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Synthesis and evaluation of hedgehog signaling inhibitor with novel core system

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ABSTRACT

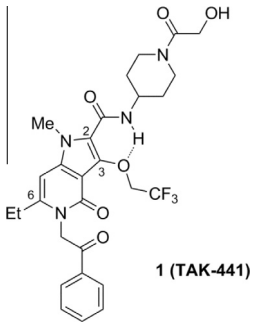
As we previously reported, *N*-methylpyrrolo[3,2-*c*]pyridine derivatives **1** (TAK-441) was discovered as a clinical candidate of hedgehog (Hh) signaling inhibitor by modification of the upper part. We next focused on modification of the lower part including core skeletons to discover new Hh signaling inhibitors with novel core rings. Efforts to find novel chemotypes by using X-ray single crystal structure analysis led to some potent Hh signaling inhibitors (**2c**, **2d**, **2e**, **2f**) with novel core ring systems, which had benzamide moiety at the 5-position as a key component for potent activity. The suppression of Gli1 expression with these new Hh signaling inhibitors were weaker than that of compound **1** (TAK-441) because of low pharmacokinetic property. We recognized again TAK-441 is a good compound as clinical candidate with good structural and pharmacokinetic advantages.

1. Introduction

The hedgehog (Hh) signaling pathway plays a significant role in the regulation of cell growth and differentiation during embryonic development. Abnormal activation of the Hh signaling pathway has been linked to several types of human cancers including pancreatic cancer,[1] prostate cancer,[2] colon cancer,[3] basal cell carcinoma[4] and medulloblastoma,[5] and the development of small-molecule inhibitors of this pathway represents a promising route toward novel anticancer therapeutics.[6]

The pyrrolo[3,2-*c*]pyridine-4-one derivative **1** (TAK-441) we had reported as a clinical candidate was discovered by modification of 2-, 3-, 6-substituents of a lead compound to improve activity and physicochemical property as hedgehog (Hh) signaling inhibitors (Fig. 1).[7,8] However, it was still unclear that exact role of the core skeleton including substituents at the 4- and 5-positions. Thus, we evaluated the modification of central core to confirm these properties and discovered new potent Hh signaling inhibitors with novel skeletons compared to that of **1**.

The pyrrole ring of **1** was 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)methoxy group.[8] Thus, we fixed this upper part and focused on the lower part of core ring, especially six-membered rings. Besides novel core ring modification, we evaluated the effect of 4- or 5-substituents to in vitro inhibitory activity. In this report, we describe syntheses and SAR of novel hedgehog signaling inhibitors with multi-substituted bicyclic core rings (Fig. 2).



1 (TAK-441)

Figure 1. Chemical structure of **1** (TAK-441).

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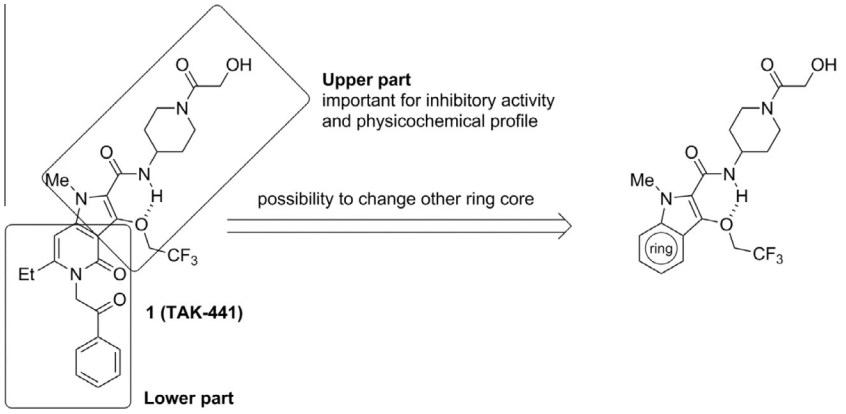


Figure 2. Change to novel core ring in **1** (TAK-441).

2. Chemistry

As we previously reported, 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.[7] Figure 3 illustrates our retrosynthetic analysis to access novel bicyclic cores with these two moieties. To construct the unique bicyclic core of **2**, it was necessary to synthesize intermediate **4**. We 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 **6** was selected for a key intermediate which was converted to di-ester **5** by addition of sarcosine ester unit.[9] We thought this strategy could be utilized for the synthesis of various useful core rings. The syntheses for five types of carboxylic acid **3** are detailed in the next section.

The synthesis of pyrrolo[2,3-*d*]pyrimidine **3a** is shown in Scheme 1. Addition of propionitrile **7** with ammonia in acidic condition provided amidine hydrochloride **8**, followed by cyclization with diethyl malonate to afford **9** in 67% yield. The preparation of a key intermediate **6a** was conducted by stepwise conversion; introduction of formyl group, oxidation to carboxylic acid and esterification. After addition of sarcosine ethyl ester hydrochloride, subsequent Dieckmann condensation provided the bicyclic compound **4a** in 91% yield. The 3-hydroxyl group of **4a** was treated with (2,2,2-trifluoroethyl)trifluoromethanesulfonate to afford **10**. The stepwise saponification of chloropyridine and ethoxycarbonyl moieties gave carboxylic acid **3a**.

In our previous papers, we reported the synthesis of **1** using an intermediate with requisite phenacyl moiety on six-membered ring of the bicyclic core. The similar synthetic route using the intermediate **11** was initially attempted (Scheme 2). The phenacylation of **11** gave **12** in 57% yield.[10] The following hydrolysis under basic

