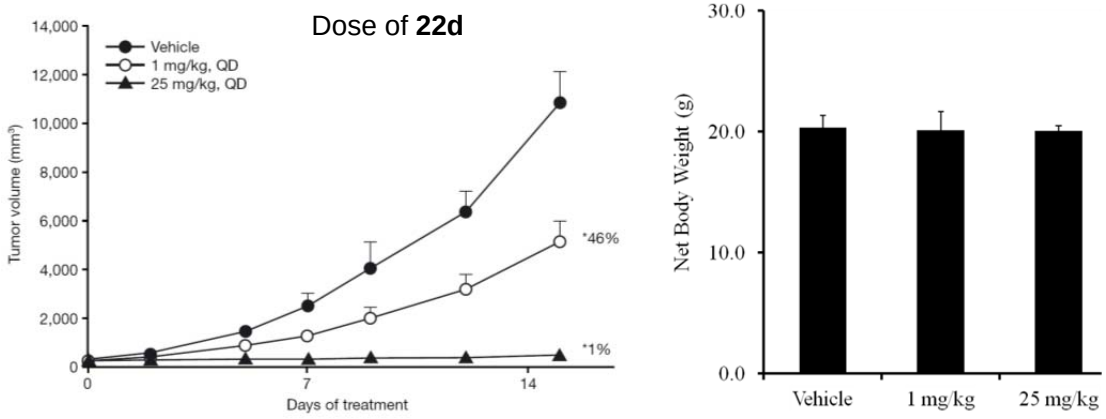


Figure 13. Efficacy of compound **22d** in medulloblastoma in a Ptc1+/- p53-/- mouse allograft model (left) and body weight after 14 days, QD (right).

Oral administration of **22d** in mice once daily (QD) for 14 days at 1 mg/kg (○) or 25 mg/kg (▲) compared with vehicle-treated animals (●). Data are shown as mean ± SD (n = 5). Antitumor effects were expressed as the ratio of treatment vs control (T/C, %) which was calculated by comparison of the mean change in tumor volume over the treatment period for the control and treated groups. *$P \leq 0.025$ by a 1-tailed Shirley-Williams test compared to vehicle control.

3.3.4 Pharmacokinetic data of 22d in other animals

The PK profiles of **22d** in other species are shown in Table 8. Compound **22d** showed favorable oral bioavailability (F), which was 32% in rats and 90% in dogs. Moreover, low drug clearance (CL) and high AUC were observed in dogs. These results suggested that compound **22d** is able to achieve sufficient exposure following oral administration in rats and dogs to enable appropriate toxicological studies.

Table 8 Pharmacokinetic profile[a] in rats and dogs

	V_{ss} (mL/kg)	CL (mL/h/kg)	$AUC_{0\text{-}24h,iv}$ (ng•h/mL)	$AUC_{0\text{-}24h,po}$ (ng•h/mL)	F (%)
Rat[b]	681.6 ± 81.6	397.9 ± 10.1	2532.3 ± 69.1	8031.8 ± 1218.6	31.7
Dog[c]	2181.3 ± 82.8	161.3 ± 35.6	5101.5 ± 685.5	45405.6 ± 5812.0	90.3 ± 8.8

[a] Dose: iv, 1 mg/kg; po, 10 mg/kg

[b] Each value except for bioavailabilty (F%) represents the mean ± SD of three non-fasted animals. The F value is calculated from mean values of $AUC_{0\text{-}24h}$ after oral and intravenous administration.

[c] Each value represents the mean ± SD of four fed animals. The F value is calculated from the individual value of $AUC_{0\text{-}24h}$ after oral and intravenous administration for the same animal.

3.4 Conclusion

With the aim of improving solubility, chemical modification of the first developed compound **12b** was examined. Decreasing the planarity by reducing the number of aromatic rings led to the identification of the novel pyrrolo[3,2-*c*]pyridine-4-one derivative **22j** which had good solubility. Further optimization of the 3-alkoxy group resulted in discovery of **22d** with good physicochemical profiles and potent activity. Compound **22d** exhibited strong antitumor activity in an *in vivo* study in Ptc1+/- p53-/- mice bearing medulloblastoma allografts; improved solubility of compound **22d** enabled dose-dependent plasma and tumor concentrations to be achieved in this model.

Chapter III

Synthesis and evaluation of hedgehog signaling inhibitor with novel core system

4.1 Introduction

In previous chapters, the modification of core ring and substituent at 3-position of pyrrole ring for improvement of solubility were discussed. In the results, 6-ethyl-*N*-(1-glycoloylpiperidin-4-yl)-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-3-(2,2,2-trifluoroethoxy)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxamide **22d** (TAK-441) was discovered. However, it was still unclear that exact role of the core skeleton including substituents at the 4- and 5-positions. Thus, further modification of central core was evaluated to confirm these properties and discovered new potent Hh signaling inhibitors with novel skeletons compared to that of **22d**.

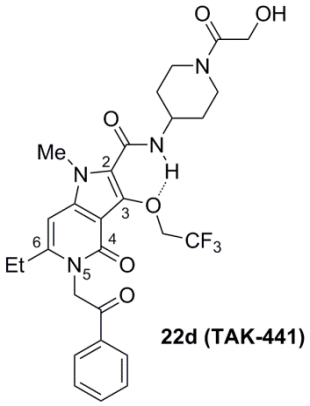


Figure 14. Chemical structure of **22d** (TAK-441).

The substituents on the *N*-methyl pyrrole ring of **22d** were essential for potent inhibitory activity because of the interaction by internal hydrogen bond between 2-amide NH and oxygen of 3-(2,2,2-trifluoro)ethoxy group. Thus this upper part was fixed, and the following modification was focused on the lower part of core ring, especially six-membered rings. Besides novel core ring modification, the effect of 4- or 5-substituents to *in vitro* inhibitory activity was evaluated. In this chapter, the syntheses and SAR of novel hedgehog signaling inhibitors with multi-substituted bicyclic core rings are described (Figure 15).

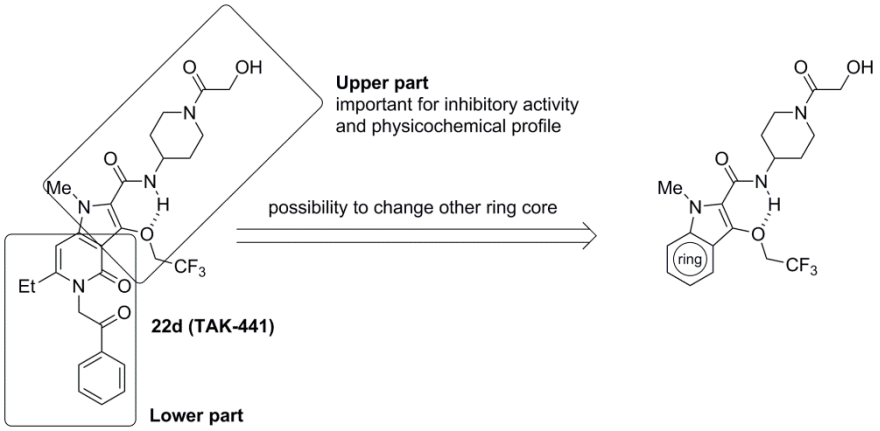


Figure 15. Change to novel core ring in **22d**.

4.2 Synthesis

As mentioned, the intramolecular hydrogen bond formed between (2,2,2-trifluoro)ethoxy group at 3-position and *N*-[1-(hydroxyacetyl)piperidinyl] amide at the 2-position on *N*-methyl pyrrole ring was important for potent *in vitro* activity. Figure 16 illustrates the retrosynthetic analysis to access novel bicyclic cores with these two moieties. To construct the unique bicyclic core of **25**, it was necessary to synthesize intermediate **27**. It was considered the Dieckmann condensation would be promising because the important substituents as mentioned before could be introduced in the desired position. Therefore, *o*-halo arylcarboxylic acid ester **29** was selected for a key intermediate which was converted to di-ester **28** by addition of sarcosine ester unit.[24] This strategy could be utilized for the synthesis of various useful core rings. The syntheses for five types of carboxylic acid **28** are detailed in the next section.

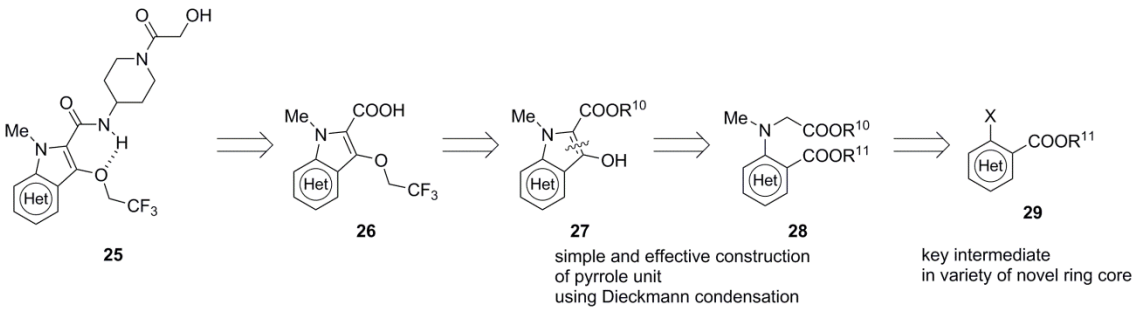
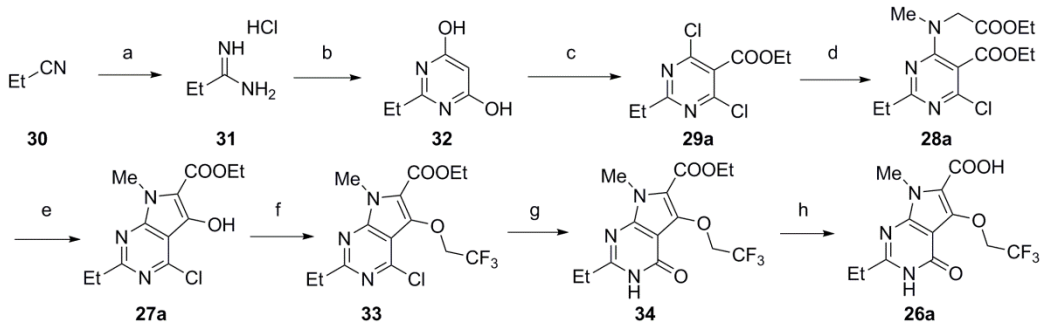


Figure 16. Retrosynthetic analysis of novel core.

The synthesis of pyrrolo[2,3-*d*]pyrimidine **26a** is shown in Scheme 5. Addition of propionitrile **30** with ammonia in acidic condition provided amidine hydrochloride **31**, followed by cyclization with diethyl malonate to afford **32** in 67% yield. The

preparation of a key intermediate **29a** was conducted by stepwise conversion; introduction of formyl group, oxidation to carboxylic acid and esterification, then chlorination. After addition of sarcosine ethyl ester hydrochloride, subsequent Dieckmann condensation provided the bicyclic compound **27a** in 91% yield. The 3-hydroxyl group of **27a** was treated with (2,2,2-trifluoro)ethyl triflate to afford **33**. The stepwise saponification of chloropyridine and ethoxycarbonyl moieties gave carboxylic acid **26a**.

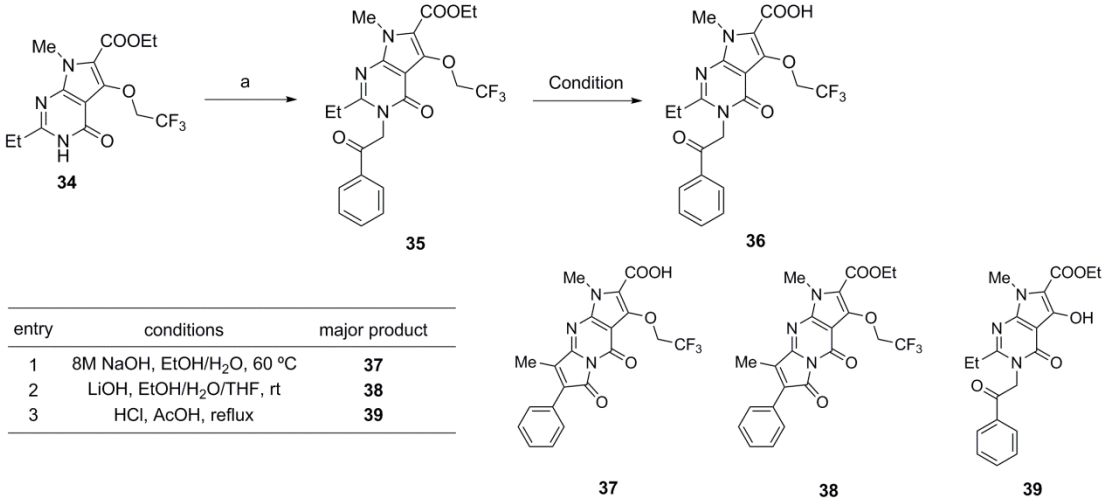
Scheme 5. Synthesis of pyrrolo[2,3-*d*]pyrimidine derivative **26a**.



Reagents and conditions : (a) (1) HCl, EtOH; (2) NH_3, MeOH, 70%; (b) diethyl malonate, NaOMe, MeOH, 67%; (c) (1) DMF, $POCl_3$, 0 ºC to reflux, 73%; (2) $NaClO_2$, sulfamic acid, *tert*-BuOH/H_2O; (3) $(COCl)_2$, DMF, THF; (4) Et_3N, EtOH, 53%; (d) $MeNHCH_2COOEt \cdot HCl$, Et_3N, THF, 99%; (e) NaOEt, EtOH, 91%; (f) CF_3CH_2OTf, Cs_2CO_3, DMF, 91%; (g) AcONa, AcOH, reflux, 96%; (h) NaOH, EtOH, 60 ºC, 79%.

In the previous chapter, the synthesis of **22d** using an intermediate with requisite phenacyl moiety on six-membered ring of the bicyclic core was described. The similar synthetic route using the intermediate **34** was initially attempted (Scheme 6). The phenacylation of **34** gave **35** in 57% yield.[26] The following hydrolysis under basic conditions did not work well and gave only tricyclic product **37** or **38**, which was supposed by LC-MS analysis. It was considered the undesired product was obtained by cyclization of 6-ethylene moiety with carbonyl at the 5-phenacyl group prior to saponification (Scheme 7).[27] It was speculated that this cyclization was triggered by higher acidity of methylene in the ethyl group of compound **35** than that of **22d**. On the other hand, the hydrolysis of **35** under acidic condition gave dealkylated compound **39** as a major product supposed by LC-MS analysis. These results strongly suggested the hydrolysis should be conducted before introduction of 5-phenacyl moiety as described above.

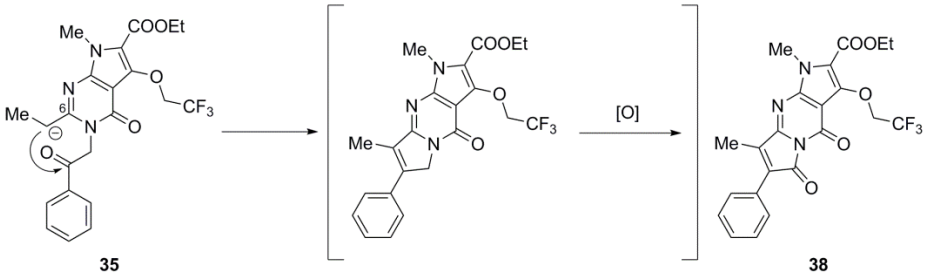
Scheme 6. Synthesis of carboxylic acid **36** using similar route to **22d**.



entry	conditions	major product
1	8M NaOH, EtOH/H_2O, 60 ºC	**37**
2	LiOH, EtOH/H_2O/THF, rt	**38**
3	HCl, AcOH, reflux	**39**

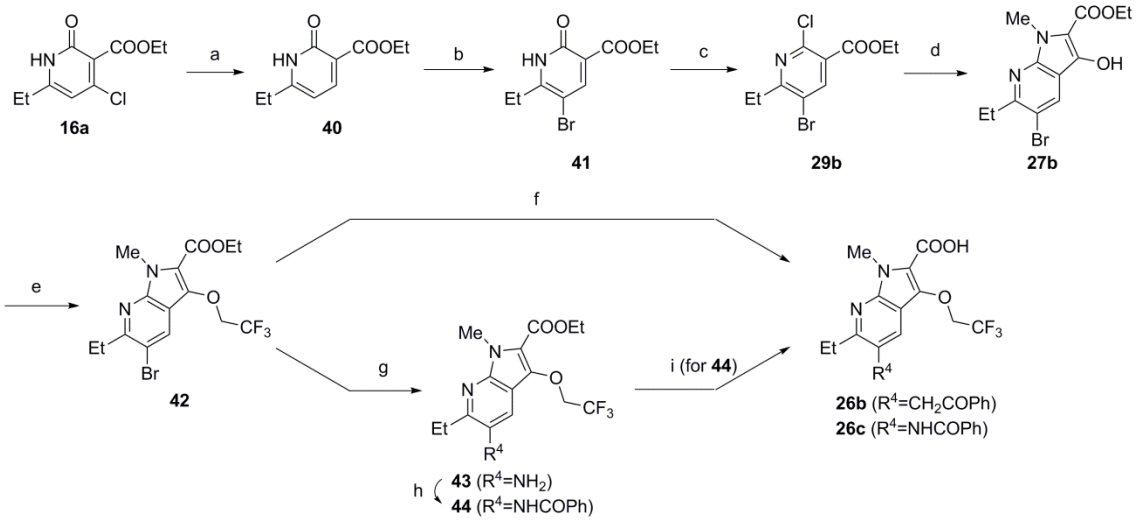
Reagents and conditions : (a) phenacyl bromide, *tert*-BuONa, LiBr, DME, DMF, 60 ºC, 57%.

Scheme 7. Presumed reaction mechanism of tricyclic compound.



Next, the pyrrolo[2,3-*b*]pyridine ring was constructed along the synthetic route below (Scheme 8). The hydrogenation of 4-chloropyridine-2-one derivative **16a** by palladium-carbon was achieved in 90% yield. Stepwise halogenation of **40** was conducted to afford a key intermediate **29b**. Addition of sarcosine ethyl ester hydrochloride and cyclization along the synthetic strategy provided **27b**. Alkylation of the 3-hydroxy group with (2,2,2-trifluoro)ethyl triflate led to **42** in 96% yield. Coupling with acetophenone in Buchwald condition gave **26b** in 22% yield, but introduction of amino group to benzophenoneimine gave **43** in 91% yield. Acylation with benzoyl chloride followed by hydrolysis led to carboxylic acid **26c**.

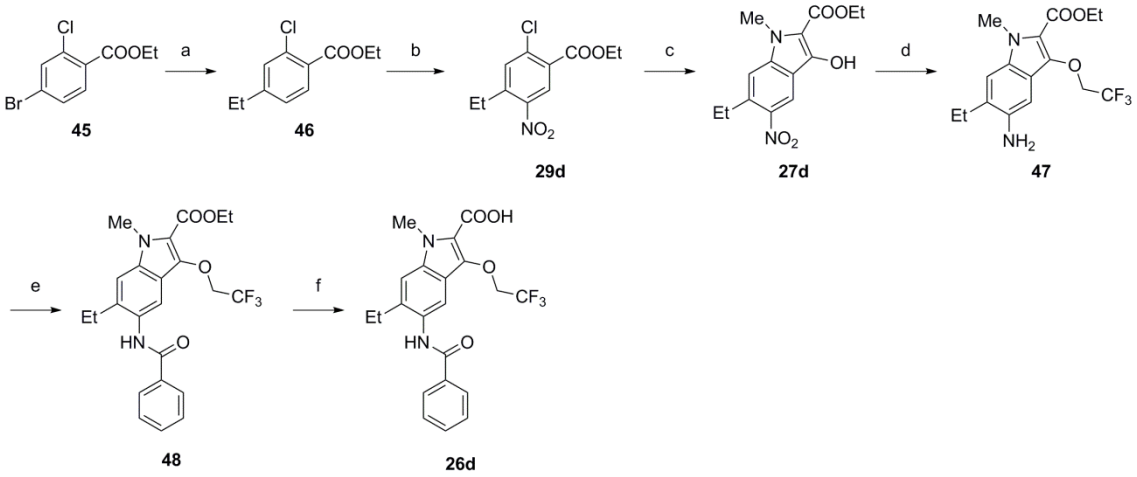
Scheme 8. Synthesis of pyrrolo[2,3-*b*]pyridine derivatives **26b** and **26c**.



Reagents and conditions : (a) Pd/C, H_2, Et_3N, EtOH, THF, 90%; (b) NBS, DMF, 86%; (c) $POCl_3$, reflux, 76%; (d) (1) $MeNHCH_2COOEt \cdot HCl$, Et_3N, THF, reflux; (2) NaOEt, EtOH, 73%; (e) CF_3CH_2OTf, Cs_2CO_3, DMF, 96%; (f) (1) acetophenone, *tert*-BuONa, $Pd(OAc)_2$, MePhos, toluene, 70 ºC; (2) NaOH, EtOH, 50 ºC, 22%; (g) (1) benzophenoneimine, $Pd_2(dba)_3$, xantphos, Cs_2CO_3, toluene, 100 ºC; (2) 2M HCl, THF, 91%; (h) BzCl, Py, THF, 94%; (i) NaOH, EtOH, 95%.

The synthesis of indole derivative **26d** is shown in Scheme 9. The key intermediate **29d** was synthesized from ethyl 4-bromo-2-chlorobenzoate **45** as follows; introduction of ethyl group by Stille coupling followed by hydrogenation, and nitration. The construction of *N*-methyl pyrrole ring proceeded directly in 41% yield. After alkylation of **27d**, subsequent hydrogenation of nitro group led to aniline **47** in 97% yield. Acylation of **47** with benzoyl chloride followed by saponification were conducted to afford the desired carboxylic acid **26d**.

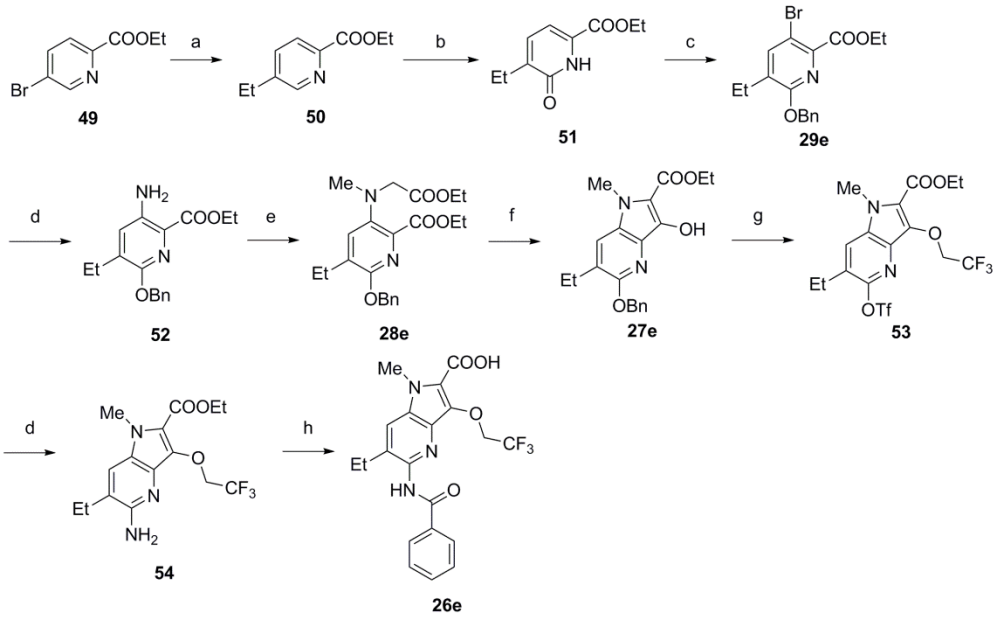
Scheme 9. Synthesis of indole derivative **26d**.



