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# Structure-based drug design of novel and highly potent pyruvate dehydrogenase kinase inhibitors

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ABSTRACT

Pyruvate dehydrogenase kinases (PDHKs) are fascinating drug targets for numerous diseases, including diabetes and cancers. In this report, we describe the result of our structure-based drug design from tricyclic lead compounds that led to the discovery of highly potent PDHK2 and PDHK4 dual inhibitors in enzymatic assay. The C3-position of the tricyclic core was explored, and the PDHK2 X-ray structure with a representative compound revealed a novel ATP lid conformation in which the phenyl ring of Phe326 mediated the interaction of the Arg258 sidechain and the compound. Compounds with amide linkers were designed to release the ATP lid by forming an intramolecular pi-pi interaction, and these compounds showed single-digit nM $IC_{50}$ values in an enzymatic assay. We also explored the C4-position of the tricyclic core to reproduce the interaction observed with the C3-position substitution, and the pyrrolidine compound showed the same level of $IC_{50}$ values. By optimizing an interaction with the Asn255 sidechain through a docking simulation, compounds with 2-carboxy pyrrole moiety also showed single-digit nM $IC_{50}$ values without having a cation-pi interaction with the Arg258 sidechain.

## 1. Introduction

Pyruvate dehydrogenase (PDH) is a primary enzyme of the pyruvate dehydrogenase complex (PDC). The PDC fulfills an essential role in energy metabolism, regulating the oxidative decarboxylation of pyruvate to form acetyl-CoA by a process called the Swanson Conversion. PDC activity is regulated by pyruvate dehydrogenase kinase (PDHK), which phosphorylates PDH E1α subunit and inactivates PDC. PDHK is a member of the GHKL ATPase/kinase superfamily,[1–2] and there are four isozymes (PDHK1, 2, 3 and 4).[3] The reduction of PDC activity, which is caused by the induction and activation of PDHK, have been observed in various diseases such as type2 diabetes, ischemic heart disease, peripheral artery disease, pulmonary hypertension, and cancers.[4–9] This decrease in PDC activity leads to uncoupling of glycolysis and carbohydrate oxidation, which is one of the factors of abnormal glucose metabolism in these disorders.

Among the four PDHK isozymes, PDHK4 has attracted extensive attention because its expression is changed considerably depending on the physiological conditions. In starvation and diabetes, PDHK4 expression is significantly upregulated in several peripheral tissues including skeletal muscle, heart, mammary gland and kidney.[10–11] Regarding the expression level in the liver, PDHK4 is increased slightly and PDHK2 is primarily upregulated.[10–11] It has also been reported that the glucose levels of PDHK2/4 double knockout mice are lower than single knockout mice bearing only PDHK2 or PDHK4.[12] These data indicate that the dual inhibition of PDHK2 and PDHK4 is a promising

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approach to enhance PDC activity for treating metabolic diseases.

Several PDHK inhibitors acting at allosteric binding domains and substrate binding domains have been reported.[13] Among these binding domains, highly potent inhibitors were discovered for the lipoyl group binding site and the ATP binding site (Fig. 1a).[14–18] AZD-7545 acts at the lipoyl group binding site and inhibits PDHK1-3 activity by impeding PDHK binding to the E2/E3bp core of PDC.[14–15] AZD-7545 binds to PDHK4 as well but stimulates PDHK4 kinase activity similar to the action of E2/E3bp on other PDHK1-3 isozymes.[16] The activation role of the lipoyl binding site on PDHK4 excludes this domain from the target site of PDHK4 inhibitors. Compounds **2** and **3** act on the ATP binding site of PDHKs, and as the ATP binding site is conserved among the isoforms, these compounds are pan-PDHK inhibitors.[17–18] These compounds use a resorcinol core structure to occupy the adenine binding site, and modification at the solvent-exposed region leads to enhanced activity. Compound **2** displayed antiproliferative activities toward cancer cells, and compound **3** improved glucose tolerance in diet-induced obese mouse.

In our previous report, we have described our fragment-based lead discovery result to identify novel inhibitors of PDHKs at the ATP binding site.[19] Several lead compounds with novel chemotypes were discovered by conducting structure-based drug design (SBDD) from two X-ray fragment hits. Fig. 1b shows the chemical structure of tricyclic lead compound with PDHK2 and PDHK4 inhibition data. Pyrimidoindole **4** were obtained through the SBDD optimization at the adenine binding site of ATP and showed $IC_{50}$ values in the low micromolar range. The selectivity profile in the GHKL ATPase/kinase superfamily was evaluated by testing inhibition activity of BCKDK, and compound **4** showed little inhibition. From the structural information from our previous report, this compound was considered to fill up the space at the adenine binding site of ATP on PDHKs. Therefore, to further improve the inhibition activity, the optimization outside of the adenine binding site was required.

In this report, we describe our efforts to discover highly potent PDHK2/4 dual inhibitors by applying SBDD and computational analysis. Several substituents were installed at the solvent-exposed site to discover inhibitors with single-digit nM $IC_{50}$ values in the enzymatic assay.

## 2. Results and discussion

### 2.1. Analysis of an X-ray structure of compound 5 with PDHK2

As mentioned above, the ATP binding site is conserved among the PDHK isoforms, and we observed a linear structure–activity relationships (SAR) between PDHK2 and PDHK4.[17–19] To advance the SBDD on the ATP binding site, we chose PDHK2 for X-ray crystallographic studies as this isoform gave higher crystallographic resolution. Firstly, we obtained an X-ray structure of compound **5**, which is a pyridoindole analogue of compound **4**. Fig. 2 shows the obtained X-ray structure, and an X-ray structure of AMP-PNP bound with PDHK2[20] is also shown for comparison. The compound **5** was bound to the adenine-binding site of ATP, which is located in the C-terminal domain of PDHKs. The nitrogen atom at the N1-position formed a water-bridged hydrogen bond interaction with the aspartic acid moiety of PDHKs (Asp290 for PDHK2). Three water molecules were displaced by hydrophobic groups, making the compound bind deeper than the adenine ring of ATP (Fig. 2b). The hydrogen atoms on the N9-position and the cyclopropyl on the benzyl position formed a polar interaction with the Asp290 sidechain, and the methyl substituent of the C2-position formed a CH-O polar interaction with Gly292 and the Gly294 backbone carbonyl. The ATP lid, which traps the phosphate of ATP, were just partially visible in the electron density on known X-ray structures of PDHKs, and the residues 313–326 were not visible in the X-ray structure of compound **5**. To target the ribose and triphosphate binding site from the tricyclic core, the C3-position and C4-position were selected for modification.

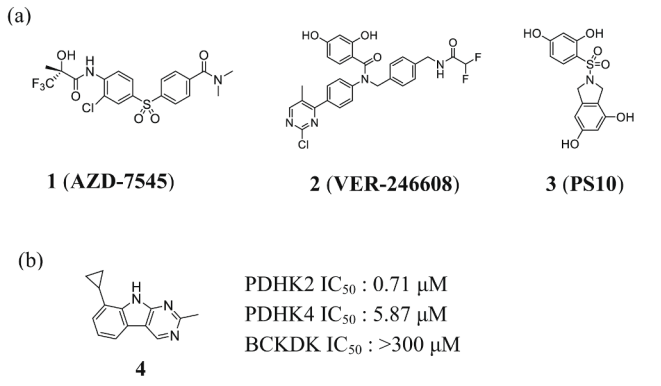



**Fig. 1.** Representative chemical structures of reported PDHKs inhibitors. (a) Highly potent compounds identified by other groups. AZD-7545 acts on the lipoyl group binding site, and VER-246608 and PS10 act on the ATP binding site. (b) Our tricyclic lead compound identified by FBDD approach on the ATP binding site.

### 2.2. SBDD optimization of the pyridoindole 5 by attaching substituents from C3-position of tricyclic core

To target the ribose and the triphosphate binding site of ATP from

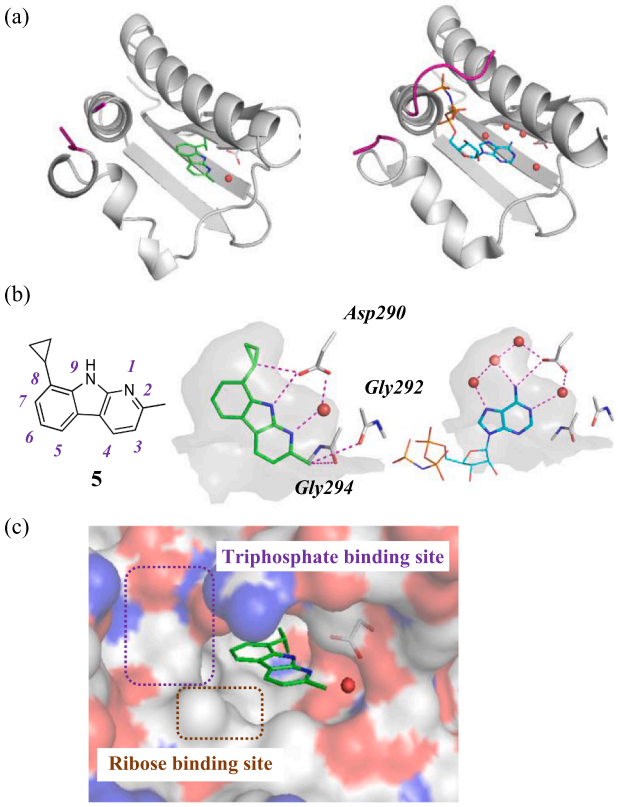



**Fig. 2.** PDHK2 X-ray structure with compound **5**. X-ray structure with AMP-PNP is shown for comparison (PDB ID: 3CRL). Compound **5** is shown in sticks with light green carbons, and AMP-PNP with cyan carbons. Water molecules are represented as red spheres. (a) PDHK2 is shown in gray cartoons, and just the GHKL domain is shown for clarity. The ATP lid from Ser309 to Gly327 is colored in magenta. (b) Detailed view around the adenine binding site of ATP. Protein interiors are shown in gray surface. Location number of compound **5** is shown in purple italic, and dashed lines indicate polar interactions. (c) Ribose and triphosphate binding sites of ATP are shown on the protein surface of PDHK2 X-ray structure of compound **5**.

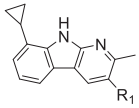
the C3-position of the tricyclic core, the SAR near the adenine binding site were firstly investigated by installing several chemical groups on compound **5**. The inhibitory activities toward PDHK2 and PDHK4 were measured by spectrophotometric assay which detected the residual PDH activity at the $K_m$ concentration of ATP (0.3 μM for PDHK2 and 10 μM for PDHK4). As shown in Table 1, the inhibitory activities of the *N*-methylamide **6**, the phenyl **8**, the 2-pyridine **9** and the 1,3,4-thiadiazole **11** were comparable to that of compound **5** that had no substituent at the C3-position. The methoxy-carbonyl compound **7** and the 1,3,4-oxadiazole **10** as a methoxy-carbonyl bioisostere enhanced PDHK2/4 inhibitory activities by 4 to 10 times.

To gain structural information for improving the inhibitory activity, we obtained the X-ray structure of compound **7** bound to PDHK2 (Fig. 3). Interestingly, two types of ATP lid structures were observed in the X-ray structure. In one type of X-ray structure, denoted as "open ATP lid conformation" in Fig. 3a, the backbone of the ATP lid occupied the space near the triphosphate binding site of ATP. In the other X-ray structure, denoted as "closed ATP lid conformation" in Fig. 3b, the ATP lid approached the adenine binding site of ATP. The phenyl ring of Phe326 on the ATP lid and the methoxy carbonyl of compound **7** formed a pi-pi interaction (Fig. 3c). The guanidino group of Arg258 on the α-helix 10 and the phenyl ring of Phe326 formed a cation-pi interaction, which potentially stabilized the interaction. As the increase of activity of **7** and **10** is more consistent with the formation of a pi-pi interaction with Phe326, the X-ray structure with the closed ATP lid conformation was selected for further SBDD analysis.

To analyze the thermodynamic properties of water molecules near the binding site, a WaterMap[21] calculation was performed toward the X-ray structure of **7** on PDHK2 with the "closed ATP lid conformation" (Fig. 4). WaterMap is a molecular dynamics (MD)-based computational method to detect hydration sites from resultant MD trajectories, and calculates the thermodynamic properties (free energy (ΔG), enthalpy (ΔH) and entropy (-TΔS)) relative to bulk water. The WaterMap calculation revealed the unstable hydration site near Lys299, Arg302 and Leu303 residues (free energy to the bulk water = +5.43 kcal/mol). Both enthalpy and entropy at this hydration site with respect to the bulk water was calculated as unstable (enthalpy =+1.38 kcal/mol, entropy = +4.05 kcal/mol), and an unstable enthalpic energy profile suggested that the polar interaction at this hydration site was not sufficient to overcome the desolvation penalty. The thermodynamic profile of the WaterMap calculation led to the strategy to displace the unstable water molecule with a hydrophobic group.

SAR results of installing several alkyl chains on the 2-position of the

**Table 1**
Influence of C3-position substituent on PDHK2 and PDHK4 activity.



| compd | $R_1$ | $IC_{50}$ (μM) | |
|---|---|---|---|
| | | PDHK2 | PDHK4 |
| **5** | H | 2.03 | 11.87 |
| **6** | CONHMe | 5.51 | >30 (35%) [a] |
| **7** | $CO_2Me$ | 0.21 | 1.54 |
| **8** | phenyl | 5.33 | 7.49 |
| **9** | 2-pyridine | 1.51 | 2.98 |
| **10** |  | 0.49 | 1.51 |
| **11** |  | 1.49 | 6.62 |

[a] Values in parentheses were percent inhibition at the indicated concentration.

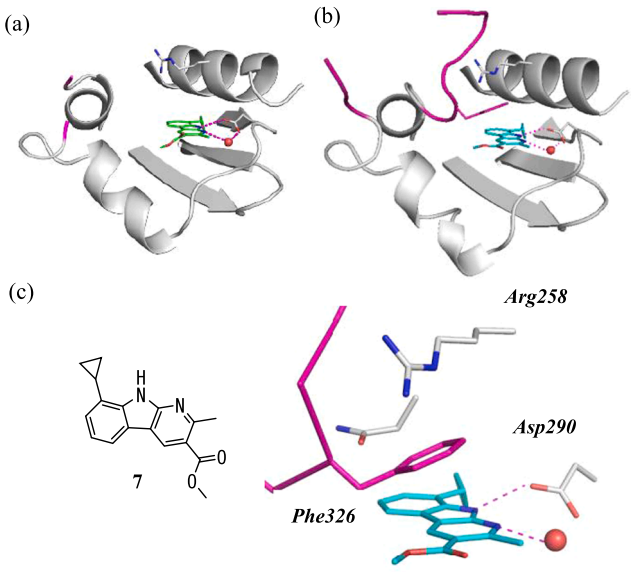



**Fig. 3.** X-ray structure of compound **7** on PDHK2. ATP lid from Ser309 to Gly327 is colored in magenta, and water molecules are represented as red spheres. (a) Chain A X-ray structure with "open ATP lid conformation", where the ATP lid is distant from the ligand. (b) Chain B X-ray structure with "closed ATP lid conformation", where ATP lid is close to the ligand. (c) Close-up view of Chain B. Arg258, Asp290, Phe326 and the ligand are shown in sticks. Purple dashed lines indicate hydrogen bonds.

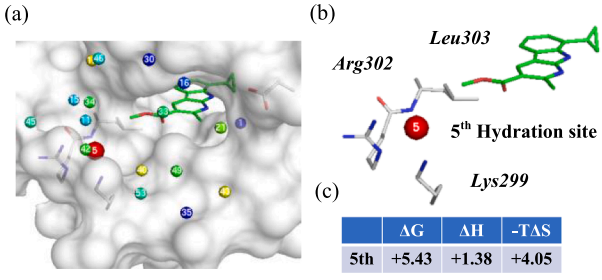
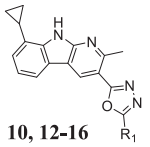
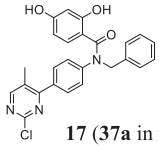



**Fig. 4.** WaterMap calculation of PDHK2 X-ray structure with compound **7.** (a) Detected hydration sites are represented as spheres. Color scheme reflects the predicted ΔG gain when the water site is displaced: from red (unstable waters) to blue (stable waters). (b) Detailed view around the most unstable water (5th hydration site). (c) Thermodynamic profile of WaterMap detected a 5th hydrationsite. Free energy (ΔG), enthalpy (ΔH) and entropy (-TΔS) relative to a bulk water were calculated through MD calculation.

**Table 2**
Inhibitory activities of substituted 1,3,4-oxadiazole compounds against PDHK2 and PDHK4 in the enzymatic assay.


**10, 12-16**


**17 (37a** in ref 17)

| compd | $R_1$ | $IC_{50}$ (μM) | |
|---|---|---|---|
| | | PDHK2 | PDHK4 |
| **10** | $CH_3$ | 0.494 | 1.510 |
| **12** | $CH_2CH_3$ | 0.190 | 2.122 |
| **13** | $CH(CH_3)_2$ | 0.130 | 0.592 |
| **14** | $(CH_2)_2CH_3$ | 0.091 | 0.292 |
| **15** | $CH_2CH(CH_3)_2$ | 0.056 | 0.462 |
| **16** | $CH(CH_3)CH(CH_3)_2$ | 0.087 | 0.494 |
| **17** | – | 0.035 | 0.350 |

1,3,4-oxadiazole ring (**12**–**16**) to displace the unstable water in the hydrophobic site are summarized in Table 2. By increasing the alkyl chain length from methyl to propyl, the inhibition activity was improved. Among the alkyl groups synthesized, the isobutyl compound **15** showed $IC_{50}$ values of 0.056 μM and 0.46 μM against PDHK2 and PDHK4, respectively. To compare the activity with the reference compound, we measured the activity of the resorcinol compound **17**, which corresponds to the substructure of compound **2** (VER-246608) (Fig. 1a). This compound was reported to display a similar $IC_{50}$ value with VER-246608 toward PDHK1 (**17**:0.072 μM, **2**:0.035 μM),[17] and in our assay system, the compound displayed PDHK2 and PDHK4 $IC_{50}$ values of 0.035 μM and 0.350 μM, respectively. These data indicated that the activity of our compound **15** was comparable to VER-246608. The weaker inhibitory activities toward PDHK4 were observed in our assay, in which the $K_m$ concentration of ATP was used for each enzyme (0.3 μM for PDHK2 and 10 μM for PDHK4). PDHK4 was reported to take a metastable open conformation to promote ATP/ADP exchange,[16] and the resulted robust basal activity of PDHK4 may cause the observed differences in inhibitory activities.

To further improve the inhibition activity, we attempted to reproduce the pi-pi interaction internally in the molecule, and to increase interaction with the guanidino group of Arg258 to form a direct cation-pi interaction. The ATP lid was originally a flexible loop, and we considered that releasing the ATP lid from the more rigid closed conformation to a flexible open conformation could be energetically favorable. This idea led to the strategy to replace the phenyl ring of Phe326 by forming a intramolecular pi-pi interaction in the ligand.

To obtain a suitable linker to connect 1,3,4-oxadiazole and phenyl ring of Phe326, a 3D geometry search toward all ligands in PDB structures was conducted (Fig. 5). The centroid distance and the angle of two rings were measured on the PDHK2 docking pose of **15**, and the 3D geometry search query was constructed. The PDB geometry search was performed using PSILO/MOE[22] software with the query (centroid distance < 4.5Å, angle of two planes < 30°, and the number of atoms between the nearest path of the two rings < 6 atoms), and the obtained hit structures were inspected manually. The amide linker of PDB code 1TCX[23] was selected as a reference for our linker design, and 2 types of amide linkers in **18** and **19** were designed to form internal pi-pi interactions.

Compound **18** and **19** were synthesized as racemate, and these compounds improved the PDHK2/4 inhibitory activities around 10-fold compared to **15** (Table 3). Selectivity profile toward BCKDK in the same GHKL ATPase/kinase superfamily was also evaluated, and compounds **18** and **19** showed little inhibition. Compound **20**, which is a chiral analogue of compound **19** with a methyl substituent on benzyl position, kept the inhibition activity.

X-ray structure of a chiral compound **20** bound to PDHK2 was obtained that confirmed our design hypothesis (Fig. 6a). Phenyl ring and 1,3,4-oxadiazole ring formed an intramolecular pi-pi interaction, and the phenyl ring formed a direct cation-pi interaction with Arg258 sidechain (Fig. 6b). The isopropyl substituent occupied the hydrophobic space near Lys299, Arg302 and Leu303, where the WaterMap calculation detected the unstable hydration site in Fig. 4. Conformation of amide linker in the X-ray structure resembles the conformation of parent PDB code 1TCX, which validated the effectivity of 3D geometry search (Fig. 6c). Amide linker formed a water mediated interaction with the sidechain of Asn255. ATP lid from residues 317–330 were just partially visible in the electron density in the "open ATP lid conformation" of this X-ray structure.

### 2.3. SBDD optimization of the pyrimidoindole **4** by attaching substituents from C4-position of tricyclic core

We also conducted the SBDD optimization from the C4-position of the pyrimidoindole **4**. As discussed in the previous section, compound **20** improved activity by forming a cation-pi interaction with Arg258 and a hydrogen bond interaction with Asn255, and these interactions were targeted from C4-position. From the result of docking simulations, the pyrrolidine linker was selected for the synthesis as it showed suitable vectors ($R_2$ and $R_3$) to target Arg258 and Asn255 (Fig. 7).

Table 4 shows the SAR result of installing several pyrrolidine derivatives from the C4-position of the tricyclic core. Although PDHK2/4 inhibitory activities of pyrrolidine compound **21** were slightly decreased

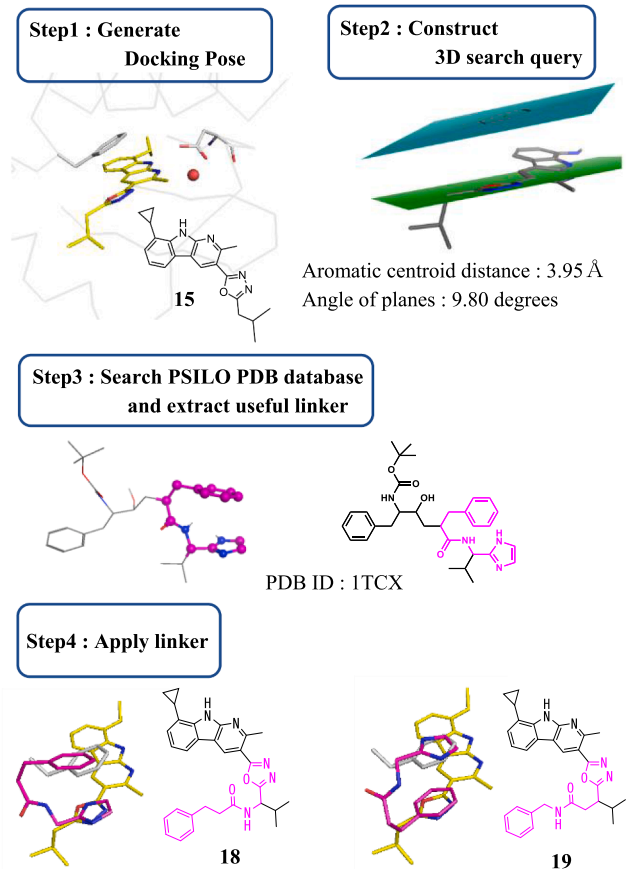
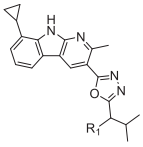
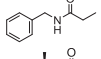
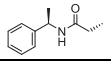



**Fig. 5.** Procedure of 3D geometry search to obtain suitable linkers to form an intramolecular pi-pi interaction.

**Table 3**
Inhibitory activities of substituted 1,3,4-oxadiazolyl compounds against PDHK2, PDHK4 and BCKDK.



| compd | $R_1$ | $IC_{50}$ (μM) PDHK2 | PDHK4 | BCKDK [a] |
|---|---|---|---|---|
| **15** | H | 0.0558 | 0.4624 | |
| **18** |  | 0.0042 | 0.0241 | >100 (3.0%) [b] |
| **19** |  | 0.0060 | 0.0329 | >100 (18%) [b] |
| **20** |  | 0.0171 | 0.0615 | |

[a] Luminescent kinase assay using ADP-Glo™.
[b] Values in parentheses were percent inhibition at the indicated concentration.