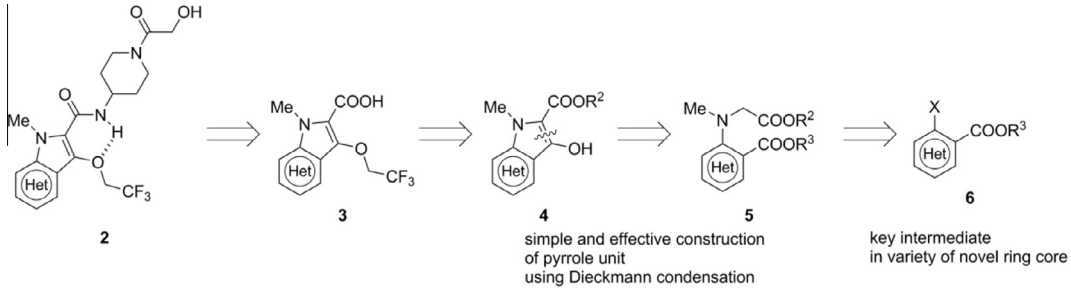
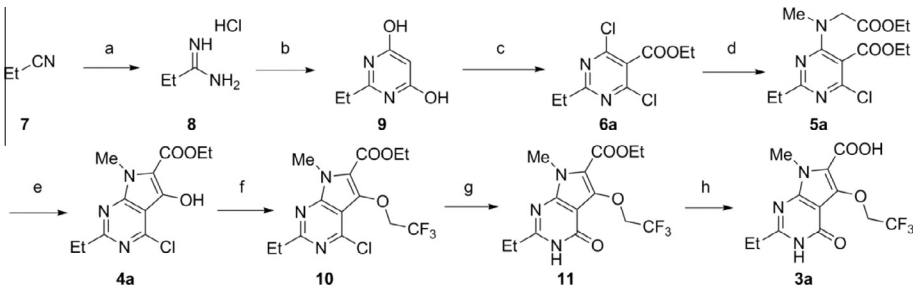
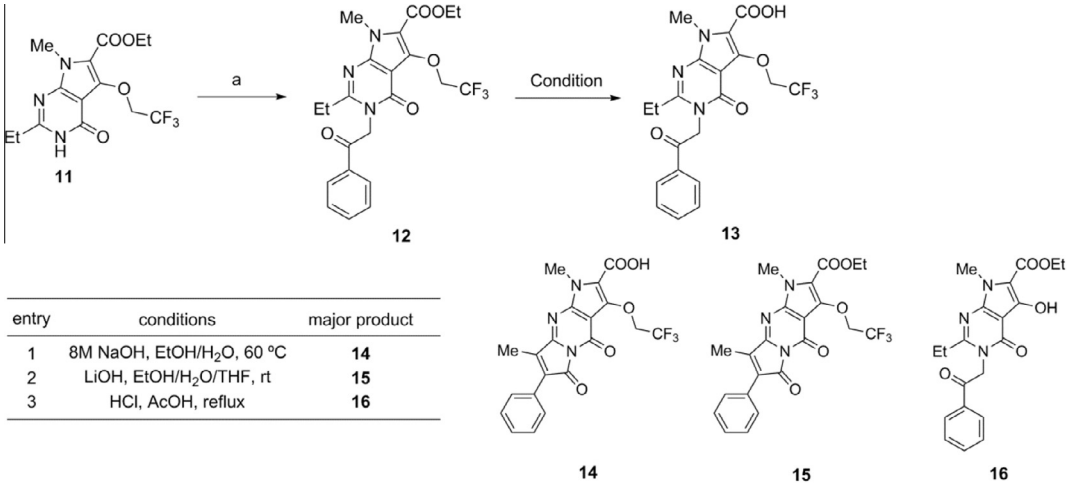


Figure 3. Retrosynthetic analysis of novel core.



Scheme 1. Synthesis of pyrrolo[2,3-*d*]pyrimidine derivative **3a**. Reagents and conditions : (a) (1) HCl, EtOH; (2) NH_3, MeOH, 70%; (b) $(COOEt)_2$, NaOMe, MeOH, 67%; (c) (1) DMF, $POCl_3$, 0 °C to reflux, 73%; (2) $NaClO_2$, NH_2SO_3H, *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 °C, 79%.

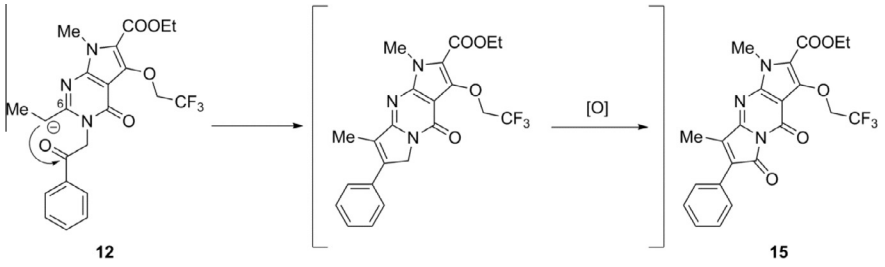


entry	conditions	major product
1	8M NaOH, EtOH/H_2O, 60 °C	**14**
2	LiOH, EtOH/H_2O/THF, rt	**15**
3	HCl, AcOH, reflux	**16**

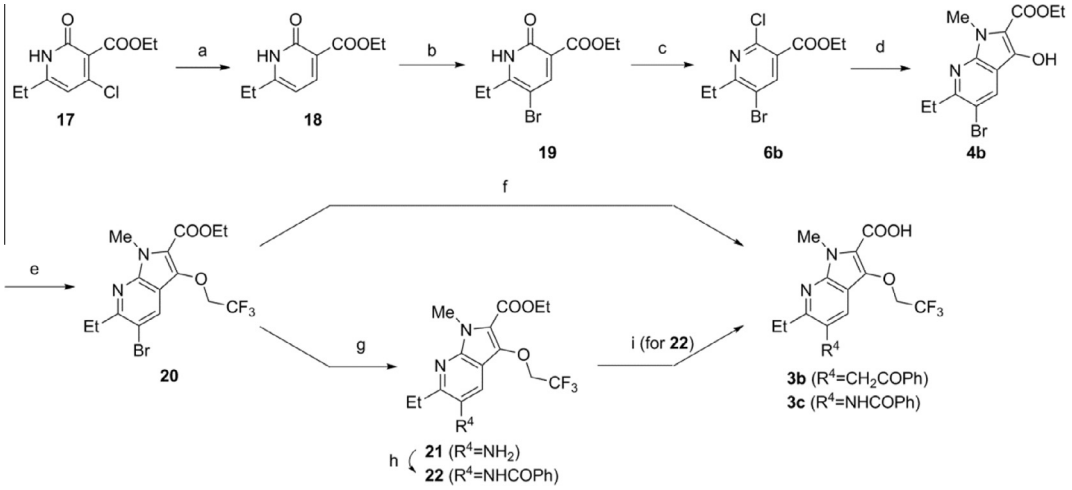
Scheme 2. Synthesis of carboxylic acid **13** using similar route to TAK-441. Reagents and conditions: (a) phenacyl bromide, *tert*-BuONa, LiBr, DME, DMF, 60 °C, 57%.

conditions did not work well and gave only tricyclic product **14** or **15**, 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 3).[11] We speculated this cyclization was triggered by higher acidity of methylene in the ethyl group of compound **12** than that of **1**. On the other hand, the hydrolysis of **12** under acidic condition gave dealkylated compound **16** 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.

Next, the pyrrolo[2,3-*b*]pyridine ring was constructed along the synthetic route below (Scheme 4). The hydrogenation of 4-chloropyridine-2-one derivative **17**[7] by palladium-carbon was achieved in 90% yield. Stepwise halogenation of **18** was conducted to afford a key intermediate **6b**. Addition of sarcosine ethyl ester hydrochloride and cyclization along our strategy provided **4b**. Alkylation of the 3-hydroxy group with (2,2,2-



Scheme 3. Presumed reaction mechanism of tricyclic compound.