Reagents and conditions : (a) (1) (vinyl)$SnBu_3$, $Pd(PPh_3)_4$, DMF, 100 ºC, 100%; (2) Pd/C, $Ba(OH)_2$, H_2, AcOEt, 98%; (b) $NaNO_3$, H_2SO_4, 0 ºC, 70%; (c) $MeNHCH_2COOEt \cdot HCl$, Et_3N, EtOH, reflux, 41%; (d) (1) CF_3CH_2OTf, Cs_2CO_3, DMF, 100%; (2) Pd/C, H_2, EtOH, THF, 97%; (e) BzCl, Py, THF, 86%; (f) NaOH, EtOH, 95%.

The synthetic route of pyrrolo[3,2-*b*]pyridine derivative **26e** is shown in Scheme 10. The key intermediate **29e** was successfully prepared from ethyl 3-bromopicolinate **49** in 3 steps; conversion of bromo to ethyl moiety, successive introduction of hydroxyl and bromo groups. The direct addition of sarcosine ethyl ester was difficult because of its low reactivity of bromo group against nucleophilic substitution, thus stepwise introduction was conducted. The amination of **29e** with benzophenoneimine afforded **52** under Buchwald condition. Following monomethylation was achieved via Boc protection. The precursor for Dieckmann condensation **28e** was prepared by addition of ethyl bromoacetate. The cyclization of **28e** with sodium ethoxide afforded **27e** in 77% yield. After the alkylation of 3-hydroxyl group, the hydrogenation was carried out to remove benzyl group. Conversion to triflate with trifluoromethanesulfonic anhydride was successively conducted to give **53**. The similar reaction in the synthesis of pyrrolo[2,3-*b*]pyridine **26c** afforded carboxylic acid **26e** with *N*-benzoyl group at the 5-position.

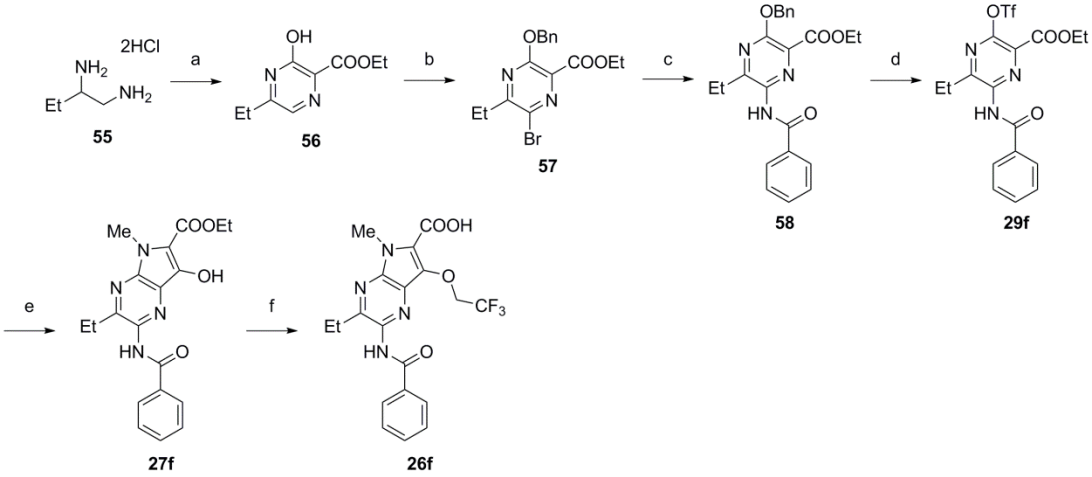
Scheme 10. Synthesis of pyrrolo[3,2-*b*]pyridine derivative **26e**.



Reagents and conditions : (a) (1) (vinyl)$SnBu_3$, $Pd(PPh_3)_4$, DMF, 100 ºC, 100%; (2) Pd/C, H_2, EtOH, 100%; (b) (1) *m*CPBA, MeCN, 83%; (2) TFAA, DMF, 96%; (c) (1) NBS, DMF, 79%; (2) BnBr, Ag_2CO_3, toluene, 40 ºC, 99%; (d) (1) benzophenoneimine, $Pd_2(dba)_3$, xantphos, Cs_2CO_3, toluene; (2) 2M HCl, THF, 98% (for **52**), 92% (for **54**); (e) (1) Boc_2O, *tert*-BuOH, 90 ºC; (2) MeI, Cs_2CO_3, DMF, 87%; (3) 4M HCl, AcOEt, 0 ºC, 87%; (4) $BrCH_2COOEt$, iPr_2NEt, DMF, 110 ºC, 79%; (f) NaOEt, EtOH, 77%; (g) (1) CF_3CH_2OTf, Cs_2CO_3, DMF, 97%; (2) Pd/C, H_2, EtOH, THF, 97%; (3) Tf_2O, pyridine, 60 ºC, 92%; (h) (1) BzCl, pyridine, THF; (2) NaOH, EtOH, 50 ºC, 88%.

The synthesis of pyrrolo[2,3-*b*]pyrazine derivative **26f** is shown in Scheme 11. Cyclization of **55** with diethyl ketomalonate gave pyrazine **56** in 25% yield. Bromination followed by benzylation of **56** provided **57**. Conversion to amino group using Buchwald condition and following acylation with benzoyl chloride afforded **58**. The benzyloxy moiety was converted to triflate by hydrogenation and triflation. Addition with sarcosine ethyl ester hydrochloride was achieved at the triflate moiety, and cyclization of pyrrole moiety was prepared in one-pot to give **27f**. The obtained alcohol was alkylated with (2,2,2-trifluoro)ethyl triflate, following hydrolysis to give carboxylic acid **26f**.

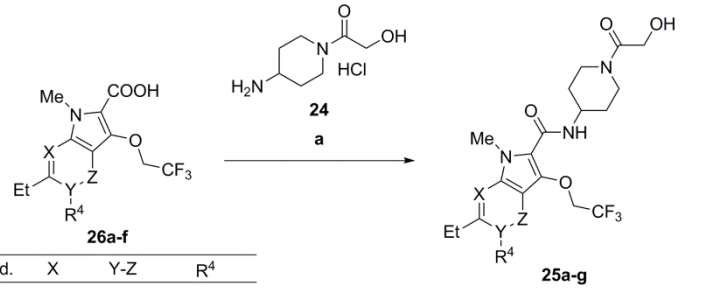
Scheme 11. Synthesis of pyrrolo[2,3-*b*]pyrazine derivative **26f**.



Reagents and conditions : (a) diethyl ketomalonate, iPr_2NEt, EtOH, reflux, 25%; (b) (1) NBS, DMF; (2) BnBr, Ag_2CO_3, acetone, 91%; (c) (1) benzophenoneimine, $Pd_2(dba)_3$, xantphos, Cs_2CO_3, toluene, 100 ºC; (2) 2M HCl, THF, 59%; (3) BzCl, pyridine, THF, 0 ºC, 93%; (d) (1) Pd/C, H_2, EtOH, 77%; (2) Tf_2O, pyridine, 0 ºC, 94%; (e) (1) $MeNHCH_2COOEtHCl$, Et_3N, EtOH; (2) NaOEt, EtOH, 80%; (f) (1) CF_3CH_2OTf, Cs_2CO_3, DMF, 0 ºC, 74%; (2) NaOH, THF, EtOH, 99%.

Finally, the amidation reaction is listed in Scheme 12. The compound **25a-f** was obtained by condensation of corresponding carboxylic acid **26a-f** with amine **24** in 46-82% yield. For the compound **25a**, the alkylation with phenacyl bromide was carried out in 14% yield.

Scheme 12. Amidation reaction in each core ring.



compd.	X	Y-Z	R^4
26a	N	N-CO	H
26b	N	C=CH	CH_2COPh
26c	N	C=CH	NHCOPh
26d	C	C=CH	NHCOPh
26e	C	C=N	NHCOPh
26f	N	C=N	NHCOPh

compd.	X	Y-Z	R^4	yield
b **25a**	N	N-CO	H	79%
25g	N	N-CO	CH_2COPh	14%
25b	N	C=CH	CH_2COPh	47%
25c	N	C=CH	NHCOPh	53%
25d	C	C=CH	NHCOPh	82%
25e	C	C=N	NHCOPh	46%
25f	N	C=N	NHCOPh	61%

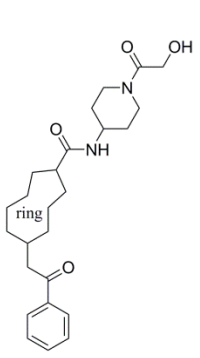
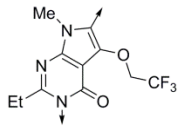
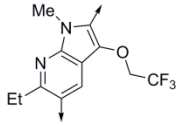
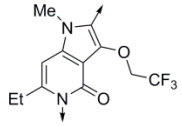
Reagents and conditions : (a) amine **24**, EDC, HOBt, Et_3N, DMF, 46-82%.; (b) $BrCH_2COPh$, LiBr, K_2CO_3, DME/DMF, 60 ºC to 80 ºC, 14%.

4.3 Results and discussion

4.3.1 Biological data for hedgehog, and SAR study

The novel two types of core ring bearing *N*-[1-(hydroxyacetyl)piperidinyl] amide, (2,2,2-trifluoro)ethoxy, phenacyl, and ethyl groups at the same positions as **22d** (TAK-441) were initially assessed (Table 9). Pyrrolo[2,3-*d*]pyrimidine **25g** maintained potent inhibitory activity compared to **22d**, but the pyrrolo[2,3-*b*]pyridine **25b** showed 10-fold drop. This result indicated it might be important for potent inhibitory activity to bear carbonyl group in the core ring. However, the compound **25b** still showed 10^{-8} order inhibition without carbonyl moiety. It was suggested that the pyrrolo[2,3-*b*]pyridine ring could be promising core as novel Hh signaling inhibitor by further modification. Next, the enhancement of inhibitory activity in compound **25b** was conducted.

Table 9. Effect of core ring with phenacyl group.

compd.	ring	Gli-luc reporter IC_{50} (nM)[a]
		
25g		6.2
25b		46
22d		4.4

[a] IC_{50} values are the mean of four measurements.

For this purpose, X-ray single crystal structure of **22d** and **25b** were obtained (Figure 17). The upper parts of these two compounds were well overlaid. On the other hand, the conformations of terminal benzenes in the 5-phenacyl group were quite different. The 5-phenacyl group of **22d** might be influenced by the steric hindrance of both 6-ethyl and 4-carbonyl groups, but lack of the 4-carbonyl group led **25b** to take the different conformation from **22d**. Based on these analyses of X-ray single crystal structures, the enhancement of inhibitory activity in **25b** could be achieved by adjusting the conformation of terminal benzene ring similar to compound **22d**. The decrease of steric hindrance by the lack of 4-carbonyl group induced flexible conformation at the

5-position, thereby an alternative regulation was necessary for enhancement of inhibitory activity as a surrogate of steric hindrance. As mentioned in the chapter I, the lack of carbonyl group in the side chain or benzene ring resulted in decrease of activity. Considering the current knowledge and necessity of regulation, the benzamide moiety would be a proper substituent at 5-position.

The benzamide was provided the planarity to whole in the 5-position, and regulated the conformation compare to phenacyl group. This structural regulation newly introduced in the 5-position as a surrogate of regulation by steric hindrance in compound **22d** brought us expectation that benzamide side chain took the appropriate conformation. Moreover, the rigid benzamide group was advantaged in entropy compared to more flexible phenacyl group because the flexibility loss in binding the target protein was small.[28] Thus, next effort was shifted to investigation of 5-benzamide derivatives.

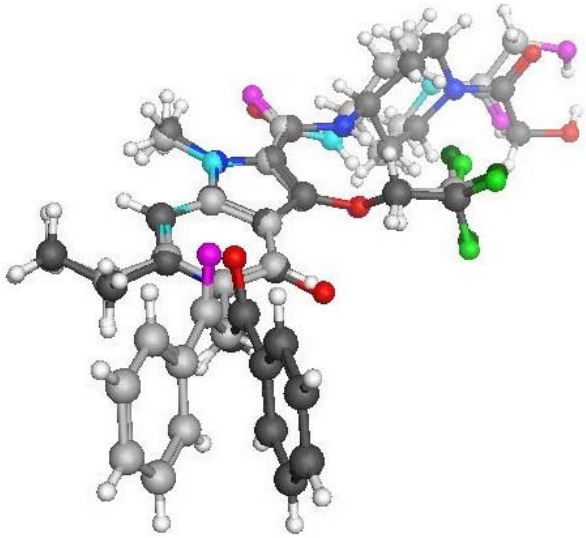


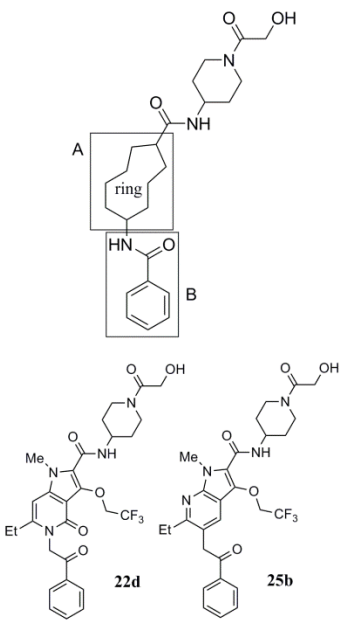
Figure 17. Overlay of **22d** (black) and **25b** (gray) in X-ray single crystal structure.[a]

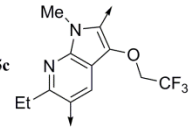
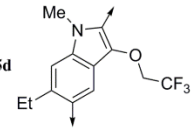
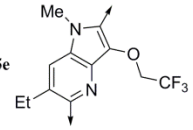
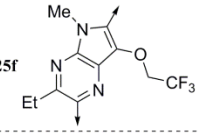
[a] The figure was described based on X-ray structural analysis data using PyMOL Software. (PyMOL version 1.4.1)

The compound **25c** with benzamide moiety instead of phenacyl group showed dramatic enhancement of Gli-luc reporter inhibitory activity compared to **25b** as expected. This finding was next adapted to other novel core skeleton without carbonyl group. Consequently, as the indole **25d**, pyrrolo[3,2-*b*]pyridine **25e** and pyrrolo[2,3-*b*]pyrazine **25f** also showed potent inhibitory activity. These results demonstrated that benzamide was quite effective side chain for these novel core skeletons without carbonyl group in the core ring (Table 10). For further consideration, the X-ray single crystal structures of **25d** and **25e** were obtained and its overlaid figure

was illustrated in Figure 18.

Table 10. The inhibitory activity and dihedral angle of each novel core ring.



compd.	ring	Gli-luc reporter IC_{50} (nM)[a]	dihedral angle between A and B (degree)[b]
25c		3.6	ND[c]
25d		2.6	90
25e		3.9	91
25f		3.1	ND[c]
25b		46	57
22d		4.4	84

[a] IC50 values are the mean of four measurements.

[b] A : least-squares surface of central core, B : least-squares surface of 5-phenacyl or benzamide group.

[c] Not detected.

They also indicated that 5-substituents of **25d** and **25e** took similar position to that of **22d** despite the absence of carbonyl moiety. This conformation was considered to be necessary for potent inhibition of Gli reporter activity as expected. The dihedral angles between central core ring and 5-substituent are also described in Table 10. To notify the relation between inhibitory activity and X-ray single structure, the potent activity requested almost vertical dihedral angle, and its value in **22d**, **25d**, and **25e** were 84, 90 and 91 degrees respectively. It was considered that amide linker formed adequate conformation with dihedral angle of about 90 degrees, and fitted well to whole molecule to the pocket of smo protein.

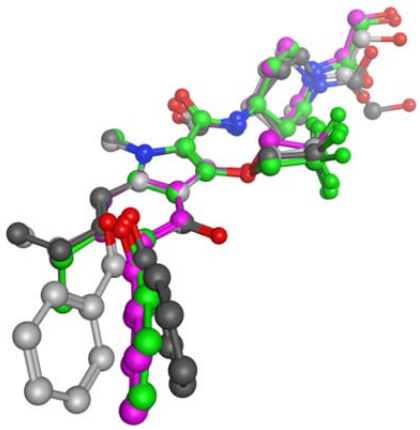


Figure 18. Overlay of **22d** (black), **25b** (gray), **25d** (pink) and **25e** (green) in X-ray single crystal structure.[a]

[a] The figure was described based on X-ray structural analysis data using PyMOL Software. (PyMOL version 1.4.1)

4.3.2 Pharmacodynamic and pharmacokinetic data of 25c-25f

The *in vivo* PD profiles of **25c-f** are shown in Table 11. PAN-04 is a human pancreatic xenograft tumor line derived from a clinical specimen established by the Central Institute for Experimental Animals (Kanagawa, Japan). This xenograft tumor significantly expressed stroma-derived Gli1 and cancer-derived Shh activity. The reduction of Gli1 *m*RNA expression levels was measured as a pharmacodynamic marker in this model. The suppression of Gli1 *m*RNA expression in novel compounds **25c-f** was weaker than **22d** despite of their potent *in vitro* inhibitory activity. It was caused by their pharmacokinetics, which was well correlated to suppression of Gli1 *m*RNA expression. Considering these results, compound **22d** (TAK-441) was well-balanced compound in the point of good PK profile while potent inhibitory activity.

Table 11. The results of in vivo PD study and corresponding PK data.

compd.	Gli-luc reporter IC_{50} (nM)[a]	in vivo PD Gli mRNA (%ctrl)[b]	AUC (μgh/mL)[c]
25c	3.6	24	10.832
25d	2.6	50	1.272
25e	3.9	87	0.984
25f	3.1	57	0.925
22d	4.4	4	28.346

[a] IC50 values are the mean of four measurements.

[b] Gli1 *m*RNA expression at 25 mg/kg BID, 24 h post-dose. The value indicates Gli1 *m*RNA expression levels compared to controls. Test compounds were dosed at 25 mg/kg, BID.

[c] Cassette dosing at 10 mg/kg, po in mice. AUC: area under the plasma concentration versus time curve from 0 to 8 h.

4.4. Conclusion

The new Hh signaling inhibitors with novel core rings were explored by the modification of 5-substituent including core ring, and pyrrolo[2,3-*b*]pyridine derivative **25b** with 10^{-8} order activity in Gli reporter assay was obtained. To enhance *in vitro* activity, the preferable conformation was considered to show potent inhibiton of Gli reporter activity with X-ray single crystal structure, and compound **25c** with benzamide moiety at the 5-position was discovered. The benzamide substituent worked well for the potent inhibitory activity in the core ring without 4-carbonyl moiety, including the indole **25d**, pyrrolo[3,2-*b*]pyridine **25e** and pyrrolo[2,3-*b*]pyrazine **25f**. In these compounds, vertical dihedral angle between 5-substituent and core ring might be important for potent inhibitory activity from X-ray crystal structure. The suppression of Gli1 *m*RNA expression in these novel compounds **25c-f** was unfortunately inefficient compare to that of **22d** in the result of pharmacokinetics. Based on the results of potent inhibition of Gli reporter activity including structural characteristics and good PK profiles, **22d** (TAK-441) is the best candidate as Hh signaling inhibitor.

Summary

The author reported the synthetic studies on novel hedgehog signaling inhibitor for cancer therapy. The knowledge which was obtained during the discovery of clinical candidate TAK-441 from hit compound are shown below.

In chapter I, the optimization of hit compound **1a** identified from the HTS campaign led to the discovery of *N*-methylpyrrolo[3,2-*c*]quinoline-4-one derivative **12b** bearing *N*-[1-(hydroxyacetyl)piperidinyl] amide at 2-position. **12b** suppresses Gli1 *m*RNA expression reflecting with the potent Gli reporter inhibiton activity and Smo binding inhibition. **12b** also shows the *in vivo* efficacy in mouse medullobrastoma allograft model at 6.25 mg/kg, twice daily. These findings suggested that the Hh signaling inhibitor could be a therapeutic reagent of cancer.

In chapter II, the optimization of **12b** for the improvement of oral absorption at higher dose was reported. The author hypothesized that the oral absorption could be attributed to low solubility, and synthesized the compounds based on reduction of planarity of the compound. As a result of modification, solubility of 6-ethyl pyrroro[3,2-*c*]pyridine-4-one derivatives was improved while retaining potent inhibition of Gli reporter activity. Further optimization at 3-position for the adjustment of lipophilicity led to the discovery of **22d**. **22d** also exhibited strong efficacy in mouse medullobrastoma allograft model at 25 mg/kg, and good pharmacokinetics in rats and dogs. On the basis of these findings, **22d** could be an orally active candidate for the treatment of cancer.

In chapter III, the modification of **22d** for clarification of the exact role at lower part including core skeleton was conducted. The modification based on X-ray single crystal structure analysis in novel core skeletons was conducted to show potent inhibition of Gli reporter activity. It was found that the vertical dihedral angles between core ring and the substituent at 5-position might be essential for the potency to fit to Smo protein tightly. Eventually, pyrrolo[2,3-*d*]pyrimidine **25c**, indole **25d**, pyrrolo[3,2-*b*]pyridine **25e** and pyrrolo[2,3-*b*]pyrazine **25f** were discovered as novel Hh signaling inhibitors with potency comparable to **22d**. Among **22d** and **25c-f**, **22d** showed the strongest suppression of Gli1 *m*RNA expression derived from good PK. On the basis of these findings, **22d** was adequate to select as a clinical candidate of Hh signaling inhibitor in the point of its structural characteristics and good PK profiles.

As mentioned above, the discovery of TAK-441 as a clinical candidate of Hh signaling inhibitor was successfully achieved. The author believes that the knowledge which has been obtained through the discovery of novel Hh signaling inhibitor contributes to the development of pharmaceutical science.

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Experimental Section

General Methods

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 a Varian Gemini-300 (300 MHz) or Bruker DPX300 (300 MHz) instrument or Bruker Avance 300 (300 MHz). Chemical shifts are reported as δ values (ppm) downfield from internal tetramethylsilane of the indicated organic solution. Peak multiplicities are expressed using the following abbreviations: 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, and the results were within 0.4% of theoretical values. LC-MS spectra were obtained on a Shimadzu Corporation LC-MS system (LCMS-2010A). Column chromatography was carried out on silica gel columns (Kieselgel 60, 63-200 mesh, Merck, Darmstadt, Germany) or basic silica gel columns (Chromatorex® NH-DM1020, 100-200 mesh, Fuji Silysia Chemical Ltd., Kasugai, Japan) or Purif-Pack® columns (SI 60 μM or NH 60 μM, Fuji Silysia). Reaction progress was determined by thin layer chromatography (TLC) analysis on silica gel $60F_{254}$plate (Merck) or NH TLC plates (Fuji Silysia Chemical Ltd.). X-ray structural analyses were determined using MOE version 2010.10; Chemical Computing Group, Inc., Montreal, Quebec, Canada. ClogP values were calculated by Daylight Software ClogP, version 4.82, Daylight Chemical Information Systems, Inc., Aliso Viejo, CA. The purities of compounds submitted for biological evaluation were ≥95% as determined by LC/MS and elemental analysis. Yields are not optimized.

Experimental section of chapter I

Ethyl 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylate (3)

A 20% solution of NaOEt in EtOH (274 g) was added to a solution of methyl anthranilate (121 g, 0.800 mol) and diethyl malonate (128 g, 0.800 mol) in EtOH (900 mL), and the mixture was stirred at room temperature for 30 min. The mixture was heated at 140 °C under stirring for 14 h removing EtOH by a Dean-Stark trap. After cooling, the residual solid was washed with Et_2O and dissolved in water. After removal of insoluble solid by filtration, the filtrate was acidified with 5M HCl, and the precipitated solid was collected by filtration. The solid was washed with water and dried *in vacuo* to give the title compound (161 g, 86%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6) δ 1.31 (3H, t, J = 7.1 Hz), 4.34 (2H, q, J = 7.1 Hz), 7.19-7.29 (2H, m), 7.63 (1H, dt, J = 1.2, 7.8 Hz), 7.94 (1H, d, J = 8.1 Hz), 11.51 (1H, br s), 13.40 (1H, br s).