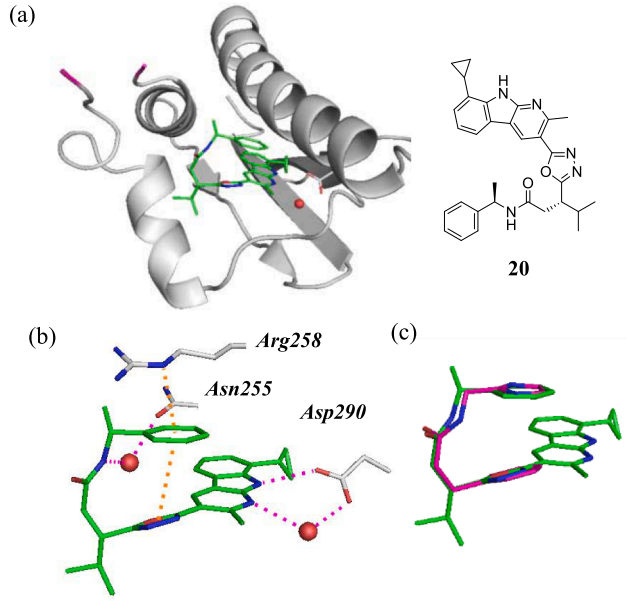



**Fig. 6.** X-ray structure of compound **20** on PDHK2. Compound **20** is shown in sticks with light green carbons, and water molecules are represented as red spheres. (a) PDHK2 is shown in gray cartoons, and just the GHKL domain is shown for clarity. ATP lid from Ser309 to Gly327 is colored in magenta. (b) Close-up view around the ligand. Purple lines indicate hydrogen bonds, and orange lines indicate potential stacking interactions. (c) Superposition of the binding pose of **20** on PDHK2 and the substructure pose of PDB 1TCX shown on Fig. 5.

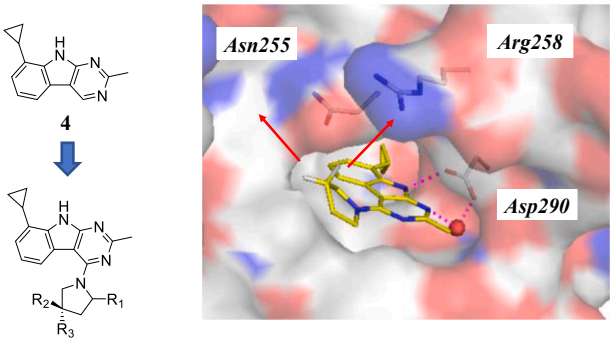



**Fig. 7.** Docking pose of the designed pyrrolidine compound on PDHK2. $R_2$ and $R_3$-position of the pyrolidine linker offered suitable vectors to target Arg258 and Asn255 sidechains.

**Table 4**
Inhibitory activities of C4-position substituted pyrimidoindole compounds against PDHK2 and PDHK4.

| compd [a] | $R_1$ | $R_2$ | $R_3$ | $IC_{50}$ (μM) | |
|---|---|---|---|---|---|
| | | | | PDHK2 | PDHK4 |
| **4** | – | – | – | 0.7080 | 5.8700 |
| **21** | H | H | H | 2.4100 | 10.3000 |
| **22** | Ethyl | H | H | 0.7580 | 8.4600 |
| **23** | (*S*)–$CH_2OH$ | H | H | 0.3520 | 4.5300 |
| **24** | (*R*)–$CH_2OH$ | H | H | 8.5200 | >30 (21%) [b] |
| **25** | (*S*)–$CH_2OH$ | Ph | H | 1.0100 | 7.7000 |
| **26** | (*S*)–$CH_2OH$ | Bn | H | 0.0730 | 1.1200 |
| **27** | (*S*)–$CH_2OH$ | Bn | $CH_2OH$ | 0.0146 | 0.2920 |
| **28** | (*S*)–$CH_2OH$ | Bn | $CONH_2$ | 0.0045 | 0.0264 |

[a] Related chemical structures are shown on Fig. 7.
[b] Values in parentheses were percent inhibition at the indicated concentration.

from **4**, the compounds that introduced ethyl and (*S*)-hydroxymethyl on $R_1$ position (compound **22** and **23)** showed same level of PDHK2/4 inhibitory activities. Hence, these substituted pyrrolidines were selected as linkers for further optimization. The phenyl and benzyl substituent were introduced on $R_2$ position to form a cation-pi interaction with Arg258 sidechain, and the benzyl compound **26** improved the activity. To further improve inhibition activities, polar substituents were attached additionally on pyrrolidine $R_3$-position to form a hydrogen bond interaction with Asn255 sidechain. Compounds **27** and **28** further improved the activity, and compound **28** showed $IC_{50}$ values of 4.5 nM and 26.4 nM against PDHK2 and PDHK4 enzymatic assay.

Fig. 8a shows the docking pose of compound **28** on PDHK2. In the docking model, benzyl substituent formed a cation-pi interaction with Arg258, and an amide formed a hydrogen-bond interaction with the sidechain carbonyl of Asn255. The one hydrogen of Asn255 sidechain $NH_2$ formed a hydrogen-bond interaction with Glu251 backbone to restrict the conformation. As the second hydrogen of Asn255 sidechain $NH_2$ located near the ligand, we designed compounds to form an additional hydrogen bond interaction on this position. 2-carboxy-pyrrole analogues were selected for synthesis as these showed the potential interaction with Asn255 in the docking model (Fig. 8b).

To attach the 2-carboxy-pyrrole moiety on the pyrrolidine ring, the benzyl substituent of compound **28** was removed to keep the compound's molecular weight under 500. Compound **29**, **30** and **31** of 2-carboxy-pyrrole analogues were synthesized, and these compounds showed $IC_{50}$ values of 5.1–9.2 nM for PDHK2 and 12.2–40.6 nM for PDHK4 (Table 5).

### 2.4. Chemistry

The synthetic methods of C3-position substituted pyridoindole **6**–**9** are illustrated in Scheme 1. The bromopyridine **35** was synthesized from the cyanide **32**, conducted the hydrolysis of the nitrile to the carboxylic group, the esterification of **33** to the methyl ester **34**, and the bromination of **34**. The palladium-catalyzed Suzuki-Miyaura cross-coupling reaction of 2-bromoaniline (**36**) and cyclopropylboronic acid gave the cyclopropylbenzene **37**. Conversion of the amino group in **37** to the iodine via the Sandmeyer reaction provided the iodobenzene **38**. The palladium-mediated Buchwald-Hartwig type amination of the iodobenzene **38** and the aminopyridine gave the compound **39** and **40**, and the subsequent intramolecular Heck type cyclization provided the pyridoindole **7** and **41**. The ester of pyridoindole **7** was hydrolyzed to give the carboxylic acid **42**, and the amidation with methyl amine using HATU gave the compound **6**. The compound **8** was prepared from **41** by conducting the Suzuki-Miyaura cross-coupling reaction with the phenyl boronic acid. The compound **41** was borated with bis(pinacolato)

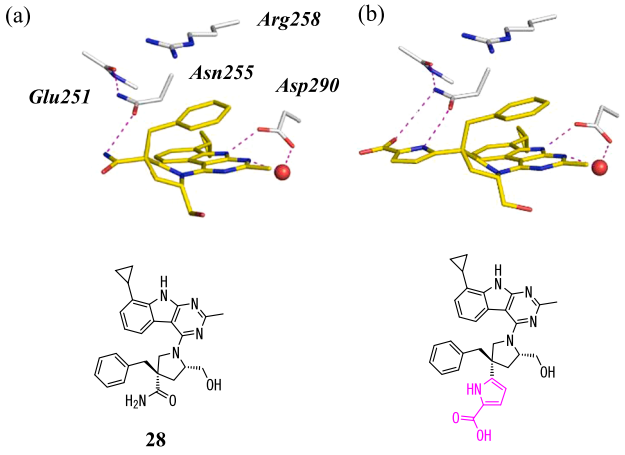
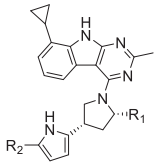



**Fig. 8.** Docking model of compound **28** and the analogue of 2-carboxy pyrole on PDHK2.

**Table 5**
Inhibitory activities of C4-position substituted pyrimidoindole compounds against PDHK2 and PDHK4.

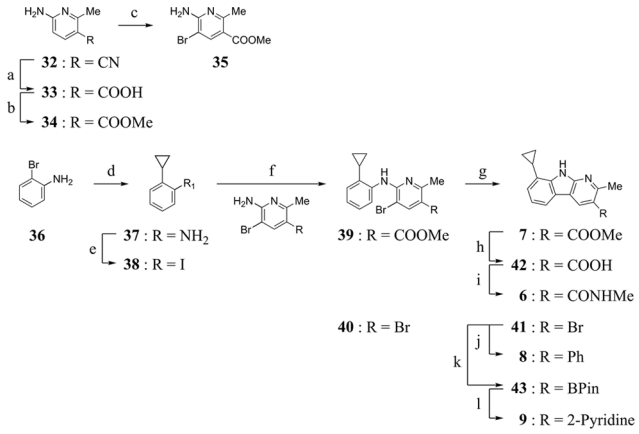


| compd | $R_1$ | $R_2$ | $IC_{50}$ (μM) | |
|---|---|---|---|---|
| | | | PDHK2 | PDHK4 |
| **29** | Ethyl | COOH | 0.0092 | 0.0406 |
| **30** | $CH_2OH$ | COOH | 0.0051 | 0.0122 |
| **31** | $CH_2OH$ | $CONH_2$ | 0.0055 | 0.0178 |

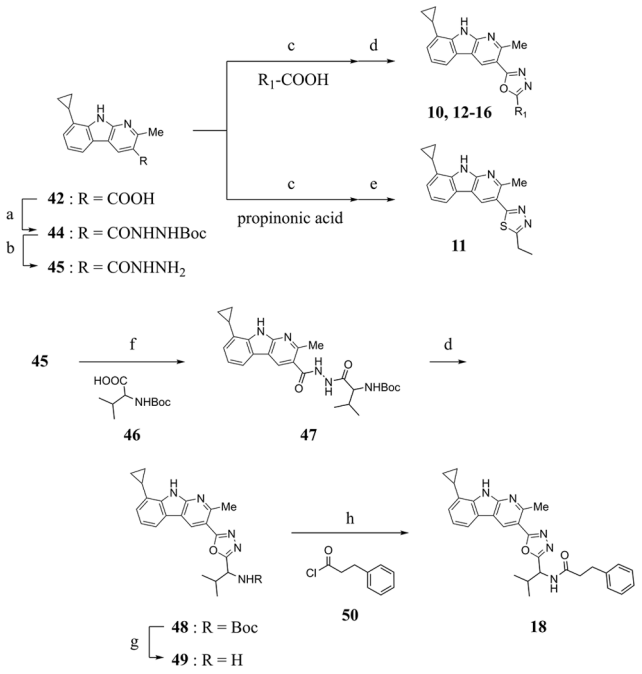
diboron to give **43**, and the subsequent cross-coupling reaction with 2-bromopyridine gave the compound **9**.

The synthetic methods of C3-position substituted pyridoindole **10**–**16** and **18** are illustrated in Scheme 2. The acylhydrazine **45** was prepared from the carboxylic acid **42** by the amidation with *tert*-butyl carbazate and the deprotection of *N*-Boc. In the next step, the compound **45** was reacted with the carboxylic acid by using the HATU condensation to obtain the intermediate diacylhydrazine, which was converted to the 1,3,4-oxadiazole **10**–**12** and **16** by addition of the methyl *N*-(triethylammoniumsulfonyl)carbamate (Burgess reagent). The thiadiazole **11** was synthesized from corresponding diacylhydrazine using the 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetan-2,4-disulfide (Lawesson reagent). The compound **45** was reacted with the carboxylic acid **46** using 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (WSC)/HOBt condensation to obtain the diacylhydrazine **47**, which was converted to the 1,3,4-oxadiazole **48** by addition of the Burgess reagent. *N*-Boc of **48** was deprotected to the target amide **49** by treating with HCl in dioxane solvent. Finally, the **49** was treated with 3-phenylpropanoyl chloride (**50**) under a basic condition to give the compound **18**

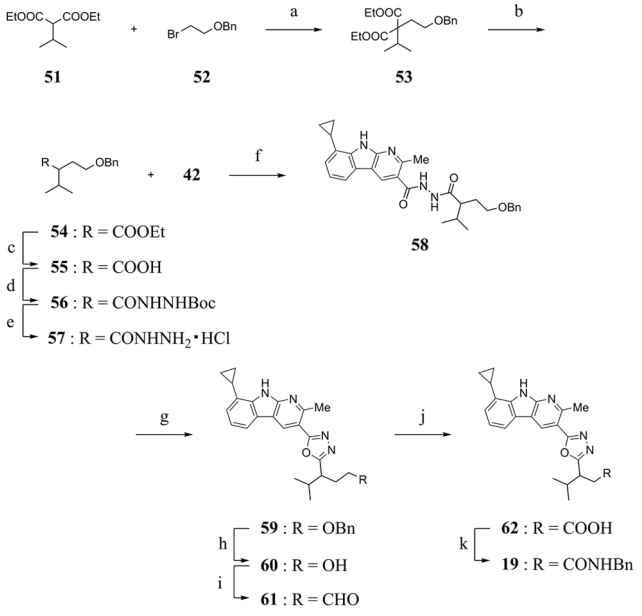
The synthetic methods of C3-position substituted pyridoindole **19** are illustrated in Scheme 3. 2-Isopropylmalonate (**51**) was reacted with




**Scheme 1.** Reagents and conditions: (a) KOH, $H_2O$, 100 °C, 8 h, 70%; (b) $SOCl_2$, MeOH, 80 °C, 15 h, 91%; (c) *N*-bromosuccinimide, THF, rt, 2 h, 82%; (d) cyclopropylboronic acid, $PdCl_2$(dppf)-$CH_2Cl_2$, $K_3PO_4$, toluene, $H_2O$, 90 °C, 5 h, 91%; (e) HCl, $H_2O$, $NaNO_2$, 0 °C, 15 min, then NaI, 0 °C, 30 min, then rt, 2 h, 86%; (f) $Pd(OAc)_2$, Xantphos, sodium *t*-pentoxide, toluene, 140 °C 4 h, 38% for **39**, 37% for **40**; (g) $Pd(OAc)_2$, CyJohnphos, DBU, DMA, 130 °C, 4 h, 66% for **7**, 26% for **41**; (h) NaOH, $H_2O$, THF, EtOH, 60 °C, 4.5 h, quant.; (i) methyl amine, HATU, $Et_3N$, DMF, rt, 2 h, 46%; (j) phenyl boronic acid, $PdCl_2$(dppf)-$CH_2Cl_2$, $K_2CO_3$, 1,4-dioxane, $H_2O$, 80 °C, 4 h, 45%; (k) $(BPin)_2$, $PdCl_2$(dppf)-$CH_2Cl_2$, KOAc, DMSO, 100 °C, 2 h, 26%; (l) 2-bromopyridine, $PdCl_2$(dppf)-$CH_2Cl_2$, $K_2CO_3$, 1,4-dioxane, $H_2O$, 100 °C, 2 h, 22%.

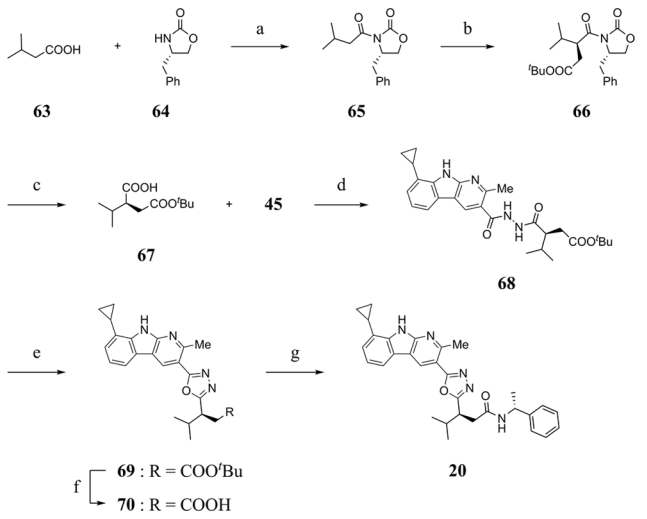



**Scheme 2.** Reagents and conditions: (a) *t*-butyl carbazate, WSC, HOBt, DMF, rt, 4 h; (b) HCl, 1,4-dioxane, 70 °C, 3 h, 84% (2 steps); (c) HATU, $Et_3N$, DMF, rt, 16 h; (d) Burgess reagent, THF, rt, 3 h, 37% for **10** (2 steps); (e) Lawesson reagent, THF, 80 °C, 19 h, 40%; (f) WSC, HOBt, $Et_3N$, DMF, rt, 3 h; (g) HCl, 1,4-dioxane, rt, 1 h, 68% (3 steps); (h) $Et_3N$, $CHCl_3$, 1 h, 96%. The synthetic yields for **12**–**16** are described in the experimental section. $R_1$ substituents of **10** and **12**–**16** are listed in Table 2.




**Scheme 3.** Reagents and conditions: (a) NaH, DMSO, 60 °C, 3 h, 76%; (b) KOH, EtOH, reflux, 17 h; (c) KOH, $H_2O$, EtOH, reflux, 8 h , 47%, 2 steps, (d) $BocNHNH_2$, WSC, HOBt, DMF, rt, 20 h, quant.; (e) HCl, 1,4-dioxane, rt, 3.5 h, quant.; (f) WSC, HOBt, *i*-$Pr_2EtN$, DMF, rt, 1 h; 76%; (g) $POCl_3$, 110 °C, 2 h, 57%; (h) Pd/C (ASCA-2), $H_2$, rt, 2 days, 52%; (i) Dess-Martin Periodinane, $CHCl_3$, rt, 4 h; (j) $NaClO_2$, $NaH_2PO_4$, 2-methyl-2-butene, *t*-BuOH, $H_2O$, THF, rt, 1.5 h; (k) benzylamine, WSC, HOBt, $CHCl_3$, rt, 1 h, 51% (3 steps).

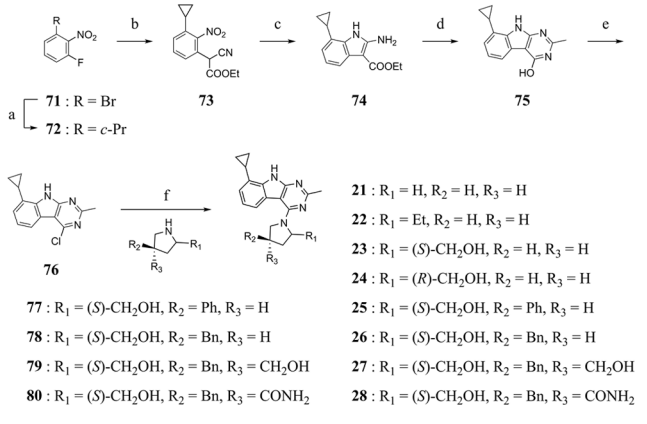
the bromide **52** in the presence of NaH to give **53**, and the compound **53** was decarboxylated using KOH, and the subsequent amidation with *tert*-butyl carbazate gave the compound **56**. *N*-Boc of **56** was deprotected to




**Scheme 4.** Reagents and conditions: (a) WSC, DMAP, MeCN, rt, 19 h, quant.; (b) *t*-butyl 2-bromoacetate, LiHMDS, THF, rt, 3.5 h, 53% (2 steps); (c) $H_2O_2$, THF, 0 °C, 1 h then LiOH, $H_2O$, rt, 1 h, 96%; (d) WSC, HOBt, *i*-$Pr_2EtN$, DMF, rt, 2.5 h, 95%; (e) Burgess reagent, THF, rt, 0.5 h, 63%; (f) TFA, rt, 1 h, 82%; (g) WSC, HOBt, DMF, rt, 1.5 h, 99%.

the acylhydrazine **57** by treating with HCl in dioxane solvent. The carboxylic acid **42** was treated with **57** under the WSC/HOBt condensation to obtain the diacylhydrazine **58**. Subsequent cyclization of **58** in the presence of $POCl_3$ gave the 1,3,4-oxadiazole derivative **59**. Deprotection of the benzyl group of **59** led to the alcohol **60**, followed by Dess-Martin oxidation and Pinnick oxidation to the aldehyde **61** and the carboxylic acid **62** in that order. And then, the target compound **19** was obtained by the amidation with **62** and benzyl amine.

The synthetic methods of C3-position substituted pyridoindole **20** are illustrated in Scheme 4. The isovaleric acid **63** was reacted with the chiral oxazolidinone **64**, and the obtained compound **65** was reacted with *tert*-butyl bromoacetate in the presence of LiHMDS to give compound **66**. The chiral carboxylic acid **67** was obtained from **66** by removing oxazolidinone using $H_2O_2$. The compound **45** was reacted with **67** by using the WSC/HOBt condensation to obtain the diacylhydrazine **68**, which was converted to the 1,3,4-oxadiazole **69** by addition of the Burgess reagent. Then, the *tert*-butyl group in **69** was deprotected by treating with an excess amount of TFA to give the carboxylic acid **70**.

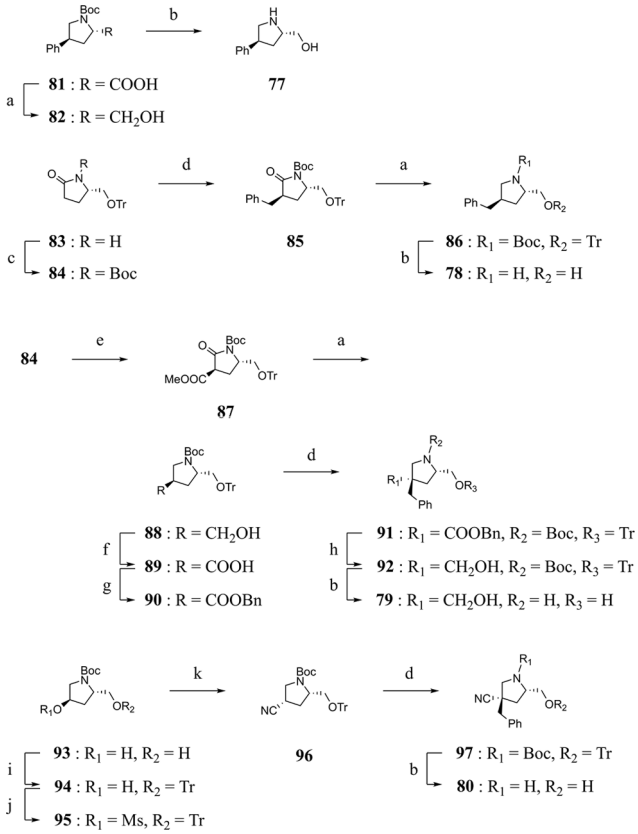



**Scheme 5.** Reagents and conditions: (a) cyclopropylboronic acid, $PdCl_2$(dppf)-$CH_2Cl_2$, $K_3PO_4$, DME, $H_2O$, 110 °C, 1 h, 89%; (b) ethyl cyanoacetate, $K_2CO_3$, DMF, 90 °C, 3 h; (c) Fe, AcOH, 90 °C, 2 h, 52% (2 steps); (d) HCl, 1,4-dioxane, MeCN, rt, 20 h, then sat. $NaHCO_3$ aq, MeOH, 75 °C, 2.5 h, 99%; (e) $SOCl_2$, $CHCl_3$, DMF, 60 °C, 2 h, 46%; (f) corresponding pyrrolidine, *i*-$PrNEt_2$, NMP, Micro-Wave 140 °C, 2 h, 69% for **21**. The synthetic yields for **22**–**28** are described in the experimental section.