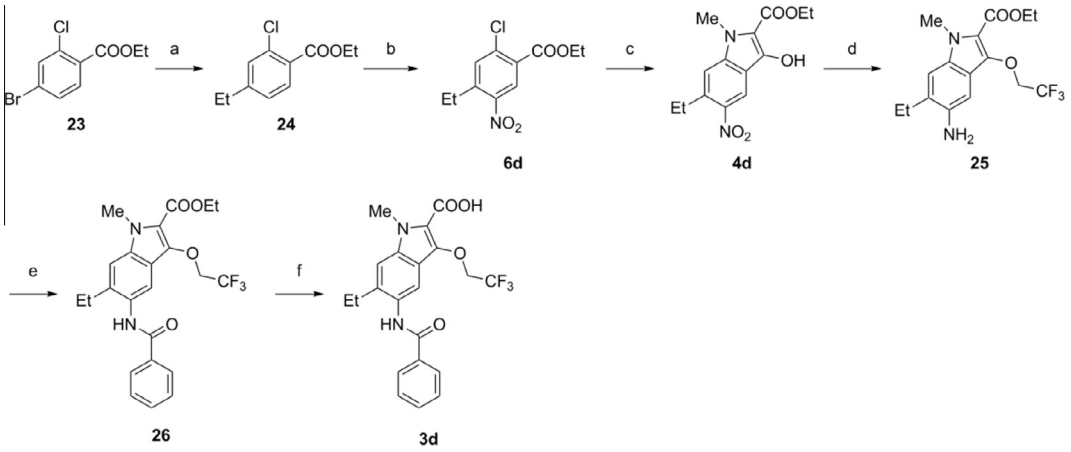
Scheme 4. Synthesis of pyrrolo[2,3-*b*]pyridine derivatives **3b** and **3c**. Reagents and conditions: (a) Pd-C, H_2, Et_3N, EtOH, THF, 90%; (b) NBS, DMF, 86%; (c) $POCl_3$, reflux, 76%; (d) (1) $MeNHCH_2COOEt{\cdot}HCl$, Et_3N, THF, reflux; (2) NaOEt, EtOH, 73%; (e) CF_3CH_2OTf, Cs_2CO_3, DMF, 96%; (f) (1) acetophenone, ^tBuONa, $Pd(OAc)_2$, MePhos, toluene, 70 °C; (2) NaOH, EtOH, 50 °C, 22%; (g) (1) benzophenoneimine, $Pd_2(dba)_3$, xantphos, Cs_2CO_3, toluene, 100 °C; (2) 2 M HCl, THF, 91%; (h) BzCl, Py, THF, 94%; (i) NaOH, EtOH, 95%.

trifluoroethyl)triflate led to **20** in 96% yield. Coupling with acetophenone in Buchwald condition gave **3b** in 22% yield, but introduction of amino group to benzophenoneimine gave **21** in 91% yield. Acylation with benzoyl chloride followed by hydrolysis led to carboxylic acid **3c**.

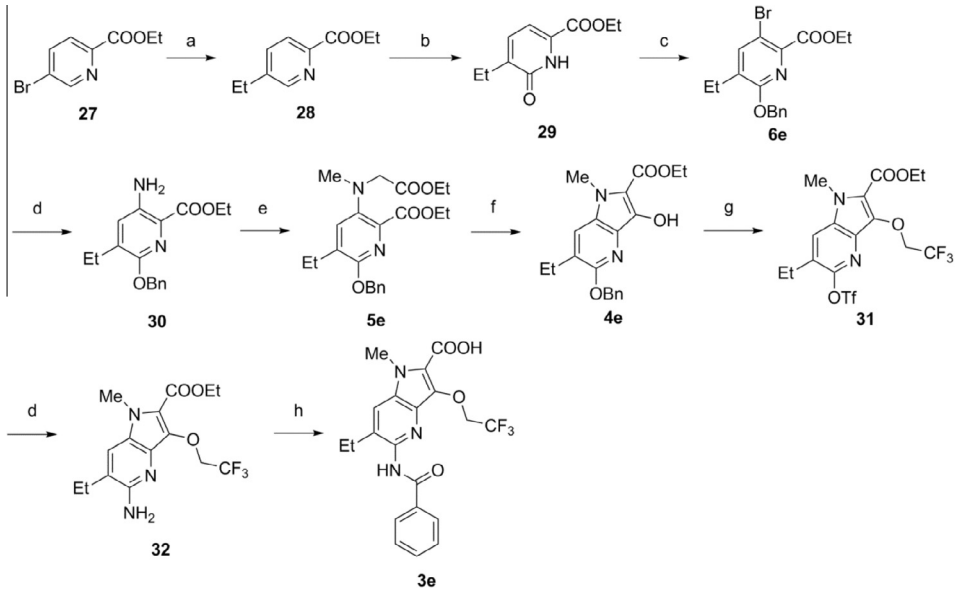
The synthesis of indole derivative **3d** is shown in Scheme 5. The key intermediate **6d** was synthesized from ethyl 4-bromo-2-chlorobenzoate **23** as follows; introduction of ethyl group by Stille coupling followed by hydrogenation, and nitration. The construction of pyrrole ring proceeded directly in 41% yield. After alkylation of **4d**, subsequent hydrogenation of nitro group led to aniline **25** in 97% yield. Acylation of **25** with benzoyl chloride followed by saponification were conducted to afford the desired carboxylic acid **3d**.

The synthetic route of pyrrolo[3,2-*b*]pyridine derivative **3e** is shown in Scheme 6. The key intermediate **6e** was successfully prepared from ethyl 3-bromopicolinate **27** 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 we conducted stepwise introduction. The amination of **6e** with benzophenoneimine afforded **30** under Buchwald condition. Following monomethylation was achieved via Boc protection. The precursor for Dieckmann condensation **5e** was prepared by addition of ethyl bromoacetate. The cyclization of **5e** with sodium ethoxide afforded **4e** 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 **31**. The similar reaction in the synthesis of pyrrolo[2,3-*b*]pyridine **3c** afforded carboxylic acid **3e** with *N*-benzoyl group at the 5-position.

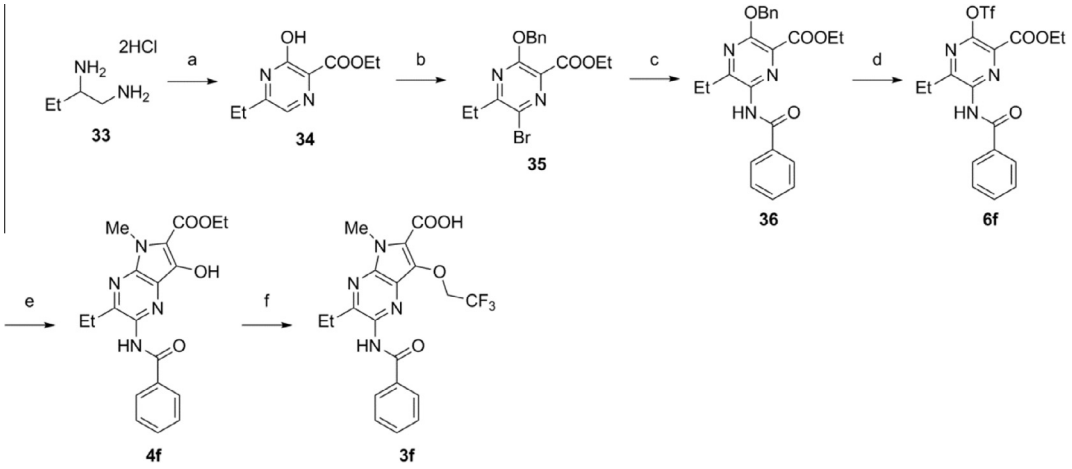
The synthesis of pyrrolo[2,3-*b*]pyrazine derivative **3f** is shown in Scheme 7. Cyclization of **33** with diethyl ketomalonate gave