Ethyl 2,4-dichloroquinoline-3-carboxylate (4)

A mixture of **3** (75.0 g, 0.320 mol) and $POCl_3$ (200 mL) was stirred at 110 °C for 6 h. After cooling, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in a small amount of AcOEt and the mixture was poured into ice water followed by extraction with AcOEt. The extract was washed with 1M NaOH, water, and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by basic silica gel column chromatography (AcOEt/hexane = 1/5) to give the title compound (68.0 g, 79%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.47 (3H, t, J = 7.2 Hz), 4.54 (2H, q, J = 7.2 Hz), 7.71 (1H, t, J = 7.5 Hz), 7.85 (1H, t, J = 7.5 Hz), 8.06 (1H, d, J = 8.2 Hz), 8.24 (1H, d, J = 8.2 Hz).

Ethyl 4-chloro-2-oxo-1,2-dihydroquinoline-3-carboxylate (5)

A mixture of **4** (68.0 g, 0.250 mol) and AcONa (21.7 g, 0.260 mol) in AcOH (200 mL) was stirred at 120 °C for 20 h. After the reaction mixture was added to water, the precipitated solid was collected and washed with water to give the title compound (58.8 g, 94%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.46 (3H, t, J = 7.2 Hz), 4.53 (2H, q, J = 7.2 Hz), 7.34 (1H, t, J = 7.8 Hz), 7.43 (1H, d, J = 8.1 Hz), 7.63 (1H, t, J = 7.8 Hz), 8.00 (1H, d, J = 8.1 Hz), 12.41 (1H, br s).

Ethyl 4-chloro-2-oxo-1-(2-oxo-2-phenylethyl)-1,2-dihydroquinoline-3-carboxylate (6a)

NaH (60% in oil, 1.70 g, 41.7 mmol) was added to a solution of compound **5**

(10.0 g, 39.7 mmol) in DMF (160 mL) under ice-cooling, and the mixture was stirred for 15 min. Phenacyl bromide (8.70 g, 43.7 mmol) was added under ice-cooling, and the resulting mixture was stirred for 1 h. The reaction mixture was added to water, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 1/2 to 1/1) to give the title compound (10.5 g, 71%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.42 (3H, t, J = 7.2 Hz), 4.47 (2H, q, J = 7.2 Hz), 5.80 (2H, s), 7.02 (1H, d, J = 8.1 Hz), 7.34 (1H, t, J = 7.8 Hz), 7.53-7.60 (3H, m), 7.65-7.70 (1H, m), 8.06-8.13 (3H, m).

Ethyl 4-chloro-1-(4-methoxybenzyl)-2-oxo-1,2-dihydroquinoline-3-carboxylate (6b)

Similar to the preparation of **6a**, the title compound (10.8 g, 79%) was obtained as a white powder from **5** (10.0 g, 39.8 mmol). ^{1}H NMR (DMSO-d_6) δ 1.34 (3H, t, J = 7.1 Hz), 3.70 (3H, s), 4.40 (2H, q, J = 7.1 Hz), 5.48 (2H, s), 6.89 (2H, d, J = 8.7 Hz), 7.19 (2H, d, J = 8.7 Hz), 7.42 (1H, t, J = 7.8 Hz), 7.61 (1H, d, J = 8.7 Hz), 7.69-7.75 (1H, m), 8.04 (1H, dd, J = 7.8, 1.2 Hz).

Ethyl 3-hydroxy-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydrothieno[3,2-*c*]quinoline-2-carboxylate (7a)

A mixture of ethyl thioglycolate (6.10 g, 50.5 mmol), 20% solution of NaOEt in EtOH (17.2 g, 50.5 mmol), and EtOH (50 mL) was stirred at room temperature for 5 min. Compound **6a** (9.30 g, 25.3 mmol) was added and the mixture was stirred at room temperature for 18 h. The mixture was acidified with 2M HCl and the resulting mixture was stirred for 30 min. The precipitated solid was collected by filtration, washed with water and Et_2O to give the title compound (10.0 g, 96%) as a white powder. ^{1}H NMR (DMSO-d_6) δ 1.32 (3H, t, J = 7.0 Hz), 4.25-4.35 (2H, m), 6.01 (2H, s), 7.40 (1H, t, J = 7.2 Hz), 7.55-7.67 (4H, m), 7.77 (1H, t, J = 7.5 Hz), 8.00-8.07 (1H, m), 8.07-8.19 (2H, m), 10.57 (1H, br s).

Ethyl 3-hydroxy-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydrofuro[3,2-*c*]quinoline-2-carboxylate (7b)

Similar to the preparation of **7a**, the title compound (1.10 g, 69%) was obtained as a white powder from **6a** (1.50 g, 4.06 mmol). ^{1}H NMR (DMSO-d_6) δ 1.34 (3H, t, J = 7.1 Hz), 4.35 (2H, q, J = 7.1 Hz), 5.96 (2H, s), 7.39 (1H, t, J = 7.7 Hz), 7.51 (1H, d, J = 8.4 Hz), 7.60-7.67 (3H, m), 7.74-7.79 (1H, m), 8.05 (1H, dd, J = 7.7, 1.4 Hz), 8.15-8.18 (2H, m), 10.55 (1H, br s).

Ethyl 3-hydroxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxylate (7c)

A mixture of **6a** (52.0 g, 0.14 mol), sarcosine ethyl ester hydrochloride (25.6 g, 0.16 mol), Et_3N (28.5 g, 0.28 mol), and EtOH (500 mL) was stirred at 85 °C for 12 h. After cooling, the mixture was diluted with water (2200 mL) and extracted with AcOEt. The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was crystallized with hexane/AcOEt to give the ethyl 4-[(2-ethoxy-2-oxoethyl)(methyl)amino]-2-oxo-1-(2-oxo-2-phenylethyl)-1,2-dihydroquinoline-3-carboxylate (51.5 g) as pale yellow crystals. A mixture of the compound (51.5 g) obtained above, 20% solution of NaOEt in EtOH (46.6 g, 0.14 mol), and EtOH (1000 mL) was stirred at 50 °C for 18 h. The mixture was diluted with water (500 mL) then acidified with 1M HCl (140 mL), the resulting mixture was stirred for 30 min. The precipitated solid was collected by filtration, washed with water and EtOH successively, and dried *in vacuo* to give the title compound (37.5 g, 66%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.33 (3H, t, J = 7.2 Hz), 4.30-4.38 (5H, m), 5.93 (2H, s), 7.30-7.40 (2H, m), 7.48-7.53 (1H, m), 7.61-7.66 (2H, m), 7.73-7.79 (1H, m), 8.15-8.18 (2H, m), 8.34-8.37 (1H, m), 9.00 (1H, s). Anal. calcd for $C_{23}H_{20}N_2O_5$: C, 68.31; H, 4.98; N, 6.93. Found: C, 68.41; H, 5.08; N, 7.11.

Ethyl 3-hydroxy-5-(4-methoxybenzyl)-1-methyl-4-oxo-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxylate (7d)

A mixture of **6b** (16.5 g, 44.47 mmol), sarcosine ethyl ester hydrochloride (10.2 g, 66.5 mmol), Et_3N (27.5 mL, 0.197 mol), and EtOH (170 mL) was stirred at 85 °C for 18 h. A 20% solution of NaOEt in EtOH (75.3 g, 0.221 mol) was added, and the mixture was stirred at 60 °C for 1 h. The mixture was concentrated *in vacuo*, and the residue was suspended with water (100 mL). The suspension was acidified with 5M HCl at 0 °C, and the mixture was stirred at room temperature for 1.5 h. The precipitate was collected by filtration, washed with water and EtOH to give the title compound (14.6 g, 81%) as a brown solid. ^{1}H NMR (DMSO-d_6) δ 1.33 (3H, t, J = 7.0 Hz), 3.69 (3H, s), 4.28 (3H, s), 4.34 (2H, q, J = 7.0 Hz), 5.48 (2H, br s), 6.80-6.93 (2H, m), 7.16 (2H, d, J = 8.7 Hz), 7.23-7.35 (1H, m), 7.43-7.52 (2H, m), 8.33 (1H, d, J = 8.1 Hz), 9.10 (1H, s).

Ethyl 3-methoxy-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydrothieno[3,2-*c*]quinoline-2-carboxylate (8a)

DBU (4.30 g, 18.3 mmol) was added at room temperature to a stirred solution of compound **7a** (7.00 g, 17.1 mmol) in DMF (150 mL). After stirring for 10 min, MeI

(4.00 g, 28.0 mmol) was added. The mixture was stirred at room temperature for 4 h, and concentrated *in vacuo*. The residue was diluted with water, and extracted with AcOEt/THF. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residual solid was collected and washed with AcOEt/Et_2O to give the title compound (5.70 g, 79%) as a pale green powder. ^{1}H NMR (DMSO-d_6) δ 1.34 (3H, t, *J* = 7.1 Hz), 3.95 (3H, s), 4.34 (2H, q, *J* = 7.1 Hz), 5.98 (2H, s), 7.37 (1H, t, *J* = 7.5 Hz), 7.49 (1H, d, *J* = 8.7 Hz), 7.57-7.67 (3H, m), 7.76 (1H, t, *J* = 7.5 Hz), 8.06 (1H, d, *J* = 7.8 Hz), 8.16-8.19 (2H, m).

The following compounds **8b-e** were prepared in a manner similar to that described for **8a**.

Ethyl 3-methoxy-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydrofuro[3,2-*c*]quinoline-2-carboxylate (8b)

Yield 23%, white powder. ^{1}H NMR (DMSO-d_6) δ 1.35 (3H, t, *J* = 7.1 Hz), 4.14 (3H, s), 4.37 (2H, q, *J* = 7.1 Hz), 5.99 (2H, s), 7.39-7.45 (1H, m), 7.55 (1H, d, *J* = 8.7 Hz), 7.62-7.68 (3H, m), 7.74-7.80 (1H, m), 8.09 (1H, dd, *J* = 7.8, 1.5 Hz), 8.16-8.18 (2H, m). Anal. calcd for $C_{23}H_{19}NO_6$: C, 68.14; H, 4.72; N, 3.46. Found: C, 67.91; H, 4.78; N, 3.42.

Ethyl 3-methoxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxylate (8c)

Yield 55%, white powder. ^{1}H NMR (DMSO-d_6) δ 1.35 (3H, t, *J* = 7.1 Hz), 3.89 (3H, s), 4.29-4.37 (5H, m), 5.96 (2H, s), 7.30-7.40 (2H, m), 7.48-7.53 (1H, m), 7.61-7.66 (2H, m), 7.73-7.78 (1H, m), 8.15-8.18 (2H, m), 8.38 (1H, dd, *J* = 8.3, 1.4 Hz). Anal. calcd for $C_{24}H_{22}N_2O_5$: C, 68.89; H, 5.30; N, 6.69. Found: C, 68.52; H, 5.26; N, 6.61.

Ethyl 3-ethoxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxylate (8d)

Yield 100%, white powder. ^{1}H NMR (DMSO-d_6) δ 1.27-1.37 (6H, m), 4.16 (2H, q, *J* = 6.9 Hz), 4.29-4.36 (5H, m), 5.95 (2H, s), 7.27-7.39 (2H, m), 7.50 (1H, t, *J* = 8.0 Hz), 7.63 (2H, t, *J* = 7.3 Hz), 7.76 (1H, t, *J* = 7.4 Hz), 8.17 (2H, d, *J* = 7.3 Hz), 8.38 (1H, d, *J* = 8.1 Hz).

Ethyl 3-methoxy-5-(4-methoxybenzyl)-1-methyl-4-oxo-4,5-dihydro-1*H*-pyrrolo [3,2-*c*]quinoline-2-carboxylate (8e)

Yield 61%, pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.35 (3H, t, J = 7.1 Hz), 3.69 (3H, s), 3.94 (3H, s), 4.26 (3H, s), 4.34 (2H, q, J = 7.1 Hz), 5.51 (2H, br s), 6.86 (2H, d, J = 8.7 Hz), 7.15 (2H, d, J = 8.7 Hz), 7.25-7.31 (1H, m), 7.47 (2H, d, J = 3.6 Hz), 8.34 (1H, d, J = 8.1 Hz).

3-Methoxy-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydrothieno[3,2-*c*]quinoline-2-carboxylic acid (9a)

A solution of **8a** (5.70 g, 13.5 mmol) and 2M NaOH (60 mL) in THF (200 mL)/EtOH (100 mL) was stirred at room temperature for 18 h. The reaction mixture was neutralized with 2M HCl, and the resulting solid was collected by filtration. The solid was washed with water and Et_2O to give the title compound (4.20 g, 79%) as a pale pink powder. ^{1}H NMR (DMSO-d_6) δ 3.93 (3H, s), 5.98 (2H, s), 7.35 (1H, t, J = 7.8 Hz), 7.48 (1H, d, J = 8.7 Hz), 7.56-7.67 (3H, m), 7.77 (1H, t, J = 7.2 Hz), 8.04 (1H, dd, J = 8.1, 1.2 Hz), 8.16-8.19 (2H, m), 13.43 (1H, s).

The following compounds **9b-e** were prepared in a manner similar to that described for **9a**.

3-Methoxy-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydrofuro[3,2-*c*]quinoline-2-carboxylic acid (9b)

Yield 72%, white powder. ^{1}H NMR (DMSO-d_6) δ 4.12 (3H, s), 5.99 (2H, s), 7.42 (1H, t, J = 7.6 Hz), 7.54 (1H, d, J = 7.8 Hz), 7.62-7.67 (3H, m), 7.77 (1H, t, J = 7.8 Hz), 8.07 (1H, dd, J = 7.6, 1.5 Hz), 8.16-8.18 (2H, m), 13.35 (1H, br s).

3-Methoxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*] quinoline-2-carboxylic acid (9c)

Yield 91%, white powder. ^{1}H NMR (DMSO-d_6) δ 3.88 (3H, s), 4.31 (3H, s), 5.95 (2H, s), 7.29-7.39 (2H, m), 7.46-7.52 (1H, m), 7.61-7.66 (2H, m), 7.73-7.78 (1H, m), 8.16-8.18 (2H, m), 8.38 (1H, dd, J = 8.3, 1.1 Hz), 12.92 (1H, s).

3-Ethoxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*] quinoline-2-carboxylic acid (9d)

Yield 89%, white powder. ^{1}H NMR (DMSO-d_6) δ 1.27 (3H, t, J = 7.0 Hz), 4.16 (2H, q, J = 7.0 Hz), 4.31 (3H, s), 5.95 (2H, s), 7.27-7.39 (2H, m), 7.49 (1H, t, J = 7.4

Hz), 7.63 (2H, t, *J* = 7.4 Hz), 7.76 (1H, t, *J* = 7.4 Hz), 8.15-8.18 (2H, m), 8.38 (1H, d, *J* = 7.2 Hz), 12.83 (1H, s). Anal. calcd for $C_{23}H_{20}N_2O_5 \bullet H_2O$: C, 65.39; H, 5.25; N, 6.63. Found: C, 65.65; H, 4.95; N, 6.73.

3-Methoxy-5-(4-methoxybenzyl)-1-methyl-4-oxo-4,5-dihydro-1*H*-pyrrolo[3,2-*c*] quinoline-2-carboxylic acid (9e)

Yield 96%, pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 3.70 (3H, s), 3.94 (3H, s), 4.28 (3H, s), 5.51 (2H, br s), 6.86 (2H, d, *J* = 8.7 Hz), 7.15 (2H, d, *J* = 8.7 Hz), 7.25-7.30 (1H, m), 7.46 (2H, d, *J* = 3.9 Hz), 8.34 (1H, d, *J* = 8.4 Hz), 12.90 (1H, br s).

3-Methoxy-4-oxo-5-(2-oxo-2-phenylethyl)-*N*-(2-pyrrolidin-1-ylethyl)-4,5-dihydro-thieno[3,2-*c*]quinoline-2-carboxamide (1a)

EDC (94 mg, 0.49 mmol) was added to a mixture of **9a** (130 mg, 0.33 mmol), 1-(2-aminoethyl)-pyrrolidine (56 mg, 0.49 mmol), and HOBt (67 mg, 0.49 mmol) in DMF (4 mL), and the mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with saturated $NaHCO_3$ aq. and extracted twice with AcOEt. The combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by basic silica gel column chromatography (hexane/AcOEt = 1/1 to 0/1), and crystallized from hexane/AcOEt to give the title compound (100 mg, 62%) as white crystals; mp. 190 °C. ^{1}H NMR (DMSO-d_6) δ 1.72 (4H, br s), 2.49 (4H, m), 2.64 (2H, t, *J* = 6.3 Hz), 3.44 (2H, t, *J* = 5.9 Hz), 4.01 (3H, s), 6.00 (2H, s), 7.35 (1H, t, *J* = 7.8 Hz), 7.49 (1H, d, *J* = 8.1 Hz), 7.55-7.67 (3H, m), 7.76 (1H, t, *J* = 7.5 Hz), 8.05 (1H, dd, *J* = 8.1, 1.5 Hz), 8.16-8.19 (3H, m). Anal. calcd for $C_{27}H_{27}N_3O_4S$: C, 66.24; H, 5.56; N, 8.58. Found: C, 66.08; H, 5.50; N, 8.58.

The following compounds **1b-h** were prepared in a manner similar to that described for **1a**.

3-Methoxy-4-oxo-5-(2-oxo-2-phenylethyl)-*N*-(2-pyrrolidin-1-ylethyl)-4,5-dihydro-furo[3,2-*c*]quinoline-2-carboxamide (1b)

Yield 66%, white crystals; mp 171 °C (recrystallized from AcOEt). ^{1}H NMR (DMSO-d_6) δ 1.69-1.73 (4H, m), 2.49-2.51 (4H, m), 2.61 (2H, t, *J* = 6.6 Hz), 3.40-3.46 (2H, m), 4.15 (3H, s), 6.00 (2H, s), 7.43 (1H, t, *J* = 8.1 Hz), 7.54 (1H, d, *J* = 8.4 Hz), 7.60-7.67 (3H, m), 7.74-7.79 (1H, m), 8.06 (1H, t, *J* = 8.6 Hz), 8.15-8.19 (3H, m). LC-MS: m/z = 474 (MH^+). Anal. calcd for $C_{27}H_{27}N_3O_5$: C, 68.48; H, 5.75; N, 8.87.

Found: C, 68.32; H, 5.68; N, 8.97.

3-Methoxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-*N*-(2-pyrrolidin-1-ylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxamide (1c)

Yield 78%, white crystals; mp 204 °C (recrystallized from AcOEt). ^{1}H NMR (DMSO-d_6) δ 1.65-1.75 (4H, m), 2.48-2.55 (4H, m), 2.62 (2H, t, J = 6.2 Hz), 3.39-3.45 (2H, m), 3.97 (3H, s), 4.37 (3H, s), 5.97 (2H, s), 7.29-7.39 (2H, m), 7.45-7.50 (1H, m), 7.61-7.66 (2H, m), 7.73-7.78 (1H, m), 8.16-8.18 (3H, m), 8.39 (1H, dd, J = 8.1, 1.2 Hz). LC-MS: m/z = 487 (MH^+). Anal. calcd for $C_{28}H_{30}N_4O_4$: C, 69.12; H, 6.21; N, 11.51. Found: C, 69.16; H, 6.20; N, 11.59.

3-Ethoxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-*N*-(2-pyrrolidin-1-ylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxamide (1d)

Yield 18%, white crystals; mp 217 °C (recrystallized from AcOEt/THF). ^{1}H NMR (DMSO-d_6) δ 1.32 (3H, t, J = 7.0 Hz), 1.65-1.75 (4H, m), 2.40-2.55 (4H, m), 2.62 (2H, t, J = 6.0 Hz), 3.05-3.47 (2H, m), 4.29 (2H, q, J = 7.0 Hz), 4.39 (3H, s), 5.96 (2H, s), 7.29-7.39 (2H, m), 7.48 (1H, t, J = 7.8 Hz), 7.63 (2H, t, J = 7.7 Hz), 7.76 (1H, t, J = 7.4 Hz), 8.12-8.19 (3H, m), 8.38 (1H, d, J = 8.4 Hz). LC-MS: m/z = 501 (MH^+). Anal. calcd for $C_{29}H_{32}N_4O_4$: C, 69.58; H, 6.44; N, 11.19. Found: C, 69.83; H, 6.26; N, 10.63.

3-Methoxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-*N*-(2-piperidin-1-ylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxamide (1e)

Yield 90%, white crystals; mp 230 °C (recrystallized from THF/EtOH). ^{1}H NMR (DMSO-d_6) δ 1.30-1.65 (6H, m), 2.30-2.60 (6H, m), 3.40-3.45 (2H, m), 4.01 (3H, s), 4.38 (3H, s), 5.97 (2H, s), 7.29-7.39 (2H, m), 7.45-7.51 (1H, m), 7.63 (2H, t, J = 7.5 Hz), 7.73-7.78 (1H, m), 8.11-8.19 (3H, m), 8.38 (1H, dd, J = 8.4, 1.2 Hz). LC-MS: m/z = 501 (MH^+). Anal. calcd for $C_{29}H_{32}N_4O_4 \bullet 0.2H_2O$: C, 69.08; H, 6.48; N, 11.11. Found: C, 69.06; H, 6.45; N, 11.09.

3-Methoxy-1-methyl-*N*-(2-morpholin-4-ylethyl)-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxamide (1f)

Yield 82%, white crystals; mp 228 °C (recrystallized from AcOEt/THF). ^{1}H NMR (DMSO-d_6) δ 2.40-2.55 (6H, m), 3.40-3.50 (2H, m), 3.61 (4H, t, J = 4.2 Hz), 4.01 (3H, s), 4.37 (3H, s), 5.97 (2H, s), 7.29-7.40 (2H, m), 7.48 (1H, t, J = 7.7 Hz), 7.64 (2H, t, J = 7.4 Hz), 7.76 (1H, t, J = 7.4 Hz), 8.12-8.19 (3H, m), 8.38 (1H, d, J = 8.4 Hz). LC-MS: m/z = 503 (MH^+). Anal. calcd for $C_{28}H_{30}N_4O_5$: C, 66.92; H, 6.02; N, 11.15.

Found: C, 67.04; H, 5.93; N, 11.28.

3-Methoxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-*N*-piperidin-4-yl-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxamide (1g)

Yield 85%, white crystals; mp 206 °C (recrystallized from AcOEt/EtOH). ^{1}H NMR (DMSO-d_6) δ 1.34-1.46 (2H, m), 1.78-1.84 (2H, m), 1.90-2.20 (1H, m), 2.50-2.60 (2H, m), 2.85-3.00 (2H, m), 3.80-3.95 (1H, m), 3.98 (3H, s), 4.32 (3H, s), 5.97 (2H, s), 7.29-7.39 (2H, m), 7.45-7.50 (1H, m), 7.63 (2H, t, J = 7.5 Hz), 7.76 (1H, t, J = 7.5 Hz), 7.86 (1H, d, J = 7.8 Hz), 8.16-8.18 (2H, m), 8.37 (1H, dd, J = 8.4, 1.2 Hz). LC-MS: m/z = 473 (MH^+). Anal. calcd for $C_{27}H_{28}N_4O_4$•1.0H_2O: C, 66.11; H, 6.16; N, 11.42. Found: C, 65.82; H, 6.05; N, 11.32.

***tert*-Butyl 4-({[3-methoxy-5-(4-methoxybenzyl)-1-methyl-4-oxo-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]quinolin-2-yl]carbonyl}amino)piperidine-1-carboxylate (1h)**

Yield 93%, white powder. ^{1}H NMR (DMSO-d_6) δ 1.33-1.58 (11H, m), 1.76-1.94 (2H, m), 2.83-3.09 (2H, m), 3.69 (3H, s), 3.79-3.95 (2H, m), 3.95-4.11 (4H, m), 4.27 (3H, s), 5.52 (2H, br s), 6.81-6.91 (2H, m), 7.14 (2H, d, J = 8.7 Hz), 7.22-7.32 (1H, m), 7.38-7.53 (2H, m), 7.97 (1H, d, J = 7.9 Hz), 8.33 (1H, d, J = 7.9 Hz). LC-MS: m/z = 575 (MH^+).