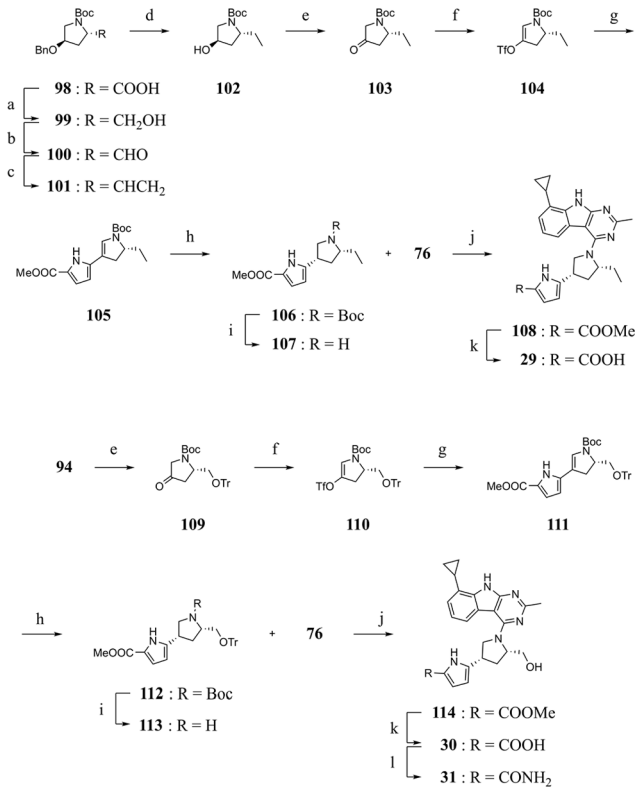
Finally, the amidation of **70** with (*R*)-1-phenylethiamine gave the corresponding amide compound **20**.

The C4-position substituted pyrimidoindole **21**–**28** were prepared by the methods as detailed in Scheme 5. The fluorine in compound **72**, which was prepared from 1-bromo-3-fluoro-2-nitrobenzene (**71**) and cyclopropyl boronic acid by conducting the Suzuki-Miyaura cross-coupling reaction, was substituted by ethyl cyanoacetate to obtain **73**. The iron-mediated reduction of the nitro group in **73** was accompanied with direct cyclization and gave the indole **74**. The reaction of **74** and acetonitrile, treated under acidic condition of HCl/dioxane first, then under basic condition of saturated $NaHCO_3$ solution, gave the pyrimidoindole intermediate **75**. The hydroxyl group in **75** was converted to the chlorine **76** by treating with thionyl chloride. The pyrrolidine compounds **21**–**28** were synthesized by substitutions of the chlorine atom of **76** by corresponding pyrrolidines using a Micro-Wave equipment. The cyano group of compound **80** was hydrolyzed during the reaction to give the compound **28** as the second major component.

The synthetic methods of pyrrolidine compounds **77**–**80** are illustrated in Scheme 6. The carboxylic acid **81** was reduced to alcohol **82** using $BH_3$, and the subsequent *N*-Boc deprotection by treating HCl in dioxane solvent gave the target pyrrolidine **77**. The pyrrolidone **85** was synthesized from the pyrrolidone **83** by benzylating 3-position after protecting lactam NH with Boc group. The pyrrolidine **78** was obtained by reducing the pyrrolidone **85** using $BH_3$ and removing the Boc/Trityl protecting group by HCl in dioxane. The pyrrolidine **88** was synthesized in the similar synthetic procedure of **86**, except using methyl




**Scheme 6.** Reagents and conditions: (a) $BH_3$, THF, 80 °C, 20 min; (b) HCl, dioxane, rt, 1.5 h, 94% for **77** (2 steps); (c) $Boc_2O$, DMAP, DMF, 70 °C, 4 h; (d) BnBr, LiHMDS, THF, rt, 1 h, 77% for **85** (2 steps); (e) Methyl chloroformate, LiHMDS, THF, $CHCl_3$, −30 °C, 1 h, 80% (2 ateps); (f) 1-Methyl-2-azaadamantane-*N*-oxyl, NaClO, $NaClO_2$, *t*-BuOH, $H_2O$, rt, 1.5 h; (g) BnBr, $K_2CO_3$, DMF, rt, 1.5 h, 69% (2 steps); (h) LAH, THF, 0 °C, 30 min, 89%; (i) TrCl, $NEt_3$, DMAP, DMF, 60 °C, 4 h, 95%; (j) MsCl, $Et_3N$, $CHCl_3$, 0 °C, 30 min; (k) $Et_4NCN$, MeCN, 100 °C, 4 h, 74% (2 steps). The synthetic yields of **86**, **78**, **88**, **91**, **79**, **97** and **80** are described in the experimental section.




**Scheme 7.** Reagents and conditions: (a) $BH_3$-THF, THF, 50 °C, 2 h, 81%; (b) Dess-Martin Periodinane, $CH_2Cl_2$, rt, 1.5 h; (c) $Ph_3BMeBr$, *t*-BuOK, THF, rt, 5.5 h, 20% (2 steps); (d) $H_2$, Pd/C, MeOH, THF, rt, 24 h; (e) Dess-Martin Periodinane, $CH_2Cl_2$, rt, 1 h; (f) $Tf_2NPh$, LiHMDS, THF, −78 °C to rt, 1 h, 50% for **104** (3 steps); (g) (5-(methoxycarbonyl)-1H-pyrrol-2-yl)boronic acid, $PdCl_2(dppf)$-$CH_2Cl_2$, $Na_2CO_3$,toluene, $H_2O$, 90 °C, 2 h, 65% for **105**, 65%; (h) $H_2$, Pd/C, MeOH, THF, rt, 24 h, 75% for **106**; (i) HCl, 1,4-dioxane, rt, 1.5 h; (j) *i*-PrNEt$_2$, NMP, Micro-Wave 140 °C, 2 h, 45% for **108** (2 steps); (k) NaOH, $H_2O$, MeOH, THF, 80 °C, 90% for **29**; (l) $NH_4Cl$, HATU, $NEt_3$, $CHCl_3$, rt, 18 h, 25%. The synthetic yields for **30** and **31** are described in the experimental section.

chloroformate as an alkylating reagent. The alcohol group of pyrrolidine **88** was oxidized to the carboxylic acid **89** using NaClO, which was then converted to the benzyl ester **90**. After benzylating 4-position of the pyrrolidine **90**, the ester was reduced to the alcohol **92** using LAH, and the removal of the Boc/Trityl protecting group gave target pyrrolidine **79**. The pyrrolidine **95** was synthesized from **93** by mesylating the 3-hydroxy with MsCl after protecting the 2-hydroxymethyl with trityl group. The methylsulfonyl group of compound **95** was converted to cyano group using the tetraethylammonium cyanide, and the pyrrolidine **97** was synthesized by benzylating 4-position. The pyrrolidine **80** was obtained after removing the Boc/Trityl protecting group of **97**.

The C4-position substituted pyrimidoindole **29**–**31** were prepared by the methods as detailed in Scheme 7. The pyrrolidine **99** was synthesized from **98** by reducing carboxylic acid to alcohol using $BH_3$, which was then oxidized to aldehyde using Dess-Martin Periodinane. The pyrrolidine **100** was reacted with $Ph_3PMeBr$ to give **101**, which was then hydrogenated to give the pyrrolidine **102**. The pyrrolidine **104** was synthesized by reacting $Tf_2NPh$ after oxidizing the hydroxyl group of compound **102** with Dess-Martin Periodinane. The palladium-mediated coupling reaction with pyrrole boronic acid gave the compound **105**, which was then hydrogenated to give the pyrrolidine **106**. After removing *N*-Boc group by HCl in dioxane, the pyrrolidine **107** was reacted with **76** using a Micro-Wave equipment to give **108.** The pyrimidoindole **29** was obtained by hydrolyzing methyl ester with NaOH. The pyrimidoindole **30** was synthesized in the similar synthetic procedure of **29**, which was then amidated with ammonium chloride using HATU to give the compound **31.**

## 3. Conclusions

Highly potent PDHK2/PDHK4 dual inhibitors were discovered through the SBDD optimization at the ribose and triphosphate binding site of ATP. Firstly, C3-position of the tricyclic core was explored, and the PDHK2 X-ray structure with compound **7** revealed a novel ATP lid conformation. The phenyl ring of Phe326 of ATP lid was induced to occupy the space between the Arg258 sidechain and the ligand, and the phenyl ring mediated the interaction through the cation-pi and pi-pi interaction. We attempted to displace the phenyl ring by forming an intramolecular pi-pi interaction, and compounds **18** and **19** with amide linkers were designed based on the 3D geometry search result. These compounds showed $IC_{50}$ values of 4.2–6.0 nM in the PDHK2 and 24.1–32.9 nM in the PDHK4 enzymatic assay, and the intramolecular pi-pi interaction was observed in the PDHK2 X-ray structure of compound **20**. The conformation of amide linker of compound **20** resembled the conformation of parent PDB code 1TCX, which demonstrated the effectivity of 3D geometry search.

We also explored the C4-position of the tricyclic core to reproduce the interaction of compound **20**. The pyrrolidine linker was utilized to form interactions with Arg258 and Asn255 sidechains, and compound **28** showed $IC_{50}$ values of 4.5 and 26.4 nM for PDHK2 and PDHK4 respectively. The docking model of compound **28** on PDHK2 suggested the potential interaction with Asn255 sidechain, and 2-carboxy pyrrole analogues were designed. Compound **30** formed two hydrogen bonds with Asn255 in the docking model, and this compound showed an $IC_{50}$ value around 10.0 nM for PDHK2/PDHK4 without having a cation-pi interaction with Arg258 sidechain. The information gained on this study provides structural insights of PDHKs on ribose and triphosphate binding site of ATP, and will be useful for developing potent PDHKs inhibitors.

## 4. Experimental section

### 4.1. Chemistry

Solvents and reagents were obtained from commercial suppliers and used as received. Flash column chromatography was performed using Merck 230–400 mesh silica gel 60. Proton nuclear magnetic resonance $^1$H NMR) spectra were recorded on an Agilent Technologies Inc. MERCURYplus-AS400 or 400-MR, JEOL Ltd. JNM-AL400 or JNM-ESZ400S/L1, or Bruker Corporation AV400 or AVANCE III 400 spectrometer in the indicated solvent. Chemical shifts (δ) are reported in parts per million relative to internal standard tetramethylsilane. All compounds tested in biological assays were >95% purity by $^1$H NMR. $^{13}$C NMR spectra were recorded on an Agilent Technologies Inc. JNM-ECZ400S or DD2 500. High-resolution mass spectra (HRMS) analyses were performed on an LC-MS system composed of Agilent Technologies Inc. 1290 Infinity LC and Thermo Fisher Orbitrap ID-X. Optical rotation ($[\alpha]_D$) was measured at 25 °C with a Rudolph Research Analytical Autopol V spectrometer.

#### 4.1.1. Synthesis of compound 7

Step 1: 6-Amino-2-methylnicotinic acid (**33**)

6-amino-2-methylnicotinonitrile (**32**, 50.0 g, $3.76 \times 10^2$ mmol) was added to a solution of KOH (120 g, 2.14 mol) and $H_2O$ (500 mL), and the mixture was stirred at 100 °C for 8 h. After cooling to room temperature, the reactant mixture was neutralized with 12 M aqueous HCl solution (178 mL, 2.14 mmol). The precipitated solid was collected by filtration, washed with $H_2O$, and dried to give the title compound **33** (40.2 g, 70% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 2.52 (s, 3H), 6.27 (d, $J$ = 8.9 Hz, 1H), 6.56 (s, 2H), 7.81 (d, $J$ = 8.9 Hz, 1H), 12.10 (s, 1H)

Step 2: Methyl 6-amino-2-methylnicotinate (**34**)

To a solution of **33** (40.0 g, 2.63 × $10^2$ mmol) in MeOH (400 mL) was added thionyl chloride (52.0 mL, 7.21 × $10^2$ mmol) at room temperature, and then the mixture was stirred at 80 °C for 15 h. Under cooling to 0 °C, the reactant solution was quenched with saturated aqueous $NaHCO_3$ solution (approx. 600 mL), dropwise. The precipitated solid was collected by filtration, washed with $H_2O$, and dried to give the title compound **34** (39.7 g, 91% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 2.52 (s, 3H), 3.73 (s, 3H), 6.29 (d, $J$ = 8.5 Hz, 1H), 6.66 (s, 2H), 7.82 (d, $J$ = 8.5 Hz, 1H)

Step3: Methyl 6-amino-5-bromo-2-methylnicotinate (**35**)

To a solution of **34** (75.7 g, 4.55 × $10^2$ mmol) in THF (500 mL) was added *N*-bromosuccinimide (89.2 mL, 5.01 × $10^2$ mmol), portionwise, with stirring and the mixture was cooled externally by using a water bath. After stirring at room temperature for 2 h, the reactant solution was quenched with $H_2O$ (500 mL). The precipitated solid was collected by filtration, washed with $H_2O$, and dried to give the title compound **35** (92.0 g, 82% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 2.51 (s, 3H), 3.73 (s, 3H), 6.97 (br s, 2H), 8.05 (s, 1H)

Step4: 2-Cyclopropylaniline (**37**)

A mixture of 2-bromoaniline (**36**, 32.0 g, 1.86 × $10^2$ mmol), cyclopropylboronic acid (23.9 g, 2.78 × $10^2$ mmol), and $K_3PO_4$ (113 g, 5.32 × $10^2$ mmol) in toluene (350 mL) and $H_2O$ (100 mL) was treated with $PdCl_2$(dppf)-$CH_2Cl_2$ (5.66 g, 6.93 mmol) and stirred at 90 °C for 5 h under argon atmosphere. The mixture was cooled to 0 °C, added ammonium pyrrolidinedithiocarbamate (APDTC, 5.06 g, 30.8 mmol) and stirred at room temperature for 1 h. The reactant mixture was filtered through Celite®, and the organic layer was sepatated and washed with $H_2O$ and brine. The resultant organic layer was loaded on silica gel column chromatography (eluted with *n*-hexane/EtOAc = 80/20 (v/v), approximately 1.6 L) for elimination of the polar component. The eluate was acidified by adding 4 M HCl in EtOAc (50 mL) without concentration. The precipitated crystals were collected by filtration, washed with EtOAc and *n*-hexane, and dried to give the hydrochloride salt of the title compound **37** as a white crystalline (28.6 g, 91% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.69–0.73 (m, 2H), 0.95–1.00 (m, 2H), 1.97–2.06 (br m, 1H), 7.09–7.05 (m, 1H), 7.22–7.26 (m, 1H), 7.31–7.39 (br m, 1H), 9.90 (br s, 3H)

Step5: 1-Cyclopropyl-2-iodobenzene (**38**)

Sodium nitrate (11.5 g, 1.67 × $10^2$ mmol) in $H_2O$ (70 mL) was dropped into a mixture of the hydrochloride salt of **37** (25.6 g, 1.51 × $10^2$ mmol) and 1 M aqueous HCl solution (20 mL) at 0 °C. After stirring at 0 °C for 15 min, the reaction mixture was added sodium iodide (24.9 g, 1.66 × $10^2$ mmol) in $H_2O$ (70 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and then room temperature for 2 h. The reactant mixture was quenched with aqueous $NaS_2O_3$, and was extracted with EtOAc. The extract was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. The solvent was evaporated *in vacuo*. The residue was purified by flash chromatography (Biotage-SNAP Ultra 100 g, eluted with *n*-hexane/EtOAc = 100/0 to 95/5 (v/v)) to give the title compound **38** (31.9 g, 86% yield).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 0.63–0.68 (m, 2H), 0.99–1.05 (m, 2H), 1.98–2.06 (m, 1H), 6.88 (t, $J$ = 7.7 Hz, 1H), 6.92 (d, $J$ = 7.7 Hz, 1H), 7.24 (t, $J$ = 7.7 Hz, 1H), 7.83 (d, $J$ = 7.7 Hz, 1H)

Step6: Methyl 5-bromo-6-((2-cyclopropylphenyl)amino)-2-methylnicotinate (**39**)

A mixture of **35** (15.1 g, 6.16 × 10 mmol), **38** (16.5 g, 6.76 × 10 mmol), and sodium *tert*-pentoxide (10.2 g, 9.26 × 10 mmol) in toluene (160 mL) was treated with $Pd(OAc)_2$ (1.40 g, 6.24 mmol) and Xantphos (3.60 g, 6.22 mmol) and stirred at 140 °C for 4 h under argon atmosphere. After cooling to room temperature, the mixture was diluted with EtOAc and $H_2O$. The insoluble solid was filtered through Celite®. The organic layer was separated from the filtrate and concentrated. The residue was purified by flash chromatography (Biotage-SNAP Ultra 100 g, eluted with *n*-hexane/EtOAc = 98/2 to 80/20 (v/v)) to give the title compound **39** (9.33 g, 38% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.62–0.66 (m, 2H), 0.92–0.97 (m, 2H), 1.82–1.89 (m, 1H), 2.57 (s, 3H), 3.79 (s, 3H), 7.06–7.14 (m, 2H), 7.23 (t, $J$ = 7.8 Hz, 1H), 8.02 (d, $J$ = 7.8 Hz, 1H), 8.25 (s, 1H), 8.39 (s, 1H)

Step7: Methyl 8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indole-3-carboxylate (**7**)

A solution of **39** (8.00 g, 2.21 × 10 mmol) and DBU (6.70 mL, 4.48 × 10 mmol) in DMA (50 mL) was treated with $Pd(OAc)_2$ (500 mg, 2.23 mmol) and (2-biphenyl)dicyclohexylphosphine (780 mg, 6.22 mmol) under argon atmosphere and stirred at 130 °C for 4 h. After cooling to room temperature, the mixture was diluted with $H_2O$ and extracted with EtOAc. The organic layer was washed twice with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 100 g, eluted with *n*-hexane/EtOAc = 94/6 to 50/50 (v/v)) to give the title compound **7** (4.79 g, 66% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.73–0.84 (m, 2H), 1.00–1.11 (m, 2H), 2.32–2.44 (m, 1H), 2.87 (s, 3H), 3.89 (s, 3H), 7.00 (d, $J$ = 7.6 Hz, 1H), 7.16 (t, $J$ = 7.6 Hz, 1H), 8.00 (d, $J$ = 7.6 Hz, 1H), 8.95 (s, 1H), 12.25 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 8.0 (2C), 10.4, 25.2, 51.7, 113.2, 115.9, 118.3, 120.0, 120.5, 121.4, 127.0, 138.9, 152.8, 156.9, 167.0, 170.2

HRMS (ESI, *m/z*, MH+) Calcd for $C_{17}H_{17}O_2N_2$: 281.1285, Found: 281.1284

### 4.1.2. Synthesis of compound *6*

Step1: 8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indole-3-carboxylic acid (**42**)

Compound **7** (4.79 g, 1.71 × 10 mmol) was dissolved in 1:1 THF-MeOH (34 mL). To the solution, 4 M aqueous NaOH solution (17.1 mL, 6.84 × 10 mmol) was added, and the mixture was stirred at 60 °C for 4.5 h. After cooling to room temperature, the reactant mixture was acidified by adding 2 M aqueous HCl solution (42.8 mL, 8.56 × 10 mmol), and was stirred at room temperature for 1 h. The precipitated solid was collected by filtration, washed with $H_2O$, and dried to give the title compound **42** (5.07 g, quant.), which was proceeded to the next step without further purification.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.76–0.81 (m, 2H), 1.03–1.08 (m, 2H), 2.34–2.41 (m, 1H), 2.88 (s, 3H), 6.99 (d, $J$ = 7.6 Hz, 1H), 7.15 (t, $J$ = 7.6 Hz, 1H), 8.00 (d, $J$ = 7.6 Hz, 1H), 8.96 (s, 1H), 12.21 (s, 1H), 12.75 (s, 1H)

Step2: 8-Cyclopropyl-*N*,2-dimethyl-9*H*-pyrido[2,3-*b*]indole-3-carboxamide (**6**)

Methylamine (45.6 mg, 6.76 × $10^{-1}$ mmol), HATU (206 mg, 5.41 × $10^{-1}$ mmol) and $Et_3N$ (137 mg, 1.35 mmol) were added to a solution of **40** (120 mg, 4.51 × $10^{-1}$ mmol) in DMF (1.2 mL), and the reactant mixture was stirred at room temperature for 2 h. The mixture was quenched with saturated aqueous $NaHCO_3$ solution and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$. After filtration and concentration, the residue was purified by preparative TLC ($CHCl_3$/MeOH = 20/1 (v/v)) to give the title compound **6** (57.5 mg, 46% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.76–0.80 (m, 2H), 1.03–1.08 (m, 2H), 2.34–2.41 (m, 1H), 2.67 (s, 3H), 2.81 (d, $J$ = 4.8 Hz, 3H), 6.96 (d, $J$ = 7.5 Hz, 1H), 7.13 (t, $J$ = 7.5 Hz, 1H), 7.92 (t, $J$ = 7.5 Hz, 1H), 8.33 (q, $J$ = 4.8 Hz, 1H), 8.47 (s, 1H), 12.00 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 8.1 (2C), 10.4, 23.3, 26.1, 79.1, 112.4, 117.9, 119.9, 120.7, 123.8, 126.7, 127.8, 138.7, 151.6, 152.9, 169.3

HRMS (ESI, *m/z*, MH+) Calcd for $C_{17}H_{18}ON_3$: 280.1444, Found: 281.1441

### 4.1.3. Synthesis of compound *8*

Step1: 3,5-Dibromo-*N*-(2-cyclopropylphenyl)-6-methylpyridin-2-amine (**40**)

A mixture of **38** (5.00 g, 2.05 × 10 mmol), 3,5-dibromo-6-

methylpyridin-2-amine (5.45 g, 2.05 × 10 mmol) and sodium *tert*-pentoxide (3.39 g, 3.08 × 10 mmol) in toluene (50 mL) was treated with $Pd(OAc)_2$ (0.46 g, 2.05 mmol) and Xantphos (1.18 g, 2.05 mmol) and stirred at 140 °C for 4 h under argon atmosphere. After cooling to room temperature, the mixture was diluted with EtOAc and $H_2O$. The insoluble solid was filtered through Celite®. The organic layer was separated from the filtrate and concentrated. The residue was purified by flash chromatography (Biotage-SNAP Ultra 100 g, eluted with *n*-hexane/EtOAc = 98/2 to 80/20 (v/v)) to give the title compound **40** (2.89 g, 37% yield).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 0.65–0.79 (m, 2H), 0.98–1.12 (m, 2H), 1.77–1.77 (m, 1H), 2.55 (s, 3H), 7.20 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.25 (td, *J* = 7.8, 1.4 Hz, 1H), 7.80 (br s, 2H), 7.83 (s, 1H), 8.02 (dd, *J* = 7.8, 1.4 Hz, 1H)

Step2: 3-Bromo-8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*] indole (**41**)