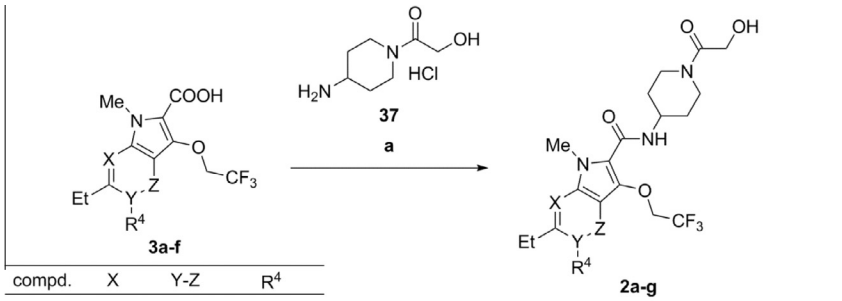
Scheme 5. Synthesis of indole derivative **3d**. Reagents and conditions: (a) (1) (vinyl)$SnBu_3$, $Pd(PPh_3)_4$, DMF, 100 °C, 100%; (2) Pd-C, $Ba(OH)_2$, H_2, AcOEt, 98%; (b) $NaNO_3$, H_2SO_4, 0 °C, 70%; (c) $MeNHCH_2COOEt{\cdot}HCl$, Et_3N, EtOH, reflux, 41%; (d) (1) CF_3CH_2OTf, Cs_2CO_3, DMF, 100%; (2) Pd-C, H_2, EtOH, THF, 97%; (e) BzCl, Py, THF, 86%; (f) NaOH, EtOH, 95%.



Scheme 6. Synthesis of pyrrolo[3,2-*b*]pyridine derivative **3e**. Reagents and conditions: (a) (1) (vinyl)$SnBu_3$, $Pd(PPh_3)_4$, DMF, 100 °C, 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 °C, 99%; (d) (1) benzophenoneimine, $Pd_2(dba)_3$, xantphos, Cs_2CO_3, toluene; (2) 2 M HCl, THF, 98% (for **30**), 92% (for **32**); (e) (1) Boc_2O, *tert*-BuOH, 90 °C; (2) MeI, Cs_2CO_3, DMF, 87%; (3) 4 M HCl, AcOEt, 0 °C, 87%; (4) $BrCH_2COOEt$, $^iPr_2NEt$, DMF, 110 °C, 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 °C, 92%; (h) (1) BzCl, pyridine, THF; (2) NaOH, EtOH, 50 °C, 88%.



Scheme 7. Synthesis of pyrrolo[2,3-*b*]pyrazine derivative **3f**. Reagents and conditions: (a) $(COOEt)_2CO$, $^iPr_2NEt$, 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 °C; (2) 2 M HCl, THF, 59%; (3) BzCl, pyridine, THF, 0 °C, 93%; (d) (1) Pd/C, H_2, EtOH, 77%; (2) Tf_2O, pyridine, 0 °C, 94%; (e) (1) $MeNHCH_2COOEtHCl$, Et_3N, EtOH; (2) NaOEt, EtOH, 80%; (f) (1) CF_3CH_2OTf, Cs_2CO_3, DMF, 0 °C, 74%; (2) NaOH, THF, EtOH, 99%.



compd.	X	Y-Z	R^4
3a	N	N-CO	H
3b	N	C=CH	CH_2COPh
3c	N	C=CH	NHCOPh
3d	C	C=CH	NHCOPh
3e	C	C=N	NHCOPh
3f	N	C=N	NHCOPh

compd.	X	Y-Z	R^4	yield
2a	N	N-CO	H	79%
2g	N	N-CO	CH_2COPh	14%
2b	N	C=CH	CH_2COPh	47%
2c	N	C=CH	NHCOPh	53%
2d	C	C=CH	NHCOPh	82%
2e	C	C=N	NHCOPh	46%
2f	N	C=N	NHCOPh	61%

Scheme 8. Amidation reaction in each core ring. Reagents and conditions: (a) amine **37**, EDC, HOBt, Et_3N, DMF, 46–82%; (b) $BrCH_2COPh$, LiBr, K_2CO_3, DME/DMF, 60–80 °C, 14%.

pyrazine **34** in 25% yield. Bromination followed by benzylation of **34** provided **35**. Conversion to amino group using Buchwald condition and following acylation with benzoyl chloride afforded **36**. 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 **4f**. The obtained alcohol was alkylated with (2,2,2-trifluoro)ethyl triflate, following hydrolysis to give carboxylic acid **3f**.

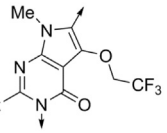
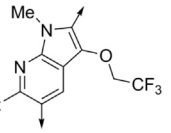
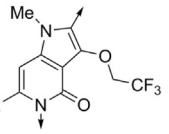
Finally, the amidation reaction is listed in Scheme 8. The compound **2a**–**f** was obtained by condensation of corresponding carboxylic acid **3a**–**f** with amine **37**[8] in 46–82% yield. For the compound **2a**, the alkylation with phenacyl bromide was carried out in 14% yield.

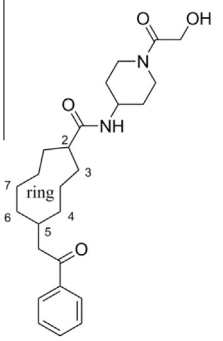
3. Results and discussions

In vitro activities of compounds **2b**–**g** were evaluated using a luciferase reporter in NIH3T3 cells carrying a stably transfected Gli-reporter construct (Gli-luc reporter cell line). An in vivo suppression assay of transcription factor Gli1 *m*RNA expression was conducted by the treatment of mice bearing PAN-04 human pancreatic xenograft tumors with the compounds after oral administration (0 h and 9 h). Quantification of mouse stromal Gli1 *m*RNA as a pharmacodynamic (PD) marker extracted from the tumors was performed 24 h after the first administration of drug (in vivo PD assay).

We initially assessed the novel two types of core ring bearing 2-{*N*-[1-(hydroxyacetyl)piperidinyl]} amide, 3-[(2,2,2-trifluoro) ethoxy], 5-phenacyl, and 6-ethyl groups at the same positions as **1** (TAK-441) (Table 1). Pyrrolo[2,3-*d*]pyrimidine **2g** maintained potent inhibitory activity compared to **1**, but the pyrrolo[2,3-*b*] pyridine **2b** showed 10-fold drop. This result indicated it might be important for potent inhibitory activity to bear carbonyl group at the 4-position in the core ring. However, the compound **2b** 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. We next focused on enhancement of inhibitory activity in compound **2b**.

Table 1
Effect of core ring with 5-phenacyl group

compd.	ring	Gli-luc reporter IC_{50} (nM)[a]
2g		6.2
2b		46
1		4.4



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

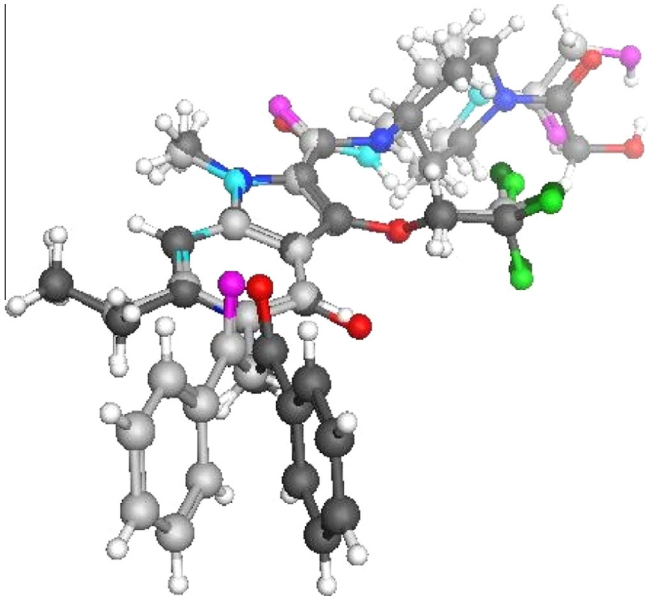


Figure 4. Overlay of **1** (black) and **2b** (gray) in X-ray single crystal structure. The figure was described based on X-ray structural analysis data using PyMOL Software. (PyMOL version 1.4.1).