3-Methoxy-5-(4-methoxybenzyl)-1-methyl-4-oxo-*N*-piperidin-4-yl-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxamide hydrochloride (1i)

4M HCl in AcOEt solution (5 mL) was added to a solution of **1h** (500 mg, 0.87 mmol) in AcOEt (15 mL), and the mixture was stirred at room temperature for 4 h. The precipitate was collected by filtration, washed with diethyl ether, and dried *in vacuo* to give the title compound (440 mg, 99%) as a pale pink powder. ^{1}H NMR (DMSO-d_6) δ 1.78 (2H, q, J = 10.3 Hz), 2.05 (2H, d, J = 10.8 Hz), 3.06 (2H, t, J = 10.7 Hz), 3.26-3.33 (2H, m), 3.63 (3H, s), 4.02 (3H, s), 4.09-4.13 (1H, m), 4.24 (3H, s), 5.40-5.60 (2H, m), 6.86 (2H, d, J = 8.7 Hz), 7.14 (2H, d, J = 8.7 Hz), 7.25-7.30 (1H, m), 7.42-7.49 (2H, m), 8.15 (1H, d, J = 7.5 Hz), 8.33 (1H, d, J = 7.8 Hz), 8.60-8.90 (2H, m). LC-MS: m/z = 475 (MH^+-HCl).

***N*-[1-(2-Hydroxyethyl)piperidin-4-yl]-3-methoxy-5-(4-methoxybenzyl)-1-methyl-4-oxo-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxamide (10)**

A mixture of **1i** (9.39 g, 18.4 mmol), 2-bromoethanol (3.25 mL, 45.6 mmol) and K_2CO_3 (10.2 g, 73.6 mmol) in DMF (250 mL) was stirred at 80 °C for 16 h. After

cooling, the reaction mixture was concentrated *in vacuo* and the residue was partitioned between saturated $NaHCO_3$ aq. and AcOEt. The aqueous layer was separated and extracted with AcOEt/THF. The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residual precipitate was collected by filtration, and washed with hexane/AcOEt to give the title compound (7.75 g, 81%) as white powder. ^{1}H NMR (DMSO-d_6) δ 1.43-1.71 (2H, m), 1.77-1.94 (2H, m), 2.08-2.26 (2H, m), 2.40 (2H, t, J = 6.3 Hz), 2.70-2.86 (2H, m), 3.50 (2H, q, J = 6.2 Hz), 3.69 (3H, s), 3.73-3.94 (1H, m), 4.04 (3H, s), 4.29 (3H, s), 4.37 (1H, t, J = 5.4 Hz), 5.52 (2H, br s), 6.78-6.91 (2H, m), 7.14 (2H, d, J = 8.7 Hz), 7.22-7.33 (1H, m), 7.38-7.53 (2H, m), 7.90 (1H, d, J = 7.7 Hz), 8.33 (1H, d, J = 7.9 Hz). LC-MS: m/z = 519 (MH^+).

2-(4-{[(3-Methoxy-1-methyl-4-oxo-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]quinolin-2-yl) carbonyl]amino}piperidin-1-yl)ethyl acetate (11)

A mixture of **10** (498 mg, 0.959 mmol), anisole (0.4 mL, 3.68 mmol), TfOH (1 mL), and TFA (4 mL) was stirred at 80 °C for 1 h. After the mixture was concentrated *in vacuo*, the residue was quenched with aqueous $NaHCO_3$ solution and diluted with AcOEt/THF = 1/1 solution (40 mL). The organic layer was separated and the aqueous layer was extracted with AcOEt/THF = 1/1 solution. NaCl (15 g) was added to the aqueous layer and was extracted with THF. The combined organic layer was dried over $MgSO_4$, and concentrated *in vacuo*. Et_3N (0.4 mL, 2.87 mmol) and acetyl chloride (164 μL, 2.31 mmol) were added at 0 °C to a suspension of the residue in THF (10 mL). The mixture was stirred at 0 °C for 2 h, Et_3N (0.4 mL, 2.87 mmol) and acetyl chloride (164 μL, 2.31 mmol) were further added. After stirring at 0 °C for 2 h, the mixture was quenched with aqueous $NaHCO_3$ solution (30 mL) and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1/2 to AcOEt/MeOH = 4/1) to give the title compound (221 mg, 52%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6) δ 1.46-1.66 (2H, m), 1.76-1.92 (2H, m), 2.02 (3H, s), 2.13-2.30 (2H, m), 2.55 (2H, t, J = 5.9 Hz), 2.69-2.86 (2H, m), 3.70-3.92 (1H, m), 4.03 (3H, s), 4.11 (2H, t, J = 5.9 Hz), 4.29 (3H, s), 7.15-7.28 (1H, m), 7.35-7.52 (2H, m), 7.85 (1H, d, J = 7.7 Hz), 8.24 (1H, d, J = 8.1 Hz), 11.33 (1H, s). LC-MS: m/z = 441 (MH^+).

***N*-[1-(2-Hydroxyethyl)piperidin-4-yl]-3-methoxy-1-methyl-4-oxo-5-(2-oxo-2-phenyl ethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxamide (12a)**

A mixture of **1g** (200 mg, 0.399 mmol), 2-bromoethanol (42.5 μL, 0.599 mmol),

K_2CO_3 (248 mg, 1.80 mmol), and DMF (5 mL) was stirred at 100 °C for 15 h. The reaction mixture was diluted with water, and extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by basic silica gel column chromatography (AcOEt) followed by crystallization from AcOEt/EtOH to give the title compound (68.0 mg, 33%) as white crystals; mp 201 °C. ^{1}H NMR (DMSO-d_6) δ 1.51-1.62 (2H, m), 1.75-1.90 (2H, m), 2.18 (2H, t, J = 10.5 Hz), 2.40 (2H, t, J = 5.9 Hz), 2.70-2.85 (2H, m), 3.47-3.52 (2H, m), 3.75-3.90 (1H, m), 3.98 (3H, s), 4.32-4.38 (4H, m), 5.97 (2H, s), 7.29-7.39 (2H, m), 7.47 (1H, t, J = 7.6 Hz), 7.63 (2H, t, J = 7.6 Hz), 7.76 (1H, t, J = 7.1 Hz), 7.87 (1H, d, J = 7.5 Hz), 8.17 (2H, d, J = 7.6 Hz), 8.37 (1H, d, J = 8.4 Hz). LC-MS: m/z = 517 (MH^+). Anal. calcd for $C_{29}H_{32}N_4O_5$: C, 67.43; H, 6.24; N, 10.85. Found: C, 67.28; H, 6.20; N, 10.79.

_N_-[1-(Hydroxyacetyl)piperidin-4-yl]-3-methoxy-1-methyl-4-oxo-5-(2-oxo-2-phenyl ethyl)-4,5-dihydro-1_H_-pyrrolo[3,2-_c_]quinoline-2-carboxamide (12b)

Acetoxyacetyl chloride (47.3 μL, 0.440 mmol) was added to a mixture of **1g** (200 mg, 0.423 mmol), Et_3N (122 μL, 0.880 mmol), and THF (10 mL) at 0 °C and the mixture was stirred at room temperature for 15 h. The reaction mixture was diluted with AcOEt, washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by basic silica gel column chromatography (AcOEt) followed by crystallization from AcOEt/THF to give 2-[4-({[3-methoxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3, 2-*c*]quinolin-2-yl]carbonyl}amino)piperidin-1-yl]-2-oxoethyl acetate (171 mg, 75%) as a white powder. The compound obtained was dissolved in 8M NaOH aq. (1 mL), THF (2 mL), and EtOH (7 mL). After stirring at room temperature for 2 h, the reaction mixture was diluted with water, acidified with 6M HCl aq., and extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by basic silica gel column chromatography (AcOEt) followed by crystallization from AcOEt/THF to give the title compound (59.2 mg, 51%) as white crystals; mp 223 °C. ^{1}H NMR (DMSO-d_6) δ 1.35-1.65 (2H, m), 1.80-2.00 (2H, m), 2.85-3.00 (1H, m), 3.05-3.25 (1H, m), 3.60-3.75 (1H, m), 3.97 (3H, s), 4.00-4.30 (4H, m), 4.31 (3H, s), 4.51 (1H, t, J = 5.3 Hz), 5.97 (2H, s), 7.29-7.39 (2H, m), 7.48 (1H, t, J = 7.6 Hz), 7.63 (2H, t, J = 7.6 Hz), 7.76 (1H, t, J = 7.4 Hz), 7.95 (1H, d, J = 8.4 Hz), 8.17 (2H, d, J = 7.6 Hz), 8.37 (1H, d, J = 8.1 Hz). LC-MS: m/z = 531 (MH^+). Anal. calcd for $C_{29}H_{30}N_4O_6$: C, 65.65; H, 5.70; N, 10.56. Found: C, 65.60; H, 5.62; N, 10.46.

_N_-[1-(2-Hydroxyethyl)piperidin-4-yl]-3-methoxy-1-methyl-4-oxo-5-(2-oxobutyl)-4,5-dihydro-1_H_-pyrrolo[3,2-_c_]quinoline-2-carboxamide (12c)

tert-BuONa (89.3 mg, 0.929 mmol) was added to a suspension of **11** (299 mg, 0.679 mmol) in DME (2.4 mL)/DMF (0.6 mL) and the mixture was stirred at 0 °C for 30 min. LiBr (142 mg, 1.64 mmol) was added, and the mixture was stirred at room temperature for 10 min. 1-Bromo-2-butanone (76.6 μL, 0.750 mmol) was added and the mixture was stirred at room temperature for 65 h. After addition of 1M NaOH aq. (1.5 mL) and MeOH (1 mL), the mixture was stirred at room temperature for 30 min, and concentrated *in vacuo*. The residue was partitioned between AcOEt/THF = 3/1 solution (20 mL) and aq. $NaHCO_3$ solution (20 mL). The organic layer was separated, and the aq. layer was extracted with AcOEt/THF = 3/1 solution (20 mL × 3). The organic layer was combined, washed with brine (10 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was suspended with AcOEt (10 mL) and DMF (1 mL) at 60 °C, and insoluble material was removed by filtration. The filtrate was concentrated *in vacuo*, and purified by basic silica gel column chromatography (hexane/AcOEt = 1/2 to AcOEt/MeOH = 9/1). Crystallization from IPE/AcOEt gave the title compound (48.7 mg, 15%) as white crystals; mp 191 °C. ^{1}H NMR (DMSO-d_6) δ 1.01 (3H, t, J = 7.3 Hz), 1.45-1.67 (2H, m), 1.75-1.91 (2H, m), 2.08-2.25 (2H, m), 2.39 (2H, t, J = 6.3 Hz), 2.70 (2H, q, J = 7.2 Hz), 2.70-2.85 (2H, m), 3.42-3.57 (2H, m), 3.71-3.91 (1H, m), 3.98 (3H, s), 4.29 (3H, s), 4.38 (1H, t, J = 5.3 Hz), 5.29 (2H, s), 7.24-7.37 (2H, m), 7.43-7.56 (1H, m), 7.87 (1H, d, J = 7.9 Hz), 8.28-8.40 (1H, m). LC-MS: m/z = 469 (MH^+). Anal. calcd for $C_{25}H_{32}N_4O_5$: C, 64.09; H, 6.88; N, 11.96. Found: C, 63.73; H, 6.83; N, 11.81.

_N_-[1-(2-Hydroxyethyl)piperidin-4-yl]-3-methoxy-1-methyl-4-oxo-5-(2-phenylethyl)-4,5-dihydro-1_H_-pyrrolo[3,2-_c_]quinoline-2-carboxamide (12d)

In a similar manner to the preparation of **12c**, the title compound (8.2 mg, 2.4%) was obtained as beige crystals from **11** (299 mg, 0.678 mmol); mp 163 °C (crystallized from IPE/AcOEt). ^{1}H NMR (DMSO-d_6) δ 1.46-1.67 (2H, m), 1.76-1.91 (2H, m), 2.09-2.25 (2H, m), 2.40 (2H, t, J = 6.3 Hz), 2.67-2.84 (2H, m), 2.86-2.99 (2H, m), 3.44-3.55 (2H, m), 3.70-3.91 (1H, m), 3.98 (3H, s), 4.28 (3H, s), 4.37 (1H, t, J = 5.4 Hz), 4.45-4.58 (2H, m), 7.17-7.28 (1H, m), 7.28-7.40 (5H, m), 7.56-7.67 (1H, m), 7.69-7.78 (1H, m), 7.86 (1H, d, J = 7.7 Hz), 8.36 (1H, dd, J = 8.4, 1.2 Hz). LC-MS: m/z = 503 (MH^+). Anal. calcd for $C_{29}H_{34}N_4O_4 \cdot 0.5H_2O$: C, 68.08; H, 6.90; N, 10.95. Found: C, 67.77; H, 6.76; N, 10.72.

Experimental section of chapter II

Methyl (2*Z*)-3-aminopent-2-enoate (14a)

A mixture of **13a** (75.0 g, 576 mmol), NH_4OAc (222 g, 2.88 mmol) and MeOH (750 mL) was stirred at room temperature for 3 days. The mixture was concentrated *in vacuo*. The residue was diluted with water (500 mL) and extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, concentrated *in vacuo*, and dried to give the title compound (68.5 g, 92%) as a pale yellow oil. ^{1}H NMR (DMSO-d_6) δ 1.06 (3H, t, J = 7.6 Hz), 2.09 (2H, q, J = 7.6 Hz), 3.49 (3H, s), 4.34 (1H, s), 6.94 (1H, s), 7.72 (1H, br s).

Ethyl 2-aminocyclohex-1-ene-1-carboxylate (14b)

In the same manner as in the preparation of **14a**, the title compound (32.3 g, 60%) was obtained as a white solid from **13b** (53.9 g, 0.317 mol). ^{1}H NMR (DMSO-d_6) δ 1.16 (3H, t, J = 7.1 Hz), 1.45-1.59 (4H, m), 2.09-2.21 (4H, m), 4.00 (2H, q, J = 7.1 Hz), 7.09 (2H, br s).

Ethyl 6-ethyl-4-hydroxy-2-oxo-1,2-dihydropyridine-3-carboxylate (15a)

A mixture of **14a** (100 g, 774 mmol), diethyl malonate (130 mL, 856 mmol), 20% solution of NaOEt in EtOH (290 g, 852 mmol), EtOH (400 mL), xylene (800 mL) was stirred at 120 °C for 2 h, at 150 °C for 17 h with a Dean-Stark trap. The precipitate was collected by filtration and washed with hexane. The filtered material was dissolved in water (800 mL) and filtered. The filtrate was acidified with 5M HCl aq. at 0 °C. The precipitate was collected by filtration, successively washed with water and hexane / IPE to give the title compound (75.4 g, 46%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6) δ 1.12 (3H, t, J = 7.6 Hz), 1.26 (3H, t, J = 7.1 Hz), 2.42 (2H, q, J = 7.6 Hz), 4.25 (2H, q, J = 7.1 Hz), 5.79 (1H, s), 11.37 (1H, br s), 12.56 (1H, s).

Ethyl 4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (15b)

In the same manner as in the preparation of **15a**, the title compound (24.4 g, 58%) was obtained as a yellow solid from **14b** (30.0 g, 0.177 mol). ^{1}H NMR (DMSO-d_6) δ 1.28 (3H, t, J = 7.1 Hz), 1.57-1.73 (4H, m), 2.24-2.35 (2H, m), 2.41-2.48 (2H, m), 4.30 (2H, q, J = 7.1 Hz), 11.19 (1H, s), 13.49 (1H, s).

Ethyl 4-chloro-6-ethyl-2-oxo-1,2-dihydropyridine-3-carboxylate (16a)

A mixture of **15a** (15.0 g, 71.0 mmol) and $POCl_3$ (19.9 mL, 213 mmol) was

stirred at 80 °C for 30 min. The mixture was concentrated *in vacuo* and ice water was added to the residue at 0 °C. The resulting solid was collected by filtration and washed with water and AcOEt to give the title compound (9.80 g, 60%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t, J = 7.5 Hz), 1.26 (3H, t, J = 7.1 Hz), 2.44-2.55 (2H, m), 4.25 (2H, q, J = 7.1 Hz), 6.26 (1H, s), 12.28 (1H, s).

Ethyl 4-chloro-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (16b)

A mixture of **15b** (26.0 g, 110 mmol) and $POCl_3$ (51.3 mL, 552 mmol) was stirred at 130 °C for 1.5 h. After cooling, the reaction mixture was concentrated *in vacuo* and ice was added to the residue. The mixture was neutralized with saturated $NaHCO_3$ aq. and extracted with AcOEt. The extract was washed with saturated $NaHCO_3$ aq. water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residual solid was collected by filtration and washed with hexane / AcOEt to give the title compound (4.65 g, 17%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.25 (3H, t, J = 7.1 Hz), 1.61-1.74 (4H, m), 2.37-2.45 (2H, m), 2.51-2.57 (2H, m), 4.24 (2H, q, J = 7.1 Hz), 12.09 (1H, s).

Ethyl 4-chloro-6-methyl-2-oxo-1,2-dihydropyridine-3-carboxylate (16c)

A mixture of **15c** (3.00 g, 15.2 mmol), $POCl_3$ (7.75 mL, 83.3 mmol), and benzyltriethylammonium chloride (13.8 g, 60.8 mmol) in MeCN (60 mL) was stirred at 40 °C for 30 min and at reflux for 30 min. After cooling, the reaction mixture was concentrated *in vacuo*, water was added to the residue, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residual solid was collected and washed with hexane / AcOEt to give the title compound (1.45 g, 44%) as a white powder. ^{1}H NMR (DMSO-d_6) δ 1.26 (3H, t, J = 7.0 Hz), 2.20 (3H, s), 4.25 (2H, q, J = 7.0 Hz), 6.26 (1H, s), 12.29 (1H, s).

Ethyl 4-chloro-6-ethyl-2-oxo-1-(2-oxo-2-phenylethyl)-1,2-dihydropyridine-3-carboxylate (17a)

A mixture of **16a** (9.50 g, 41.4 mmol), K_2CO_3 (13.7 g, 99.2 mmol), phenacyl bromide (9.88 g, 49.6 mmol) and DMF (100 mL) was stirred at room temperature for 15 h. The reaction mixture was diluted with water and extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by basic silica gel column chromatography (hexane/AcOEt = 9/1 to 3/7) to give the title compound (1.00 g, 7%) as a white powder. ^{1}H NMR (DMSO-d_6) δ 1.14 (3H, t, J = 7.4 Hz), 1.24 (3H, t, J = 7.1 Hz), 2.62 (2H, q, J = 7.4 Hz), 4.25 (2H, q, J = 7.1 Hz), 5.61 (2H, s), 6.44 (1H, s), 7.54-7.66 (2H, m), 7.70-7.79 (1H, m), 8.05-8.13 (2H, m).

Ethyl 4-chloro-2-oxo-1-(2-oxo-2-phenylethyl)-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (17b)

Compound **16b** (3.00 g, 11.7 mmol) was added to a suspension of NaH (60% in oil, 516 mg, 12.9 mmol) in DMA (30 mL) and the mixture was stirred at room temperature for 30 min. Phenacyl bromide (2.57 g, 12.9 mmol) was added, and the mixture was stirred at room temperature for 15 h. The mixture was diluted water, extracted with AcOEt, and washed with water and brine. The organic layer was dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by basic silica gel column chromatography (hexane/AcOEt = 9/1 to AcOEt) to give the title compound (710 mg, 16%) as a white powder. ^{1}H NMR (DMSO-d_6) δ 1.25 (3H, t, J = 7.1 Hz), 1.60-1.80 (4H, m), 2.50-2.65 (4H, m), 4.26 (2H, q, J = 7.1 Hz), 5.66 (2H, s), 7.61 (2H, t, J = 7.4 Hz), 7.74 (1H, t, J = 7.4 Hz), 8.09 (2H, d, J = 7.4 Hz).

Ethyl 4-chloro-6-methyl-2-oxo-1-(2-oxo-2-phenylethyl)-1,2-dihydropyridine-3-carboxylate (17c)

In the same manner as in the preparation of **17a**, the title compound (3.18 g, 23%) was obtained as a white powder from **16c** (9.00 g, 41.7 mmol). ^{1}H NMR (DMSO-d_6) δ 1.25 (3H, t, J = 7.0 Hz), 2.30 (3H, s), 4.25 (2H, q, J = 7.0 Hz), 5.63 (2H, s), 6.54 (1H, d, J = 0.6 Hz), 7.61 (2H, t, J = 7.5 Hz), 7.72-7.78 (1H, m), 8.07-8.10 (2H, m).

Ethyl 6-ethyl-3-hydroxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (18a)

A mixture of **17a** (7.00 g, 19.3 mmol), sarcosine ethyl ester hydrochloride (5.94 g, 38.7 mmol), Et_3N (29.7 mL, 193 mmol), and EtOH (100 mL) was stirred at reflux for 2 days. After cooling, the mixture was diluted with water and acidified with 5M HCl aq. The resulting solid was collected and washed with water and hexane / AcOEt to give the title compound (5.70 g, 74%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, t, J = 7.4 Hz), 1.31 (3H, t, J = 7.1 Hz), 2.56 (2H, q, J = 7.4 Hz), 3.80 (3H, s), 4.30 (2H, q, J = 7.1 Hz), 5.55 (2H, s), 6.43 (1H, s), 7.60 (2H, t, J = 7.6 Hz), 7.68-7.79 (1H, m), 8.00-8.21 (2H, m), 8.90 (1H, s).

Ethyl 3-hydroxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5,6,7,8,9-hexahydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxylate (18b)

A mixture of the compound **17b** (270 mg, 0.724 mmol), sarcosine ethyl ester hydrochloride (222 mg, 1.45 mmol), Et_3N (1.00 mL, 7.24 mmol), and EtOH (5 mL) was stirred at reflux for 2 days. After cooling, water (10 mL) was added to the mixture and

the resulting solid was collected. This was used for the next reaction without further purification.

Ethyl 3-hydroxy-1,6-dimethyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (18c)

In the same manner as in the preparation of **18a**, the title compound (1.50 g, 68%) was obtained as a white powder from **17c** (2.00 g, 5.99 mmol). ^{1}H NMR (DMSO-d_6) δ 1.31 (3H, t, J = 7.1 Hz), 2.26 (3H, s), 3.77 (3H, s), 4.30 (2H, q, J = 7.1 Hz), 5.57 (2H, s), 6.52 (1H, s), 7.54-7.67 (2H, m), 7.69-7.79 (1H, m), 8.03-8.18 (2H, m), 8.90 (1 H, s).

Ethyl 3-ethoxy-6-ethyl-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (19a)

K_2CO_3 (10.6 g, 76.8 mmol) and Et_2SO_4 (4.03 mL, 30.1 mmol) were added to a solution of **18a** (9.80 g, 25.6 mmol) in acetone (196 mL) and the mixture was refluxed for 1 h. Et_2SO_4 (4.03 mL, 30.1 mmol) and acetone (70 mL) were added, and the mixture was refluxed for 17 h. The mixture was diluted with water (400 mL) and the resulting precipitate was collected by filtration and washed with water and hexane / AcOEt to give the title compound (8.58 g, 82%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.13-1.27 (6H, m), 1.31 (3H, t, J = 7.1 Hz), 2.57 (2H, q, J = 7.3 Hz), 3.84 (3H, s), 4.15 (2H, q, J = 7.1 Hz), 4.26 (2H, q, J = 7.1 Hz), 5.57 (2H, s), 6.49 (1H, s), 7.56-7.66 (2H, m), 7.69-7.77 (1H, m), 8.07-8.16 (2H, m).