A solution of **40** (2.89 g, 7.56 mmol) and DBU (2.28 mL, 1.51 × 10 mmol) in DMA (29 mL) was treated with $Pd(OAc)_2$ (693 mg, 7.56 × $10^{-1}$ mmol) and (2-biphenyl)dicyclohexylphosphine (1.06 g, 3.03 mmol) under argon atmosphere and stirred at 130 °C for 4 h. After cooling to room temperature, the mixture was diluted with $H_2O$ and extracted with EtOAc. The organic layer was washed twice with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 100 g, eluted with *n*-hexane/EtOAc = 94/6 to 50/50 (v/v)) to give the title compound **41** (590 mg, 26% yield).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 0.75–0.82 (m, 2H), 0.99–1.08 (m, 2H), 2.03–2.14 (m, 1H), 2.78 (s, 3H), 7.15–7.26 (m, 2H), 7.81 (d, *J* = 7.5 Hz, 1H), 8.41 (s, 1H), 8.69 (br s, 1H)

Step 3: 8-Cyclopropyl-2-methyl-3-phenyl-9*H*-pyrido[2,3-*b*]indole (**8**)

A mixture of the compound **41** (170 mg, 5.64 × $10^{-1}$ mmol), phenyl boronic acid (103 mg, 8.47 × $10^{-1}$ mmol), and $K_2CO_3$ (156 mg, 1.13 mmol) in 1,4-dioxane (2.5 mL) and $H_2O$ (2.5 mL) was treated with $PdCl_2$(dppf)-$CH_2Cl_2$ (461 mg, 5.60 × $10^{-2}$ mmol) and stirred under 80 °C for 4 h. After cooling to room temperature, the mixture was diluted with EtOAc and $H_2O$, filtered through Celite®, and the organic layer was sepatated and washed with $H_2O$ and brine. After filtration and concentration, the residue was purified by flashchromatography (Yamazen-Fuji Silysia Q-PACK SI50 SIZE400, eluted with *n*-hexane/EtOAc = 92/8 to 25/75 (v/v)) to give the title compound **8** (75.3 mg, 45% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.75–0.83 (m, 2H), 1.01–1.11 (m, 2H), 2.34–2.45 (m, 1H), 2.56 (s, 3H), 6.94 (d, *J* = 7.5 Hz, 1H), 7.09 (t, *J* = 7.5 Hz, 1H), 7.35–7.44 (m, 1H), 7.44–7.54 (m, 4H), 7.91 (d, *J* = 7.5 Hz, 1H), 8.29 (s, 1H), 11.87 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 8.1 (2C), 10.4, 23.7, 113.5, 118.0, 119.5, 119.9, 120.5, 126.5, 126.7, 128.1, 128.2 (2C), 129.4, 129.5 (2C), 138.6, 140.8, 151.1, 151.8

HRMS (ESI, *m/z*, MH+) Calcd for $C_{21}H_{19}N_2$: 299.1543, Found: 299.1542

#### 4.1.4. Synthesis of compound *9*

Step1: 8-Cyclopropyl-2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9*H*-pyrido[2,3-*b*]indole (**43**)

A mixture of 3-bromo-8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*] indole (**41**, 100 mg, 3.32 × $10^{-1}$ mmol), $(Bpin)_2$ (126 mg, 4.96 × $10^{-1}$ mmol), and KOAc (98.0 mg, 9.99 × $10^{-1}$ mmol) in DMSO (2.0 mL) was treated with $PdCl_2$(dppf)-$CH_2Cl_2$ (28.0 mg, 3.43 × $10^{-2}$ mmol) under argon atmosphere. The mixture was heated at 100 °C for 2 h. After cooling to room temperature, the reactant mixture was diluted with $H_2O$ and extracted with AcOEt, and the organic layer was washed with $H_2O$ and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was purified by flashchromatography (Biotage-SNAP Ultra 10 g, eluted with *n*-hexane/EtOAc = 66/34 (v/v)) to give the title compound **43** (30.0 mg, 26% yield).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 0.77–0.81 (m, 2H), 1.02–1.07 (m, 2H), 1.40 (s, 12H), 2.07–2.14 (m, 1H), 2.88 (s, 3H), 7.16–7.21 (m, 2H), 7.85–7.89 (m, 1H), 8.71 (s, 1H), 9.36 (s, 1H)

Step2: 8-Cyclopropyl-2-methyl-3-(pyridin-2-yl)-9*H*-pyrido[2,3-*b*] indole (**9**)

A mixture of **43** (57.8 mg, 1.66 × $10^{-1}$ mmol), 2-bromopyridine (52.2 mg, 3.32 × $10^{-1}$ mmol), and $K_2CO_3$ (69.0 mg, 4.98 × $10^{-1}$ mmol) in 1,4-dioxane (2.0 mL) was treated with $PdCl_2$(dppf)-$CH_2Cl_2$ (14.0 mg, 1.71 × $10^{-2}$ mmol) under argon atmosphere. The mixture was heated at 100 °C for 2 h. After cooling to room temperature, the reactant mixture was diluted with $H_2O$ and extracted with AcOEt, and the organic layer was washed with $H_2O$ and brine and dried over $MgSO_4$. After filtration and concentration, the residue was purified by preparative TLC (*n*-hexane/AcOEt = 1/1 (v/v)) to give the title compound **9** (11.0 mg, 22% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.77–0.81 (m, 2H), 1.04–1.09 (m, 2H), 2.37–2.44 (m, 1H), 2.67 (s, 3H), 6.96 (d, *J* = 7.5 Hz, 1H), 7.12 (t, *J* = 7.5 Hz, 1H), 7.40 (ddd, *J* = 7.5, 4.9, 0.9 Hz, 1H), 7.69 (dt, *J* = 7.8, 0.9 Hz, 1H), 7.93 (td, *J* = 7.8, 1.8 Hz, 1H), 7.95 (d, *J* = 7.5 Hz, 1H), 8.51 (s, 1H), 8.72 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 1H), 11.96 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 8.0 (2C), 10.4, 23.8, 113.4, 118.0, 119.7, 120.0, 120.6, 121.6, 124.3, 126.6, 127.3, 129.8, 136.5, 138.7, 149.0, 151.3, 152.5, 158.8

HRMS (ESI, *m/z*, MH+) Calcd for $C_{20}H_{18}N_3$: 300.1495, Found: 300.1496

#### 4.1.5. Synthesis of compound *10*

Step1: *tert*-Butyl 2-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indole-3-carbonyl)hydrazine-1-carboxylate (**44**)

To a solution of **42** (5.00 g, 1.70 × 10 mmol) and *tert*-butyl carbazate (3.37 g, 2.55 × 10 mmol) in DMF (50 mL) was added WSC·HCl (4.89 g, 2.55 × 10 mmol) and HOBt·$H_2O$ (3.91 g, 2.55 × 10 mmol), and the mixture was stirred at room temperature for 4 h. The reactant solution was dropped into saturated aqueous $NaHCO_3$ solution under stirring. The precipitated solid was collected by filtration, washed with $H_2O$, and dried to give the title compound **44** (6.61 g, overweight), which was proceeded to the next step without further purification.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.76–0.80 (m, 2H), 1.03–1.08 (m, 2H), 1.45 (s, 9H), 2.34–2.41 (m, 1H), 2.71 (s, 3H), 6.97 (d, *J* = 7.5 Hz, 1H), 7.13 (t, *J* = 7.5 Hz, 1H), 7.93 (d, *J* = 7.5 Hz, 1H), 8.46 (s, 1H), 8.94 (br s, 1H), 9.99 (br s, 1H), 12.00 (br s, 1H)

Step2: 8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indole-3-carbohydrazide (**45**)

A suspension of **44** (6.60 g, approx. 1.7 × 10 mmol) in 1,4-dioxane (40 mL) was treated with 4 M HCl in 1,4-dioxane (40 mL) and stirred at 70 °C for 3 h. The resultant suspension was filtrated and washed with 1,4-dioxane, and dried to give the hydrochloride salt of the title compound **45** (5.43 g, 84% yield, 2steps), which was proceeded to the next step without further purification.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.77–0.82 (m, 2H), 1.04–1.09 (m, 2H), 2.38–2.45 (m, 1H), 2.76 (s, 3H), 7.01 (d, *J* = 7.6 Hz, 1H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.96 (d, *J* = 7.6 Hz, 1H), 8.69 (s, 1H), 10.79 (br s, 2H), 11.56 (s, 1H), 12.24 (s, 1H)

Step3 : 2-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-5-methyl-1,3,4-oxadiazole (**10**)

AcOH (45.5 mg, 7.58 × $10^{-1}$ mmol), HATU (288 mg, 7.58 × $10^{-1}$ mmol) and $Et_3N$ (191 mg, 1.89 mmol) were added to a solution of **45** (200 mg, 6.31 × $10^{-1}$ mmol) in DMF (1.2 mL), and the mixture was stirred at room temperature for 16 h. The mixture was quenched with saturated aqueous $NaHCO_3$ solution and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$. After concentration, the residue was suspended in 1:1 *n*-hexane-EtOAc and stirred. The precipitated solid was collected by filtration, washed with *n*-hexane, and dried to give the intermediate diacyl hydrazide compound. After adding THF (4.0 mL) and the Burgess reagent (300 mg, 1.26 mmol), the mixture was stirred at room temperature for 3 h. The mixture was diluted with $H_2O$ and extracted with EtOAc. The organic layer was washed twice with $H_2O$ and

brine, and dried over $MgSO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 100 g, eluted with *n*-hexane/EtOAc = 50/50 (v/v)) to give the title compound **10** (71.7 mg, 37% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.78–0.82 (m, 2H), 1.04–1.09 (m, 2H), 2.36–2.43 (m, 1H), 2.63 (s, 3H), 2.95 (s, 3H), 7.02 (d, $J$ = 7.5 Hz, 1H), 7.17 (t, $J$ = 7.5 Hz, 1H), 8.05 (t, $J$ = 7.5 Hz, 1H), 8.94 (s, 1H), 12.29 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 8.1 (2C), 10.4, 10.6, 25.2, 110.2, 113.6, 118.4, 119.7, 120.5, 121.5, 127.0, 129.0, 139.0, 152.4, 154.1, 163.2, 164.3

HRMS (ESI, *m/z*, MH+) Calcd for $C_{18}H_{17}ON_4$: 305.1397, Found: 305.1397

#### 4.1.6. *Synthesis of compound 12–16*

2-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-5-ethyl-1,3,4-oxadiazole (**12**)

Compound **12** was synthesized in the same procedure described above for compound **10**, except using the propionic acid instead of AcOH to obtain the corresponding diacyl hydrazide intermediate (8-cyclopropyl-2-methyl-*N'*-propionyl-9*H*-pyrido[2,3-*b*]indole-3-carbohydrazide). Starting from the compound **42** (200 mg, 6.31 × $10^{-1}$ mmol), the title compound **12** (87.4 mg, 46% yield) was obtained.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.78–0.82 (m, 2H), 1.04–1.09 (m, 2H), 1.38 (t, $J$ = 7.5 Hz, 3H), 2.36–2.43 (m, 1H), 2.95 (s, 3H), 2.99 (q, $J$ = 7.5H z, 2H), 7.02 (d, $J$ = 7.6 Hz, 1H), 7.18 (t, $J$ = 7.6 Hz, 1H), 8.06 (t, $J$ = 7.6 Hz, 1H), 8.96 (s, 1H), 12.29 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 8.1 (2C), 10.4, 10.5, 18.4, 25.2, 110.3, 113.6, 118.5, 119.7, 120.5, 121.5, 127.0, 129.1, 139.0, 152.4, 154.1, 164.2, 167.0

HRMS (ESI, *m/z*, MH+) Calcd for $C_{19}H_{19}ON_4$: 319.1553, Found: 319.1556

2-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-5-isopropyl-1,3,4-oxadiazole (**13**)

Compound **13** was synthesized in the same procedure described above for compound **10**, except using the isobutyric acid instead of AcOH. Starting from the compound **42** (200 mg, 6.31 × $10^{-1}$ mmol), the title compound **13** (97.0 mg, 46% yield) was obtained.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.78–0.82 (m, 2H), 1.05–1.09 (m, 2H), 1.43 (d, $J$ = 7.2 Hz, 6H), 2.36–2.43 (m, 1H), 2.94 (s, 3H), 3.28–3.38 (m, 1H), 7.02 (d, $J$ = 7.6 Hz, 1H), 7.18 (t, $J$ = 7.6 Hz, 1H), 8.07 (t, $J$ = 7.6 Hz, 1H), 8.98 (s, 1H), 12.29 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 8.1 (2C), 10.4, 19.8 (2C), 25.2, 25.7, 110.3, 113.6, 118.5, 119.8, 120.5, 121.5, 127.0, 129.1, 139.0, 152.4, 154.1, 164.2, 170.0

HRMS (ESI, *m/z*, MH+) Calcd for $C_{20}H_{21}ON_4$: 333.1710, Found: 333.1711

2-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-5-propyl-1,3,4-oxadiazole (**14**)

Compound **14** was synthesized in the same procedure described above for compound **10**, except using the butyric acid instead of AcOH. Starting from the compound **42** (200 mg, 6.31 × $10^{-1}$ mmol), the title compound **14** (97.6 mg, 47% yield) was obtained.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.78–0.82 (m, 2H), 1.03 (t, $J$ = 7.5 Hz, 3H), 1.05–1.11 (m, 2H), 1.80–1.90 (m, 2H), 2.36–2.43 (m, 1H), 2.95 (s, 3H), 2.95 (t, $J$ = 7.5 Hz, 3H), 7.02 (d, $J$ = 7.6 Hz, 1H), 7.18 (t, $J$ = 7.6 Hz, 1H), 8.06 (t, $J$ = 7.6 Hz, 1H), 8.96 (s, 1H), 12.29 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 8.1 (2C), 10.4, 13.4, 19.5, 25.2, 26.4, 110.3, 113.6, 118.5, 119.7, 120.5, 121.5, 127.0, 129.1, 139.0, 152.4, 154.1, 164.2, 166.0

HRMS (ESI, *m/z*, MH+) Calcd for $C_{20}H_{21}ON_4$: 333.1710, Found: 333.1711

2-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-5-isobutyl-1,3,4-oxadiazole (**15**)

Compound **15** was synthesized in the same procedure described above for compound **10**, except using the isovaleric acid instead of AcOH. Starting from the compound **42** (200 mg, 6.31 × $10^{-1}$ mmol), the title compound **15** (73.6 mg, 34% yield) was obtained.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.78–0.82 (m, 2H), 1.03 (d, $J$ = 6.6 Hz, 6H), 1.04–1.09 (m, 2H), 2.17–2.27 (m, 1H), 2.36–2.43 (m, 1H), 2.87 (d, $J$ = 6.9 Hz, 2H), 2.94 (s, 3H), 7.02 (d, $J$ = 7.6 Hz, 1H), 7.18 (t, $J$ = 7.6 Hz, 1H), 8.06 (t, $J$ = 7.6 Hz, 1H), 8.95 (s, 1H), 12.29 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 8.1 (2C), 10.4, 22.0 (2C), 25.2, 26.6, 33.2, 110.3, 113.6, 118.5, 119.7, 120.5, 121.5, 127.0, 129.1, 139.0, 152.4, 154.1, 164.3, 165.4

HRMS (ESI, *m/z*, MH+) Calcd for $C_{21}H_{23}ON_4$: 347.1866, Found: 347.1870

2-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-5-(3-methylbutan-2-yl)-1,3,4-oxadiazole (**16**)

Compound **16** was synthesized in the same procedure described above for compound **10**, except using the 2,3-dimethyl butanoic acid instead of AcOH. Starting from the compound **42** (200 mg, 6.31 × $10^{-1}$ mmol), the title compound **16** (116 mg, 51% yield) was obtained.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.78–0.82 (m, 2H), 0.95 (d, $J$ = 6.8 Hz, 3H), 0.98 (d, $J$ = 6.8 Hz, 3H), 1.04–1.09 (m, 2H), 1.37 (d, $J$ = 6.9 Hz, 3H), 2.13 (td, $J$ = 13.4, 6.7 Hz, 1H), 2.36–2.43 (m, 1H), 2.94 (s, 3H), 3.06–3.12 (m, 1H), 7.02 (d, $J$ = 7.6 Hz, 1H), 7.18 (t, $J$ = 7.6 Hz, 1H), 8.07 (t, $J$ = 7.6 Hz, 1H), 8.96 (s, 1H), 12.28 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 8.1 (2C), 10.4, 14.2, 19.1, 19.8, 25.2, 31.4, 36.9, 110.3, 113.6, 118.5, 119.7, 120.4, 121.5, 127.0, 129.1, 139.0, 152.4, 154.1, 164.2, 168.7

HRMS (ESI, *m/z*, MH+) Calcd for $C_{22}H_{25}ON_4$: 361.2023, Found: 361.2027

#### 4.1.7. *Synthesis of compound 11*

2-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-5-ethyl-1,3,4-thiadiazole (**11**)

8-Cyclopropyl-2-methyl-*N'*-propionyl-9*H*-pyrido[2,3-*b*]indole-3-carbohydrazide (15.0 mg, 4.46 × $10^{-2}$ mmol) was prepared in the same method listed above for compound **12**·THF (2.0 mL) and Lawesson reagent (38.0 mg, 9.41 × $10^{-2}$ mmol) was added, and the reactant mixture was stirred at 80 °C for 19 h. After cooling to room temperature, the mixture was diluted with $H_2O$ and extracted with EtOAc. After filtration and concentration, the residue was purified by preparative TLC (*n*-hexane/AcOEt = 2/1 (v/v)) to give the title compound **11** (6.0 mg, 40% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.78–0.82 (m, 2H), 1.04–1.09 (m, 2H), 1.41 (t, $J$ = 7.5 Hz, 3H), 2.36–2.43 (m, 1H), 2.84 (s, 3H), 3.19 (q, $J$ = 7.5 Hz, 2H), 7.00 (d, $J$ = 7.6 Hz, 1H), 7.16 (t, $J$ = 7.6 Hz, 1H), 8.02 (d, $J$ = 7.6 Hz, 1H), 8.80 (s, 1H), 12.21 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 8.1 (2C), 10.4, 14.0, 22.9, 24.7, 113.6, 116.4, 118.4, 119.7, 120.2, 121.3, 126.8, 130.7, 138.9, 152.0, 153.0, 166.9, 171.6

HRMS (ESI, *m/z*, MH+) Calcd for $C_{19}H_{19}N_4S$: 335.1325, Found: 335.1330

#### 4.1.8. *Synthesis of compound 18*

Step1: *tert*-Butyl (1-(2-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indole-3-carbonyl)hydrazineyl)-3-methyl-1-oxobutan-2-yl)carbamate (**47**)

To a solution of the hydrochloride salt of **45** (200 mg, 6.31 × $10^{-1}$ mmol) and *N*-(*tert*-butoxycarbonyl)-DL-valine (**46**; 164 mg, 7.57 × $10^{-1}$ mmol) in DMF (2.0 mL) was added WSC·HCl (145 mg, 7.56 × $10^{-1}$ mmol), HOBt·$H_2O$ (116 mg, 7.58 × $10^{-1}$ mmol) and $Et_3N$ (105 μL, 7.53 × $10^{-1}$ mmol), and the mixture was stirred at room temperature for 3 h. The mixture was quenched with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The organic layer was washed twice with $H_2O$ and brine, and dried over $MgSO_4$. After filtration, the solvent was evaporated *in vacuo* to give the title compound **47** (310 mg, overweight) as a crude product. This was used to the next step without further purification.

Step2: *tert*-Butyl (1-(5-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]

indol-3-yl)-1,3,4-oxadiazol-2-yl)-2-methylpropyl)carbamate (**48**)

To a solution of **47** (310 mg, approx. $6.3 \times 10^{-1}$ mmol) in THF (5.0 mL) was added Burgess reagent (300 mg, 1.26 mmol), and the mixture was stirred at room temperature for 3 h. The mixture was diluted with $H_2O$ and extracted with EtOAc. The organic layer was washed twice with $H_2O$ and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was suspended in 1:2 *n*-hexane-EtOAc and slurried for a while. The precipitated solid was collected by filtration, washed with *n*-hexane, and dried to give the title compound **48** (200 mg), which was used to the next step without further purification.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.77–0.83 (m, 2H), 0.93 (d, $J = 6.7$ Hz, 3H), 1.02 (d, $J = 6.7$ Hz, 3H), 1.04–1.10 (m, 2H), 1.42 (s, 9H), 2.22–2.31 (m, 1H), 2.36–2.44 (m, 1H), 2.93 (s, 3H), 4.69–4.76 (m, 1H), 7.03 (d, $J = 7.5$ Hz, 1H), 7.19 (t, $J = 7.5$ Hz, 1H), 7.71 (d, $J = 8.3$ Hz, 1H) 8.00 (d, $J = 7.5$ Hz, 1H), 8.94 (s, 1H), 12.31 (s, 1H)

Step3: 1-(5-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazol-2-yl)-2-methylpropan-1-amine (**49**)

Compound **48** (200 mg, approx. $4.3 \times 10^{-1}$ mmol) was treated with 4 M HCl in 1,4-dioxane (2.0 mL) and stirred at room temperature for 1 h. The solvent was removed by *in vacuo*, and then the residue was suspended in EtOAc and slurried for a while. The precipitated solid was collected by filtration, washed with EtOAc, and dried to give the title compound **49** (170 mg, 68%, 3 steps).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.78–0.83 (m, 2H), 1.02 (d, $J = 6.8$ Hz, 3H), 1.04–1.11 (m, 2H), 1.13 (d, $J = 6.8$ Hz, 3H), 2.35–2.48 (m, 2H), 2.98 (s, 3H), 4.71–4.83 (m, 1H), 7.04 (d, $J = 7.5$ Hz, 1H), 7.20 (t, $J = 7.5$ Hz, 1H), 8.02 (d, $J = 7.5$ Hz, 1H), 9.04 (s, 1H), 8.94–9.08 (br m, 3H), 12.37 (s, 1H)

Step4 : *N*-(1-(5-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazol-2-yl)-2-methylpropyl)-3-phenylpropanamide (**18**)

To a solution of **49** (20.0 mg, $5.03 \times 10^{-2}$ mmol) and 3-phenylpropanoyl chloride (**50**; 9.00 μL, $6.36 \times 10^{-2}$ mmol) in $CHCl_3$ (1.0 mL) was added $Et_3N$ (18.0 μL, $1.28 \times 10^{-1}$ mmol), and the reactant mixture was stirred at room temperature for 1 h. The mixture was quenched with saturated aqueous $NaHCO_3$ solution, and extracted with EtOAc. The organic layer was washed with $H_2O$ and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was suspended in *n*-hexane and slurried for a while. The precipitated solid was collected by filtration, washed with *n*-hexane and dried to give the title compound **18** (24.0 mg, 96%).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.77–0.83 (m, 2H), 0.90 (d, $J = 6.7$ Hz, 3H), 0.96 (d, $J = 6.7$ Hz, 3H), 1.04–1.10 (m, 2H), 2.21–2.31 (m, 1H), 2.35–2.44 (m, 1H), 2.48–2.61 (m, 2H), 2.86 (t, $J = 7.6$ Hz, 1H), 2.92 (s, 3H), 5.07 (dd, $J = 8.3$, 7.9 Hz, 1H), 7.03 (d, $J = 7.6$ Hz, 1H), 7.06–7.13 (m, 1H), 7.15–7.31 (m, 5H), 8.02 (d, $J = 7.6$ Hz, 1H), 8.58 (d, $J = 8.6$ Hz, 1H), 8.92 (s, 1H), 12.32 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 8.1 (2C), 10.4, 18.4, 18.9, 25.1, 30.8, 30.9, 36.5, 50.4, 109.9, 113.5, 118.3, 119.6, 120.5, 125.7, 127.1, 128.06 (2C), 128.10 (2C), 128.18, 128.23, 129.1, 141.0, 152.4, 154.1, 164.2, 165.4, 171.6

HRMS (ESI, *m/z*, MH+) Calcd for $C_{30}H_{32}O_2N_5$: 494.2551, Found: 494.2550

### *4.1.9. Synthesis of compound **19***

Step1: Diethyl 2-(2-(benzyloxy)ethyl)-2-isopropylmalonate (**53**)

To a suspension of sodium hydride (60% dispersion in mineral oil, 480 mg, $1.20 \times 10$ mmol) in DMSO (10 mL), diethyl 2-isopropylmalonate (**51**, 2.00 mL, $1.00 \times 10$ mmol) and ((2-bromoethoxy)methyl) benzene (**52**, 1.60 mL, $1.00 \times 10$ mmol) were added at room temperature. Then the mixture was stirred at 60 °C for 3 h. The mixture was quenched with aqueous $NH_4Cl$ solution and extracted with AcOEt, and the organic layer was washed with brine, and dried over $MgSO_4$. After filtration and concentration, the residue was purified by silica gel column chromatography (eluted with *n*-hexane/EtOAc = 90/10 (v/v)) to give the title compound **53** (2.56 g, 76% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.91 (d, $J = 7.0$ Hz, 6H), 1.14 (d, $J = 7.0$ Hz, 6H), 2.10 (t, $J = 6.9$ Hz, 2H), 2.20–2.30 (m, 1H), 3.44 (t, $J = 6.9$ Hz, 2H), 4.40 (s, 2H), 7.25–7.36 (m, 5H)

Step2 : Ethyl 4-(benzyloxy)-2-isopropylbutanoate (**54**)

To a solution of **53** (2.56 g, 7.60 mmol) in EtOH (26 mL), EtOH (4.0 mL) solution of KOH (2.55 g, $4.56 \times 10$ mmol) was added at 0 °C, and the reactant mixture was stirred at reflux temperature for 17 h. The reactant solution was acidified by adding 2 M aqueous HCl solution (30 mL), extracted with EtOAc, and the organic layer was washed with brine. The solvent was evaporated *in vacuo* to give the title compound **54** (2.50 g, overweight) as a crude product. That was used in the next step without further purification.