For this purpose, we obtained X-ray single crystal structure of **1** and **2b** (Fig. 4). 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 **1** might be influenced by the steric hindrance of both 6-ethyl and 4-carbonyl groups, but lack of the 4-carbonyl group led **2b** to take the different conformation from that of **1**. Based on these analyses of X-ray single crystal structures, the enhancement of inhibitory activity in **2b** could be achieved by adjusting the conformation of terminal benzene ring similar to compound **1**. 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. In our previous report, the lack of carbonyl group in the side chain or benzene ring resulted in decrease of activity.[8] Considering our previous knowledge and necessity of regulation, the benzamide moiety would be a proper substituent at 5-position.

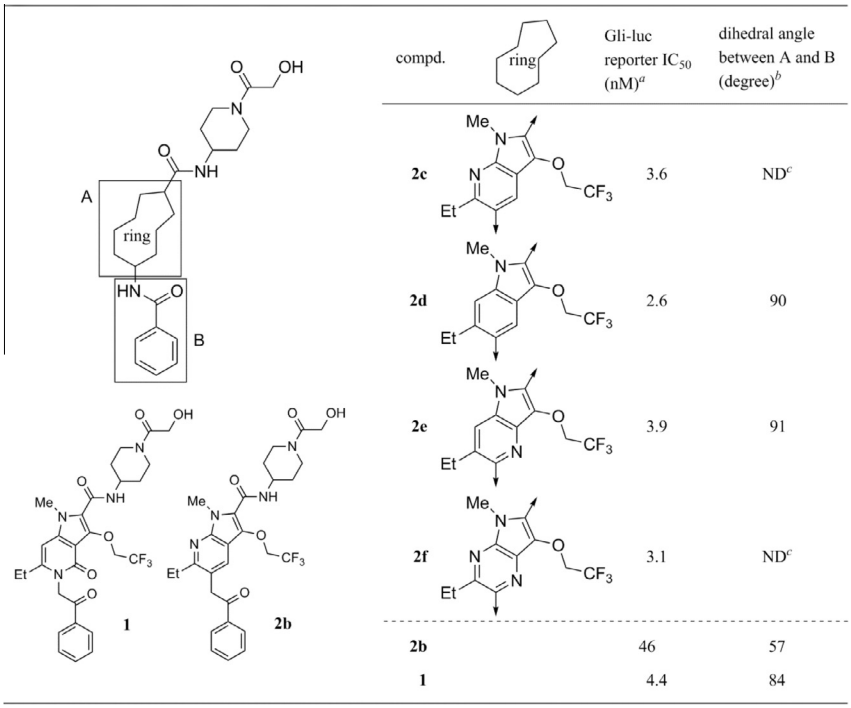
The benzamide was provided the planarity to whole in the 5-position, and regulated the conformation compare to phenacyl group. This structural regulation newly introduced in the 5-position as a surrogate of regulation by steric hindrance in compound **1** brought us expectation that benzamide side chain 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.[12] Thus, our next effort was shifted to investigation of 5-benzamide derivatives.

The compound **2c** with benzamide moiety instead of phenacyl group showed dramatic increase of Gli reporter inhibitory activity compared to that of **2b** as we expected. We next adapted this finding to other novel core skeleton without 4-carbonyl group. Consequently, as the indole **2d**, pyrrolo[3,2-*b*]pyridine **2e** and pyrrolo[2,3-*b*]pyrazine **2f** also showed potent inhibitory activity. These results demonstrated that 5-benzamide was quite effective side chain for our novel core skeletons without 4-carbonyl group (Table 2). For further consideration, the X-ray single crystal structures of **2d** and **2e** were obtained and its overlaid figure was illustrated in Figure 5.

They also indicated that 5-substituents of **2d** and **2e** took similar position to that of **1** despite the absence of 4-carbonyl moiety. This conformation was considered to be necessary for potent Gli reporter inhibitory activity as we expected. The dihedral angles between central core ring and 5-substituent are also described in Table 2. To notify the relation between inhibitory activity and X-ray single structure, the potent activity requested almost vertical dihedral angle, and its value in **1**, **2d**, and **2e** were 84°, 90° and 91°, respectively. We considered that amide linker formed adequate conformation with dihedral angle of about 90°, and fitted well to whole molecule to the pocket of smo protein.

The in vivo PD profiles of **2c**–**f** 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 expressed significant stroma-derived Gli1 and cancer-derived Shh activity. We measured the reduction of Gli1 *m*RNA expression levels as a pharmacodynamic marker in this model. The suppression of Gli1 *m*RNA

Table 2
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]
2c		3.6	ND[c]
2d		2.6	90
2e		3.9	91
2f		3.1	ND[c]
2b		46	57
1		4.4	84

[a] IC_{50} 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.

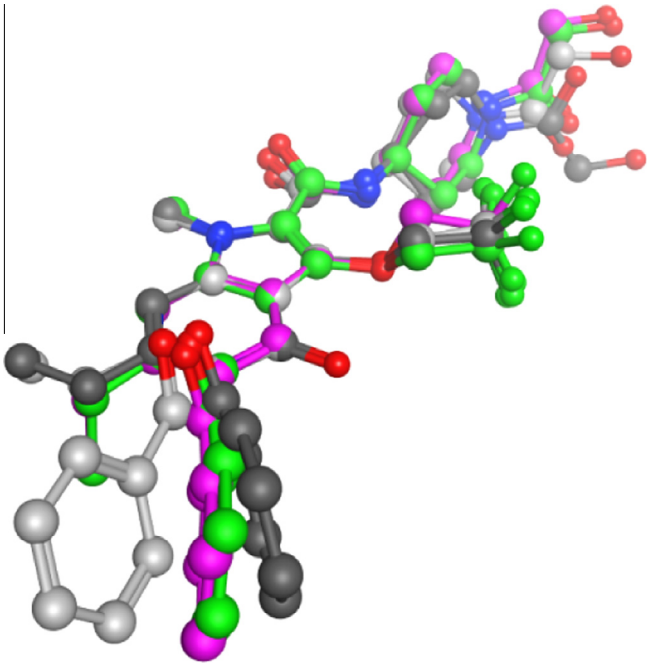


Figure 5. Overlay of **1** (black), **2b** (gray), **2d** (pink) and **2e** (green) in X-ray single crystal structure. The figure was described based on X-ray structural analysis data using PyMOL Software. (PyMOL version 1.4.1).

expression in novel compounds **2c**–**f** was weaker than **1** despite of their potent in vitro inhibitory activity. We considered it was caused by their pharmacokinetics, which was well correlated to suppression of Gli1 *m*RNA expression. Considering these results, compound **1** (TAK-441) was well-balanced compound in the point of good PK profile while potent inhibitory activity.