Ethyl 6-ethyl-3-(2-fluoroethoxy)-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (19b)

2-Fluoroethyl iodide (512 mg, 2.94 mmol) was added to a mixture of **18a** (750 mg, 1.96 mmol) and DBU (0.440 mL, 2.94 mmol) in DMF (10 mL) and the mixture was stirred at room temperature for 15 h. The mixture was diluted with water and extracted with AcOEt. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by basic silica gel column chromatography (AcOEt) to give the title compound (649 mg, 77%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.3 Hz), 1.30 (3H, t, J = 7.1 Hz), 2.58 (2H, q, J = 7.3 Hz), 3.85 (3H, s), 4.26 (2H, q, J = 7.1 Hz), 4.32-4.36 (1H, m), 4.42-4.47 (1H, m), 4.53-4.59 (1H, m), 4.69-4.75 (1H, m), 5.58 (2H, s), 6.51 (1H, s), 7.61 (2H, t, J = 7.6 Hz), 7.70-7.77 (1H, m), 8.08-8.14 (2H, m).

Ethyl 3-(2,2-difluoroethoxy)-6-ethyl-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (19c)

2,2-Difluoroethyl trifluoromethanesulfonate (336 mg, 1.57 mmol) was added to a mixture of **18a** (500 mg, 1.31 mmol) and Cs_2CO_3 (554 mg, 1.70 mmol) in DMF (5 mL) and the mixture was stirred at room temperature for 2 h. The mixture was diluted with water (15 mL) and the precipitate was collected by filtration. The collected material was washed with water, EtOH and Et_2O, and dried *in vacuo* to give the title compound (0.45 g, 77%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.4 Hz), 1.30 (3H, t, J = 7.2 Hz), 2.59 (2H, q, J = 7.4 Hz), 3.86 (3H, s), 4.26 (2H, q, J = 7.2 Hz), 4.42 (2H, td, J = 14.9, 3.9 Hz), 5.61 (2H, s), 6.27 (1H, tt, J = 54.9, 3.9 Hz), 6.54 (1H, s), 7.61 (2H, t, J = 7.5 Hz), 7.74 (1H, t, J = 7.5 Hz), 8.10-8.13 (2H, m).

Ethyl 6-ethyl-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-3-(2,2,2-trifluoroethoxy)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (19d)

In the same manner as in the preparation of **19c**, the title compound (7.81 g, 86%) was obtained as a white solid from **18a** (7.47 g, 19.5 mmol). ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.4 Hz), 1.29 (3H, t, J = 7.1 Hz), 2.59 (2H, q, J = 7.4 Hz), 3.86 (3H, s), 4.26 (2H, q, J = 7.1 Hz), 4.83 (2H, q, J = 9.3 Hz), 5.61 (2H, s), 6.56 (1H, s), 7.61 (2H, t, J = 7.6 Hz), 7.69-7.79 (1H, m), 8.06-8.18 (2H, m).

Ethyl 6-ethyl-3-methoxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (19e)

In the same manner as in the preparation of **19b**, the title compound (156 mg, 23%) was obtained as a white powder from **18a** (650 mg, 1.70 mmol). ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.3 Hz), 1.30 (3H, t, J = 7.1 Hz), 2.58 (2H, q, J = 7.4 Hz), 3.84 (3H, s), 3.85 (3H, s), 4.27 (2H, q, J = 7.0 Hz), 5.58 (2H, s), 6.50 (1H, s), 7.61 (2H, t, J = 7.6 Hz), 7.67-7.82 (1H, m), 8.03-8.19 (2H, m).

Ethyl 6-ethyl-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-3-{[(trifluoromethyl) sulfonyl]oxy}-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (19f)

A mixture of **18a** (300 mg, 0.784 mmol), Tf_2O (158 μL, 0.941 mmol), and pyridine (6 mL) was stirred at 60 °C for 3 h under N_2 atmosphere. The mixture was diluted with water (50 mL) and extracted with AcOEt (100 mL). The extract was washed with brine (50 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt=1/1 to AcOEt) to give the title compound (239 mg, 59%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6) δ

1.20 (3H, t, *J* = 7.3 Hz), 1.33 (3H, t, *J* = 7.2 Hz), 2.60 (2H, q, *J* = 7.3 Hz), 3.95 (3H, s), 4.34 (2H, q, *J* = 7.2 Hz), 5.64 (2H, s), 6.64 (1H, s), 7.57-7.68 (2H, m), 7.70-7.81 (1H, m), 8.07-8.19 (2H, m).

Ethyl 3-ethenyl-6-ethyl-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (19g)

A mixture of **19f** (218 mg, 0.424 mmol), vinyltributyltin (328 μL, 1.28 mmol), $Pd(PPh_3)_4$ (148 mg, 0.128 mmol) and DMF (5.9 mL) was stirred at 100 °C for 3 h under Ar atmosphere. The mixture was diluted with water (50 mL) and extracted with AcOEt (100 mL). The extract was washed with brine (50 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The insoluble material was removed by filtration and the filtrate was concentrated *in vacuo*. The precipitate was collected by filtration and washed with IPE to give the title compound (120 mg, 72%) as a grey solid. ^{1}H NMR (DMSO-d_6) δ 1.20 (3H, t, *J* = 7.3 Hz), 1.33 (3H, t, *J* = 7.1 Hz), 2.61 (2H, q, *J* = 7.3 Hz), 3.85 (3H, s), 4.33 (2H, q, *J* = 7.1 Hz), 5.29-5.43 (1H, m), 5.59 (2H, s), 6.41-6.59 (2H, m), 7.23 (1H, dd, *J* = 17.7, 11.8 Hz), 7.55-7.67 (2H, m), 7.68-7.81 (1H, m), 8.01-8.20 (2H, m).

Ethyl 3,6-diethyl-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (19h)

A mixture of **19g** (1.33 g, 3.40 mmol), 10% Pd-C (270 mg) and THF (50 mL) / MeOH (25 mL) was stirred at room temperature for 7 h under H_2 atmosphere. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo*. The precipitate was collected by filtration to give the title compound (1.35 g, 100%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.09 (3H, t, *J* = 7.2 Hz), 1.19 (3H, t, *J* = 7.5 Hz), 1.33 (3H, t, *J* = 7.2 Hz), 2.58 (2H, q, *J* = 7.2 Hz), 3.07-3.19 (2H, m), 3.87 (3H, s), 4.30 (2H, q, *J* = 7.2 Hz), 5.59 (2H, s), 6.48 (1H, s), 7.58-7.64 (2H, m), 7.70-7.77 (1H, m), 8.08-8.14 (2H, m).

Ethyl 3-methoxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5,6,7,8,9-hexahydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxylate (19i)

In the same manner as in the preparation of **19a**, the title compound (34.0 mg, 11% in 2 steps) was obtained as a white powder from **18b**. ^{1}H NMR (DMSO-d_6) δ 1.31 (3H, t, *J* = 7.1 Hz), 1.60-1.77 (4H, m), 2.45-2.55 (2H, m), 2.94-3.02 (2H, m), 3.81 (3H, s), 4.03 (3H, s), 4.27 (2H, q, *J* = 7.1 Hz), 5.62 (2H, s), 7.61 (2H, t, *J* = 7.5 Hz), 7.73 (1H, t, *J* = 7.5 Hz), 8.10 (2H, d, *J* = 7.5 Hz).

Ethyl 3-methoxy-1,6-dimethyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (19j)

In the same manner as in the preparation of **19b**, the title compound (390 mg, 26%) was obtained as a white powder from **18c** (1.45 g, 3.94 mmol). ^{1}H NMR (DMSO-d_6) δ 1.30 (3H, t, J = 7.1 Hz), 2.28 (3H, s), 3.81 (3H, s), 3.86 (3H, s), 4.27 (2H, q, J = 7.1 Hz), 5.60 (2H, s), 6.59 (1H, s), 7.54-7.66 (2H, m), 7.69-7.79 (1H, m), 8.05-8.16 (2H, m).

3-Ethoxy-6-ethyl-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid (20a)

8M NaOH aq. (42.9 mL) was added to a solution of **19a** (8.58 g, 20.9 mmol) in EtOH (257 mL) and the mixture was stirred at 60 °C for 30 min. The mixture was diluted with water and acidified with 5M HCl aq. The resulting precipitate was collected by filtration, and washed with water and Et_2O to give the title compound (7.12 g, 89%) as a beige powder. ^{1}H NMR (DMSO-d_6) δ 1.12-1.27 (6H, m), 2.57 (2H, q, J = 7.4 Hz), 3.84 (3H, s), 4.16 (2H, q, J = 7.1 Hz), 5.58 (2H, s), 6.48 (1H, s), 7.55-7.66 (2H, m), 7.68-7.78 (1H, m), 8.05-8.19 (2H, m), 12.41 (1H, br s).

The following compounds **20b-j** were prepared in a same manner similar to that described for **20a**.

6-Ethyl-3-(2-fluoroethoxy)-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid (20b)

Yield 56%, white powder. ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.4 Hz), 2.58 (2H, q, J = 7.4 Hz), 3.85 (3H, s), 4.32-4.36 (1H, m), 4.42-4.47 (1H, m), 4.54-4.59 (1H, m), 4.70-4.74 (1H, m), 5.58 (2H, s), 6.50 (1H, s), 7.61 (2H, t, J = 7.6 Hz), 7.70-7.77 (1H, m), 8.09-8.15 (2H, m), 12.48 (1H, br s).

3-(2,2-Difluoroethoxy)-6-ethyl-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid (20c)

Yield 83%, white powder. ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.2 Hz), 2.58 (2H, q, J = 7.3 Hz), 3.85 (3H, s), 4.40 (2H, td, J = 14.6, 1.2 Hz), 5.60 (2H, s), 6.26 (1H, tt, J = 55.0, 3.9 Hz), 6.77 (1H, s), 7.58-7.76 (3H, m), 8.12 (2H, d, J = 7.5 Hz), 12.50-12.70 (1H, m).

6-Ethyl-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-3-(2,2,2-trifluoroethoxy)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid (20d)

Yield 93%, white powder. ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.4 Hz), 2.58 (2H, q, J = 7.4 Hz), 3.86 (3H, s), 4.82 (2H, q, J = 9.2 Hz), 5.61 (2H, s), 6.54 (1H, s), 7.61 (2H, t, J = 7.6 Hz), 7.70-7.77 (1H, m), 8.07-8.16 (2H, m), 12.74 (1H, br s).

6-Ethyl-3-methoxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid (20e)

Yield 79%, white powder. ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.3 Hz), 2.57 (2H, q, J = 7.3 Hz), 3.84 (3H, s), 3.85 (3H, s), 5.58 (2H, s), 6.48 (1H, s), 7.61 (2H, t, J = 7.6 Hz), 7.67-7.86 (1H, m), 7.97-8.31 (2H, m), 12.51 (1H, br s).

3,6-Diethyl-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*] pyridine-2-carboxylic acid (20h)

Yield 99%, white powder. ^{1}H NMR (DMSO-d_6) δ 1.07 (3H, t, J = 7.3 Hz), 1.19 (3H, t, J = 7.4 Hz), 2.57 (2H, q, J = 7.4 Hz), 3.11 (2H, q, J = 7.3 Hz), 3.87 (3H, s), 5.58 (2H, s), 6.46 (1H, s), 7.56-7.66 (2H, m), 7.68-7.78 (1H, m), 8.05-8.17 (2H, m), 12.80 (1H, br s).

3-Methoxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5,6,7,8,9-hexahydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxylic acid (20i)

Yield 23%, white powder. ^{1}H NMR (DMSO-d_6) δ 1.67 (4H, m), 2.44-2.52 (2H, m), 2.94-3.02 (2H, m), 3.81 (3H, s), 4.05 (3H, s), 5.62 (2H, s), 7.56-7.65 (2H, m), 7.70-7.78 (1H, m), 8.10 (2H, d, J = 7.6 Hz), 12.56 (1H, br s).

3-Methoxy-1,6-dimethyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo [3,2-*c*]pyridine-2-carboxylic acid (20j)

Yield 89%, white powder. ^{1}H NMR (DMSO-d_6) δ 2.28 (3H, s), 3.81 (3H, s), 3.86 (3H, s), 5.60 (2H, s), 6.58 (1H, s), 7.61 (2H, t, J = 7.4 Hz), 7.74 (1H, t, J = 7.3 Hz), 8.11 (2H, d, J = 7.7 Hz), 12.50 (1H, br s).

3-Methoxy-1,6-dimethyl-4-oxo-5-(2-oxo-2-phenylethyl)-*N*-piperidin-4-yl-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxamide hydrochloride (21a)

A mixture of **20j** (290 mg, 0.818 mmol), EDC (236 mg, 1.23 mmol), HOBt (166 mg, 1.23 mmol), and DMF (5 mL) was stirred at room temperature for 15 h, after which 4-amino-1-Boc-piperidine (212 mg, 1.06 mmol) was added and the mixture was stirred

at room temperature for 15 h. The mixture was diluted with water and extracted with AcOEt. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by basic silica gel column chromatography (AcOEt) to give *tert*-butyl 4-({[3-methoxy-1,6-dimethyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridin-2-yl]carbonyl}amino)piperidine-1-carboxylate. 4M HCl in AcOEt (4 mL) was added to a solution of the compound obtained above in AcOEt (4 mL) and the mixture was stirred at room temperature for 2 h. The precipitated solid was collected by filtration, and washed with AcOEt to give the title compound (240 mg, 62%) as a white powder. ^{1}H NMR (DMSO-d_6) δ 1.61-1.84 (2H, m), 1.95-2.11 (2H, m), 2.28 (3H, s), 2.92-3.12 (2H, m), 3.20-3.32 (2H, m), 3.83 (3H, s), 3.94-4.15 (4H, m), 5.62 (2H, s), 6.59 (1H, s), 7.61 (2H, t, J = 7.6 Hz), 7.68-7.81 (2H, m), 8.04-8.19 (2H, m), 8.64 (1H, br s.), 8.87 (1H, br s).

6-Ethyl-3-methoxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-*N*-piperidin-4-yl-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxamide hydrochloride (21b)

In the same manner as in the preparation of **21a**, the title compound (97.2 mg, 74%) was obtained as a white powder from **20e** (100 mg, 0.271 mmol). ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.3 Hz), 1.66-1.85 (2H, m), 1.97-2.09 (2H, m), 2.58 (2H, q, J = 7.4 Hz), 2.95-3.10 (2H, m), 3.19-3.30 (2H, m), 3.87 (3H, s), 3.96-4.14 (4H, m), 5.60 (2H, s), 6.49 (1H, s), 7.55-7.66 (2H, m), 7.69-7.79 (2H, m), 8.12 (2H, d, J = 7.7 Hz), 8.92 (2H, br s).

3-Ethoxy-6-ethyl-*N*-[1-(hydroxyacetyl)piperidin-4-yl]-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxamide (22a)

EDC (55.6 mg, 0.290 mmol) at 0 ºC was added to a mixture of the compound **21a** (74.0 mg, 0.194 mmol), **24** (49.1 mg, 0.252 mmol), HOBt (39.2 mg, 0.290 mmol), and Et_3N (34.9 mL, 0.252 mmol) in DMF (3 mL) and the mixture was stirred at room temperature for 15 h. The reaction mixture was diluted with water and extracted twice with AcOEt. The combined extract was washed with water and saturated brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by basic silica gel column chromatography (AcOEt) and the obtained solid was recrystallized from AcOEt to give the title compound (61.2 mg, 60%) as white crystals; mp 204 °C. ^{1}H NMR (DMSO-d_6) δ 1.13-1.29 (6H, m), 1.32-1.56 (2H, m), 1.83-1.96 (2H, m), 2.57 (2H, q, J = 7.4 Hz), 2.80-2.94 (1H, m), 3.04-3.19 (1H, m), 3.59-3.72 (1H, m), 3.90 (3H, s), 3.95-4.14 (3H, m), 4.17-4.27 (1H, m), 4.34 (2H, q, J = 7.2 Hz), 4.51 (1H, t, J = 5.4 Hz),

5.59 (2H, s), 6.49 (1H, s), 7.56-7.65 (2H, m), 7.66-7.77 (2H, m), 8.07-8.14 (2H, m). Anal. calcd for $C_{28}H_{34}N_4O_6$: C 64.35; H 6.56; N 10.72. Found: C, 64.20; H, 6.52; N, 10.61. LC-MS: m/z = 523 (MH^+).

The following compounds **22b-d, h, i** were prepared in a same manner similar to that described for **22a**.

6-Ethyl-3-(2-fluoroethoxy)-*N*-[1-(hydroxyacetyl)piperidin-4-yl]-1-methyl-4-oxo-5-(2-oxo-2- phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxamide (22b)

Yield 80%, white crystals; mp 208 °C (recrystallized from AcOEt / THF). ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.4 Hz), 1.26-1.48 (2H, m), 1.87 (2H, s), 2.59 (2H, q, J = 7.4 Hz), 2.76-2.91 (1H, m), 3.01-3.17 (1H, m), 3.57-3.73 (1H, m), 3.92 (3H, s), 3.97-4.13 (3H, m), 4.18-4.32 (1H, m), 4.45-4.81 (5H, m), 5.59 (2H, s), 6.49-6.56 (1H, m), 7.55-7.66 (3H, m), 7.69-7.78 (1H, m), 8.06-8.16 (2H, m). Anal. calcd for $C_{28}H_{33}FN_4O_6$: C, 62.21; H, 6.15; N, 10.36. Found: C, 62.03; H, 6.24; N, 10.18. LC-MS: m/z = 541 (MH^+).

3-(2,2-Difluoroethoxy)-6-ethyl-*N*-[1-(hydroxyacetyl)piperidin-4-yl]-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxamide (22c)

Yield 65%, white crystals; mp 188 °C (recrystallized from acetone / H_2O). ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.4 Hz), 1.20-1.50 (2H, m), 1.90 (2H, d, J = 9.9 Hz), 2.59 (2H, q, J = 7.4 Hz), 2.83 (1H, t, J = 12.2 Hz), 3.09 (1H, t, J = 11.7 Hz), 3.68 (1H, d, J = 12.9 Hz), 3.90 (3H, s), 3.95-4.20 (3H, m), 4.26 (1H, d, J = 11.7 Hz), 4.51 (1H, t, J = 5.4 Hz), 4.67 (2H, td, J = 16.1, 3.0 Hz), 5.23 (1H, s), 5.61 (2H, s), 6.33 (1H, tt, J = 54.2, 3.0 Hz), 7.49 (1H, d, J = 7.8 Hz), 7.61 (2H, t, J = 7.5 Hz), 7.73 (1H, t, J = 7.5 Hz), 8.10-8.12 (2H, m). Anal. calcd for $C_{28}H_{32}F_2N_4O_6$: C, 60.21; H, 5.77; N, 10.03. Found: C, 60.23; H, 5.78; N, 10.01. LC-MS: m/z = 559 (MH^+).

6-Ethyl-*N*-[1-(hydroxyacetyl)piperidin-4-yl]-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-3-(2,2,2-trifluoroethoxy)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxamide (22d)

Yield 76%, white crystals; mp 169 °C (recrystallized from EtOH / H_2O). ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.3 Hz), 1.24-1.51 (2H, m), 1.79-1.98 (2H, m), 2.59 (2H, q, J = 7.3 Hz), 2.75-2.94 (1H, m), 2.98-3.20 (1H, m), 3.57-3.77 (1H, m), 3.86 (3H, s), 3.94-4.17 (3H, m), 4.17-4.38 (1H, m), 4.53 (1H, t, J = 5.3 Hz), 5.05 (2H, q, J = 9.3 Hz),

5.63 (2H, s), 6.54 (1H, s), 7.51 (1H, d, J = 7.7 Hz), 7.55-7.67 (2H, m), 7.68-7.82 (1H, m), 8.01-8.21 (2H, m). Anal. calcd for $C_{28}H_{31}F_3N_4O_6$: C, 58.33; H, 5.42; N, 9.72. Found: C, 58.32; H, 5.55; N, 9.63. LC-MS: m/z = 577 (MH^+).

3,6-Diethyl-*N*-[1-(hydroxyacetyl)piperidin-4-yl]-1-methyl-4-oxo-5-(2-oxo-2-phenyl ethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxamide (22h)

Yield 47%, white crystals; mp 141 °C (recrystallized from hexane / AcOEt). ^{1}H NMR (DMSO-d_6) δ 1.06 (3H, t, J = 7.2 Hz), 1.18 (3H, t, J = 7.3 Hz), 1.30-1.54 (2H, m), 1.81-1.93 (2H, m), 2.54-2.63 (2H, m), 2.78-2.92 (3H, m), 3.01-3.17 (1H, m), 3.59-3.73 (4H, m), 3.99-4.16 (3H, m), 4.20-4.31 (1H, m), 4.51 (1H, t, J = 5.5 Hz), 5.58 (2H, s), 6.42 (1H, s), 7.56-7.65 (2H, m), 7.69-7.77 (1H, m), 8.07-8.15 (2H, m), 8.21 (1H, d, J = 7.4 Hz). Anal. calcd for $C_{28}H_{34}N_4O_5$·0.5AcOEt: C, 65.44; H, 6.96; N, 10.17. Found: C, 65.46; H, 7.01; N,10.27. LC-MS: m/z = 507 (MH^+).

***N*-[1-(Hydroxyacetyl)piperidin-4-yl]-3-methoxy-1-methyl-4-oxo-5-(2-oxo-2-phenyl ethyl)-4,5,6,7,8,9-hexahydro-1*H*-pyrrolo[3,2-*c*]quinoline-2-carboxamide (22i)**

Yield 76%, colorless oil. ^{1}H NMR (DMSO-d_6) δ 1.22-1.25 (2H, m), 1.35-1.56 (2H, m), 1.60-1.74 (4H, m), 1.80-1.92 (2H, m), 2.83-3.22 (4H, m), 3.59-3.70 (1H, m), 3.90 (3H, s), 3.98-4.12 (7H, m), 4.51 (1H, t, J = 5.4 Hz), 5.63 (2H, s), 7.56-7.65 (2H, m), 7.69-7.82 (2H, m), 8.10 (2H, d, J = 8.3 Hz). LC-MS: m/z = 535 (MH^+).

***N*-[1-(Hydroxyacetyl)piperidin-4-yl]-3-methoxy-1,6-dimethyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxamide (22j)**

Acetoxyacetyl chloride (61.4 μL, 0.571 mmol) at 0 ºC was added to a mixture of **21a** (225 mg, 0.476 mmol) and Et_3N (198 μL, 1.43 mmol) in THF (5 mL) and the mixture was stirred at room temperature for 15 h. The mixture was diluted with AcOEt, washed with water and brine, and dried over $MgSO_4$. After removal of $MgSO_4$ by filtration, the filtrate was concentrated *in vacuo*. The residue was purified by basic silica gel column chromatography (AcOEt) to give 2-[4-({[3-methoxy-1,6-dimethyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrol o[3,2-*c*]- pyridin-2-yl]carbonyl}amino)piperidin-1-yl]-2-oxoethyl acetate. 8M NaOH aq. (0.5 mL), THF (2.5 mL) and EtOH (5 mL) were added to a mixture of the compound obtained above and stirred at room temperature for 2 h. The mixture was neutralized with 1M HCl aq. and diluted with water, and extracted with AcOEt / THF. The organic layer was washed with water and brine and dried over $MgSO_4$. After removal of $MgSO_4$ by filtration, the filtrate was concentrated *in vacuo*. The residue was purified by basic

silica gel column chromatography (AcOEt) to give the title compound (156 mg, 66%) as a white solid; mp 237 °C (recrystallized from hexane / AcOEt / THF). ^{1}H NMR (DMSO-d_6) δ 1.32-1.61 (2H, m), 1.79-1.93 (2H, m), 2.28 (3H, s), 2.82-2.98 (1H, m), 3.04-3.20 (1H, m), 3.57-3.71 (1H, m), 3.86 (3H, s), 3.96-4.25 (7H, m), 4.50 (1H, d, *J* = 5.3 Hz), 5.61 (2H, s), 6.58 (1H, s), 7.55-7.67 (3H, m), 7.69-7.78 (1H, m), 8.07-8.16 (2H, m). Anal. calcd for $C_{26}H_{30}N_4O_6$: C, 63.15; H, 6.11; N, 11.33. Found: C, 63.03; H, 6.15; N, 11.07. LC-MS: m/z = 495 (MH^+).