Step3: 4-(Benzyloxy)-2-isopropylbutanoic acid (**55**)

To a solution of **54** (2.50 g, approx. 7.6 mmol) in EtOH (25 mL), 8 M aqueous KOH solution (4.80 mL, $3.84 \times 10$ mmol) and $H_2O$ (10 mL) were added, and the reactant mixture was stirred at reflux temperature for 8 h. The reactant solution was quenched with 1 M aqueous HCl solution (40 mL) and extracted with EtOAc. The organic layer was washed with brine, and dried over $MgSO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 50 g, eluted with *n*-hexane/EtOAc = 66/34 (v/v)) to give the title compound **55** (850 mg, 47% yield, 2 steps).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.89 (d, $J = 6.7$ Hz, 6H), 1.64–1.86 (m, 3H), 2.13–2.20 (m, 1H), 3.32–3.47 (m, 2H), 4.43 (s, 2H), 7.24–7.37 (m, 5H), 12.08 (br s, 1H)

Step4: *tert*-Butyl 2-(4-(benzyloxy)-2-isopropylbutanoyl)hydrazine-1-carboxylate (**56**)

To a solution of **55** (536 mg, 2.27 mmol) and *tert*-butyl carbazate (300 mg, 2.27 mmol) in DMF (3.0 mL) were added WSC·HCl (522 mg, 2.27 mmol) and HOBt·$H_2O$ (263 mg, 2.27 mmol), and the mixture was stirred overnight at room temperature. The reactant solution was diluted with aqueous $NaHCO_3$ solution and extracted with *n*-hexane/EtOAc (3/1 (v/v)). The organic layer was washed with brine, and dried over $MgSO_4$. After filtration, the solvent was evaporated *in vacuo* to give the title compound **56** (801 mg, quant.) as a crude product.

Step5: 4-(Benzyloxy)-2-isopropylbutanehydrazide hydrochloride (**57**)

The compound **56** (800 mg, approx. 2.2 mmol) was dissolved in 4 N HCl dioxane solution (8.0 mL), and the mixture was stirred at room temperature for 3.5 h. The reaction solution was concentrated *in vacuo* to give the title compound **57** (700 mg, quant.), which was used for the next step without further purification.

Step6: *N*'-(4-(Benzyloxy)-2-isopropylbutanoyl)-8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indole-3-carbohydrazide (**58**)

To the solution of **42** (160 mg, $6.01 \times 10^{-1}$ mmol) and **57** (350 mg, approx. 1.1 mmol) in DMF (2.0 mL) was added WSC·HCl (176 mg, $9.18 \times 10^{-1}$ mmol), HOBt·$H_2O$ (140 mg, $9.14 \times 10^{-1}$ mmol) and *i*-$Pr_2$EtN (260 μL, 1.53 mmol), and the mixture was stirred at room temperature for 1 h. The reactant solution was dropped into saturated aqueous $NaHCO_3$ solution under stirring. The precipitated solid was collected by filtration, washed with $H_2O$, and dried to give the title compound **58** (232 mg, 76%) as a crude product.

Step7: 2-(1-(Benzyloxy)-4-methylpentan-3-yl)-5-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazole (**59**)

A solution of **58** (460 mg, $9.23 \times 10^{-1}$ mmol) in $POCl_3$ (3.0 mL) stirred at 110 °C for 2 h. The reactant solution was poured into $H_2O$, and then the mixture was extracted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$ solution, and dried over $MgSO_4$. After filtration and concentration, the residue was purified by silica gel column chromatography (eluted with *n*-hexane/EtOAc = 75/25 to 67/33 (v/v)) to give the title compound **59** (250 mg, 57% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.78–0.83 (m, 2H), 0.91 (d, $J = 6.7$ Hz, 3H), 1.03 (d, $J = 6.7$ Hz, 3H), 1.04–1.10 (m, 2H), 2.15–2.25 (m, 1H), 2.35–2.44 (m, 1H), 2.91 (s, 3H), 3.84–3.95 (m, 2H), 4.52 (dd, $J = 27.5$, 12.3 Hz, 2H), 7.03 (t, $J = 7.5$ Hz, 1H), 7.16–7.32 (m, 6H), 8.03 (d, $J = 7.5$ Hz, 1H), 8.91 (s, 1H), 12.28 (br s, 1H)

Step8: 3-(5-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-

1,3,4-oxadiazol-2-yl)-4-methylpentan-1-ol (**60**)

A solution of **59** (230 mg, $4.79 \times 10^{-1}$ mmol) in THF (3.0 mL) was treated with ASCA-2 (120 mg) and stirred under 1.0 atm of hydrogen at room temperature for 2 days. After removal of the catalyst by Celite® filtration and concentration, the residue was purified by silica gel column chromatography (eluted with *n*-hexane/EtOAc = 67/33 (v/v)) to give the title compound **60** (97.0 mg, 52% yield).

$^1$H NMR (400 MHz, $CDCl_3$) δ: 0.79–0.84 (m, 2H), 1.02 (d, $J$ = 6.7 Hz, 3H), 1.08 (d, $J$ = 6.7 Hz, 3H), 1.04–1.11 (m, 2H), 2.06–2.24 (m, 4H), 3.02 (s, 3H), 3.16–3.24 (m, 1H), 3.65–3.81 (m, 2H), 7.21–7.26 (m, 2H), 7.87–7.93 (m, 1H), 8.79 (s, 1H), 9.45 (br s, 1H)

Step9: 3-(5-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazol-2-yl)-4-methylpentanal (**61**)

To a solution of **60** (97.0 mg, $2.48 \times 10^{-1}$ mmol) in $CHCl_3$ (2.0 mL) was added Dess-Martin Periodinane (158 mg, $3.73 \times 10^{-1}$ mmol), and the mixture was stirred at room temperature for 4 h. The reaction was quenched with saturated aqueous $NaHCO_3$ solution. The mixture was extracted with $CHCl_3$, and the organic layer was washed with aqueous $Na_2CO_3$ and brine. After dried over $Na_2SO_4$ and filtration, the solvent was evaporated *in vacuo* to give the title compound **61** (108 mg, overweight) as a crude product. That was used to the next step without further purification.

Step10: 3-(5-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazol-2-yl)-4-methylpentanoic acid (**62**)

To the solution of **61** (108 mg, approx. $2.5 \times 10^{-1}$ mmol) in 8:2:1 THF-*t*-BuOH-$H_2O$ (5.5 mL) was added 2-methyl-2-butene (300 μg, 2.78 mmol), $NaH_2PO_4$ (130 mg, $8.33 \times 10^{-1}$ mmol) and $NaClO_2$ (75.0 mg, $8.33 \times 10^{-1}$ mmol), and the mixture was stirred at room temperature for 1.5 h. The reaction was quenched with aqueous $Na_2S_2O_4$ solution, and then acidified by adding 6 M aqueous HCl solution under stirring. The mixture was extracted with EtOAc, and the organic layer was washed with brine. After dried over $MgSO_4$ and filtration, the solvent was evaporated *in vacuo*. The residue was resolved into $CHCl_3$, and the insoluble residue was removed by filtration. The organic layer was concentrated to give the title compound **62** (132 mg, overweight).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.76–0.84 (m, 2H), 0.94 (d, $J$ = 6.7 Hz, 3H), 0.99 (d, $J$ = 6.7 Hz, 3H), 1.04–1.12 (m, 2H), 2.05–2.23 (m, 1H), 2.30–2.47 (m, 1H), 2.93 (s, 3H), 2.74–2.97 (m, 2H), 3.32–3.45 (m, 1H), 7.03 (d, $J$ = 7.7 Hz, 1H), 7.18 (t, $J$ = 7.7 Hz, 1H), 8.07 (d, $J$ = 7.7 Hz, 1H), 8.96 (s, 1H), 12.29 (s, 1H), 12.34 (br s, 1H)

Step11 : *N*-Benzyl-3-(5-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazol-2-yl)-4-methylpentanamide (**19**)

To a solution of **62** (25.0 mg, approx. $4.7 \times 10^{-2}$ mmol) and benzylamine (15.0 μL, $1.37 \times 10^{-2}$ mmol) in $CHCl_3$ (2.0 mL) was added WSC·HCl (20.0 mg, $1.04 \times 10^{-1}$ mmol) and HOBt·$H_2O$ (16.0 mg, $1.04 \times 10^{-1}$ mmol), and the mixture was stirred at room temperature for 1 h. The mixture was quenched with saturated aqueous $NaHCO_3$ solution and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$. After filtration and concentration, the residue was purified by preparative TLC (*n*-hexane/EtOAc = 1/2 (v/v)) twice to give the title compound **19** (11.0 mg, 51%, 3 steps).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.76–0.84 (m, 2H), 0.94 (d, $J$ = 6.9 Hz, 3H), 1.01 (d, $J$ = 6.9 Hz, 3H), 1.03–1.12 (m, 2H), 2.06–2.19 (m, 1H), 2.35–2.45 (m, 1H), 2.68–2.90 (m, 2H), 2.93 (s, 3H), 3.45–3.53 (m, 1H), 4.25 (ddd, $J$ = 35.3, 15.4, 5.8 Hz, 2H), 7.03 (d, $J$ = 7.7 Hz, 1H), 7.11–7.23 (m, 6H), 8.05 (d, $J$ = 7.7 Hz, 1H), 8.56 (t, $J$ = 7.7 Hz, 1H), 8.93 (s, 1H), 12.28 (br s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 8.1 (2C), 10.4, 19.1 (2C), 20.0, 25.1, 30.6, 35.3, 41.9, 110.2, 113.5, 118.4, 119.6, 120.4, 121.5, 126.5, 126.9 (2C), 127.0, 128.0 (2C), 129.0, 138.9, 139.2, 152.3, 154.0, 164.0, 167.2, 169.9

HRMS (ESI, *m/z*, MH+) Calcd for $C_{30}H_{32}O_2N_5$: 494.2551, Found: 494.2549

#### *4.1.10. Synthesis of compound **20***

Step1: (*S*)-4-Benzyl-3-(3-methylbutanoyl)oxazolidin-2-one (**65**)

To a solution of isovaleric acid (**63**, 10.0 g, $5.65 \times 10$ mmol) and (*S*)-4-benzyl-2-oxazolidinone (**64**, 7.40 mL, $6.78 \times 10$ mmol) in MeCN (100 mL) were added DMAP (3.40 g, $2.79 \times 10$ mmol) and WSC·HCl (12.0 g, $6.28 \times 10$ mmol), and the mixture was stirred at room temperature for 19 h. The reaction was quenched with saturated aqueous $NaHCO_3$ solution. The mixture was extracted with toluene, and the organic layer was washed with $H_2O$, 1 M aqueous HCl solution, $H_2O$ and brine. After drying over $MgSO_4$ and filtration, the solvent was evaporated *in vacuo* to give the title compound **65** (15.0 g, quant.), which was applied to the next step without further purification.

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.01 (d, $J$ = 6.6 Hz, 3H), 1.03 (t, $J$ = 6.6 Hz, 3H), 2.17–2.28 (m, 1H), 2.75 (dd, $J$ = 13.2, 9.6 Hz, 1H), 2.84 (ddd, $J$ = 44.9, 16.2, 6.9 Hz, 2H), 3.32 (dd, $J$ = 13.2, 3.4 Hz, 1H), 4.13–4.22 (m, 2H), 4.65–4.72 (m, 1H), 7.12–7.38 (m, 5H)

Step2: *tert*-Butyl (*S*)-3-((*S*)-4-benzyl-2-oxooxazolidine-3-carbonyl)-4-methylpentanoate (**66**)

The compound **65** (2.00 g, approx. 7.7 mmol) was dissolved in THF (20 mL). To the solution, 1.0 M LiHMDS in THF (8.43 mL, 8.43 mmol) was added at −78 °C. *tert*-Butyl bromoacetate (1.66 mL, $1.15 \times 10$ mmol) was added to the reactant mixture, and then the mixture was stirred at room temperature for 3.5 h. The mixture was quenched with aqueous $NH_4Cl$ solution and extracted with AcOEt, and the organic layer was washed with aqueous $NaHCO_3$ solution and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was crystallize in $CHCl_3$-*n*-hexane. The precipitated solid was collected by filtration, washed with *n*-hexane, and dried to give the title compound **66** (1.52 g, 53% yield, 2 steps).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.80 (d, $J$ = 6.8 Hz, 3H), 0.92 (d, $J$ = 6.8 Hz, 3H), 1.39 (s, 9H), 1.89–2.00 (m, 1H), 2.45 (dd, $J$ = 16.5, 3.7 Hz, 1H), 2.64 (dd, $J$ = 16.5, 11.6 Hz, 1H), 2.85 (dt, $J$ = 13.4, 8.0 Hz, 1H), 2.99 (dd, $J$ = 13.4, 3.0 Hz, 1H), 4.00 (ddd, $J$ = 11.6, 3.7, 3.0 Hz, 1H), 4.14 (dd, $J$ = 8.8, 2.5 Hz, 1H), 4.32 (t, $J$ = 8.0 Hz, 1H), 4.65 (tt, $J$ = 8.0, 2.5 Hz, 1H), 7.23–7.33 (m, 5H)

Step3 : (*S*)-4-(*tert*-Butoxy)-2-isopropyl-4-oxobutanoic acid (**67**)

The compound **66** (1.52 g, 4.05 mmol) was dissolved in THF (40 mL). To the solution, 30% aqueous $H_2O_2$ solution (1.65 mL, $1.62 \times 10$ mmol) and 0.4 M aqueous LiOH solution ($2.03 \times 10$ mL, 8.10 mmol) was added at 0 °C, and then the mixture was stirred at room temperature for 1 h. After cooling to 0 °C, the reactant mixture was quenched with aqueous $Na_2S_2O_3$ solution. After concentration of the organic layer, the aqueous layer was washed with $CHCl_3$ three times, and then acidified by adding 6 N HCl solution. The mixture was extracted with EtOAc, and the organic layer was washed with $H_2O$ and brine. After drying over $MgSO_4$ and filtration, the solvent was evaporated *in vacuo* to give the title compound **67** (840 mg, 96%).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.85 (d, $J$ = 6.8 Hz, 3H), 0.88 (d, $J$ = 6.8 Hz, 3H), 1.37 (s, 9H), 1.82–1.94 (m, 1H), 2.30 (dd, $J$ = 16.0, 3.9 Hz, 1H), 2.36–2.49 (m, 2H), 12.16 (br s, 1H)

Step4: *tert*-Butyl (*S*)-3-(2-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indole-3-carbonyl)hydrazine-1-carbonyl)-4-methylpentanoate (**68**)

To a solution of the hydrochloride salt of **45** (500 mg, 1.58 mmol) and (*S*)-4-(*tert*-butoxy)-2-isopropyl-4-oxobutanoic acid (**67**, 512 mg, 2.37 mmol) in DMF (5.0 mL) was added WSC·HCl (290 mg, 1.89 mmol), HOBt·$H_2O$ (363 mg, 1.90 mmol) and *i*-$Pr_2$EtN (670 μL, 3.94 mmol), and the mixture was stirred at room temperature for 2.5 h. The reactant solution was dropped into saturated aqueous $NaHCO_3$ solution under stirring. The precipitated solid was collected by filtration, washed with $H_2O$, and dried to give the title compound **68** (716 mg, 95% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.77–0.83 (m, 2H), 0.94 (d, $J$ = 6.7 Hz, 3H), 0.99 (d, $J$ = 6.7 Hz, 3H), 1.03–1.10 (m, 2H), 1.32 (s, 9H), 2.07–2.18 (m, 1H), 2.35–2.44 (m, 1H), 2.78 (dd, $J$ = 16.3, 5.2 Hz, 1H), 2.86 (dd, $J$ = 16.3, 10.0 Hz, 1H), 2.93 (s, 3H), 3.33–3.40 (m, 1H), 7.03 (d, $J$ = 7.6 Hz, 1H), 7.18 (t, $J$ = 7.6 Hz, 1H), 8.06 (d, $J$ = 7.6 Hz, 1H), 8.96 (s, 1H), 12.30 (s, 1H)

Step5 : *tert*-Butyl (*S*)-3-(5-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazol-2-yl)-4-methylpentanoate (**69**)

To a solution of **68** (716 mg, 1.50 mmol) in THF (14 mL) was added Burgess reagent (1.07 g, 4.49 mmol), and the mixture was stirred at room temperature for 30 min. The mixture was diluted with $H_2O$ and extracted with EtOAc. The organic layer was washed twice with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by silica gel column chromatography (eluted with *n*-hexane/EtOAc = 85/15 to 67/33 (v/v)) to give the title compound **69** (432 mg, 63% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.77–0.83 (m, 2H), 0.94 (d, $J$ = 6.8 Hz, 3H), 0.99 (d, $J$ = 6.8 Hz, 3H), 1.04–1.10 (m, 2H), 1.32 (s, 9H), 2.08–2.18 (m, 1H), 2.35–2.44 (m, 1H), 2.78 (dd, $J$ = 16.2, 5.3 Hz, 1H), 2.86 (dd, $J$ = 16.2, 10.2 Hz, 1H), 2.93 (s, 3H), 3.33–3.40 (m, 1H), 7.02 (d, $J$ = 7.4 Hz, 1H), 7.18 (t, $J$ = 7.4 Hz, 1H), 8.06 (d, $J$ = 7.4 Hz, 1H), 8.96 (s, 1H), 12.30 (s, 1H), 12.38 (br s, 1H)

Step6: (*S*)-3-(5-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazol-2-yl)-4-methylpentanoic acid (**70**)

The compound **69** (432 mg, 9.38 × $10^{-1}$ mmol) was dissolved in TFA (2.0 mL), and the mixture was stirred at room temperature for 1 h. The reaction solution was concentrated *in vacuo* and azeotroped twice with toluene. The residue was purified by twice precipitation in $CHCl_3$/*n*-hexane/EtOAc (1/1/1 (v/v)) and MeOH/$H_2O$ (1/2 (v/v). The precipitated solid was collected by filtration and dried to give the title compound **70** (311 mg, 82% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.77–0.84 (m, 2H), 0.94 (d, $J$ = 6.9 Hz, 3H), 0.99 (d, $J$ = 6.9 Hz, 3H), 1.03–1.11 (m, 2H), 2.09–2.20 (m, 1H), 2.35–2.44 (m, 1H), 2.79 (dd, $J$ = 16.7, 4.8 Hz, 1H), 2.93 (s, 3H), 2.86–2.94 (m, 1H), 3.34–3.45 (m, 1H), 7.02 (d, $J$ = 7.6 Hz, 1H), 7.18 (t, $J$ = 7.6 Hz, 1H), 8.07 (d, $J$ = 7.6 Hz, 1H), 8.97 (s, 1H), 12.29 (s, 1H), 12.38 (br s, 1H)

Step7: (*S*)-3-(5-(8-Cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-3-yl)-1,3,4-oxadiazol-2-yl)-4-methyl-*N*-((*R*)-1-phenylethyl)pentanamide (**20**)

To the solution of **70** (20.0 mg, 4.94 × $10^{-2}$ mmol) and (*R*)-1-phenylethan-1-amine (9.50 μL, 7.45 × $10^{-2}$ mmol) in DMF (1.0 mL) was added WSC·HCl (14.0 mg, 7.30 × $10^{-2}$ mmol) and HOBt·$H_2O$ (12.0 mg, 7.84 × $10^{-2}$ mmol), and the reactant mixture was stirred at room temperature for 1.5 h. The reactant solution was dropped into saturated aqueous $NaHCO_3$ solution under stirring. The precipitated solid was collected by filtration, washed with $H_2O$, and dried to give the title compound **20** (25.0 mg, 99% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.77–0.83 (m, 2H), 0.94 (d, $J$ = 6.9 Hz, 3H), 1.00 (d, $J$ = 6.9 Hz, 3H), 1.03–1.11 (m, 2H), 1.32 (d, $J$ = 7.2 Hz, 3H), 2.05–2.16 (m, 1H), 2.35–2.44 (m, 1H), 2.66–2.74 (m, 2H), 2.84 (dd, $J$ = 15.1, 10.1 Hz, 1H), 2.90 (s, 3H), 3.40–3.48 (m, 1H), 4.81–4.91 (m, 1H), 7.00–7.12 (m, 4H), 7.14–7.21 (m, 3H), 8.04 (dd, $J$ = 7.8, 1.0 Hz, 1H), 8.92 (s, 1H), 12.28 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 8.1 (2C), 10.5, 19.2 (2C), 20.1, 22.5, 25.2, 30.8, 35.5, 47.7, 110.3, 113.5, 118.4, 119.7, 120.4, 121.5, 125.6 (2C), 126.3, 127.1, 127.9 (2C), 129.1, 139.0, 144.4, 152.4, 154.1, 164.0, 167.2, 169.1

$[\alpha]_D^{25}$ = +44.0 ($c$ = 0.10, DMSO)

HRMS (ESI, *m/z*, MH+) Calcd for $C_{31}H_{34}O_2N_5$: 508.2707, Found: 508.2709

### 4.1.11. Synthesis of compound 21

Step1: 1-Cyclopropyl-3-fluoro-2-nitrobenzene (**72**)

A mixture of 1-bromo-3-fluoro-2-nitrobenzene (**71**; 49.8 g, 2.26 × $10^2$ mmol), cyclopropylboronic acid (21.4 g, 249 mmol), and $K_3PO_4$ (106 g, 4.99 × $10^2$ mmol) in DME (300 mL) and $H_2O$ (150 mL) was treated with $PdCl_2$(dppf)-$CH_2Cl_2$(9.25 g, 1.13 × 10 mmol) and stirred under reflux for 1 h. After cooling to room temperature, the mixture was diluted with EtOAc and $H_2O$, filtered through Celite®, and the organic layer was sepatated and washed with $H_2O$ and brine. After filtration and concentration, the residue was purified by flashchromatography (Yamazen-Fuji Silysia Q-PACK SI50 SIZE400, eluted with *n*-hexane/EtOAc = 95/5 to 75/25 (v/v)) to give the title compound **72** (36.8 g, 89% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.77–0.81 (m, 2H), 0.99–1.04 (m, 2H), 1.85–1.92 (m, 1H), 7.03–7.05 (m, 1H), 7.35–7.40 (m, 1H), 7.52–7.58 (m, 1H)

Step2: Ethyl 2-cyano-2-(3-cyclopropyl-2-nitrophenyl)acetate (**73**)

Compound **72** (3.00 g, 1.66 × 10 mmol) and ethyl 2-cyanoacetate (3.50 mL, 3.31 × 10 mmol) was dissolved in DMF (15 mL). To the solution, $K_2CO_3$ (6.90 g, 4.97 × 10 mmol) was added, and the mixture was stirred at 90 °C for 3 h. After cooling to 0 °C, the reactant mixture was diluted with $H_2O$ (50 mL), acidified by adding 6 M aqueous HCl solution (25 mL), and extracted with 1:1 *n*-hexane-EtOAc. The organic layer was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration, the solvent was evaporated *in vacuo* to give the title compound **73** (5.0 g, overweight) as a crude product. That was used to the next step without further purification.