Table 3
The results of in vivo PD study and corresponding PK data

Compd	Gli-luc reporter IC_{50} (nM)[a]	In vivo PD Gli *m*RNA (% ctrl)[b]	AUC (μgh/mL)[c]
2c	3.6	24	10.832
2d	2.6	50	1.272
2e	3.9	87	0.984
2f	3.1	57	0.925
1	4.4	4	28.346

[a] IC_{50} 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. Conclusions

We explored new Hh signaling inhibitors with novel core rings by the modification of 5-substituent including core ring, and found pyrrolo[2,3-*b*]pyridine derivative **2b** with 10^{-8} order activity in Gli reporter assay. To enhance in vitro activity, we considered the preferable conformation in X-ray single crystal structure, and discovered **2c** with benzamide moiety at the 5-position. This substituent worked well in the core ring without 4-carbonyl moiety, the indole **2d**, pyrrolo[3,2-*b*]pyridine **2e** and pyrrolo[2,3-*b*]pyrazine **2f** also showed potent inhibitory activity. 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 **2c**–**f** was unfortunately inefficient compare to that of **1** in the result of pharmacokinetics. Based on the results of potent Gli reporter inhibitory activity including structural characteristics and good PK profiles, we confirmed that **1** (TAK-441) is the best candidate as our Hh signaling inhibitor.

5. Experimental section

Melting points were determined on a Büchi melting point apparatus and were not corrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker Avance 300 (300 MHz) or Bruker DPX300 (300 MHz) instruments. Chemical shifts are reported as δ values (ppm) down-field from internal tetramethylsilane of the indicated organic solution. Peak multiplicities are expressed as follows. Abbreviations are used as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; ddd, doublet of doublet of doublets; dt, doublet of triplet; br s, broad singlet; m, multiplet. Coupling constants (J values) are given in hertz (Hz). Element analyses were carried out by Takeda Analytical Laboratories, 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) or basic silica gel columns (Chromatorex® NH-DM1020, 100–200 mesh, Fuji Silysia Chemical Ltd.) or Purif-Pack® (SI 60 μM or NH 60 μM, Fuji Silysia Chemical Ltd.). 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). The purities of compounds submitted for biological evaluation were $\geqslant$95% as determined by LC/MS and elemental analysis. Yields are not optimized.

5.1. Propanimidamide hydrochloride (8)

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 −10 °C. To the suspension was added 8 M 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).

5.2. 2-Ethylpyrimidine-4,6-diol (9)

To a solution of **8** (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 concd 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 (3H, t, J = 7.6 Hz), 2.36–2.60 (2H, m), 5.03 (1H, s), 11.62 (2H, br s).

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

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. **9** (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 (6H, m), 2.97 (2H, q, J = 7.6 Hz), 4.48 (2H, q, J = 7.2 Hz).

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

A solution of **6a** (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 (6H, m), 1.41 (3H, t, J = 7.2 Hz), 2.72 (2H, q, J = 7.6 Hz), 3.11 (3H, s), 4.21 (2H, q, J = 7.1 Hz), 4.28 (2H, s), 4.40 (2H, q, J = 7.2 Hz).

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

A solution of **5a** (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 1 M HCl aq, and stirred at 0 °C 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 (6H, m), 2.90 (2H, q, J = 7.6 Hz), 3.88 (3H, s), 4.37 (2H, q, J = 7.0 Hz), 9.50 (1H, br s).

5.6. Ethyl 4-chloro-2-ethyl-7-methyl-5-(2,2,2-trifluoroethoxy)-7*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylate (10)

To a mixture of **4a** (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 °C, 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 (6H, m), 3.02 (2H, q, J = 7.6 Hz), 4.06 (3H, s), 4.39–4.57 (4H, m).

5.7. Ethyl 2-ethyl-7-methyl-4-oxo-5-(2,2,2-trifluoroethoxy)-4,7-dihydro-3H-pyrrolo[2,3-*d*]pyrimidine-6-carboxylate (11)

A solution of **10** (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 (6H, m), 2.63 (2H, q, J = 7.6 Hz), 3.83 (3H, s), 4.25 (2H, q, J = 7.1 Hz), 5.01 (2H, q, J = 9.4 Hz), 12.02 (1H, br s).

5.8. 2-Ethyl-7-methyl-4-oxo-5-(2,2,2-trifluoroethoxy)-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylic acid (3a)

A mixture of **11** (4.0 g, 11.5 mmol) and 8 M NaOH aq (8 mL) in EtOH was stirred at room temperature for 15 min and at 60 °C for 4 h. After cooling at 0 °C, the mixture was neutralized with 6 M 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 (3H, t, J = 7.6 Hz), 2.62 (2H, q, J = 7.4 Hz), 3.83 (3H, s), 5.01 (2H, q, J = 9.3 Hz), 12.07 (1H, s), 12.71 (1H, br s).

5.9. 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 (12)

To a suspension of **11** (124 mg, 0.357 mmol) in DME (4 mL)/DMF (1 mL) was added portionwise potassium *tert*-butoxide (44 mg, 0.462 mmol) and the mixture was stirred at 0 °C 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 °C 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 (6H, m), 2.74 (2H, q, J = 7.2 Hz), 3.90 (3H, s), 4.27 (2H, q, J = 7.2 Hz), 4.91 (2H, q, J = 9.1 Hz), 5.72 (2H, s), 7.56–7.68 (2H, m), 7.69–7.83 (1H, m), 8.03–8.24 (2H, m).

5.10. Ethyl 6-ethyl-2-oxo-1,2-dihydropyridine-3-carboxylate (18)

A mixture of **17** (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 6 M 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 (3H, t, J = 7.6 Hz), 1.38 (3H, t, J = 7.2 Hz), 2.74 (2H, q, J = 7.6 Hz), 4.37 (2H, q, J = 7.2 Hz), 6.29 (1H, d, J = 7.2 Hz), 8.18 (1H, d, J = 7.6 Hz), 12.50 (1H, br s).

5.11. Ethyl 5-bromo-6-ethyl-2-oxo-1,2-dihydropyridine-3-carboxylate (19)

To a solution of **18** (17.5 g, 89.7 mmol) in DMF (100 mL) was added *N*-bromosuccinimide (16.0 g, 89.9 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. To the mixture was added dropwise water (250 mL) at 0 °C, 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 (3H, t, J = 7.6 Hz), 1.26 (3H, t, J = 7.2 Hz), 2.64 (2H, q, J = 7.6 Hz), 4.20 (2H, q, J = 7.2 Hz), 8.06 (1H, s), 12.42 (1H, br s).

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

A mixture of **19** (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 °C, and the mixture was extracted with AcOEt (100 mL × 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 (3H, t, J = 7.5 Hz), 1.33 (3H, t, J = 7.2 Hz), 2.91 (2H, q, J = 7.5 Hz), 4.34 (2H, q, J = 7.2 Hz), 8.42 (1H, s).

5.13. Ethyl 5-bromo-6-ethyl-3-hydroxy-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylate (4b)

A mixture of **6b** (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 5 M 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 (3H, t, J = 7.6 Hz), 1.33 (3H, t, J = 7.1 Hz), 2.96 (2H, q, J = 7.6 Hz), 3.88 (3H, s), 4.33 (2H, q, J = 7.1 Hz), 8.36 (1H, s), 9.76 (1H, br s).