6-Ethyl-*N*-[1-(hydroxyacetyl)piperidin-4-yl]-3-methoxy-1-methyl-4-oxo-5-(2-oxo-2-phenylethyl)-4,5-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxamide (22e)

In the same manner as in the preparation of **22j**, the title compound (61.2 mg, 65%) was obtained as a white powder from **21b** (90.0 mg, 0.185 mmol); mp 146 °C (recrystallized from AcOEt). ^{1}H NMR (DMSO-d_6) δ 1.13-1.23 (3H, m), 1.31-1.59 (2H, m), 1.80-1.94 (2H, m), 2.58 (2H, q, *J* = 7.4 Hz), 2.83-2.99 (1H, m), 3.04-3.21 (1H, m), 3.59-3.70 (1H, m), 3.89 (3H, s), 3.97-4.26 (7H, m), 4.49 (1H, t, *J* = 5.4 Hz), 5.59 (2H, s), 6.49 (1H, s), 7.56-7.67 (3H, m), 7.70-7.78 (1H, m), 8.06-8.15 (2H, m). Anal. calcd for $C_{27}H_{32}N_4O_6$: C, 63.77; H, 6.34; N, 11.02. Found: C, 63.58; H, 6.31; N, 10.87. LC-MS: m/z = 509 (MH^+).

2-(4-Aminopiperidin-1-yl)-2-oxoethanol hydrochloride (24)

Acetoxyacetyl chloride (3.10 mL, 28.8 mmol) was added dropwise at 0 °C to a mixture of **23** (4.80 g, 24.0 mmol) and Et_3N (9.96 mL, 71.9 mmol) in THF (50 mL) and the mixture was stirred for 2 h. The mixture was diluted with AcOEt, washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residual solid was collected by filtration and washed with hexane / AcOEt solution. A mixture of the solid obtained, 8M NaOH aq. (5 mL) and EtOH (35 mL) was stirred at room temperature for 2 h. The mixture was acidified with 6M HCl aq. and concentrated *in vacuo*. The residue was dissolved in AcOEt (30 mL) and 4M HCl in AcOEt (30 mL) was added. After stirring at room temperature for 6 h, the resulting solid was collected by filtration and washed with AcOEt to give the title compound (3.73 g, 80%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.31-1.51 (2H, m), 1.93 (2H, d, *J* = 12.9 Hz), 2.69 (1H, t, *J* = 12.2 Hz), 3.00 (1H, t, *J* = 12.3 Hz), 3.10-3.30 (1H, m), 3.73 (1H, d, *J* = 13.8 Hz), 4.08 (2H, q, *J* = 13.9 Hz), 4.33 (1H, d, *J* = 12.9 Hz), 8.38 (3H, br s).

Experimental section of chapter III

Propanimidamide hydrochloride (31)

To a solution of propionitrile (10 g, 142 mmol) in EtOH (8.4 mL) was blown hydrogen chloride gas (total amount increase 7.9 g), and the mixture was stirred at room temperature for 21.5 h. The mixture was concentrated *in vacuo*, the residue was suspended in EtOH (5.5 mL), and cooled to -10ºC. To the suspension was added 8M MeOH solution of ammonia (17.8 mL), and the mixture was stirred at room temperature for 28 h. The insoluble material was filtered off, and the filtrate was concentrated *in vacuo* to give the title compound (10.8 g, 70%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.17 (3H, t, J = 7.6 Hz), 2.40 (2H, q, J = 7.7 Hz), 8.77 (2H, br s), 9.07 (2H, br s).

2-Ethylpyrimidine-4,6-diol (32)

To a solution of **31** (11 g, 99 mmol) in MeOH (20 mL) was added 28% solution of NaOMe in MeOH (57 g, 296 mmol), and the mixture was stirred at room temperature for 10 min. Diethyl malonate (15 mL, 99 mmol) was added dropwise, and the mixture was further stirred at room temperature for 16 h. The reaction mixture was concentrated *in vacuo*, the residue was dissolved in water and acidified with conc. HCl. The precipitated solid was collected by filtration, washed with water and Et_2O to give the title compound (9.3 g, 67%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.16 (3 H, t, J = 7.6 Hz), 2.36-2.60 (2 H, m), 5.03 (1 H, s), 11.62 (2 H, br s).

Ethyl 4,6-dichloro-2-ethylpyrimidine-5-carboxylate (29a)

DMF (5.5 mL) was added dropwise to $POCl_3$ (60 mL, 641 mmol) at 0 °C, the mixture was stirred at 0 °C for 1 h. **32** (10 g, 71 mmol) was added, the mixture was stirred at room temperature for 1 h and heated under reflux for 16 h. The mixture was concentrated *in vacuo*, the residue was added to ice water by small portions. The mixture was extracted three times with a mixed solvent of Et_2O/AcOEt. The extracts were combined, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was suspended in AcOEt, and the insoluble material was filtered off. The filtrate was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (hexane/AcOEt = 99/1 to 9/1) to give 4,6-dichloro-2-ethylpyrimidine-5-carbaldehyde (10.6 g, 73%) as a pale yellow powder. To a mixture of obtained aldehyde (11 g, 52 mmol), amidosulfuric acid (7.0 g, 72 mmol), *tert*-butanol (100 mL) and water (40 mL) was added dropwise a solution of sodium chlorite (6.6 g, 72 mmol) in water (20 mL). After stirring at room temperature for 30

min, the reaction mixture was diluted with water, and extracted twice with AcOEt. The extracts were combined, washed with water and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was dissolved in THF (60 mL), oxalyl chloride (9.7 mL, 111 mmol) was added dropwise at 0 °C, then DMF (1 drop) was added. After stirring at room temperature for 3 h, the reaction mixture was concentrated *in vacuo*. EtOH (60 mL) and Et_3N (16 mL, 111 mmol) were added under ice-cooling, and the mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with saturated $NaHCO_3$ aq. and water, and extracted twice with AcOEt. The extracts were combined, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 97/3 to 17/3) to give the title compound (6.7 g, 53%) as pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.31-1.48 (6 H, m), 2.97 (2 H, q, *J* = 7.6 Hz), 4.48 (2 H, q, *J* = 7.2 Hz).

Ethyl 4-chloro-6-[(2-ethoxy-2-oxoethyl)(methyl)amino]-2-ethylpyrimidine-5-carboxylate (28a)

A solution of **29a** (425 mg, 1.7 mmol), ethyl sarcosinate hydrochloride (262 mg, 1.7 mmol) and Et_3N (0.57 mL, 4.1 mmol) in THF (13 mL) was stirred at room temperature for 19.5 h. The reaction mixture was diluted with saturated $NaHCO_3$ aq., and extracted twice with AcOEt. The extracts were combined, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give the title compound (560 mg, 99%) as colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.17-1.32 (6 H, m), 1.41 (3 H, t, *J* = 7.2 Hz), 2.72 (2 H, q, *J* = 7.6 Hz), 3.11 (3 H, s), 4.21 (2 H, q, *J* = 7.1 Hz), 4.28 (2 H, s), 4.40 (2 H, q, *J* = 7.2 Hz).

Ethyl 4-chloro-2-ethyl-5-hydroxy-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylate (27a)

A solution of **28a** (810 mg, 2.5 mmol) and 20% solution of NaOEt in EtOH (836 mg, 2.5 mmol) in EtOH (25 mL) was stirred at room temperature for 15 min. The reaction mixture was diluted with water, neutralized with 1M HCl aq., and stirred at 0 ºC for 30 min. The precipitated solid was collected by filtration, washed with water, and dried *in vacuo* to give the title compound (637 mg, 91%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6) δ 1.21-1.43 (6 H, m), 2.90 (2 H, q, *J* = 7.6 Hz), 3.88 (3 H, s), 4.37 (2 H, q, *J* = 7.0 Hz), 9.50 (1 H, br s).

Ethyl 4-chloro-2-ethyl-7-methyl-5-(2,2,2-trifluoroethoxy)-7*H*-pyrrolo[2,3-*d*] pyrimidine-6-carboxylate (33)

To a mixture of **27a** (1.0 g, 3.7 mmol) and Cs_2CO_3 (1.3 g, 4.0 mmol) in DMF (27 mL) was added dropwise 2,2,2-trifluoroethyl trifluoromethanesulfonate (0.64 mL, 4.4 mmol) at 0 ºC, and the mixture was stirred at room temperature for 4 h. The mixture was diluted with water, and the precipitated solid was collected by filtration. The obtained solid was washed with water, and dried *in vacuo* to give the title compound (1.2 g, 91%) as a pale yellow powder. ^{1}H NMR ($CDCl_3$) δ 1.34-1.49 (6 H, m), 3.02 (2 H, q, J = 7.6 Hz), 4.06 (3 H, s), 4.39-4.57 (4 H, m).

Ethyl 2-ethyl-7-methyl-4-oxo-5-(2,2,2-trifluoroethoxy)-4,7-dihydro-3*H*-pyrrolo [2,3-*d*]pyrimidine-6-carboxylate (34)

A solution of **33** (1.2 g, 3.3 mmol) and AcONa (272 mg, 3.3 mmol) in AcOH (20 mL) was heated under reflux for 2.5 h. After allowing to cool to room temperature, the reaction mixture was poured into water, and the precipitated solid was collected by filtration. The obtained solid was washed with water, and dried *in vacuo* to give the title compound (1.10 g, 96%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.16-1.37 (6 H, m), 2.63 (2 H, q, J = 7.6 Hz), 3.83 (3 H, s), 4.25 (2 H, q, J = 7.1 Hz), 5.01 (2 H, q, J = 9.4 Hz), 12.02 (1 H, br s).

2-Ethyl-7-methyl-4-oxo-5-(2,2,2-trifluoroethoxy)-4,7-dihydro-3*H*-pyrrolo[2,3-*d*] pyrimidine-6-carboxylic acid (36a)

A mixture of **34** (4.0 g, 11.5 mmol) and 8M NaOH aq. (8 mL) in EtOH was stirred at room temperature for 15 min and at 60 ºC for 4 h. After cooling at 0 ºC, the mixture was neutralized with 6M HCl aq.. The solvent was evaporated, the precipitate was collected by filtration washed with water, and dried *in vacuo* to give the title compound (2.91 g, 79%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6) δ 1.22 (3 H, t, J = 7.6 Hz), 2.62 (2 H, q, J = 7.4 Hz), 3.83 (3 H, s), 5.01 (2 H, q, J = 9.3 Hz), 12.07 (1 H, s), 12.71 (1 H, br s).

Ethyl 2-ethyl-7-methyl-4-oxo-3-(2-oxo-2-phenylethyl)-5-(2,2,2-trifluoroethoxy)-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylate (35)

To a suspension of **34** (124 mg, 0.357 mmol) in DME (4 mL) / DMF (1mL) was added portionwise potassium *tert*-butoxide (44 mg, 0.462 mmol) and the mixture was stirred at 0 ºC for 15 min. LiBr (62 mg, 0.714 mmol) was added, and the mixture was stirred at room temperature for 15 min. Phenacyl bromide (142 mg, 0.714 mmol) was

added, and the mixture was stirred at 60 ºC for 16 h. The mixture was diluted with brine, and extracted twice with AcOEt. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 97/3 to 7/3) to give the title compound (95 mg, 57%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.17-1.36 (6 H, m), 2.74 (2 H, q, *J* = 7.2 Hz), 3.90 (3 H, s), 4.27 (2 H, q, *J* = 7.2 Hz), 4.91 (2 H, q, *J* = 9.1 Hz), 5.72 (2 H, s), 7.56-7.68 (2 H, m), 7.69-7.83 (1 H, m), 8.03-8.24 (2 H, m).

Ethyl 6-ethyl-2-oxo-1,2-dihydropyridine-3-carboxylate (40)

A mixture of **16a** (32.1 g, 140 mmol), Et_3N (39 mL, 280 mmol), 10% Pd-C (1.60 g) in EtOH (180 mL) / THF (180 mL) was stirred at room temperature for 5 h under H_2 atmosphere. The catalyst was filtered off, washed with MeOH, and the filtrate was concentrated *in vacuo*. To the residue was added AcOEt, the insoluble material was filtered off, and washed with AcOEt. The filtrate was washed with water containing 6M HCl aq. (1 ml), and the aqueous layer was extracted with AcOEt. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The precipitate was collected by filtration, and washed with mixed solvent of hexane/AcOEt to give the title compound (24.7 g, 90%) as a brown powder. ^{1}H NMR ($CDCl_3$) δ 1.32 (3 H, t, *J* = 7.6 Hz), 1.38 (3 H, t, *J* = 7.2 Hz), 2.74 (2 H, q, *J* = 7.6 Hz), 4.37 (2 H, q, *J* = 7.2 Hz), 6.29 (1 H, d, *J* = 7.2 Hz), 8.18 (1 H, d, *J* = 7.6 Hz), 12.50 (1 H, br s).

Ethyl 5-bromo-6-ethyl-2-oxo-1,2-dihydropyridine-3-carboxylate (41)

To a solution of **40** (17.5 g, 89.7 mmol) in DMF (100 mL) was added *N*-bromosuccinimide (16.0 g, 89.9 mmol) at 0 ºC, and the mixture was stirred at room temperature for 1 h. To the mixture was added dropwise water (250 mL) at 0 ºC, and the mixture was stirred at room temperature for 30 min. The precipitate was collected by filtration, and washed with water to give the title compound (21.2 g, 86%) as a brown powder. ^{1}H NMR (DMSO-d_6) δ 1.14 (3 H, t, *J* = 7.6 Hz), 1.26 (3 H, t, *J* = 7.2 Hz), 2.64 (2 H, q, *J* = 7.6 Hz), 4.20 (2 H, q, *J* = 7.2 Hz), 8.06 (1 H, s), 12.42 (1 H, br s).

Ethyl 5-bromo-2-chloro-6-ethylpyridine-3-carboxylate (29b)

A mixture of **41** (28.0 g, 102 mmol) and $POCl_3$ (28 mL, 309 mmol) was heated under reflux for 12 h. The mixture was concentrated *in vacuo*, ice water (100 mL) was added at 0 ºC, and the mixture was extracted with AcOEt (100 mL x 3). The extract was washed successively with saturated $NaHCO_3$ aq. (50 mL) and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column

chromatography (hexane/AcOEt = 99/1 to 9/1) to give the title compound (22.8 g, 76%) as pale yellow oil. ^{1}H NMR (DMSO-d_6) δ 1.22 (3 H, t, J = 7.5 Hz), 1.33 (3 H, t, J = 7.2 Hz), 2.91 (2 H, q, J = 7.5 Hz), 4.34 (2 H, q, J = 7.2 Hz), 8.42 (1 H, s).

Ethyl 5-bromo-6-ethyl-3-hydroxy-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylate (27b)

A mixture of **29b** (22.8 g, 78.0 mmol), ethyl sarcosinate hydrochloride (18.0 g, 117 mmol), Et_3N (54 mL, 387 mmol) and EtOH (200 mL) was heated under reflux for 22 h. Then, ethyl sarcosinate hydrochloride (6.00 g, 39.1 mmol) and Et_3N (22 mL, 158 mmol) were added, and the mixture was heated under reflux for 17 h. To the reaction mixture was added water (250 mL), and the mixture was extracted three times with AcOEt (300 mL). The extract was washed successively with water (100 mL) and brine (100 mL), and dried over $MgSO_4$ and concentrated *in vacuo*. To the residue were added EtOH (200 mL) and a 20% solution of NaOEt in EtOH (32.0 g, 94.0 mmol), and the mixture was stirred at room temperature for 1 h. The mixture was concentrated *in vacuo*, diluted with water (250 mL), and the mixture was acidified with 5M HCl aq. (20 mL). The precipitate was collected by filtration, and washed with water to give the title compound (18.8 g, 73%) as a pale orange solid. ^{1}H NMR (DMSO-d_6) δ 1.27 (3 H, t, J = 7.6 Hz), 1.33 (3 H, t, J = 7.1 Hz), 2.96 (2 H, q, J = 7.6 Hz), 3.88 (3 H, s), 4.33 (2 H, q, J = 7.1 Hz), 8.36 (1 H, s), 9.76 (1 H, br s).

Ethyl 5-bromo-6-ethyl-1-methyl-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylate (42)

In the same manner as in the preparation of **33**, the title compound (1.80 g, 96%) was obtained as a pale yellow powder from **27b** (1.50 g, 4.58 mmol). ^{1}H NMR (DMSO-d_6) δ 1.22-1.41 (6 H, m), 3.00 (2 H, q, J = 7.4 Hz), 3.96 (3 H, s), 4.35 (2 H, q, J = 7.1 Hz), 4.86 (2 H, q, J = 9.1 Hz), 8.36 (1 H, s).

Ethyl 5-amino-6-ethyl-1-methyl-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylate (43)

To a mixture of **42** (2.53 g, 6.18 mmol), benzophenoneimine (1.5 mL, 8.94 mmol), Cs_2CO_3 (3.98 g, 12.2 mmol) and toluene (30 mL) were added $Pd_2(dba)_3$ (389 mg, 0.425 mmol) and xantphos (499 mg, 0.862 mmol), and the mixture was stirred at 100 ºC for 22 h under Ar atmosphere. The mixture was filtered through celite pad, and washed with AcOEt. The filtrate was washed with water (20 mL) and brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column

chromatography (hexane/AcOEt = 99/1 to 9/1) to give crude ethyl 5-[(diphenylmethylidene)amino]-6-ethyl-1-methyl-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo [2,3-*b*]pyridine-2-carboxylate. The obtained crude compound was dissolved in THF (20 mL), then 2M HCl aq. (5 mL) was added, and the mixture was stirred at room temperature for 1 h. To the mixture was added aqueous $NaHCO_3$ solution (40 mL), and the mixture was extracted three times with mixed solution of AcOEt/THF (50 mL). The extract was washed with brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 99/1 to 2/1) to give the title compound (1.95 g, 91%) as a yellow powder. ^{1}H NMR (DMSO-d_6) δ 1.25 (3 H, t, J = 7.4 Hz), 1.34 (3 H, t, J = 7.1 Hz), 2.75 (2 H, q, J = 7.4 Hz), 3.91 (3 H, s), 4.32 (2 H, q, J = 7.1 Hz), 4.66 (2 H, q, J = 9.1 Hz), 4.86 (2 H, s), 7.16 (1 H, s).

Ethyl 6-ethyl-1-methyl-5-[(phenylcarbonyl)amino]-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylate (44)

To a mixture of **43** (135 mg, 0.391 mmol), pyridine (63.4 μL, 0.784 mmol) and THF (3 mL) was added benzoyl chloride (54.4 μL, 0.469 mmol) at 0 ºC, and the mixture was stirred at room temperature for 1.5 h. To the reaction mixture was added water (5 mL), and the mixture was extracted four times with AcOEt (5 mL). The extracts were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The precipitate was collected by filtration to give the title compound (140 mg, 80%) as a white powder. The filtrate was concentrated *in vacuo*, and the residue was purified by basic silica gel column chromatography (hexane/AcOEt = 49/1 to 2/1) to give the title compound (24.9 mg, 14%) as a white powder. ^{1}H NMR (DMSO-d_6) δ 1.25 (3 H, t, J = 7.4 Hz), 1.36 (3 H, t, J = 7.1 Hz), 2.89 (2 H, q, J = 7.4 Hz), 4.01 (3 H, s), 4.37 (2 H, q, J = 7.1 Hz), 4.82 (2 H, q, J = 9.1 Hz), 7.46-7.72 (3 H, m), 7.90-8.13 (3 H, m), 10.13 (1 H, s).

6-Ethyl-1-methyl-5-(2-oxo-2-phenylethyl)-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylic acid (26b)

To a mixture of sodium *tert*-butoxide (264 mg, 2.75 mmol), $Pd(OAc)_2$ (25.9 mg, 0.115 mmol), 2-(dicyclohexylphosphino)-2'-methylbiphenyl (86.1 mg, 0.236 mmol) and toluene (6 mL) were added a solution of **42** (450 mg, 1.10 mmol) and acetophenone (0.256 mL, 2.20 mmol) in toluene (4 mL), and the mixture was stirred at 70 ºC for 17 h. After allowing to cool to room temperature, 1M NaOH aq. (3 mL) and EtOH (5 mL) were added, and the mixture was stirred at 50 ºC for 2 h. To the reaction mixture was added aqueous NH_4Cl solution (30 mL), and the mixture was extracted four times with AcOEt (20 mL). The extracts were combined, washed with brine (10 mL), dried over

anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1/1 to AcOEt/MeOH = 19/1) and preparative HPLC (0.1% TFA-containing MeCN/0.1% TFA-containing water = 1/1 to 7/3) to give the title compound (104 mg, 22%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6) δ 1.22 (3 H, t, J = 7.5 Hz), 2.70 (2 H, q, J = 7.5 Hz), 3.98 (3 H, s), 4.63 (2 H, s), 4.77 (2 H, q, J = 9.3 Hz), 7.51-7.63 (2 H, m), 7.64-7.74 (1 H, m), 7.82 (1 H, s), 8.04-8.17 (2 H, m), 13.35 (1 H, br s).

6-Ethyl-1-methyl-5-[(phenylcarbonyl)amino]-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo [2,3-*b*]pyridine-2-carboxylic acid (26c)

In the same manner as in the preparation of **26a**, the title compound (75.4 mg, 95%) was obtained as a white powder from **44** (84.5 mg, 0.188 mmol). ^{1}H NMR (DMSO-d_6) δ 1.25 (3 H, t, J = 7.6 Hz), 2.88 (2 H, q, J = 7.6 Hz), 4.01 (3 H, s), 4.78 (2 H, q, J = 9.1 Hz), 7.47-7.73 (3 H, m), 7.96 (1 H, s), 7.98-8.09 (2 H, m), 10.12 (1 H, s), 13.28 (1 H, br s).

Ethyl 2-chloro-4-ethylbenzoate (46)

A mixture of **45** (1.00 g, 3.79 mmol), tributyl(vinyl)tin (2.41 g, 7.59 mmol) and $Pd(PPh_3)_4$ (439 mg, 0.38 mmol) in DMF (15 mL) was stirred at 100 ºC for 1 h. After cooling, the mixture was diluted with water (150 mL), and extracted twice with AcOEt. The extracts were combined, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 20/1) to give ethyl 2-chloro-4-ethenylbenzoate (820 mg, 100%) as colorless oil. A mixture of ethyl 2-chloro-4-ethenylbenzoate (7.70 g, 36.55 mmol), 5% barium hydroxide on palladium (1.5 g) in AcOEt (150 mL) was stirred at room temperature for 8 h under H_2 atmosphere. The catalyst was filtered off, and the filtrate was concentrated *in vacuo* to give the title compound (7.61 g, 98%) as pale yellow liquid. ^{1}H NMR ($CDCl_3$) δ 1.24 (3 H, t, J = 7.5 Hz), 1.40 (3 H, t, J = 7.2 Hz), 2.65 (2 H, q, J = 7.5 Hz), 4.38 (2 H, q, J = 7.2 Hz), 7.13 (1 H, dd, J = 8.1, 1.2 Hz), 7.28 (1 H, d, J = 1.2 Hz), 7.76 (1 H, d, J = 8.1 Hz).

Ethyl 2-chloro-4-ethyl-5-nitrobenzoate (29d)

To a solution of **46** (3.0 g, 14.1 mmol) in conc. H_2SO_4 (10 mL) was added dropwise a solution of $NaNO_3$ (1.20 g, 14.1 mmol) in conc. H_2SO_4 (10 mL) at 0 ºC, and the mixture was stirred at 0 ºC for 30 min. The mixture was poured into water (300 mL) and extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$ and

concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 20/1) to give the title compound (2.56 g, 70%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.22 (3 H, t, J = 7.5 Hz), 1.34 (3 H, t, J = 7.1 Hz), 2.88 (2 H, q, J = 7.5 Hz), 4.36 (2 H, q, J = 7.1 Hz), 7.82 (1 H, s), 8.38 (1 H, s).