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 0.64–0.77 (m, 2H), 0.96–1.06 (m, 2H), 1.30 (t, $J$ = 7.3 Hz, 3H), 1.98–2.06 (m, 1H), 4.26 (q, $J$ = 7.3 Hz, 2H), 5.00 (s, 1H), 7.21 (dd, $J$ = 7.7, 0.8 Hz, 1H), 7.04 (t, $J$ = 7.7 Hz, 1H), 7.54 (dd, $J$ = 7.7,1.6 Hz, 1H)

Step3: Ethyl 2-amino-7-cyclopropyl-1*H*-indole-3-carboxylate (**74**)

To a solution of **73** (5.0 g, approx. 1.6 × 10 mmol) in AcOH (30 mL) was added iron powder (5.09 g, 9.11 × 10 mmol), and the mixture was stirred at 90 °C for 2 h. After cooling to room temperature, the reactant mixture was diluted with 1:1 toluene-$H_2O$ (60 mL), filtered through Celite®, and the organic layer was sepatated and washed with $H_2O$, saturated $NaHCO_3$ solution and brine sequentially, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flashchromatography (Yamazen-Universal Prenium L, eluted with *n*-hexane/EtOAc = 85/15 to 40/60 (v/v)) to give the title compound **74** (2.10 g, 52% two-step yield from **72**).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 0.71 (br s, 2H), 0.93 (br s, 2H), 1.42 (t, $J$ = 7.1 Hz, 3H), 1.91 (br s, 1H), 4.36 (q, $J$ = 7.1 Hz, 2H), 5.68 (br s, 1H), 6.79 (d, $J$ = 7.4 Hz, 1H), 7.04 (t, $J$ = 7.4 Hz, 1H), 7.64 (d, $J$ = 7.4 Hz, 1H), 7.92 (br s, 2H)

Step4: 8-Cyclopropyl-2-methyl-9*H*-pyrimido[4,5-*b*]indol-4-ol (**75**)

Compound **74** (5.00 g, 2.05 × 10 mmol) was dissolved in 4 M aqueous HCl solution (25 mL). To the solution, MeCN (7.5 mL) was added, and the mixture was stirred at room temperature for 20 h. The resultant suspension was diluted with 1:1 *n*-hexane-dioxane (50 mL), and the precipitated solid was collected by filtration, washed with *n*-hexane, and dried *in vacuo*. The obtained solid was redissolved in MeOH (65 mL) and $H_2O$ (20 mL). Saturated $NaHCO_3$ solution (40 mL) was added to the solution, and the reactant mixture was stirred at 75 °C for 2.5 h. After cooling to 0 °C, the reactant mixture was acidified by adding 6 M aqueous HCl solution (7.5 mL), diluted with $H_2O$ (100 mL), and then stirred at 0 °C for 15 min. The precipitated solid was collected by filtration, washed with $H_2O$, and dried to give the title compound **75** (4.88 g, 99% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.77–0.73 (m, 2H), 0.99–1.04 (m, 2H), 2.30–2.37 (m, 1H), 2.42 (s, 3H), 6.80 (d, $J$ = 7.5 Hz, 1H), 7.10 (t, $J$ = 7.5 Hz, 1H), 7.73 (dd, $J$ = 7.5, 0.9 Hz, 1H), 12.07 (s, 1H), 12.12 (s, 1H)

Step5: 4-Chloro-8-cyclopropyl-2-methyl-9*H*-pyrimido[4,5-*b*]indole (**76**)

To a suspension of **75** (4.88 g, 2.04 × 10 mmol) in $CHCl_3$ (66 mL) was added thionyl chloride (7.42 mL, 1.02 × $10^2$ mmol) and DMF (33 mL), and the mixture was stirred at 60 °C for 2 h. The resultant solution was cooled to 0 °C, quenched with $H_2O$. The mixture was extracted with EtOAc, and the organic layer was washed twice with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flashchromatography (Yamazen-Universal Prenium 3L, eluted with *n*-hexane/EtOAc = 90/10 to 20/80 (v/v)) to give the title compound **76** (2.44 g, 46% yield).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 2.05–2.09 (m, 2H), 2.27–2.31 (m, 2H), 3.08–3.14 (m, 1H), 3.68 (s, 3H), 7.26–7.31 (m, 2H), 7.93–7.98 (m, 1H), 8.84 (s, 1H)

Step6 : 8-Cyclopropyl-2-methyl-4-(pyrrolidin-1-yl)-9*H*-pyrimido

[4,5-*b*]indole (**21**)

A microwave vial was charged with **76** (150 mg, $5.82 \times 10^{-1}$ mmol), pyrrolidine (49.7 mg, $6.98 \times 10^{-1}$ mmol), *i*-$Pr_2NEt$ (301 mg, 2.328 mmol), and NMP (3.00 mL). The vial was heated to 140 °C and then irradiated with the microwave apparatus for 2 h. The mixture was diluted with $H_2O$ and extracted with AcOEt, and the organic layer was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 100 g, eluted with *n*-hexane/EtOAc = 50/50 (v/v)) to give the title compound **21** (117 mg, 69% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.70–0.73 (m, 2H), 0.97–1.02 (m, 2H), 1.90–1.94 (m, 4H), 2.28–2.35 (m, 1H), 2.44 (s, 3H), 3.82–3.85 (m, 4H), 6.82 (d, $J$ = 7.7 Hz, 1H), 7.03 (t, $J$ = 7.7 Hz, 1H), 7.81 (d, $J$ = 7.7 Hz, 1H), 11.88 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 7.8 (2C), 10.4, 25.1 (2C), 25.8, 49.4 (2C), 94.6, 118.5, 119.2, 119.5, 120.1, 126.2, 136.1, 156.9, 157.5, 161.8

HRMS (ESI, *m/z*, MH+) Calcd for $C_{18}H_{21}N_4$: 293.1761, Found: 293.1761

*4.1.12. Synthesis of compound **22–28***

8-Cyclopropyl-4-(2-ethylpyrrolidin-1-yl)-2-methyl-9*H*-pyrimido [4,5-*b*]indole (**22**)

Compound **22** was synthesized in the same procedure described above for compound **21**, except using the 2-ethyl pyrrolidine instead of the pyrrolidine. Starting from the compound **76** (150 mg, 5.82 × 10 mmol), the title compound **22** (175 mg, 94% yield) was obtained.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.70–0.74 (m, 2H), 0.87 (t, $J$ = 7.4 Hz, 3H), 0.98–1.03 (m, 2H), 1.49 (dq, $J$ = 22.0, 6.7 Hz, 1H), 1.58–1.71 (m, 2H), 1.85–1.95 (m, 2H), 2.10–2.18 (m, 1H), 2.29–2.36 (m, 1H), 2.46 (s, 3H), 3.75–3.79 (m, 1H), 3.96–4.02 (m, 1H), 4.50–4.57 (m, 1H), 6.83 (d, $J$ = 7.7 Hz, 1H), 7.06 (t, $J$ = 7.7 Hz, 1H), 7.70 (d, $J$ = 7.7 Hz, 1H), 11.89 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 7.8, 7.9, 9.8, 10.4, 25.2, 25.9, 26.3, 29.6, 51.9, 58.7, 95.4, 118.5, 119.1, 119.5, 120.1, 126.2, 136.2, 157.2, 157.7, 161.7

HRMS (ESI, *m/z*, MH+) Calcd for $C_{20}H_{25}N_4$: 321.2074, Found: 321.2076

(*S*)-(1-(8-Cyclopropyl-2-methyl-9*H*-pyrimido[4,5-*b*]indol-4-yl)pyrrolidin-2-yl)methanol (**23**)

Compound **23** was synthesized in the same procedure described above for compound **21**, except using the (*S*)-2-pyrrolidine methanol instead of the pyrrolidine. Starting from the compound **76** (150 mg, 5.82 × 10 mmol), the title compound **23** (111 mg, 59% yield) was obtained.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.70–0.77 (m, 2H), 0.99–1.04 (m, 2H), 1.24–1.25 (m, 2H) 1.87–2.21 (m, 3H), 2.30–2.37 (m, 1H), 2.47 (s, 3H), 3.53–3.59 (m, 1H), 3.68–3.73 (m, 1H), 3.85 (t, $J$ = 7.6 Hz, 1H), 4.65–4.71 (m, 1H), 4.96 (t, $J$ = 5.1 Hz, 1H), 6.84 (d, $J$ = 7.8 Hz, 1H), 7.06 (t, $J$ = 7.8 Hz, 1H), 7.76 (d, $J$ = 7.8 Hz, 1H), 11.93 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 7.8, 7.9, 10.4, 24.9, 25.7, 27.6, 51.9, 59.4, 62.6, 95.1, 118.6, 119.4, 119.5, 120.1, 126.2, 136.2, 157.3, 157.7, 161.6

$[\alpha]_D^{25}$ = -272 ($c$ = 1.00, DMSO)

HRMS (ESI, *m/z*, MH+) Calcd for $C_{19}H_{23}ON_4$: 323.1866, Found: 323.1866

(*R*)-(1-(8-Cyclopropyl-2-methyl-9*H*-pyrimido[4,5-*b*]indol-4-yl)pyrrolidin-2-yl)methanol (**24**)

Compound **24** was synthesized in the same procedure described above for compound **21**, except using the (*R*)-2-pyrrolidine methanol instead of the pyrrolidine. Starting from the compound **76** (150 mg, 5.82 × 10 mmol), the title compound **24** (88.6 mg, 47% yield) was obtained.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.70–0.77 (m, 2H), 0.99–1.04 (m, 2H), 1.69 (td, $J$ = 17.6, 9.2 Hz, 1H), 1.87–2.21 (m, 3H), 2.30–2.37 (m, 1H), 2.47 (s, 3H), 3.53–3.59 (m, 1H), 3.68–3.73 (m, 1H), 3.85 (t, $J$ = 7.6 Hz, 1H), 4.03 (td, $J$ = 9.2, 6.4 Hz, 1H), 4.65–4.71 (m, 1H), 4.96 (t, $J$ = 5.2 Hz, 1H), 6.84 (d, $J$ = 7.8 Hz, 1H), 7.06 (t, $J$ = 7.8 Hz, 1H), 7.76 (d, $J$ = 7.8 Hz, 1H), 11.93 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 7.8, 7.9, 10.4, 24.9, 25.7, 27.6, 51.9, 59.4, 62.6, 95.1, 118.6, 119.4, 119.5, 120.1, 126.2, 136.2, 157.3, 157.7, 161.6

$[\alpha]_D^{25}$ = +284 ($c$ = 1.00, DMSO)

HRMS (ESI, *m/z*, MH+) Calcd for $C_{19}H_{23}ON_4$: 323.1866, Found: 323.1867

((2*S*,4*S*)-1-(8-Ccyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-4-yl)-4-phenylpyrrolidin-2-yl)methanol (**25**)

Compound **25** was synthesized in the same procedure described above for compound **21**, except using pyrrolidine **77** instead of the pyrrolidine. Starting from the compound **76** (20.0 mg, $7.76 \times 10^{-2}$ mmol), the title compound **25** (13.0 mg, 42% yield) was obtained.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.67–0.77 (m, 2H), 0.96–1.05 (m, 2H), 2.13–2.22 (m, 1H), 2.28–2.37 (m, 1H), 2.40–2.48 (m, 1H), 2.48 (s, 3H), 3.84 (dd, 1H, $J$ = 9.9, 5.3 Hz, 1H), 4.57 (dd, $J$ = 10.1, 7.1 Hz, 1H), 4.87–4.94 (m, 1H), 4.98 (t, $J$ = 5.3 Hz, 1H), 6.84 (d, $J$ = 7.8 Hz, 1H), 7.05 (t, $J$ = 7.8 Hz, 1H), 7.11–7.27 (m, 6H), 7.79 (d, $J$ = 7.8 Hz, 1H), 11.96 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 7.7, 7.8, 10.3, 25.7, 34.5, 41.7, 57.3, 59.0, 62.1, 94.8, 118.6, 119.2, 119.3, 120.1, 126.2 (2C), 126.6 (2C), 128.4 (2C), 136.1, 143.3, 156.8, 157.7, 161.7

$[\alpha]D^{25}$ = -321 (c = 0.40, DMSO)

HRMS (ESI, *m/z*, MH+) Calcd for $C_{25}H_{27}N_4O$: 399.2179, Found: 399.2181

((2*S*,4*R*)-4-Benzyl-1-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*] indol-4-yl)pyrrolidin-2-yl)methanol (**26**)

Compound **26** was synthesized in the same procedure described above for compound **21**, except using pyrrolidine **78** instead of the pyrrolidine. Starting from the compound **76** (20.0 mg, $7.76 \times 10^{-2}$ mmol), the title compound **26** (26.0 mg, 81% yield) was obtained.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.75 (td, $J$ = 5.4, 3.2 Hz, 2H), 1.03 (ddd, $J$ = 9.1, 5.4, 3.2 Hz, 2H), 1.81–1.90 (m, 1H), 2.03–2.11 (m, 1H), 2.31–2.40 (m, 1H), 2.54 (s, 3H), 2.53–2.57 (m, 1H), 2.65–2.76 (m, 1H), 3.58 (dd, $J$ = 10.7, 6.0 Hz, 1H), 3.66 (dd, $J$ = 10.7, 4.5 Hz, 1H), 3.73–3.65 (m, 1H), 4.21 (dd, $J$ = 9.8, 6.4 Hz, 1H), 4.77–4.85 (m, 1H), 6.88 (d, $J$ = 7.4 Hz, 1H), 7.18–7.25 (m, 2H), 7.70 (d, $J$ = 8.1 Hz, 1H), 12.28 (br s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 8.0, 8.1, 10.4, 24.5, 32.2, 38.2, 38.7, 56.5, 59.4, 62.3, 94.9, 119.1, 119.3, 119.5, 120.8, 126.0, 126.8, 128.2 (2C), 128.7 (2C), 136.2, 140.2, 155.7 (br, 2C), 159.9 (br)

$[\alpha]_D^{25}$ = -295 ($c$ = 0.20, DMSO)

HRMS (ESI, *m/z*, MH+) Calcd for $C_{26}H_{29}N_4O$: 413.2336, Found: 413.2338

((2*S*,4*S*)-4-Benzyl-1-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*] indol-4-yl)pyrrolidine-2,4-diyl)dimethanol (**27**)

Compound **27** was synthesized in the same procedure described above for compound **21**, except using pyrrolidine **79** instead of the pyrrolidine. Starting from the compound **76** (20.0 mg, $7.76 \times 10^{-2}$ mmol), the title compound **27** (19.0 mg, 55% yield) was obtained.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.71–0.78 (m, 2H), 0.98–1.07 (m, 2H), 1.20–1.32 (m, 1H), 1.20–1.32 (m, 1H), 1.71–1.85 (m, 2H), 2.28–2.40 (m, 2H), 2.48 (s, 3H), 3.32–3.43 (m, 2H), 3.53–3.60 (m, 1H), 3.62–3.68 (m, 1H), 3.68 (d, $J$ = 10.5 Hz, 1H), 3.94 (d, $J$ = 10.5 Hz, 1H), 4.76–4.85 (m, 1H), 4.87 (t, $J$ = 5.4 Hz, 1H), 4.95 (t, $J$ = 5.0 Hz, 1H), 6.84 (d, $J$ = 7.6 Hz, 1H), 6.89–6.95 (m, 2H), 7.02–7.10 (m, 4H), 7.68 (d, $J$ = 7.6 Hz, 1H), 11.96 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 7.8 (2C), 10.4, 25.7, 33.4, 39.1, 48.2, 57.9, 59.8, 62.8, 64.2,106.0, 118.4, 119.1, 119.5, 120.2, 125.8, 126.5, 127.6 (2C), 129.9 (2C), 130.2, 137.0, 138.2, 157.6 (2C), 161.8

$[\alpha]_D^{25}$ = -344 ($c$ = 0.04, DMSO)

HRMS (ESI, *m/z*, MH+) Calcd for $C_{27}H_{31}N_4O_2$: 443.2442, Found: 443.2438

(3*S*,5*S*)-3-Benzyl-1-(8-cyclopropyl-2-methyl-9*H*-pyrido[2,3-*b*]indol-

4-yl)-5-(hydroxymethyl)pyrrolidine-3-carboxamide (**28**)

Compound **28** was synthesized in the same procedure described above for compound **21**, except using pyrrolidine **80** instead of the pyrrolidine. Starting from the compound **76** (103 mg, 4.00 × $10^{-1}$ mmol), the title compound **28** (34.0 mg, 19% yield) was obtained.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.71–0.79 (m, 2H), 0.99–1.08 (m, 2H), 2.03–2.27 (m, 2H), 2.30–2.41 (m, 1H), 2.67 (s, 3H), 2.83–3.06 (m, 1H), 3.52–3.70 (m, 2H), 3.85 (d, $J$ = 10.5 Hz, 1H), 4.13 (d, $J$ = 10.5 Hz, 1H), 4.81–4.94 (br m, 2H), 6.80–6.87 (m, 3H), 7.00–7.10 (m, 4H), 7.16 (br s, 1H), 7.19–7.38 (m, 2H), 7.47 (br s, 1H), 7.65 (d, $J$ = 8.1 Hz, 1H), 11.98 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 7.8, 7.9, 10.4, 25.8, 34.5, 40.0, 53.8, 57.5, 58.1, 62.2, 95.4, 118.6, 119.1, 119.3, 120.1, 126.1 (2C), 127.7 (2C), 129.2 (2C), 136.2, 137.2, 157.6, 157.7, 161.8, 174.9

$[\alpha]_D^{25}$ = -359 ($c$ = 0.30, DMSO)

HRMS (ESI, $m/z$, MH+) Calcd for $C_{27}H_{30}N_2O_5$: 456.2394, Found: 456.2397

#### 4.1.13. Synthesis of pyrrolidine 77

Step1: *tert*-Butyl (2*S*,4*S*)-2-(hydroxymethyl)-4-phenylpyrrolidine-1-carboxylate (**82**)

To a solution of (2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-phenylpyrrolidine-2-carboxylic acid (**81**, 100 mg, 3.43 × $10^{-1}$ mmol) in THF (1.0 mL) was added 1.0 M $BH_3$-THF in THF (1.00 mL, 1.00 mmol) at room temperature, and then the mixture was stirred at 80 °C for 20 min. The reactant mixture was quenched with saturated aqueous $NaHCO_3$ solution, and the mixture was extracted with EtOAc. The extract was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. The solvent was evaporated *in vacuo* to give the title compound **82** (105 mg, overweight), which was advanced to the next step without further purification.

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.48 (s, 9H), 1.96–2.07 (br m, 1H), 2.18 (dd, $J$ = 20.3, 10.6 Hz, 1H), 3.34–3.52 (br m, 2H), 3.66–3.85 (m, 3H), 4.15–4.27 (br m, 2H), 7.19–7.25 (m, 3H), 7.29–7.36 (m, 2H)

Step2: ((2*S*,4*S*)-4-Phenylpyrrolidin-2-yl)methanol (**77**)

Compound **82** (105 mg, approx. 3.4 × $10^{-1}$ mmol) was treated with 4 M HCl in 1,4-dioxane (500 μL) and stirred at room temperature for 1.5 h. The reaction mixture was evaporated and dried *in vacuo* to give the hydrochloride salt of the title compound **77** (75.0 mg, 94% yield (2 steps)).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.48 (s, 9H), 1.96–2.07 (br m, 1H), 2.18 (dd, $J$ = 20.3, 10.6 Hz, 1H), 3.34–3.52 (br m, 2H), 3.66–3.85 (m, 3H), 4.15–4.27 (br m, 2H), 7.19–7.25 (m, 3H), 7.29–7.36 (m, 2H)

#### 4.1.14. Synthesis of pyrrolidine 78

Step1 : *tert*-Butyl (*S*)-2-oxo-5-((trityloxy)methyl)pyrrolidine-1-carboxylate (**84**)

To a solution of (*S*)-5-((trityloxy)methyl)pyrrolidin-2-one (**83**, 9.00 g, 2.52 × 10 mmol) in DMF (50 mL) were added $Boc_2O$ (6.60 mL, 3.02 × 10 mmol) and DMAP (3.70 g, 3.03 × 10 mmol) at room temperature. The reaction mixture was stirred at 70 °C for 4 h. After cooling to room temperature, the reaction was quenched by an addition of TMEDA (1.63 mL, 1.25 × 10 mmol). After stirring for 30 min at room temperature, the mixture was diluted with 1 M HCl solution and extracted with EtOAc. The organic layer was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. Evaporation of the solvent *in vacuo* gave the title compound **84** (11.9 g, overweight), which was applied to the next step without further purification.