5.14. Ethyl 5-bromo-6-ethyl-1-methyl-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylate (20)

In the same manner as in the preparation of **10**, the title compound (1.80 g, 96%) was obtained as a pale yellow powder from **4b** (1.50 g, 4.58 mmol). ^{1}H NMR (DMSO-d_6) δ 1.22–1.41 (6H, m), 3.00 (2H, q, J = 7.4 Hz), 3.96 (3H, s), 4.35 (2H, q, J = 7.1 Hz), 4.86 (2H, q, J = 9.1 Hz), 8.36 (1H, s).

5.15. Ethyl 5-amino-6-ethyl-1-methyl-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylate (21)

To a mixture of **20** (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 °C 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 2 M 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 (3H, t, J = 7.4 Hz), 1.34 (3H, t, J = 7.1 Hz), 2.75 (2H, q, J = 7.4 Hz), 3.91 (3H, s), 4.32 (2H, q, J = 7.1 Hz), 4.66 (2H, q, J = 9.1 Hz), 4.86 (2H, s), 7.16 (1H, s).

5.16. Ethyl 6-ethyl-1-methyl-5-[(phenylcarbonyl)amino]-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylate (22)

To a mixture of **21** (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 °C, 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 (3H, t, J = 7.4 Hz), 1.36 (3H, t, J = 7.1 Hz), 2.89 (2H, q, J = 7.4 Hz), 4.01 (3H, s), 4.37 (2H, q, J = 7.1 Hz), 4.82 (2H, q, J = 9.1 Hz), 7.46–7.72 (3H, m), 7.90–8.13 (3H, m), 10.13 (1H, s).

5.17. 6-Ethyl-1-methyl-5-(2-oxo-2-phenylethyl)-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylic acid (3b)

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 **20** (450 mg, 1.10 mmol) and acetophenone (0.256 mL, 2.20 mmol) in toluene (4 mL), and the mixture was stirred at 70 °C for 17 h. After allowing to cool to room temperature, 1 M NaOH aq (3 mL) and EtOH (5 mL) were added, and the mixture was stirred at 50 °C 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 (3H, t, J = 7.5 Hz), 2.70 (2H, q, J = 7.5 Hz), 3.98 (3H, s), 4.63 (2H, s), 4.77 (2H, q, J = 9.3 Hz), 7.51–7.63 (2H, m), 7.64–7.74 (1H, m), 7.82 (1H, s), 8.04–8.17 (2H, m), 13.35 (1H, br s).

5.18. 6-Ethyl-1-methyl-5-[(phenylcarbonyl)amino]-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylic acid (3c)

In the same manner as in the preparation of **3a**, the title compound (75.4 mg, 95%) was obtained as a white powder from **22** (84.5 mg, 0.188 mmol). ^{1}H NMR (DMSO-d_6) δ 1.25 (3H, t, J = 7.6 Hz), 2.88 (2H, q, J = 7.6 Hz), 4.01 (3H, s), 4.78 (2H, q, J = 9.1 Hz), 7.47–7.73 (3H, m), 7.96 (1H, s), 7.98–8.09 (2H, m), 10.12 (1H, s), 13.28 (1H, br s).

5.19. Ethyl 2-chloro-4-ethylbenzoate (24)

A mixture of **23** (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 °C 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 (3H, t, J = 7.5 Hz), 1.40 (3H, t, J = 7.2 Hz), 2.65 (2H, q, J = 7.5 Hz), 4.38 (2H, q, J = 7.2 Hz), 7.13 (1H, dd, J = 8.1, 1.2 Hz), 7.28 (1H, d, J = 1.2 Hz), 7.76 (1H, d, J = 8.1 Hz).

5.20. Ethyl 2-chloro-4-ethyl-5-nitrobenzoate (6d)

To a solution of **24** (3.0 g, 14.1 mmol) in concd H_2SO_4 (10 mL) was added dropwise a solution of $NaNO_3$ (1.20 g, 14.1 mmol) in concd H_2SO_4 (10 mL) at 0 °C, and the mixture was stirred at 0 °C 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 (3H, t, J = 7.5 Hz), 1.34 (3H, t, J = 7.1 Hz), 2.88 (2H, q, J = 7.5 Hz), 4.36 (2H, q, J = 7.1 Hz), 7.82 (1H, s), 8.38 (1H, s).

5.21. Ethyl 6-ethyl-3-hydroxy-1-methyl-5-nitro-1*H*-indole-2-carboxylate (4d)

A mixture of **6d** (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 (3H, t, J = 7.5 Hz), 1.33 (3H, t, J = 7.1 Hz), 2.99 (2H, q, J = 7.5 Hz), 3.90 (3H, s), 4.33 (2H, q, J = 7.1 Hz), 7.53 (1H, s), 8.57 (1H, s), 9.92 (1H, br s).

5.22. Ethyl 5-amino-6-ethyl-1-methyl-3-(2,2,2-trifluoroethoxy)-1*H*-indole-2-carboxylate (25)

To a mixture of **4d** (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).

5.23. Ethyl 6-ethyl-1-methyl-5-[(phenylcarbonyl)amino]-3-(2,2,2-trifluoroethoxy)-1*H*-indole-2-carboxylate (26)

In the same manner as in the preparation of **22**, the title compound (89 mg, 86%) was obtained as a beige powder from **25** (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).

5.24. 6-Ethyl-1-methyl-5-[(phenylcarbonyl)amino]-3-(2,2,2-trifl uoroethoxy)-1*H*-indole-2-carboxylic acid (3d)

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

5.25. Ethyl 5-ethylpyridine-2-carboxylate (28)

To a solution of **27** (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 °C 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).

5.26. Ethyl 5-ethyl-6-oxo-1,6-dihydropyridine-2-carboxylate (29)

To a solution of **28** (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).

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

To a solution of **29** (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 °C 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).

5.28. Ethyl 3-amino-6-(benzyloxy)-5-ethylpyridine-2-carboxylate (30)

In the same manner as in the preparation of **21**, the title compound (17.8 g, 98%) was obtained as yellow oil from **6e** (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).

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

To a solution of **30** (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 °C 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 4 M HCl/AcOEt (5.4 mL), and the mixture was stirred at 0 °C 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 °C 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).

5.30. Ethyl 5-(benzyloxy)-6-ethyl-3-hydroxy-1-methyl-1*H*-pyrrolo[3,2-*b*]pyridine-2-carboxylate (4e)

To a solution of **5e** (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 1 M 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).

5.31. Ethyl 6-ethyl-1-methyl-3-(2,2,2-trifluoroethoxy)-5-{[(trifluoromethyl)sulfonyl]oxy}-1*H*-pyrrolo [3,2-*b*]pyridine-2-carboxylate (31)

To a solution of **4e** (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*]pyridine -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 °C, and the mixture was stirred at 60 °C 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).

5.32. Ethyl 5-amino-6-ethyl-1-methyl-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo[3,2-*b*]pyridine-2-carboxylate (32)

In the same manner as in the preparation of **21**, the title compound (141 g, 90%) was obtained as a white powder from **31** (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).

5.33. 6-Ethyl-1-methyl-5-[(phenylcarbonyl)amino]-3-(2,2,2-trifluoroethoxy)-1*H*-pyrrolo[3,2-*b*]pyridine-2-carboxylic acid (3e)

To a solution of **32** (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 °C 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 8 M NaOH aq (0.366 mL), and the mixture was stirred at 50 °C for 1 h. The mixture was concentrated in vacuo, the precipitate was diluted with water (20 mL) and acidified with aqueous 1 M 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).