Ethyl 6-ethyl-3-hydroxy-1-methyl-5-nitro-1*H*-indole-2-carboxylate (27d)

A mixture of **29d** (1.2 g, 4.66 mmol), ethyl sarcosinate hydrochloride (2.86 g, 18.6 mmol) and Et_3N (3.77 g, 37.3 mmol) in EtOH (20 mL) was heated under reflux for 20 h. After cooling, the mixture was partitioned between HCl aq. and AcOEt. The organic layer was separated, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 3/1) to give the title compound (557 mg, 41%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.25 (3 H, t, J = 7.5 Hz), 1.33 (3 H, t, J = 7.1 Hz), 2.99 (2 H, q, J = 7.5 Hz), 3.90 (3 H, s), 4.33 (2 H, q, J = 7.1 Hz), 7.53 (1 H, s), 8.57 (1 H, s), 9.92 (1 H, br s).

Ethyl 5-amino-6-ethyl-1-methyl-3-(2,2,2-trifluoroethoxy)-1*H*-indole-2-carboxylate (47)

To a mixture of **27d** (150 mg, 0.51 mmol) and Cs_2CO_3 (200 mg, 0.62 mmol) in DMF (3 mL) was added 2,2,2-trifluoroethyl trifluoromethanesulfonate (131 mg, 0.56 mmol), and the mixture was stirred at room temperature for 1 h. The mixture was diluted with saturated $NaHCO_3$ aq. and extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 3/1) to give the ethyl 6-ethyl-1-methyl-5-nitro-3-(2,2,2-trifluoroethoxy)-1*H*-indole-2-carboxylate (190 mg, 100%) as a yellow solid. A mixture of obtained compound above (180 mg, 0.48 mmol) and 10% Pd-C (40 mg) in EtOH (10 mL) / THF (4 mL) was stirred at room temperature for 3 h under H_2 atmosphere. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to give the title compound (160 mg, 97%) as a pale brown solid. ^{1}H NMR (DMSO-d_6) δ 1.21 (3H, t, J = 7.4 Hz), 1.33 (3H, t, J = 7.1 Hz), 2.59 (2H, q, J = 7.4 Hz), 3.84 (3H, s), 4.31 (2H, q, J = 7.1 Hz), 4.61 (2H, q, J = 9.3 Hz), 4.67 (2H, s), 6.77 (1H, s), 7.16 (1H, s).

Ethyl 6-ethyl-1-methyl-5-[(phenylcarbonyl)amino]-3-(2,2,2-trifluoroethoxy)-1*H*-indole-2-carboxylate (48)

In the same manner as in the preparation of **44**, the title compound (89 mg, 86%) was obtained as a beige powder from **47** (80 mg, 0.23 mmol). ^{1}H NMR (DMSO-d_6) δ

1.20 (3H, t, *J* = 7.4 Hz), 1.36 (3H, t, *J* = 7.1 Hz), 2.74 (2H, q, *J* = 7.4 Hz), 3.96 (3H, s), 4.36 (2H, q, *J* = 7.1 Hz), 4.74 (2H, q, *J* = 9.0 Hz), 7.49-7.61 (5H, m), 8.01 (2H, d, *J* = 7.2 Hz), 9.97 (1H, s).

6-Ethyl-1-methyl-5-[(phenylcarbonyl)amino]-3-(2,2,2-trifluoroethoxy)-1*H*-indole -2-carboxylic acid (26d)

In the same manner as in the preparation of **26a**, the title compound (72 mg, 95%) was obtained as a white powder from **48** (80 mg, 0.18 mmol). ^{1}H NMR (DMSO-d_6) δ 1.20 (3 H, t, *J* = 7.5 Hz), 2.73 (2 H, q, *J* = 7.5 Hz), 3.96 (3 H, s), 4.72 (2 H, q, *J* = 9.1 Hz), 7.46-7.60 (5 H, m), 8.01 (2 H, d, *J* = 6.9 Hz), 9.96 (1 H, s), 13.20-13.40 (1H, br).

Ethyl 5-ethylpyridine-2-carboxylate (50)

To a solution of **49** (20.0 g, 86.9 mmol) in DMF (100 mL) were added tributyl(vinyl)tin (28.1 mL, 95.6 mmol) and $Pd(PPh_3)_4$ (2. 01 g, 1.74 mmol), and the mixture was stirred at 100 ºC for 2 h under Ar atmosphere. The mixture was diluted with water (200 mL), and extracted with AcOEt (400 mL). The extracts were combined, washed with brine (200 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 49/1 to 4/1) to give ethyl 5-ethenylpyridine-2-carboxylate (16.5 g, quant.) as pale yellow oil. To a solution of obtained compound above (7.71 g, 43.5 mmol) in EtOH (77 mL) was added 10% Pd-C (1.54 g) and the mixture was stirred at room temperature for 3 h under H_2 atmosphere. The mixture was filtered through membrane filter, and the filtrate was concentrated *in vacuo* to give the title compound (7.92 g, quant.) as pale yellow oil. ^{1}H NMR (DMSO-d_6) δ 1.22 (3H, t, *J* = 7.6 Hz), 1.33 (3H, t, *J* = 7.1 Hz), 2.71 (2H, q, *J* = 7.6 Hz), 4.33 (2H, q, *J* = 7.1 Hz), 7.83 (1H, dd, *J* = 8.0, 2.2 Hz), 7.98 (1H, d, *J* = 8.0 Hz), 8.58 (1H, d, *J* = 2.2 Hz).

Ethyl 5-ethyl-6-oxo-1,6-dihydropyridine-2-carboxylate (51)

To a solution of **50** (1.28 g, 7.14 mmol) in MeCN (13 mL) was added 3-chloroperoxybenzoic acid (2.84 g, 10.7 mmol), and the mixture was stirred at room temperature for 20 h. The mixture was concentrated to half volume, and diluted with AcOEt (50 mL). The solution was poured into saturated $NaHCO_3$ solution (100 mL), and extracted with AcOEt (200 mL). The organic layer was washed with brine (50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 9/1 to AcOEt) to give ethyl 5-ethylpyridine-2-carboxylate 1-oxide (1.15 g, 83%) as yellow oil. To a solution of

obtained compound in DMF (12 mL) was added trifluoroacetic anhydride (8.19 mL, 58.9 mmol), and the mixture was stirred at room temperature for 12 h under Ar atmosphere. The mixture was concentrated *in vacuo*, and diluted with water (50 mL). The mixture was extracted with AcOEt (100 mL). The organic layer was washed with brine (50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 19/1 to 1/1) to give the title compound (1.10 g, 96%) as yellow oil. ^{1}H NMR (DMSO-d_6) δ 1.11 (3H, t, J = 7.5 Hz), 1.30 (3H, t, J = 7.1 Hz), 2.41-2.49 (2H, m), 4.29 (2H, q, J = 7.1 Hz), 6.99 (1H, d, J = 7.0 Hz), 7.41 (1H, d, J = 7.0 Hz), 11.53 (1H, br s).

Ethyl 6-(benzyloxy)-3-bromo-5-ethylpyridine-2-carboxylate (29e)

To a solution of **51** (1.10 g, 5.63 mmol) in DMF (11 mL) was added *N*-bromosuccinimide (1.30 g, 7.33 mmol), and the mixture was stirred at room temperature for 1 h. The mixture was diluted with water (50 mL), and the resulting precipitate was collected by filtration to give ethyl 3-bromo-5-ethyl-6-oxo-1,6-dihydropyridine-2-carboxylate (1.21 g, 79%) as a white solid. To a solution of obtained bromide (17.1 g, 62.3 mmol) in toluene (342 mL) were added Ag_2CO_3 (29.2 g, 106 mmol) and benzyl bromide (14.8 mL, 125 mmol), and the mixture was stirred at 40 ºC for 2 h. The insoluble material was filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane to hexane/AcOEt = 47/3) to give the title compound (22.4 g, 99%) as yellow oil. ^{1}H NMR (DMSO-d_6) δ 1.14 (3H, t, J = 7.4 Hz), 1.34 (3H, t, J = 7.0 Hz), 2.60 (2H, q, J = 7.4 Hz), 4.36 (2H, q, J = 7.0 Hz), 5.35 (2H, s), 7.31-7.43 (3H, m), 7.45-7.53 (2H, m), 7.92 (1H, s).

Ethyl 3-amino-6-(benzyloxy)-5-ethylpyridine-2-carboxylate (52)

In the same manner as in the preparation of **43**, the title compound (17.8 g, 98%) was obtained as yellow oil from **29e** (22.0 g, 60.4 mmol). ^{1}H NMR (DMSO-d_6) δ 1.12 (3H, t, J = 7.5 Hz), 1.33 (3H, t, J = 7.1 Hz), 2.45-2.58 (2H, m), 4.27 (2H, q, J = 7.1 Hz), 5.26 (2H, s), 6.33 (2H, s), 7.08 (1H, s), 7.27-7.40 (3H, m), 7.48-7.57 (2H, m).

Ethyl 6-(benzyloxy)-3-[(2-ethoxy-2-oxoethyl)(methyl)amino]-5-ethylpyridine-2-carboxylate (28e)

To a solution of **52** (771 mg, 2.57 mmol) in *tert*-butanol (7.7 mL) was added di-*tert*-butyl dicarbonate (1.77 mL, 7.71 mmol), and the mixture was stirred at 90 ºC for 18 h. The mixture was concentrated *in vacuo*, and the residue was dissolved in DMF (10

mL). To the solution were added Cs_2CO_3 (2.93 g, 9.00 mmol) and iodomethane (480 μL, 7.71 mmol), and the mixture was stirred at room temperature for 4 h. The mixture was diluted with water (50 mL), and extracted with AcOEt (100 mL). The organic layer was washed with brine (50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. To this residue was added 4M HCl/AcOEt (5.4 mL), and the mixture was stirred at 0 ºC for 2 h. The mixture was diluted with saturated $NaHCO_3$ aq. (50 mL) and extracted with AcOEt (100 mL). The organic layer was washed with brine (50 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (hexane/AcOEt = 49/1 to 47/3) to give ethyl 6-(benzyloxy)-5-ethyl-3-(methylamino)pyridine-2-carboxylate (705 mg, 87%) as yellow oil. To a solution of the compound obtained above in DMF (14 mL) were added diisopropylethylamine (2.35 mL, 13.5 mmol) and ethyl bromoacetate (1.49 mL, 13.5 mmol), and the mixture was stirred at 110 ºC for 24 h. The mixture was diluted with water (50 mL), and extracted with AcOEt (100 mL). The organic layer was washed with brine (50 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane to hexane/AcOEt = 4/1) to give the title compound (711 mg, 79%) as yellow oil. ^{1}H NMR (DMSO-d_6) δ 1.09-1.21 (6H, m), 1.30 (3H, t, J = 7.1 Hz), 2.57 (2H, q, J = 7.4 Hz), 2.81 (3H, s), 3.88 (2H, s), 4.07 (2H, q, J = 7.1 Hz), 4.28 (2H, q, J = 7.1 Hz), 5.28 (2H, s), 7.26-7.42 (3H, m), 7.42-7.51 (3H, m).

Ethyl 5-(benzyloxy)-6-ethyl-3-hydroxy-1-methyl-1*H*-pyrrolo[3,2-*b*]pyridine-2-carboxylate (27e)

To a solution of **28e** (711 mg, 1.78 mmol) in EtOH (14 mL) was added 20% solution of NaOEt in EtOH (1.42 mL), and the mixture was stirred at room temperature for 1 h. The mixture was concentrated *in vacuo*, acidified with 1M HCl aq., and extracted with AcOEt (100 mL). The organic layer was washed with brine (50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The precipitate was collected by filtration, and washed with IPE to give the title compound (485 mg, 77%) as a pale orange powder. ^{1}H NMR (DMSO-d_6) δ 1.22 (3H, t, J = 7.4 Hz), 1.34 (3H, t, J = 7.1 Hz), 2.69 (2H, q, J = 7.4 Hz), 3.84 (3H, s), 4.35 (2H, q, J = 7.1 Hz), 5.44 (2H, s), 7.27-7.44 (3H, m), 7.46-7.56 (2H, m), 7.78 (1H, s), 8.84 (1H, s).

Ethyl 6-ethyl-1-methyl-3-(2,2,2-trifluoroethoxy)-5-{[(trifluoromethyl)sulfonyl]oxy}-1*H*-pyrrolo[3,2-*b*]pyridine-2-carboxylate (53)

To a solution of **27e** (485 mg, 1.37 mmol) in DMF (4.9 mL) were added Cs_2CO_3 (534 mg, 1.64 mmol) and 2,2,2-trifluoroethyl trifluoromethanesulfonate (217 μL, 1.51

mmol), and the mixture was stirred at room temperature for 1 h. The mixture was diluted with water (20 mL), and the precipitate was collected by filtration, washed with water and IPE to give ethyl 5-(benzyloxy)-6-ethyl-1-methyl-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo[3,2-*b*]pyridine-2-carboxylate (580 mg, 97%) as a pale orange powder. To a solution of obtained powder (428 mg, 0.981 mmol) in EtOH (0.87 mL) / THF (0.43 mL) was added 10% Pd-C (128 mg), and the mixture was stirred at room temperature for 1 h under H_2 atmosphere. The mixture was filtered through membrane filter, and the filtrate was concentrated *in vacuo*. The resulting solid was collected by filtration to give ethyl 6-ethyl-1-methyl-5-oxo-3-(2,2,2-trifluoroethoxy)-4,5-dihydro-1*H*-pyrrolo[3,2-*b*]pyridin e -2-carboxylate (330 mg, 97%) as a white solid. To a solution of obtained solid (330 mg, 0.953 mmol) in pyridine (9.9 mL) was added Tf_2O (384 μL, 2.28 mmol) at 0 ºC, and the mixture was stirred at 60 ºC for 16 h under N_2 atmosphere. The mixture was concentrated *in vacuo*, the residue was diluted with saturated $NaHCO_3$ aq. (50 mL), and extracted with AcOEt (100 mL). The organic layer was washed with brine (50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The resulting solid was collected by filtration, and washed with hexane to give the title compound (418 mg, 92%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6) δ 1.24-1.39 (6H, m), 2.79 (2H, q, J = 7.6 Hz), 3.99 (3H, s), 4.36 (2H, q, J = 7.2 Hz), 5.05 (2H, q, J = 8.9 Hz), 8.35 (1H, s).

Ethyl 5-amino-6-ethyl-1-methyl-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo[3,2-*b*] pyridine-2-carboxylate (54)

In the same manner as in the preparation of **43**, the title compound (141 g, 90%) was obtained as a white powder from **53** (218 mg, 0.456 mmol). ^{1}H NMR (DMSO-d_6) δ 1.20 (3H, t, J = 7.5 Hz), 1.29 (3H, t, J = 7.1 Hz), 2.51-2.61 (2H, m), 3.83 (3H, s), 4.26 (2H, q, J = 7.1 Hz), 5.10 (2H, q, J = 9.1 Hz), 5.75 (2H, s), 7.55 (1H, s).

6-Ethyl-1-methyl-5-[(phenylcarbonyl)amino]-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo [3,2-*b*]pyridine-2-carboxylic acid (26e)

To a solution of **54** (141 mg, 0.408 mmol) in THF (1.4 mL) were added pyridine (85.8 μL, 1.06 mmol) and benzoyl chloride (104 μL, 0.898 mmol), and the mixture was stirred at 50 ºC for 2 h. The mixture was diluted with water (30 mL) and extracted with AcOEt (50 mL). The organic layer was washed with brine (30 mL), dried over Na_2SO_4 and concentrated *in vacuo*. To a solution of the residue in EtOH (3.7 mL) was added aqueous 8M NaOH aq. (0.366 mL), and the mixture was stirred at 50 ºC for 1 h. The mixture was concentrated *in vacuo*, the precipitate was diluted with water (20 mL) and

acidified with aqueous 1M HCl aq.. The resulting precipitate was collected by filtration, and washed with water to give the title compound (152 mg, 88%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.23 (3H, t, J = 7.5 Hz), 2.71 (2H, q, J = 7.5 Hz), 3.97 (3H, s), 5.10 (2H, q, J = 9.1 Hz), 7.49-7.65 (3H, m), 7.97-8.06 (3H, m), 10.56 (1H, s), 13.17 (1H, br s).

Ethyl 5-ethyl-3-hydroxypyrazine-2-carboxylate (56)

To a suspension of **55** (6.40 g, 39.7 mmol) in EtOH (60 mL) was added diisopropylethylamine (14 mL, 80.4 mmol), and the mixture was stirred at room temperature for 10 min. To this solution was added dropwise diethyl ketomalonate (6 mL, 39.3 mmol) at 0 °C, and the mixture was stirred at room temperature for 1.5 h and with heating under reflux for 20 h. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in a small amount of EtOH. The solution was passed through silica gel (hexane/AcOEt = 1/1 to AcOEt). The eluate was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (hexane/AcOEt = 2/1 to AcOEt) to give the title compound (1.94 g, 25%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6) δ 1.19 (3 H, t, J = 7.6 Hz), 1.27 (3 H, t, J = 7.2 Hz), 2.53-2.62 (2 H, m), 4.26 (2 H, q, J = 7.2 Hz), 7.42 (1 H, br s), 12.68 (1 H, br s).

Ethyl 3-(benzyloxy)-6-bromo-5-ethylpyrazine-2-carboxylate (57)

To a solution of **56** (3.75 g, 19.1 mmol) in DMF (40 mL) was added *N*-bromosuccinimide (3.43 g, 19.3 mmol) at 0 °C. The mixture was diluted with water (80 mL), and the precipitate was collected by filtration washed with water to give ethyl 6-bromo-5-ethyl-3-hydroxypyrazine-2-carboxylate. The filtrate was extracted with Et_2O/AcOEt =1/1 solution, the extracts were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 99/1 to 4/1) to give ethyl 6-bromo-5-ethyl-3-hydroxypyrazine-2-carboxylate. To a solution of the obtained compound above (750 mg, 2.73 mmol) in toluene (10 mL) were added Ag_2CO_3 (1.05 g, 3.81 mmol) and benzyl bromide (390 μL, 3.28 mmol) at 0 °C, the mixture was stirred at room temperature for 3 h. The insoluble material was removed by filtration through celite pad, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 99/1 to 4/1) to give the title compound (908 mg, 91%) as colorless oil. ^{1}H NMR (DMSO-d_6) δ 1.25 (3 H, t, J = 7.5 Hz), 1.29 (3 H, t, J = 7.1 Hz), 2.89 (2 H, q, J = 7.5 Hz), 4.33 (2 H, q, J = 7.1 Hz), 5.50 (2 H, s), 7.28-7.44 (3 H, m), 7.44-7.56 (2 H, m).

Ethyl 3-(benzyloxy)-5-ethyl-6-[(phenylcarbonyl)amino]pyrazine-2-carboxylate (58)

To a mixture of **57** (906 mg, 2.48 mmol), benzophenoneimine (624 μL, 3.72 mmol), Cs_2CO_3 (1.62 g, 4.97 mmol) in toluene (13 mL) were added xantphos (294 mg, 0.508 mmol) and $Pd_2(dba)_3$ (228 mg, 0.249 mmol), and the mixture was stirred at 100 °C for 14 h. Benzophenoneimine (416 μL, 2.48 mmol), $Pd_2(dba)_3$ (120 mg, 0.131 mmol) and xantphos (148 mg, 0.256 mmol) were further added, the mixture was stirred at 100 °C for 3.5 h. The mixture was filtered through celite pad using AcOEt, and the filtrate was washed with water and brine. The organic layer was dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in THF (10 mL) /EtOH (2 mL), and 2M HCl aq. (3 mL) was added. After stirring for 3h, the mixture was quenched with aqueous $NaHCO_3$ solution, and extracted with AcOEt. The extracts were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 19/1 to 1/2) to give ethyl 6-amino-3-(benzyloxy)-5-ethylpyrazine-2-carboxylate (441 mg, 59%) as a brown powder. To a solution of obtained product (250 mg, 0.828 mmol) in THF (3 mL) were added pyridine (100 μL, 1.24 mmol) and benzoyl chloride (106 μL, 0.913 mmol) at 0 °C, and the mixture was stirred at 0 °C for 2.5 h. Benzoyl chloride (53 μL, 0.457 mmol) was added, and the mixture was stirred at 0 °C for 1 h. Further benzoyl chloride (53 μL, 0.457 mmol) and pyridine (55 μL, 0.680 mmol) were added, the mixture was stirred at 0 °C for 1 h. The mixture was quenched with aqueous $NaHCO_3$ solution, and the mixture was extracted with AcOEt. The organic layer was dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 19/1 to 1/1) to give the title compound (312 mg, 93%) as an orange oil. ^{1}H NMR (DMSO-d_6) δ 1.22 (3 H, t, J = 7.5 Hz), 1.28 (3 H, t, J = 7.1 Hz), 2.73 (2 H, q, J = 7.5 Hz), 4.33 (2 H, q, J = 7.1 Hz), 5.55 (2 H, s), 7.28-7.47 (3 H, m), 7.47-7.71 (5 H, m), 7.95-8.09 (2 H, m), 10.76 (1 H, s).

Ethyl 5-ethyl-6-[(phenylcarbonyl)amino]-3-{[(trifluoromethyl)sulfonyl]oxy} pyrazine-2-carboxylate (29f)

To a solution of **58** (310 mg, 0.765 mmol) in EtOH (5 mL) was added 10% Pd-C (32.7 mg), and the mixture was stirred at room temperature for 3 h under H_2 atmosphere. The catalyst was removed by filtration through celite pad, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 9/1 to 1/4) to give ethyl 5-ethyl-3-hydroxy-6-[(phenylcarbonyl)amino]pyrazine-2-carboxylate (185 mg, 77%) as a pale yellow powder. To a solution of the compound obtained above (183 mg, 0.580

mmol) in pyridine (2 mL) was added dropwise Tf_2O (147 μL, 0.871 mmol) at 0 °C, and the mixture was stirred at 0 °C for 1 h. Further Tf_2O (30 μL, 0.178 mmol) was added, and the mixture was stirred at 0 °C for 1 h. The mixture was diluted with water (15 mL), and the mixture was extracted three times with AcOEt (15 mL). The extracts were combined, washed with water (20 mL) and brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 49/1 to 1/1) to give the title compound (244 mg, 94%) as yellow oil. ^{1}H NMR (DMSO-d_6) δ 1.21 (3 H, t, J = 7.3 Hz), 1.34 (3 H, t, J = 7.1 Hz), 2.83 (2 H, q, J = 7.3 Hz), 4.43 (2 H, q, J = 7.1 Hz), 7.49-7.74 (3 H, m), 7.99-8.10 (2 H, m), 11.42 (1 H, s).

Ethyl 3-ethyl-7-hydroxy-5-methyl-2-[(phenylcarbonyl)amino]-5*H*-pyrrolo[2,3-*b*] pyrazine-6-carboxylate (27f)

In the same manner as in the preparation of **27b**, the title compound (159 mg, 80%) was obtained as a pale yellow powder from **29f** (242 mg, 0.541 mmol). ^{1}H NMR (DMSO-d_6) δ 1.28 (3 H, t, J = 7.6 Hz), 1.35 (3 H, t, J = 7.2 Hz), 2.88 (2 H, q, J = 7.6 Hz), 3.95 (3 H, s), 4.37 (2 H, q, J = 7.2 Hz), 7.45-7.72 (3 H, m), 7.94-8.12 (2 H, m), 9.91 (1 H, br s), 10.72 (1 H, s).