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.44 (s, 9H), 1.90–1.99 (m, 1H), 2.05–2.17 (m, 1H), 2.42 (ddd, $J$ = 17.7, 9.8, 1.8 Hz, 1H), 2.80 (ddd, $J$ = 19.3, 9.8, 8.2 Hz, 1H), 3.11 (dd, $J$ = 9.5, 2.8 Hz, 1H), 3.48 (dd, $J$ = 9.5, 4.6 Hz, 1H), 4.22–4.30 (m, 1H), 7.18–7.41 (m, 15H)

Step2: *tert*-Butyl (3*R*,5*S*)-3-benzyl-2-oxo-5-((trityloxy)methyl)pyrrolidine-1-carboxylate (**85**)

To a solution of **84** (250 mg, approx. 5.4 × $10^{-1}$ mmol) in THF (7.0 mL) was added 1 M LiHMDS in THF (655 μL, 6.55 × $10^{-1}$ mmol) at −78 °C. The reaction mixture was stirred at −30 °C for 15 min before adding benzyl bromide (84.0 μL, 7.07 × $10^{-1}$ mmol) at −78 °C and stirred 1 h at room temperature. The mixture was diluted with saturated $NH_4Cl$ solution and extracted with EtOAc. The organic layer was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 25 g, eluted with *n*-hexane/EtOAc = 90/10 to 60/40 (v/v)) to give the title compound **85** (232 mg, 77% yield (2 steps)).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.43 (s, 9H), 1.78 (td, $J$ = 12.4, 8.8 Hz, 1H), 1.78 (dd, $J$ = 12.4, 8.8 Hz, 1H), 1.94 (dd, $J$ = 12.4, 8.8 Hz, 1H), 2.57 (dd, $J$ = 14.0, 9.9 Hz, 1H), 3.09 (dd, $J$ = 19.5, 3.0 Hz, 1H), 3.11–3.21 (m, 1H), 3.33 (dd, $J$ = 14.0, 4.0 Hz, 1H), 3.41 (dd, $J$ = 9.5, 4.6 Hz, 1H), 4.11–4.16 (m, 1H), 7.14–7.35 (m, 20H)

Step3 : *tert*-Butyl (2*S*,4*R*)-4-benzyl-2-((trityloxy)methyl)pyrrolidine-1-carboxylate (**86**)

To a solution of **85** (232 mg, 4.23 × $10^{-1}$ mmol) in THF (2.0 mL) was added 1.0 M $BH_3$-THF in THF (2.10 mL, 2.10 mmol) at room temperature, and then the mixture was stirred at 80 °C for 30 min. The reactant mixture was quenched with saturated aqueous $NaHCO_3$ solution and $H_2O$ at 0 °C, and the mixture was extracted with EtOAc. The extract was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 25 g, eluted with *n*-hexane/EtOAc = 100/0 to 70/30 (v/v)) to give the title compound **86** (175 mg, 78%).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.24–1.51 (m, 9H), 1.58–1.77 (m, 1H), 1.87–2.13 (m, 1H), 2.49–2.90 (m, 4H), 2.96–3.15 (m, 2H), 3.24–3.52 (m, 1H), 3.91–4.09 (m, 1H), 7.08–7.47 (m, 9H)

Step4: ((2*S*,4*R*)-4-Benzylpyrrolidin-2-yl)methanol (**78**)

Compound **86** (175 mg, 3.28 × $10^{-1}$ mmol) was treated with 4 M HCl in 1,4-dioxane (800 μL) and stirred at room temperature for 2 h. The reaction mixture was evaporated and dried *in vacuo*, and the residue was decanted three times with *n*-hexane to give the hydrochloride salt of the title compound **78** (75.0 mg, overweight).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 1.67–1.80 (m, 2H), 2.49–2.60 (m, 1H), 2.62–2.91 (m, 3H), 3.14–3.24 (m, 3H), 3.49 (dd, $J$ = 11.6, 7.3 Hz, 1H), 3.60 (t, $J$ = 11.6, 4.2 Hz, 1H), 3.65–3.76 (m, 1H), 5.33 (br s, 1H), 7.19–7.26 (m, 3H), 7.28–7.34 (m, 2H), 8.69 (br s, 1H), 9.54 (br s, 1H)

#### 4.1.15. Synthesis of pyrrolidine 79

Step1 : 1-(*tert*-Butyl) 3-methyl (3*R*,5*S*)-2-oxo-5-((trityloxy)methyl) pyrrolidine-1,3-dicarboxylate (**87**)

To a solution of **84** (3.60 g, approx. 6.6 mmol) in THF (50 mL) was added 0.93 M LiHMDS in THF (8.50 mL, 7.87 mmol) at −78 °C. The reaction mixture was stirred at −30 °C for 20 min before adding methyl chloroformate (755 μL, 9.83 mmol) at −78 °C followed by stirring 1 h at −30 °C. The mixture was diluted with $H_2O$ and extracted with EtOAc. The organic layer was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 50 g, eluted with *n*-hexane/acetone = 90/10 to 65/35 (v/v)) to give the title compound **87** (2.70 g, 80% yield (2 steps)) as a mixture of diastereomers.

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.42 (s, 4.5H), 1.47 (s, 4.5H), 2.11 (ddd, $J$ = 13.0, 9.0, 1.0 Hz, 0.5H), 2.49–2.59 (m, 0.5H), 2.83 (dd, $J$ = 14.1, 3.3 Hz, 0.5H), 2.92 (dd, $J$ = 14.1, 8.4 Hz, 0.5H), 3.03–3.11 (m, 1H), 3.35 (dd, $J$ = 9.0, 3.9 Hz, 0.5H), 3.54–3.60 (m, 0.5H), 3,79 (s, 1.5H), 3.81 (s, 1.5H), 3.79–3.84 (m, 1H), 3.92 (dd, $J$ = 11.2, 9.1 Hz, 0.5H), 4.38–4.26 (m, 1H), 7.14–7.43 (m, 15H)

Step2 : *tert*-Butyl (2*S*,4*R*)-4-(hydroxymethyl)-2-((trityloxy)methyl) pyrrolidine-1-carboxylate (**88**)

To a solution of **87** (2.70 g, 5.24 mmol) in THF (10 mL) was added 1.0 M $BH_3$-THF in THF (30.0 mL, 2.62 × 10 mmol) at room temperature, and then the mixture was stirred at 80 °C for 2.5 h. The reactant was cooled at 0 °C and quenched with $H_2O$, and the mixture was extracted with EtOAc. The extract was washed with 1 M HCl solution, saturated aqueous $NaHCO_3$ solution and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 50 g, eluted with *n*-hexane/acetone = 90/

10 to 65/35 (v/v)) to give the title compound **88** (1.15 g, 46% yield) as a mixture of diastereomers.

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.32 (br s, 4.5H), 1.47 (br s, 4.5H), 1.59–1.99 (br m, 2H), 2.07–2.63 (br m, 2H), 2.88–3.74 (br m, 5H), 3.96–4.17 (br m, 1H), 7.17–7.46 (m, 15H)

Step3 : (3*R*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-((trityloxy)methyl)pyrrolidine-3-carboxylic acid (**89**)

To a solution of **88** (1.15 g, 2.43 mmol) in 3:1 *t*-BuOH-$H_2O$ (8.0 mL) were added 5% NaClO solution (362 μL, 2.42 × $10^{-1}$ mmol), 1-Methyl-2-azaadamantane-*N*-oxyl (41.0 mg, 2.47 × $10^{-1}$ mmol) and 80% $NaClO_2$ (549 mg, 4.86 mmol) at 0 °C, and then the mixture was stirred for 1.5 h at room temperature. The reactant was quenched with $Na_2SO_3$ (918 mg, 7.28 mmol) and $H_2O$, and then the mixture was extracted with EtOAc. The extract was washed with brine, and dried over $Na_2SO_4$. Evaporation of the solvent *in vacuo* gave the title compound **89** (1.20 g, overweight), which was advanced to the next step without further purification.

Step4: 3-Benzyl 1-(*tert*-butyl) (3*R*,5*S*)-5-((trityloxy)methyl)pyrrolidine-1,3-dicarboxylate (**90**)

To a solution of **89** (500 mg, approx. 1.0 mmol) in DMF (5.0 mL) were added $K_2CO_3$ (212 mg, 1.53 mmol) and benzyl bromide (146 μL, 1.23 mmol) at room temperature and stirred 1.5 h. The mixture was diluted with $H_2O$ and extracted with EtOAc. The organic layer was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 25 g, eluted with *n*-hexane/EtOAc = 95/5 to 60/40 (v/v)) to give the title compound **90** (410 mg, 69% yield (2 steps)) as a mixture of diastereomers.

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (br s, 4.5H), 1.47 (br s, 4.5H), 1.81–1.89 (br m, 1H), 2.08–2.33 (br m, 1H), 2.96–3.43 (br m, 2H), 3.59–4.78 (br m, 3H), 3.93–4.18 (br m, 1H), 5.07–5.16 (m, 2H), 7.18–7.43 (m, 20H)

Step5 : 3-Benzyl 1-(*tert*-butyl) (3*S*,5*S*)-3-benzyl-5-((trityloxy)methyl) pyrrolidine-1,3-dicarboxylate (**91**)

To a solution of **90** (410 mg, 7.10 × $10^{-1}$ mmol) in THF (8.0 mL) was added 1.0 M LiHMDS in THF (852 μL, 8.52 × $10^{-1}$ mmol) at −78 °C. The reaction mixture was stirred at −30 °C for 10 min before adding benzyl bromide (127 μL, 1.07 mmol) at −78 °C and stirred for 30 min at room temperature. The mixture was diluted with $H_2O$ and extracted with EtOAc. The organic layer was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 25 g, eluted with *n*-hexane/EtOAc = 95/5 to 60/40 (v/v)) to give the title compound **91** (325 mg, 69% yield) as a mixture of diastereomers.

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (br s, 4.5H), 1.31–1.43 (br m, 1H), 1.52 (br s, 4.5H), 2.00–2.30 (br m, 2H), 2.35–2.57 (br m, 1H), 2.76–2.92 (br m, 1H), 2.94–3.06 (m, 2H), 3.21–3.51 (br m, 1H), 3.54–3.90 (br m, 2H), 3.92–4.17 (br m, 1H), 4.91–5.17 (m, 2H), 6.97–7.07 (m, 2H), 7.16–7.42 (m, 18H)

Step6 : *tert*-Butyl (2*S*,4*S*)-4-Benzyl-4-(hydroxymethyl)-2-((trityloxy) methyl)pyrrolidine-1-carboxylate (**92**)

To a solution of **91** (325 mg, 4.87 × $10^{-1}$ mmol) in THF (8.0 mL) was added LAH (37.0 mg, 9.75 × $10^{-1}$ mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min before quenching by $H_2O$ (37 μL), 4 M NaOH solution (37 μL) and $H_2O$ (74 μL). After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 25 g, eluted with *n*-hexane/EtOAc = 90/10 to 60/40 (v/v)) to give the title compound **92** (244 mg, 89% yield) as a single diastereomer.

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.24–1.59 (br m, 9H), 1.75–2.06 (br m, 3H), 2.63–2.76 (m, 2H), 2.94–3.78 (br m, 6H), 3.88–4.16 (br m, 1H), 7.17–7.33 (br m, 14H), 7.38–7.43 (m, 6H)

Step7 : ((2*S*,4*S*)-4-Benzylpyrrolidine-2,4-diyl)dimethanol (**79**)

Compound **91** (100 mg, 1.77 × $10^{-1}$ mmol) was treated with 4 M HCl in 1,4-dioxane (500 μL) and stirred at room temperature for 2 h. The reaction mixture was evaporated and dried *in vacuo*, and the residue was decanted three times by 1:1 *n*-hexane-AcOEt to give the hydrochloride salt of the title compound **79** (46.0 mg, quant.).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 1.45 (dd, *J* = 13.4, 10.5 Hz, 1H), 1.81 (dd, *J* = 13.4, 7.0 Hz, 1H), 2.75 (dd, *J* = 29.1, 13.4 Hz, 1H), 3.01 (dd, *J* = 43.0, 11.7 Hz, 1H), 3.23 (s, 2H), 3.42–3.70 (m, 2H), 5.11 (br s, 1H), 5.34 (br s, 1H), 7.20–7.36 (m, 5H), 8.93 (br s, 2H)

#### *4.1.16. Synthesis of pyrrolidine **80***

Step1 : *tert*-Butyl (2*S*,4*R*)-4-methoxy-2-((trityloxy)methyl)pyrrolidine-1-carboxylate hydrate (**94**)

To a solution of *tert*-butyl (2*S*,4*R*)-4-hydroxy-2-(hydroxymethyl) pyrrolidine-1-carboxylate (**93**, 5.00 g, 2.30 × 10 mmol) in DMF (30 mL) were added TrCl (7.10 g, 2.55 × 10 mmol), $Et_3N$ (9.60 mL, 6.90 × 10 mmol) and DMAP (845 mg, 6.92 mmol) under cooling in a water bath, and the mixture was stirred at 60 °C for 4 h. After cooling to room temperature, the reactant mixture was quenched with 2 M aqueous HCl solution, and the mixture was extracted with EtOAc. The extract was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 200 g, eluted with *n*-hexane/EtOAc = 30/70 to 0/100 (v/v)) to give the title compound **94** (10.1 g, 95% yield).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.29–1.48 (br m, 9H), 1.62 (br s, 1H), 1.90–2.11 (br m, 1H), 2.15–2.22 (m, 1H), 2.92–3.64 (br m, 4H), 4.10 (br s, 1H), 4.51 (br s, 1H), 7.18–7.29 (m, 9H), 7.29–7.36 (m, 6H)

Step2 : *tert*-Butyl (2*S*,4*R*)-4-((methylsulfonyl)oxy)-2-((trityloxy) methyl)pyrrolidine-1-carboxylate (**95**)

To a solution of **94** (2.00 g, 4.35 mmol) in $CHCl_3$ (10 mL) were added MsCl (510 μL, 7.55 mmol) and $Et_3N$ (1.20 mL, 8.71 mmol) at 0 °C, and the mixture was stirred at 0 °C for 30 min. The reactant mixture was quenched with 1 M aqueous HCl solution, and the mixture was extracted with EtOAc. The extract was washed with saturated aqueous $NaHCO_3$ solution and brine, and dried over $Na_2SO_4$. The solvent was evaporated *in vacuo* to give the title compound **95** (2.40 mg, overweight), which was applied to the next step without further purification.

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.30–1.53 (br m, 9H), 2.16–2.51 (br m, 2H), 3.01 (s, 1H), 2.89–3.34 (br m, 2H), 3.56–4.00 (br m, 2H), 4.14 (br d, *J* = 24.7 Hz, 1H), 5.38 (br d, *J* = 31.7 Hz, 1H), 7.20–7.33 (m, 9H), 7.35–7.40 (m, 6H)

Step3 : *tert*-Butyl (2*S*,4*S*)-4-cyano-2-((trityloxy)methyl)pyrrolidine-1-carboxylate (**96**)

A mixture of **95** (2.40 g, approx. 4.3 mmol) and tetraethylammonium cyanide (2.1 g, 1.34 × 10 mmol) in MeCN (10 mL) was stirred at 100 °C for 4 h. After cooling to room temperature, the mixture was diluted with $H_2O$, and the mixture was extracted with EtOAc. The extract was washed with saturated aqueous $NaHCO_3$ solution and brine, and dried over $Na_2SO_4$. The organic layer was separated and concentrated. The residue was purified by flash chromatography (Biotage-SNAP Ultra 50 g, eluted with *n*-hexane/EtOAc = 85/15 to 50/50 (v/v)) to give the title compound **96** (1.50 g, 74% yield (2 steps)).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.25–1.53 (br m, 9H), 2.26–2.61 (br m, 2H), 2.89–3.01 (br m, 1H), 3.07–3.60 (br m, 3H), 4.06 (br dd, *J* = 11.0, 7.5 Hz, 1H), 7.20–7.34 (m, 9H), 7.39–7.43 (m, 6H)

Step4 : *tert*-Butyl (2*S*,4*S*)-4-benzyl-4-cyano-2-((trityloxy)methyl) pyrrolidine-1-carboxylate (**97**)

To a solution of **96** (900 mg, 1.92 mmol) in THF (7.0 mL) was added 1 M LiHMDS in THF (2.30 mL, 2.30 mmol) at −78 °C. The reaction mixture was stirred at −30 °C for 10 min before adding benzyl bromide (342 mL, 2.88 mmol) at −78 °C and stirred 30 min at room temperature. The mixture was diluted with $H_2O$ and extracted with EtOAc. The organic layer was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 25 g, eluted with *n*-hexane/EtOAc = 99/1 to 65/35 (v/v)) to give the title compound **97** (383 mg, 35% yield).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.25–1.53 (br m, 9H), 2.26–2.61 (br m, 2H), 2.89–3.01 (br m, 1H), 3.07–3.60 (br m, 3H), 4.06 (br dd, *J* = 11.0, 7.5 Hz, 1H), 7.20–7.34 (m, 9H), 7.39–7.43 (m, 6H)

Step5 : (3*S*,5*S*)-3-Benzyl-5-(hydroxymethyl)pyrrolidine-3-carbonitrile (**80**)

Compound **97** (383 mg, 6.85 × $10^{-1}$ mmol) was treated with 4 M HCl in 1,4-dioxane (2.0 mL) and stirred at room temperature for 2 h. The reaction mixture was evaporated and dried *in vacuo*, and the residue was decanted three times by *n*-hexane to give the hydrochloride salt of the title compound **80** (152 mg, 88% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 1.94–2.18 (m, 2H), 2.95–3.19 (m, 3H), 3.41–3.56 (m, 2H), 3.57–3.68 (m, 1H), 3.69–3.88 (br m, 1H), 5.34–5.41 (br m, 1H), 7.09–7.44 (m, 5H), 9.52 (br s, 2H)

#### *4.1.17. Synthesis of compound **29***

Step1 : *tert*-Butyl (2*S*,4*R*)-4-(benzyloxy)-2-(hydroxymethyl)pyrrolidine-1-carboxylate (**99**)

To a solution of *tert*-butyl (2*S*,4*R*)-4-(benzyloxy)-2-(1-hydroxyvinyl) pyrrolidine-1-carboxylate (**98**, 25.0 g, 7.80 × 10 mmol) in THF (100 mL) was added 0.94 mol/L THF solution of $BH_3$-THF (99.0 mL, 9.30 × 10 mmol) at temperature, and the mixture was stirred at 50 °C for 2 h. The reactant mixture was quenched with saturated aqueous $NaHCO_3$ solution, and the mixture was extracted with EtOAc. The extract was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration, the solvent was evaporated *in vacuo* to give the title compound **99** (19.4 g, 81%).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.47 (s, 9H), 1.58–1.68 (m, 1H), 2.13–2.24 (m, 1H), 3.40 (dd, *J* = 12.1, 4.0 Hz, 1H), 3.56 (ddd, *J* = 11.3, 7.2, 2.9 Hz, 1H), 3.59–3.78 (m, 2H), 4.05 (br s, 1H), 4.08–4.19 (m, 1H), 4.50 (dd, *J* = 16.3, 11.3 Hz, 1H), 4.88 (d, *J* = 8.1 Hz, 1H), 7.28–7.38 (m, 5H)

Step2 : *tert*-Butyl (2*S*,4*R*)-4-(benzyloxy)-2-formylpyrrolidine-1-carboxylate (**100**)

To a solution of **99** (9.15 g, 2.98 × 10 mmol) in $CH_2Cl_2$ (92 mL) was added Dess-Martin Periodinane (4.20 g, 9.79 mmol) at temperature, and the mixture was stirred at room temperature for 1.5 h. The reaction was quenched with aqueous $NaHCO_3$ solution and aqueous $Na_2S_2O_3$ solution. The mixture was extracted with $CHCl_3$ using a Phase Separator. The solvent was evaporated *in vacuo* to give the title compound **100** (9.18 g, overweight), which was advanced to the next step without further purification.

Step3 : *tert*-Butyl (2*S*,4*R*)-4-(benzyloxy)-2-vinylpyrrolidine-1-carboxylate (**101**)

$Ph_3PMeBr$ (31.6 g, approx. 8.8 × 10 mmol) was dissolved in THF (90 mL). To the solution, *t*-BuOK (9.92 g, 8.80 × 10 mmol) was added at 0 °C, and the mixture was stirred at 0 °C for 15 min. And then, the solution of **100** (9.00 g, 2.95 × 10 mmol) in THF (45 mL) was added to the reactant mixture at 0 °C followed by the stirring 5.5 h at room temperature. The mixture was diluted with $H_2O$ and extracted with AcOEt, and the organic layer was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 100 g, eluted with *n*-hexane/EtOAc = 97/3 to 70/30 (v/v)) to give the title compound **101** (7.18 g, 20% yield (2 steps)).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.44 (s, 9H), 1.88 (dt, *J* = 13.1, 5.8 Hz, 1H), 2.15–2.27 (m, 1H), 3.48 (dd, *J* = 11.5, 4.6 Hz, 1H), 3.70 (br s, 1H), 4.07–4.14 (m, 1H), 4.36 (br s, 1H), 4.50 (dd, *J* = 17.5, 12.1 Hz, 2H), 4.96–5.19 (m, 2H), 5.72 (br s, 1H), 7.26–7.38 (m, 5H)

Step 4: *tert*-Butyl (2*R*,4*R*)-2-ethyl-4-hydroxypyrrolidine-1-carboxylate (**102**)

A solution of **101** (7.18 g, 2.37 × 10 mmol) in THF (36 mL) and MeOH (36 mL) was treated with 5% palladium on activated carbon (N.E. Chemcat PE-type, 2.5 g) and stirred under 1.0 atm of hydrogen at room temperature for 24 h. After removal of the palladium catalyst by Celite® filtration, the filtrate was concentrated to give the title compound **102** (5.20 g, overweight), which was advanced to the next step without further purification.

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 0.84 (t, *J* = 7.5 Hz, 3H), 1.46 (s, 9H), 1.64–1.97 (m, 3H), 1.99–2.10 (m, 1H), 3.39 (dd, *J* = 12.0, 4.2 Hz, 1H), 3.48 (s, 1H), 3.54 (br s, 1H), 3.87 (br s, 1H), 4.35–4.42 (m, 2H)

Step5 : *tert*-Butyl (*R*)-2-ethyl-4-oxopyrrolidine-1-carboxylate (**103**)

To a solution of **102** (5.20 g, approx. 2.4 × 10 mmol) in $CH_2Cl_2$ (104 mL) was added Dess-Martin Periodinane (15.4 g, 3.62 × 10 mmol) at temperature of a water bath, and the mixture was stirred at room temperature for 1 h. The reaction was quenched with aqueous $NaHCO_3$ solution and aqueous $Na_2S_2O_3$ solution. The mixture was extracted with $CHCl_3$ using a Phase Separator. The solvent was evaporated *in vacuo* to give the title compound **103** (6.02 g, overweight), which was advanced to the next step without further purification.

Step6 : *tert*-Butyl (*R*)-2-ethyl-4-(((trifluoromethyl)sulfonyl)oxy)-2,3-dihydro-1*H*-pyrrole-1-carboxylate (**104**)

Compound **103** (5.15 g, approx. 2.0 × 10 mmol) was dissolved in THF (155 mL). To the solution, 1.1 M LiHMDS in THF (26.3 mL, 2.90 × 10 mmol) was added at −78 °C, and the mixture was stirred at −30 °C for 15 min. After cooling to −78 °C, $Tf_2NPh$ (10.8 g, 3.02 × 10 mmol) was added to the reactant mixture, and then the mixture was stirred at room temperature for 1 h. The mixture was diluted with aqueous $NH_4Cl$ solution and extracted with AcOEt, and the organic layer was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 50 g, eluted with *n*-hexane/EtOAc = 100/0 to 80/20 (v/v)) to give the title compound **104** (3.66 g, 50% yield (3 steps)).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 0.85 (t, *J* = 7.5 Hz, 3H), 1.49 (s, 9H), 1.76–1.90 (m, 2H), 4.14 (dd, *J* = 14.9, 5.1 Hz, 1H), 4.30 (dd, *J* = 29.7, 14.9 Hz, 1H), 4.53–4.70 (m, 1H), 5.66 (d, *J* = 19.7 Hz, 1H)

Step7 : 1′-(*tert*-Butyl) 5-methyl (*R*)-5′-ethyl-4′,5′-dihydro-1*H*,1′*H*-[2,3′-bipyrrole]-1′,5-dicarboxylate (**105**)

A mixture of **104** (1.80 g, 5.21 mmol), (5-(methoxycarbonyl)-1*H*-pyrrol-2-yl)boronic acid (1.32 g, 7.82 mmol) and $Na_2CO_3$ (600 μL, 1.20 mmol) in toluene (14 mL) and $H_2O$ (7.0 mL) was treated with $PdCl_2$(dppf)-$CH_2Cl_2$ (426 mg, 5.21 × $10^{-1}$ mmol) and stirred at 90 °C for 2 h under argon atmosphere. After cooling to room temperature, the mixture was diluted with AcOEt and $H_2O$, and the organic layer was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flash chromatography (Biotage-SNAP Ultra 25 g, eluted with *n*-hexane/EtOAc = 96/4 to 80/20 (v/v)) to give the title compound **105** (1.09 g, 65%) as a mixture of isomers on the olefine part.