5.34. Ethyl 5-ethyl-3-hydroxypyrazine-2-carboxylate (34)

To a suspension of **33** (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 (3H, t, J = 7.6 Hz), 1.27 (3H, t, J = 7.2 Hz), 2.53–2.62 (2H, m), 4.26 (2H, q, J = 7.2 Hz), 7.42 (1H, br s), 12.68 (1H, br s).

5.35. Ethyl 3-(benzyloxy)-6-bromo-5-ethylpyrazine-2-carboxylate (35)

To a solution of **34** (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 (3H, t, J = 7.5 Hz), 1.29 (3H, t, J = 7.1 Hz), 2.89 (2H, q, J = 7.5 Hz), 4.33 (2H, q, J = 7.1 Hz), 5.50 (2H, s), 7.28–7.44 (3H, m), 7.44–7.56 (2H, m).

5.36. Ethyl 3-(benzyloxy)-5-ethyl-6-[(phenylcarbonyl)amino]-pyrazine-2-carboxylate (36)

To a mixture of **35** (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 2 M HCl aq (3 mL) was added. After stirring for 3 h, 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 (3H, t, J = 7.5 Hz), 1.28 (3H, t, J = 7.1 Hz), 2.73 (2H, q, J = 7.5 Hz), 4.33 (2H, q, J = 7.1 Hz), 5.55 (2H, s), 7.28–7.47 (3H, m), 7.47–7.71 (5H, m), 7.95–8.09 (2H, m), 10.76 (1H, s).

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

To a solution of **36** (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 (3H, t, J = 7.3 Hz), 1.34 (3H, t, J = 7.1 Hz), 2.83 (2H, q, J = 7.3 Hz), 4.43 (2H, q, J = 7.1 Hz), 7.49–7.74 (3H, m), 7.99–8.10 (2H, m), 11.42 (1H, s).

5.38. Ethyl 3-ethyl-7-hydroxy-5-methyl-2-[(phenylcarbonyl)amino]-5*H*-pyrrolo[2,3-*b*]pyrazine-6-carboxylate (4f)

In the same manner as in the preparation of **4b**, the title compound (159 mg, 80%) was obtained as a pale yellow powder from **6f** (242 mg, 0.541 mmol). ^{1}H NMR (DMSO-d_6) δ 1.28 (3H, t, J = 7.6 Hz), 1.35 (3H, t, J = 7.2 Hz), 2.88 (2H, q, J = 7.6 Hz), 3.95 (3H, s), 4.37 (2H, q, J = 7.2 Hz), 7.45–7.72 (3H, m), 7.94–8.12 (2H, m), 9.91 (1H, br s), 10.72 (1H, s).

5.39. 3-Ethyl-5-methyl-2-[(phenylcarbonyl)amino]-7-(2,2,2-trifluoroethoxy)-5*H*-pyrrolo[2,3-*b*]pyrazine-6-carboxylic acid (3f)

To a solution of **4f** (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 1 M NaOH aq (0.5 mL), and the mixture was stirred at room temperature for overnight. The mixture was acidified with 1 N 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 (3H, t, J = 7.4 Hz), 2.89 (2H, q, J = 7.4 Hz), 4.01 (3H, s), 5.10 (2H, q, J = 8.9 Hz), 7.45–7.72 (3H, m), 7.96–8.10 (2H, m), 10.82 (1H, s), 13.47 (1H, br s).

5.40. 2-Ethyl-*N*-[1-(hydroxyacetyl)piperidin-4-yl]-7-methyl-4-oxo-5-(2,2,2-trifluoroethoxy)-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxamide (2a)

To a mixture of **3a** (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 (5H, m), 1.76–1.98 (2H, m), 2.63 (2H, q, J = 7.5 Hz), 2.75–2.95 (1H, m), 3.00–3.19 (1H, 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 (1H, t, J = 5.5 Hz), 5.20 (2H, q, J = 9.1 Hz), 7.43 (1H, d, J = 7.7 Hz), 12.10 (1H, s).

The following compounds **2b**–**f** were prepared in a same manner similar to that described for **2a**.

5.41. 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 (2b)

Yield 47%, white crystals. Mp 150 °C (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^+).

5.42. 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 (2c)

Yield 53%, white crystals. Mp 200 °C (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^+).

5.43. 6-Ethyl-*N*-[1-(hydroxyacetyl)piperidin-4-yl]-1-methyl-5-[(phenylcarbonyl)amino]-3-(2,2,2-trifluoroethoxy)-1*H*-indole-2-carboxamide (2d)

Yield 82%, white crystals. Mp 227 °C (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^+).

5.44. 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 (2e)

Yield 46%, white crystals. Mp 165 °C (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 $C_{27}H_{30}F_3N_5O_5{\cdot}0.30H_2O$: 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^+).

5.45. 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 (2f)

Yield 61%, pale yellow crystals. Mp 142 °C (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 $C_{26}H_{29}N_6O_5F_3{\cdot}H_2O$: 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^+).

5.46. 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 (2g)

A mixture of **2a** (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 °C for 13.5 h followed by addition of DMF (1.0 mL). The mixture was stirred at 60 °C for 1.5 h, at 70 °C for 2.5 h, and at 80 °C 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 (5H, m), 1.77–2.00 (2H, m), 2.66–2.93 (3H, m), 3.03–3.20 (1H, m), 3.60–3.78 (1H, m), 3.83–3.95 (3H, m), 3.97–4.16 (3H, m), 4.16–4.35 (1H, m), 4.53 (1H, t, J = 5.5 Hz), 5.09 (2H, q, J = 9.1 Hz), 5.73 (2H, s), 7.50 (1H, d, J = 7.7 Hz), 7.56–7.68 (2H, m), 7.69–7.83 (1H, m), 8.12 (2H, 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^+).

5.47. 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 7.5×10^3 cells/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× solution (0.0003–10 μM in DMEM) were added to the culture to achieve the final concentrations of 5.8% FBS, 1-μg/mL of Shh-N, and 0.03–1000 nM of the compounds (n = 4 wells per concentration). The cells were then incubated for an additional 48 h. 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]).

5.48. In vivo PD 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).

5.49. Pharmacokinetic studies

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.

5.50. X-ray structure analysis

Crystal data for **1**: $C_{28}H_{31}F_3N_4O_6{\cdot}0.1H_2O$, MW = 578.37; crystal size, 0.41 × 0.20 × 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 **2b**: $C_{28}H_{31}F_3N_4O_5{\cdot}2H_2O$, MW = 596.60; crystal size, 0.22 × 0.09 × 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 **2d**: $C_{28}H_{31}F_3N_4O_5$, MW = 560.57; crystal size, 0.17 × 0.13 × 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 **2e**: $C_{27}H_{30}F_3N_5O_5$, MW = 561.56; crystal size, 0.16 × 0.08 × 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[13] and was refined using full-matrix least-squares on F^2 with SHELXL-97.[13] All non-H atoms were refined with anisotropic displacement parameters.

CCDC 865214 for compound **1**, 1027998 for compound **2b**, 1027990 for compound **2d** and 1028036 for compound **2e** 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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