3-Ethyl-5-methyl-2-[(phenylcarbonyl)amino]-7-(2,2,2-trifluoroethoxy)-5*H*-pyrrolo [2,3-*b*]pyrazine-6-carboxylic acid (26f)

To a solution of **27f** (59.2 mg, 0.161 mmol) in DMF were added Cs_2CO_3 (72.0 mg, 0.220 mmol) and 2,2,2-trifluoroethyl trifluoromethanesulfonate (28 μL, 0.166 mmol) at 0 °C, and the mixture was stirred at 0 °C for 2 h. The mixture was diluted with water, and the precipitate was collected by filtration. The collection was dissolved in hexane/AcOEt, and purified by silica gel column chromatography (hexane/AcOEt = 49/1 to 2/1) to give ethyl 3-ethyl-5-methyl-2-[(phenylcarbonyl)amino]-7-(2,2,2-trifluoroethoxy)-5*H*-pyrrolo[2,3-*b*]pyrazine-6-carboxylate (53.2 mg, 74%) as a pale yellow powder. To a solution of obtained compound above (107 mg, 0.238 mmol) in THF (1 mL) / EtOH (2 mL) was added 1M NaOH aq. (0.5 mL), and the mixture was stirred at room temperature for overnight. The mixture was acidified with 1N HCl aq., and diluted with water. The precipitate was collected by filtration using water to give the title compound (99.6 mg, 99%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6) δ 1.29 (3 H, t, J = 7.4 Hz), 2.89 (2 H, q, J = 7.4 Hz), 4.01 (3 H, s), 5.10 (2 H, q, J = 8.9 Hz), 7.45-7.72 (3 H, m), 7.96-8.10 (2 H, m), 10.82 (1 H, s), 13.47 (1 H, br s).

2-Ethyl-*N*-[1-(hydroxyacetyl)piperidin-4-yl]-7-methyl-4-oxo-5-(2,2,2-trifluoro-ethoxy)-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxamide (25a)

To a mixture of **26a** (1.0 g, 3.1 mmol), 2-(4-aminopiperidin-1-yl)-2-oxoethanol hydrochloride (732 mg, 3.7 mmol) and HOBt (635 mg, 4.7 mmol) in DMF (15 mL) were added EDC (900 mg, 4.7 mmol) and Et_3N (1.2 mL, 8.5 mmol) at 0 °C, and the mixture was stirred at room temperature for 4.5 h. The reaction mixture was diluted with saturated $NaHCO_3$ aq., and the precipitated solid was collected by filtration. The solid was washed with water, and dried *in vacuo* to give the title compound (1.1 g, 79%) as a white powder. ^{1}H NMR (DMSO-d_6) δ 1.13-1.52 (5 H, m), 1.76-1.98 (2 H, m), 2.63 (2 H, q, J = 7.5 Hz), 2.75-2.95 (1 H, m), 3.00-3.19 (1 H, m), 3.61-3.75 (1H, m), 3.83 (3H, s), 3.93-4.17 (3H, m), 4.17-4.34 (1H, m), 4.53 (1 H, t, J = 5.5 Hz), 5.20 (2 H, q, J = 9.1 Hz), 7.43 (1 H, d, J = 7.7 Hz), 12.10 (1 H, s).

The following compounds **25b-f** were prepared in a same manner similar to that described for **25a**.

6-Ethyl-*N*-[1-(hydroxyacetyl)piperidin-4-yl]-1-methyl-5-(2-oxo-2-phenylethyl)-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxamide (25b)

Yield 47%, white crystals. mp. 150 ºC (recrystallized from hexane/AcOEt) ^{1}H NMR (DMSO-d_6) δ 1.22 (3H, t, J = 7.4 Hz), 1.28-1.58 (2H, m), 1.82-2.02 (2H, m), 2.70 (2H, q, J = 7.2 Hz), 2.78-2.94 (1H, m), 3.02-3.21 (1H, m), 3.60-3.78 (1H, m), 3.93 (3H, s), 4.00-4.19 (3H, m), 4.20-4.36 (1H, m), 4.53 (1H, t, J = 5.4 Hz), 4.63 (2H, s), 4.91 (2H, q, J = 8.9 Hz), 7.52-7.64 (2H, m), 7.64-7.74 (1H, m), 7.79 (1H, d, J = 7.7 Hz), 8.03 (1H, s), 8.11 (2H, d, J = 7.4 Hz). Anal. Calcd for $C_{28}H_{31}N_4O_5F_3$: C, 59.99; H, 5.57; N, 9.99. Found: C, 59.75; H, 5.51; N, 9.81. LC-MS: m/z = 561 (MH^+).

6-Ethyl-*N*-[1-(hydroxyacetyl)piperidin-4-yl]-1-methyl-5-[(phenylcarbonyl)amino]-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxamide (25c)

Yield 53%, white crystals. mp. 200 ºC (recrystallized from hexane/AcOEt). ^{1}H NMR (DMSO-d_6) δ 1.24 (3H, t, J = 7.5 Hz), 1.32-1.55 (2H, m), 1.84-1.99 (2H, m), 2.79-2.92 (1H, m), 2.86 (2H, q, J = 7.5 Hz), 3.02-3.19 (1H, m), 3.63-3.78 (1H, m), 3.94 (3H, s), 4.00-4.18 (1H, m), 4.10 (2H, t, J = 5.7 Hz), 4.21-4.37 (1H, m), 4.53 (1H, t, J = 5.5 Hz), 4.94 (2H, q, J = 8.8 Hz), 7.46-7.71 (3H, m), 7.86 (1H, d, J = 7.6 Hz), 8.03 (2H, d, J = 6.8 Hz), 8.13 (1H, s), 10.11 (1H, s). Anal. Calcd for $C_{27}H_{30}N_5O_5F_3$: C,57.75; H,5.38; N,12.47. Found: C,58.03; H,5.47; N,12.44. LC-MS: m/z = 562 (MH^+).

6-Ethyl-*N*-[1-(hydroxyacetyl)piperidin-4-yl]-1-methyl-5-[(phenylcarbonyl)amino] -3-(2,2,2-trifluoroethoxy)-1*H*-indole-2-carboxamide (25d)

Yield 82%, white crystals. mp. 227 ºC (recrystallized from hexane/AcOEt). ^{1}H NMR (DMSO-d_6) δ 1.20 (3H, t, J = 7.5 Hz), 1.30-1.50 (2H, m), 1.91 (2H, d, J = 9.9 Hz), 2.72 (2H, q, J = 7.5 Hz), 2.82 (1H, t, J = 11.9 Hz), 3.10 (1H, t, J = 11.9 Hz), 3.71 (1H, d, J = 12.9 Hz), 3.89 (3H, s), 4.04-4.12 (3H, m), 4.30 (1H, d, J = 13.8 Hz), 4.54 (1H, t, J = 5.4 Hz), 4.85 (2H, q, J = 8.9 Hz), 7.44 (1H, s), 7.51-7.62 (4H, m), 7.84 (1H, d, J = 7.5 Hz), 8.01 (2H, d, J = 6.9 Hz), 9.96 (1H, s). Anal. Calcd for $C_{28}H_{31}N_4O_5F_3$: C; 59.99, H; 5.57, N; 9.99. Found: C; 59.79, H; 5.71, N; 9.73. LC-MS: m/z = 561 (MH^+).

6-Ethyl-*N*-[1-(hydroxyacetyl)piperidin-4-yl]-1-methyl-5-[(phenylcarbonyl)amino] -3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo[3,2-*b*]pyridine-2-carboxamide (25e)

Yield 46%, white crystals. mp. 165 ºC (recrystallized from hexane/AcOEt). ^{1}H NMR (DMSO-d_6) δ 1.23 (3H, t, J = 7.5 Hz), 1.31-1.53 (2H, m), 1.84-1.96 (2H, m), 2.71 (2H, q, J = 7.5 Hz), 2.80-2.96 (1H, m), 3.04-3.20 (1H, m), 3.62-3.77 (1H, m), 3.94 (3H, s), 4.02-4.16 (3H, m), 4.19-4.30 (1H, m), 4.54 (1H, t, J = 5.5 Hz), 5.23 (2H, q, J = 8.9 Hz), 7.47-7.65 (3H, m), 7.80 (1H, d, J = 7.7 Hz), 7.95-8.05 (3H, m), 10.52 (1H, s). Anal. Calcd for $C_{27}H_{30}F_3N_5O_5$•0.30H_2O: C, 57.20; H, 5.44; N, 12.35. Found: C, 57.32; H, 5.58; N, 12.12. LC-MS: m/z = 562 (MH^+).

3-Ethyl-*N*-[1-(hydroxyacetyl)piperidin-4-yl]-5-methyl-2-[(phenylcarbonyl)amino] -7-(2,2,2-trifluoroethoxy)-5*H*-pyrrolo[2,3-*b*]pyrazine-6-carboxamide (25f)

Yield 61%, pale yellow crystals. mp. 142 ºC (recrystallized from hexane/AcOEt). ^{1}H NMR (DMSO-d_6) δ 1.28 (3H, t, J = 7.5 Hz), 1.33-1.58 (2H, m), 1.84-1.99 (2H, m), 2.81-2.98 (3H, m), 3.06-3.21 (1H, m), 3.62-3.76 (1H, m), 3.97 (3H, s), 4.01-4.18 (3H, m), 4.18-4.32 (1H, m), 4.54 (1H, t, J = 5.5 Hz), 5.20 (2H, q, J = 8.9 Hz), 7.49-7.69 (3H, m), 7.95 (1H, d, J = 7.6 Hz), 7.99-8.10 (2H, m), 10.79 (1H, s). Anal. Calcd for $C_{26}H_{29}N_6O_5F_3$•H_2O: C, 54.64; H, 5.29; N, 14.70. Found: C, 55.02; H, 5.18; N, 14.81. LC-MS: m/z = 563 (MH^+).

2-Ethyl-*N*-[1-(hydroxyacetyl)piperidin-4-yl]-7-methyl-4-oxo-3-(2-oxo-2-phenylethyl)-5-(2,2,2-trifluoroethoxy)-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxamide (25g)

A mixture of **25a** (300 mg, 0.65 mmol), K_2CO_3 (135 mg, 0.98 mmol), DMF (1.0 mL) / DME (4.0 mL) was stirred at 0 °C for 30 min. LiBr (114 mg, 1.3 mmol) was added, and the mixture was stirred at room temperature for 30 min. Phenacyl bromide

(260 mg, 1.3 mmol) was added, and the reaction mixture was stirred at 60 ºC for 13.5 h followed by addition of DMF (1.0 mL). The mixture was stirred at 60 ºC for 1.5 h, at 70 ºC for 2.5 h, and at 80 ºC for 20 h. The mixture was diluted with water, and extracted twice with AcOEt. The extracts were combined, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt = 2/3 to AcOEt) and then aminosilica gel column chromatography (hexane/AcOEt = 2/3 to AcOEt), and recrystallized from hexane/AcOEt to give the title compound (53 mg, 14%) as white crystals. mp 154 °C. ^{1}H NMR (DMSO-d_6) δ 1.16-1.55 (5 H, m), 1.77-2.00 (2 H, m), 2.66-2.93 (3 H, m), 3.03-3.20 (1 H, m), 3.60-3.78 (1 H, m), 3.83-3.95 (3 H, m), 3.97-4.16 (3 H, m), 4.16-4.35 (1 H, m), 4.53 (1 H, t, J = 5.5 Hz), 5.09 (2 H, q, J = 9.1 Hz), 5.73 (2 H, s), 7.50 (1 H, d, J = 7.7 Hz), 7.56-7.68 (2 H, m), 7.69-7.83 (1 H, m), 8.12 (2 H, d, J = 7.2 Hz). Anal. Calcd for $C_{27}H_{30}F_3N_5O_6$: C; 56.15, H; 5.24, N; 12.13. Found: C; 55.87, H; 5.28, N; 11.98. LC-MS: m/z = 578 (MH^+).

Assay protocols

Gli-luciferase assay

NIH3T3/Gli-luc cells were maintained in DMEM containing 10% FBS, 500-μg/mL G418, and 0.1% gentamicin solution (Invitrogen Corp [Carlsbad, CA, USA]). The cells were plated onto collagen-coated 384-well plates at 7.5 x 10^3cells/well and cultured overnight in 25 μL of DMEM containing 10% FBS under 5% CO_2 at 37 °C. After incubation, 20 μL of recombinant mouse Shh-N (2.5 μg/mL in DMEM containing 2% FBS) and 5 μL of a serially diluted the compounds 10 x solution (0.0003 to 10 μM in DMEM) were added to the culture to achieve the final concentrations of 5.8% FBS, 1-μg/mL of Shh-N, and 0.03 to 1000 nM of the compounds (n = 4 wells per concentration). The cells were then incubated for an additional 48 hours. To determine the window of the assay, the cells were incubated in the media containing 0.1% DMSO with or without 1 μg/mL Shh-N (0% or 100% inhibition control, respectively (n = 10 wells). The luciferase activities of reporter cells were measured by Bright-Glo™ (Promega Corp [Madison, WI, USA]) using the EnVision® plate leader (PerkinElmer, Inc [Waltham, MA, USA]).

Smo binding assay

293T cells were transfected with pCMV-HA/hSmo using Lipofectamine reagent (Lipofectamine™ 2000, Invitrogen). In brief, 45 μL of Lipofectamine 2000 and 22.5 μg of pCMV-HA/hSmo were each incubated in 1.5 mL of Opti-MEM I media (Invitrogen) at room temperature for 5 minutes and were mixed together. The mixture was incubated for 20 minutes at room temperature after which it was added to 6 x 10^6 293T cells in 15 mL of culture medium in a 75 cm^2 cell culture flask. After overnight incubation under 5% CO_2 at 37 °C, cells were detached using Versene (Invitrogen) and resuspended in culture medium. The suspended cells were added to a 96-well plate at 6 x 10^4 cells/well. After removing the media from each well, 25 μL of a 2× stock solution of the compounds and 25 μL of BODIPY-cyclopamine (4 nM in DMEM containing 1% FBS) were added to the culture which was incubated for 1 h (n = 3 wells per concentration). Final concentrations were 0.03–1000 nM for the compounds and 2 nM for BODIPY-cyclopamine. To define the assay window, 25 μL of media or 25 μL of 2× cyclopamine solution (final concentration 1 μM) was added to the culture in addition to 25 μL of BODIPY cyclopamine to serve as 0% or 100% inhibition controls (n = 5 wells), respectively. After incubation, cells were detached using Versene and were resuspended in PBS supplemented with 2% FBS. Cyclopamine binding

competition with the compounds was determined by measuring the intensity of cell fluorescence using the Guava easyCyte System (Millipore Corp, Billerica, MA, USA).

***In vivo* pharmacodynamic assay**

In vivo PD assay was conducted by using the nude mice bearing human primary pancreatic tumor (PAN-04). The tumor line was established by Central Institute for Experimental Animals. The test compounds were orally administered twice a day. After 24 h from the first administration, the tumors were extirpated and treated with RNAlater (Ambion). Total RNA samples were isolated using RNeasy Mini kit (Qiagen), and the first strand cDNA samples were prepared using the high capacity cDNA transcription kit (Applied Biosystems). The primer sets ofqPCR for quantification of stromal Gli1 mRNA were as follows (Applied Biosystems): Mm00494645_m1 (mouse Gli1), 4352339E (mouse GAPDH).

***In vivo* anti-tumor test**

Anti-tumor effects of compounds were evaluated using a mouse medulloblastoma allogeneic transplantation model[29] in which the medulloblastoma spontaneously occurred in the cerebellum of 7–9-week-old Patch 1 (+/-) and p53 (-/-) double mutant mice. Patch 1 gene mutant mice (Ptch1tm1Mps/J) were purchased from The Jackson Laboratory (Bar Harbor, ME) and p53 gene mutant mice (P53N4-M, B6.129-Trp53tm/BrdN4) were purchased from Taconic (Hudson, NY). Medulloblastoma tumors were subcutaneously transplanted into nude mice from Charles river laboratories (Yokohama, Japan; CAnN.Cg-Foxn1nu/CrlCrlj), and allograft tumors, following several serial passages *in vivo*, were used for compound testing. To examine the anti-tumor activity of compound **22d**, animals bearing tumors with an average size of 150-250 mm^3 were treated with compound **22d** (0.5% methylcellulose suspension) twice daily for 2 weeks. The tumor size was measured with an electronic vernier caliper, and tumor volume was calculated based on the longest (a) and shortest (b) tumor dimensions using the formula $V = (a \times b^2)/2$. The tumor growth rate (T/C %) was calculated as the mean values for [Treated $(V_{end}-V_{start})$/Control $(V_{end}-V_{start})$]×100.

***In vitro* metabolism with hepatic microsomes**

Metabolic stability was evaluated both in mouse and human microsomes and used according to the manufacturer's instructions. Liver microsomes were purchased from Xenotech, LLC, Lenexa, KS, USA. An incubation mixture consisted of microsomes in 50 mmol/L of KH_2PO_4/K_2HPO_4 phosphate buffer (pH 7.4) and 1 μmol/L of test

compound. The concentration of microsomes was 0.2 mg protein/mL. An NADPH-generating system containing 25 mmol/L $MgCl_2$, 25 mmol/L glucose-6-phosphate, 2.5 mmol/L beta-NADP+, and 7.5 unit/mL glucose-6-phosphate dehydrogenase was added to the incubation mixture with a 20 % volume of the reaction mixture to initiate the enzyme reaction. After the addition of the NADPH-generating system, the mixture was incubated at 37 °C. The reaction was terminated by the addition of MeCN equivalent to the volume of the reaction mixture. Test compounds in the reaction mixtures were quantified by HPLC equipped with a UV detector or LC-MS/MS. To determine metabolic stabilities, chromatograms were analyzed for parent compound disappearance from the reaction mixtures.

Pharmacokinetic studies in mice

Test compounds were administered at a dose of 10 mg/kg as a cassette dosing to nonfasted mice. After the oral administration, blood samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with MeCN containing an internal standard. After centrifugation, the supernatant was diluted with a mixture of 10 mmol/L $HCOONH_4$ containing 0.2% formic acid and MeCN containing 0.2% formic acid (9:1, v/v), and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

Pharmacokinetic studies in rats and dogs

Compounds **22d** was administered to fed rats and dogs. Plasma samples were collected after oral (10 mg/kg) and intravenous (1 mg/kg) administration and were deproteinized with methanol containing an internal standard. After centrifugation, the supernatant was diluted with a mixture of 0.01 mol/L ammonium formate solution and [MeCN/formic acid (100:0.2, v/v)] (7:3, v/v) and centrifuged again. The compound concentrations in the supernatant were measured by LC-MS/MS.

X-ray structure analysis

Crystal data for **22d**: $C_{28}H_{31}F_3N_4O_6 \cdot 0.1H_2O$, MW = 578.37; crystal size, 0.41 x 0.20 x 0.14 mm; colorless, prism; monoclinic, space group $P2_1/c$, a = 11.6375(2) Å, b = 18.6948(3) Å, c = 25.8181(5) Å, $\alpha = \gamma = 90°$, β = 100.8640(7)°, V = 5516.33(17) Å^3, Z = 8, Dx = 1.393 g/cm^3, T = 100 K, μ = 0.956 mm^{-1}, λ = 1.54187 Å, R_1 = 0.064, wR_2 = 0.181.

Crystal data for **25b**: $C_{28}H_{31}F_3N_4O_5 \cdot 2H_2O$, MW = 596.60; crystal size, 0.22 x 0.09 x 0.08 mm; colorless, block; triclinic, space group P-1, a = 9.88809(18) Å, b =

11.9889(3) Å, c = 13.0792(3) Å, α = 112.612(8)°, β = 100.466(7)°, γ = 91.845(7)°, V = 1398.57(11) Å^3, Z = 2, Dx = 1.417 g/cm^3, T = 100 K, μ = 0.982 mm^{-1}, λ = 1.54187 Å, R_1 = 0.041, wR_2 = 0.095.

Crystal data for **25d**: $C_{28}H_{31}F_3N_4O_5$, MW = 560.57; crystal size, 0.17 x 0.13 x 0.04 mm; colorless, platelet; triclinic, space group P-1, a = 9.29308(17) Å, b = 9.62000(17) Å, c = 16.0576(3) Å, α = 75.445(6)°, β = 81.749(6)°, γ = 74.270(6)°, V = 1333.05(7) Å^3, Z = 2, Dx = 1.396 g/cm^3, T = 100 K, μ = 0.938 mm^{-1}, λ = 1.54187 Å, R_1 = 0.043, wR_2 = 0.111.

Crystal data for **25e**: $C_{27}H_{30}F_3N_5O_5$, MW = 561.56; crystal size, 0.16 x 0.08 x 0.03 mm; colorless, platelet; triclinic, space group P-1, a = 9.2592(3) Å, b = 9.6488(3) Å, c = 16.0297(4) Å, α = 76.213(6)°, β = 82.549(6)°, γ = 73.877(6)°, V = 1333.03(8) Å^3, Z = 2, Dx = 1.399 g/cm^3, T = 100 K, μ = 0.951 mm^{-1}, λ = 1.54187 Å, R_1 = 0.055, wR_2 = 0.155.

All measurements were made on a Rigaku R-AXIS RAPID-191R diffractometer using graphite monochromated Cu-Kα radiation. The structure was solved by direct methods with SHELXS-97[30] and was refined using full-matrix least-squares on F^2 with SHELXL-97[30]. All non-H atoms were refined with anisotropic displacement parameters. CCDC 865214 for compound **22d**, 1027998 for compound **25b**, 1027990 for compound **25d** and 1028036 for compound **25e** contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/Community/Requestastructure/Pages/DataRequest.aspx?.

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List of publications (first author)

1) Discovery of pyrrolo[3,2-*c*]quinoline-4-one derivatives as novel hedgehog signaling inhibitors
   Ohashi, T.; Oguro, Y.; Tanaka, T.; Shiokawa, Z.; Shibata, S.; Satoh, Y.; Yamakawa, H.; Hattori, H.; Yamamoto, Y.; Kondo, S.; Miyamoto, M.; Tojo, H.; Baba, A.; Sasaki, S. *Bioorg. Med. Chem.* **2012**, 20, 5496-5506.
2) Discovery of the investigational drug TAK-441, a pyrrolo[3,2-*c*]pyridine derivative, as a highly potent and orally active hedgehog signaling inhibitor: Modification of the core skeleton for improved solubility
   Ohashi, T.; Oguro, Y.; Tanaka, T.; Shiokawa, Z.; Tanaka, Y.; Shibata, S.; Satoh, Y.; Yamakawa, H.; Hattori, H.; Yamamoto, Y.; Kondo, S.; Miyamoto, M.; Nishihara, M.; Ishimura, Y.; Tojo, H.; Baba, A.; Sasaki, S. *Bioorg. Med. Chem.* **2012**, *20*, 5507-5517.
3) Synthesis and evaluation of hedgehog signaling inhibitor with novel core system
   Ohashi, T.; Tanaka, Y.; Shiokawa, Z.; Banno, H.; Tanaka, T.; Shibata, S.; Satoh, Y.; Yamakawa, H.; Yamamoto, Y.; Hattori, H.; Kondo, S.; Miyamoto, M.; Tojo, H.; Baba, A.; Sasaki, S. *Bioorg. Med. Chem.* **2015**, *23*, 4777-4791.

List of other publications (including name)

1) Design and Synthesis of Pyrrolo[3,2-*d*]pyrimidine Human Epidermal Growth Factor Receptor 2 (HER2)/Epidermal Growth Factor Receptor (EGFR) Dual Inhibitors: Exploration of Novel Back-Pocket Binders
   Kawakita, Y.; Banno, H.; Ohashi, T.; Tamura, T.; Yusa, T.; Nakayama, A.; Miki, H.; Iwata, H.; Kamiguchi, H.; Tanaka, T.; Habuka, N.; Sogabe, S.; Ohta, Y.; Ishikawa, T. *J. Med. Chem.* **2012**, *55*, 3975-3991.
2) Design and synthesis of novel pyrimido[4,5-*b*]azepine derivatives as HER2/EGFR dual inhibitors
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3) Design and Synthesis of Novel DFG-Out RAF/Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) Inhibitors. 1. Exploration of [5,6]-Fused Bicyclic Scaffolds
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