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 0.85 (t, *J* = 7.5 Hz, 3H), 1.50 (s, 4.5H), 1.51 (m, 4.5H), 1.71–1.94 (m, 3H), 3.86 (s, 1.5H), 3.87 (br s, 1H), 4.29–4.70 (m, 2H), 5.03 (d, *J* = 28.1 Hz, 0.5H), 6.21 (dd, *J* = 6.4, 3.7 Hz, 0.5H), 6.27 (dt, *J* = 4.6, 1.9 Hz, 0.5H), 6.86–6.89 (m, 0.5H), 6.90–6.98 (m, 1H), 9.24 (br s, 1H)

Step8 : Methyl 5-((3*S*,5*R*)-1-(*tert*-butoxycarbonyl)-5-ethylpyrrolidin-3-yl)-1*H*-pyrrole-2-carboxylate (**106**)

A solution of **105** (1.00 g, 3.12 mmol) in THF (5.0 mL) and MeOH (5.0 mL) was treated with 10% palladium on activated carbon (200 mg) and stirred under 1.0 atm of hydrogen at room temperature for 24 h. After removal of the palladium catalyst by Celite® filtration, the filtrate was concentrated and purified by flash chromatography (Biotage-SNAP Ultra 25 g, eluted with *n*-hexane/EtOAc = 96/4 to 80/20 (v/v)) to give the title compound **106** (762 mg, 75%).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 0.86 (t, *J* = 7.5 Hz, 3H), 1.47 (s, 9H), 1.64–2.14 (m, 3H), 2.44–2.56 (m, 1H), 3.14–3.31 (m, 2H), 3.79 (br s, 1H), 3.84 (s, 3H), 3.93–4.20 (m, 1H), 6.04 (t, *J* = 3.5 Hz, 1H), 6.84 (dd, *J* = 3.5, 2.5 Hz, 1H), 9.27 (br s, 1H)

Step9 : Methyl 5-((3*S*,5*R*)-5-ethylpyrrolidin-3-yl)-1*H*-pyrrole-2-carboxylate (**107**)

Compound **106** (760 mg, 2.36 mmol) was dissolved in 4 M HCl in 1,4-dioxane solution (7.6 mL) and stirred at room temperature for 1.5 h. The reactant mixture was dried under vacuum to give the title compound **107** (615 mg, overweight) as a hydrochloride salt, which was applied to the next step without further purification.

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.96 (t, *J* = 7.5 Hz, 3H), 1.61–1.85 (m, 3H), 2.41–2.50 (m, 1H), 3.04–3.23 (m, 1H), 3.38 (br s, 1H), 3.42–3.59 (s, 3H), 3.74 (s, 3H), 6.11 (dd, *J* = 3.6, 2.6 Hz, 1H), 6.72 (dd,

$J$ = 3.6, 2.6 Hz, 1H), 9.02 (br s, 1H), 9.73 (br s, 1H), 11.86 (s, 1H)

Step10 : Methyl 5-((3*S*,5*R*)-1-(8-cyclopropyl-2-methyl-9*H*-pyrimido[4,5-*b*]indol-4-yl)-5-ethylpyrrolidin-3-yl)-1*H*-pyrrole-2-carboxylate (**108**)

A microwave vial was charged with **76** (250 mg, 9.70 × $10^{-1}$ mmol), **107** (251 mg, 9.70 × $10^{-1}$ mmol), *i*-$Pr_2NEt$ (800 μL, 4.40 mmol), and NMP (8.0 mL). The vial was heated to 140 °C, irradiated the microwave for 2 h. The mixture was diluted with $H_2O$ and extracted with EtOAc, and the organic layer was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by preparative TLC (EtOAc/MeOH = 20/1 (v/v)) to give the title compound **108** (191 mg, 45% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.68–0.78 (m, 2H), 0.95 (t, $J$ = 7.3 Hz, 3H), 1.00–1.04 (m, 2H), 1.57–1.69 (m, 1H), 1.92–2.03 (m, 2H), 2.31–2.38 (m, 1H), 2.51 (s, 3H), 2.46–2.55 (m, 1H), 3.12–3.21 (m, 1H), 3.73 (s, 3H), 3.92 (t, $J$ = 10.3 Hz, 1H), 4.16 (t, $J$ = 8.1 Hz, 1H), 4.67–4.75 (m, 1H), 6.15 (dd, $J$ = 3.6, 2.4 Hz, 1H), 6.73 (dd, $J$ = 3.6, 2.4 Hz, 1H), 6.85 (d, $J$ = 7.7 Hz, 1H), 7.08 (t, $J$ = 7.7 Hz, 1H), 7.63 (d, $J$ = 7.7 Hz, 1H), 11.78 (br s, 1H), 11.97 (s, 1H)

Step11 : 5-((3*S*,5*R*)-1-(8-Cyclopropyl-2-methyl-9*H*-pyrimido[4,5-*b*]indol-4-yl)-5-ethylpyrrolidin-3-yl)-1H-pyrrole-2-carboxylic acid (**29**)

Compound **108** (190 mg, 4.32 × $10^{-1}$ mmol) was dissolved in 1:1 THF-MeOH (6.0 mL). To the solution, 2 M aqueous NaOH solution (1.74 mL, 3.60 mmol) was added, and the mixture was stirred at 80 °C for 4 h. After cooling to room temperature, the reactant mixture was neutralized with 2.0 M aqueous HCl solution and diluted with $H_2O$. The precipitated solid was collected by filtration, washed with $H_2O$, and dried to give the title compound **29** (165 mg, 90% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.68–0.78 (m, 2H), 0.95 (t, $J$ = 7.3 Hz, 3H), 1.00–1.04 (m, 2H), 1.57–1.69 (m, 1H), 1.92–2.05 (m, 2H), 2.31–2.38 (m, 1H), 2.51 (s, 3H), 2.47–2.54 (m, 1H), 3.11–3.20 (m, 1H), 3.92 (t, $J$ = 10.3 Hz, 1H), 4.16 (t, $J$ = 8.1 Hz, 1H), 4.67–4.74 (m, 1H), 6.11 (dd, $J$ = 3.5, 2.5 Hz, 1H), 6.67 (dd, $J$ = 3.5, 2.4 Hz, 1H), 6.85 (d, $J$ = 7.8 Hz, 1H), 7.08 (t, $J$ = 7.8 Hz, 1H), 7.64 (d, $J$ = 7.8 Hz, 1H), 11.64 (s, 1H), 11.98 (s, 1H), 12.09 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 7.8, 8.0, 9.7, 10.5, 25.9, 26.5, 35.7, 37.4, 57.9, 58.5, 95.6, 106.6, 114.9, 118.7, 119.0, 119.4, 120.2, 122.3, 126.3, 136.2, 137.0, 157.2, 157.6, 161.8 (2C)

$[\alpha]_D^{25}$ = -310 ($c$ = 1.00, DMSO)

HRMS (ESI, *m/z*, MH+) Calcd for $C_{25}H_{28}O_2N_5$: 430.2238, Found: 430.2239

#### *4.1.18. Synthesis of compound **30** and **31***

Step1 : *tert*-Butyl (*S*)-4-oxo-2-((trityloxy)methyl)pyrrolidine-1-carboxylate (**109**)

To a solution of **94** (3.00 g, 6.52 mmol) in $CHCl_3$ (15 mL) was added Dess-Martin Periodinane (4.20 g, 9.79 mmol) at temperature of a water bath, and the mixture was stirred at room temperature for 1 h. The reaction was quenched with $NaHCO_3$ (2.50 g, 19.8 mmol) and $H_2O$ (25 mL). The mixture was diluted with $H_2O$ and extracted with AcOEt, and the organic layer was washed with aqueous $Na_2CO_3$ and brine. After drying over $Na_2SO_4$ and filtration, the solvent was evaporated *in vacuo* to give the title compound **109** (2.90 g, 97%).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.38–1.51 (br m, 9H), 2.51–2.42 (br m, 1H), 2.65–3.03 (br m, 2H), 3.46–3.69 (br m, 1H), 3.89–4.05 (br m, 2H), 4.35–4.51 (br m, 1H), 7.33–7.20 (m, 15H)

Step2 : *tert*-Butyl (*S*)-4-(((trifluoromethyl)sulfonyl)oxy)-2-((trityloxy)methyl)-2,3-dihydro-1*H*-pyrrole-1-carboxylate (**110**)

The compound **109** (1.40 g, 3.17 mmol) was dissolved in THF (10 mL). To the solution, 1.0 M LiHMDS in THF (3.80 mL, 3.80 mmol) was added at −78 °C, and the mixture was stirred at −30 °C for 15 min. After cooling to −78 °C, *N*-phenyltrifluoromethanesulfonimide ($Tf_2NPf$, 1.50 g, 4.00 mmol) was added to the reactant mixture, and then the mixture was stirred at room temperature for 1 h. The mixture was diluted with aqueous $NH_4Cl$ solution and extracted with AcOEt, and the organic layer was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by flashchromatography (Yamazen-Universal Premium L, eluted with *n*-hexane/EtOAc = 95/5 to 65/35 (v/v)) to give the title compound **110** (1.66 mg, 89% yield).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.26–1.53 (br m, 9H), 3.18–3.53 (br m, 2H), 4.19–4.36 (br m, 2H), 4.63–4.70 (br m, 1H), 5.66–5.84 (br m, 1H), 7.21–7.30 (m, 9H), 7.36–7.41 (m, 6H)

Step3 : 1′-(*tert*-Butyl) 5-methyl (*S*)-5′-((trityloxy)methyl)-4′,5′-dihydro-1*H*,1′*H*-[2,3′-bipyrrole]-1′,5-dicarboxylate (**111**)

A mixture of **110** (300 mg, 5.09 × $10^{-1}$ mmol), (5-(methoxycarbonyl)-1*H*-pyrrol-2-yl)boronic acid (172 mg, 1.02 mmol) and 2.0 M aqueous $Na_2CO_3$ solution (600 μL, 1.20 mmol) in DMA (1.5 mL) was treated with $PdCl_2$(dppf)-$CH_2Cl_2$ (42.0 mg, 5.14 × $10^{-2}$ mmol) and stirred at 90 °C for 1 h under argon atmosphere. After cooling to room temperature, the mixture was diluted with AcOEt and $H_2O$, and the organic layer was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by preparative TLC (*n*-hexane/AcOEt = 2/1 (v/v)) to give the title compound **111** (167 mg, 58% yield).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.32–1.51 (br m, 9H), 3.13–3.50 (m, 2H), 3.88 (s, 3H), 4.34–4.58 (br m, 2H), 4.75–4.82 (br m, 1H), 5.82–6.02 (br m, 1H), 6.23 (br s, 1H), 6.88 (br s, 1H), 7.20–7.30 (m, 9H), 7.37–7.41 (m, 6H), 9.14–9.21 (br m, 1H)

Step4 : Methyl 5-((3*S*,5*S*)-1-(*tert*-butoxycarbonyl)-5-((trityloxy)methyl)pyrrolidin-3-yl)-1*H*-pyrrole-2-carboxylate (**112**)

A solution of **111** (167 mg, 2.96 × $10^{-1}$ mmol) in THF (8.0 mL) and MeOH (8.0 mL) was treated with 10% palladium on activated carbon (80.0 mg) and stirred under 1.0 atm of hydrogen at room temperature for 5 h. After removal of the palladium catalyst by Celite® filtration, the filtrate was concentrated and purified by preparative TLC (*n*-hexane/AcOEt = 2/1 (v/v)) to give the title compound **112** (129 mg, 77% yield).

$^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.32–1.48 (br m, 9H), 2.11–2.21 (m, 1H), 2.38–2.56 (m, 1H), 3.09–3.52 (br m, 4H), 3.84 (s, 3H), 4.06–4.13 (br m, 2H), 6.04 (br s, 1H), 6.83 (br s, 1H), 7.20–7.30 (m, 9H), 7.39–7.42 (m, 6H), 9.01 (br s, 1H)

Step5 : Methyl 5-((3*S*,5*S*)-5-(hydroxymethyl)pyrrolidin-3-yl)-1*H*-pyrrole-2-carboxylate (**113**)

Compound **112** (129 mg, 2.28 × $10^{-1}$ mmol) was dissolved in 4 N HCl in 1,4-dioxane solution (2.0 mL) and stirred at room temperature for 2 h. The reactant mixture was diluted with $H_2O$ (250 μL) and washed with *n*-hexane (1.0 mL) three times. The aqueous layer was dried under vacuum to give the title compound **113** (56.0 mg, overweight), which was applied to the next step without further purification.

Step6 : Methyl 5-((3*S*,5*S*)-1-(8-cyclopropyl-2-methyl-9*H*-pyrimido[4,5-*b*]indol-4-yl)-5-(hydroxymethyl)pyrrolidin-3-yl)-1*H*-pyrrole-2-carboxylate (**114**)

A microwave vial was charged with **76** (58.0 mg, 2.25 × $10^{-1}$ mmol), **113** (56.0 mg, approx. 2.3 × $10^{-1}$ mmol), *i*-$Pr_2NEt$ (200 μL, 1.1 mmol), and NMP (2.0 mL). The vial was heated to 140 °C, irradiated the microwave for 2 h. The mixture was diluted with $H_2O$ and extracted with EtOAc, and the organic layer was washed with $H_2O$ and brine, and dried over $Na_2SO_4$. After filtration and concentration, the residue was purified by preparative TLC (EtOAc/MeOH = 20/1 (v/v)) to give the title compound **114** (68.8 mg, 69% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.67–0.79 (m, 2H), 1.00–1.04 (m, 2H), 2.13–2.22 (m, 1H), 2.31–2.38 (m, 1H), 2.43–2.49 (m, 1H), 2.51 (s, 3H), 3.13–3.22 (m, 1H), 3.64–3.70 (m, 1H), 3.72 (s, 3H), 3.75–3.80 (m, 1H), 3.89 (t, $J$ = 10.3 Hz, 1H), 4.24 (t, $J$ = 8.1 Hz, 1H), 4.81–4.88 (br m, 1H), 4.94 (t, $J$ = 5.4 Hz, 1H), 6.13 (dd, $J$ = 3.3, 2.5 Hz, 1H), 6.74 (dd, $J$ = 3.7, 2.5 Hz, 1H), 6.85 (d, $J$ = 7.8 Hz, 1H), 7.07 (t, $J$ = 7.8 Hz, 1H), 7.64 (d, $J$ = 7.8 Hz, 1H), 11.77 (s, 1H), 11.98 (s, 1H)

Step7 : 5-((3*S*,5*S*)-1-(8-Cyclopropyl-2-methyl-9*H*-pyrimido[4,5-*b*]indol-4-yl)-5-(hydroxymethyl)pyrrolidin-3-yl)-1*H*-pyrrole-2-carboxylic acid (**30**)

Compound **114** (57 mg, 1.3 × $10^{-1}$ mmol) was dissolved in 1:1 THF-MeOH (2.0 mL). To the solution, 2 M aqueous NaOH solution (580 μL, 1.2 mmol) was added, and the mixture was stirred at 60 °C for 3 h. After

cooling to room temperature, the reactant mixture was neutralized with 2.0 M aqueous HCl solution and diluted with $H_2O$. The precipitated solid was collected by filtration, washed with $H_2O$, and dried to give the title compound **30** (41 mg, 73% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.67–0.79 (m, 2H), 0.98–1.06 (m, 2H), 2.13–2.22 (m, 1H), 2.31–2.38 (m, 1H), 2.42–2.48 (m, 1H), 2.50 (s, 3H), 3.12–3.21 (m, 1H), 3.67 (dd, $J$ = 10.5, 5.7 Hz, 1H), 3.78 (dd, $J$ = 10.5, 3.9 Hz, 1H), 3.89 (t, $J$ = 10.3 Hz, 1H), 4.24 (t, $J$ = 8.1 Hz, 1H), 4.81–4.88 (m, 1H), 4.95 (br s, 1H), 6.09 (dd, $J$ = 3.4, 2.6 Hz, 1H), 6.68 (dd, $J$ = 3.4, 2.4 Hz, 1H), 6.85 (d, $J$ = 7.7 Hz, 1H), 7.08 (t, $J$ = 7.7 Hz, 1H), 7.65 (d, $J$ = 7.7 Hz, 1H), 11.62 (s, 1H), 11.98 (s, 1H), 12.07 (br s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 7.7, 8.1, 10.5, 25.8, 33.6, 37.3, 58.0, 59.1, 62.8, 95.4, 106.3, 114.9, 118.7, 119.2, 119.4, 120.3, 122.3, 126.3, 136.3, 137.1, 157.4, 157.7, 161.8 (2C)

$[\alpha]_D^{25}$ = -342 ($c$ = 1.00, DMSO)

HRMS (ESI, $m/z$, MH+) Calcd for $C_{24}H_{26}O_3N_5$: 432.2030, Found: 432.2032

Step8 : 5-((3*S*,5*S*)-1-(8-Cyclopropyl-2-methyl-9*H*-pyrimido[4,5-*b*]indol-4-yl)-5-(hydroxymethyl)pyrrolidin-3-yl)-1*H*-pyrrole-2-carboxylic acid (**31**)

Ammonium chloride (7.2 mg, 1.4 × $10^{-1}$ mmol), HATU (39 mg, 1.0 × $10^{-1}$ mmol) and $Et_3N$ (47 μL, 3.4 × $10^{-1}$ mmol) were added to a solution of **30** (29 mg, 6.7 × $10^{-2}$ mmol) in $CHCl_3$ (1.0 mL), and the reactant mixture was stirred at room temperature for 18 h. The mixture was diluted with $H_2O$ and extracted with $CHCl_3$, using ISOLUTE® Phase Separator manufactured by Biotage AB. After concentration, the residue was suspended in 1:1 *n*-hexane-EtOAc and stirred. The precipitated solid was collected by filtration, washed with *n*-hexane, and dried to give the title compound **31** (7.2 mg, 25% yield).

$^1$H-NMR (400 MHz, DMSO-$d_6$) δ: 0.66–0.77 (m, 2H), 0.98–1.02 (m, 2H), 2.14 (dd, $J$ = 22.5, 12.1 Hz, 1H), 2.29–2.36 (m, 1H), 2.39–2.44 (m, 1H), 2.48 (s, 3H), 3.08–3.17 (m, 1H), 3.63–3.68 (m, 1H), 3.74–3.78 (m, 1H), 3.84 (t, $J$ = 10.2 Hz, 1H), 4.22 (t, $J$ = 8.2 Hz, 1H), 4.78–4.85 (m, 1H), 4.92 (t, $J$ = 5.4 Hz, 1H), 5.99 (t, $J$ = 2.7 Hz, 1H), 6.69 (dd, $J$ = 3.5, 2.7 Hz, 1H), 6.79 (br s, 1H), 6.83 (d, $J$ = 7.7 Hz, 1H), 7.06 (t, $J$ = 7.7 Hz, 1H), 7.38 (br s, 1H), 7.62 (d, $J$ = 7.7 Hz, 1H), 11.30 (s, 1H), 11.96 (s, 1H)

$^{13}$C-NMR (400 MHz, DMSO-$d_6$) δ: 7.7, 8.1, 10.5, 25.8, 33.6, 37.2, 58.2, 59.1, 62.8, 95.3, 105.4, 110.7, 118.7, 119.2, 119.5, 120.2, 125.7, 126.3, 135.0, 136.2, 157.4, 157.7, 161.8, 162.3

$[\alpha]_D^{25}$ = –332 ($c$ = 1.00, DMSO)

HRMS (ESI, $m/z$, MH+) Calcd for $C_{24}H_{27}O_2N_6$: 431.2190, Found: 431.2191

### 4.2. Crystallographic methods

Protein production and purification of PDHK2 kinase domain (residues 16–407) were carried out as previously reported.[24] Briefly, this PDHK kinase domain was produced in *Escherichia coli*. and purified by Ni-NTA affinity column, protease digestion and gel filtration. The purified PDHK2 protein was crystallized in complex with compounds **5**, **7** and **20**. Co-crystals were obtained at 4 °C using the hanging-drop vapor diffusion method with reservoir containing 50 mM Na acetate pH 5.5, 100 mM magnesium chloride and 8% (v/v) isopropanol.

Diffraction data of the co-crystals were collected at Photon Factory (Japan) and SPring-8 (Japan). The data were integrated with DIALS[25] and scaled using aimless[26]. The structures of the PDHK-compound complex were solved by molecular replacement with MOLREP in CCP4 suite[27–28] using the coordinates of the PDHK2 kinase domain (PDB ID 2BTZ[24] as the model. The structural models were built in Coot[29] and refined using REFMAC5 in CCP4 suite[30] and Phenix[31]. Figures were created using PyMOL[32].

### 4.3. Spectrophotometry assay to measure the inhibitory activity on PDHKs

PDHK activity was assessed indirectly by measuring the residual PDH activity after PDHK reaction essentially as described previously[33]. Firstly, the composition of PDH/PDHK was determined based on the enzymatic activity of a purchased porcine PDH complex (Sigma-Aldrich). PDH activity was set to 25 mU/mL, and each of the recombinant human PDHK enzyme (0.5 μmol/L for PDHK2 and 3.0 μmol/L for PDHK4) were mixed and incubated overnight at 4 °C to obtain PDH/PDHK complex. The PDH/PDHK complex and PDHK inhibitors were incubated for 45 min at room temperature after adding the $K_m$ concentration of ATP (0.3 μmol/L for PDHK2 and 10 μmol/L for PDHK4) to start the PDHK reaction. Then, the substrates for PDH (5.0 mmol/L coenzyme A, 5.0 mmol/L sodium pyruvate and 12 mmol/L β-nicotinamide adenine dinucleotide) were added to start the PDH reaction and incubated for 90 min at room temperature. The PDH reaction was analyzed by measuring reaction products, NADH. The amount of NADH was determined measuring the absorbance at 340 nm before and after the PDH reaction to exclude the possible interference of test compounds. Concentration − response data were fitted to the following equation using Spotfire (TIBCO):

$$\%\text{inhibition} = (\text{max - min})/\ 1 + 10^{(\log IC50 - \log [comp]) \times Hill} + \text{min}$$

where min is the 100% enzymatic activity control, max is the 0% enzymatic activity control.

### 4.4. Luminescent assay to measure the inhibitory activity on BCKDK

BCKDK activity was assessed by ADP-Glo™ Kinase Assay (Promega Corporation). Human recombinant BCKDK and PDHK inhibitors were incubated for 60 min at 37℃ after adding 0.2 μmol/L ATP to start the BCKDK reaction. ADP-Glo™ Reagent were added and incubated for 40 min at room temperature to terminate the kinase reaction and deplete the remaining ATP. Then, Kinase Detection Reagent was added and incubated for 40 min at room temperature to convert ADP to ATP. The amount of ATP was determined measuring luminescence.

Concentration − response data were fitted to the following equation using Spotfire (TIBCO):

$$\%\text{inhibition} = (\text{max - min})/\ 1 + 10^{(\log IC50 - \log [comp]) \times Hill} + \text{min}$$

where min is the 100% enzymatic activity control, max is the 0% enzymatic activity control.

### 4.5. Computational study

WaterMap (Schrödinger Release 2019-1: Schrödinger, LLC, New-York, NY, 2019) calculations were performed on the co-crystal structure of compound **7** bound to PDHK2. Input structure was prepared by Protein Preparation Wizard in Maestro (Schrödinger Release 2019–1) using default options. WaterMap was run in default mode with OPLS3e forcefields, existing waters deleted, and a 2 ns MD simulation. The compound **7** was used to define the binding site.

Docking models on PDHK2 were created as follows. The co-crystal structure of compound **20** on PDHK2 was selected as a receptor input, and the structure was prepared by Protein Preparation Wizard in Maestro using default options. Designed compounds were docked into the ATP binding site of the receptor using the Glide SP docking protocol. The resulting binding poses were minimized using MacroModel with the OPLS3e force field in the default settings. Figures were created using PyMOL[32].

## 5. Accession codes

Atomic coordinates and structure factors were deposited in the Protein Data Bank with codes 7VBU for **5**/PDHK2, 7VBV for **7**/PDHK2 and 7VBX for **20**/PDHK2.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2021.116514.

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