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Discovery of 3,5-Dimethylpyridin-4(1H)-one Derivatives as Activators of AMP-Activated Protein Kinase (AMPK)

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Novel 3,5-dimethylpyridin-4(1H)-one scaffold compounds were synthesized and evaluated as AMP-activated protein kinase (AMPK) activators. Unlike direct AMPK activators, this series of compounds showed selective cell growth inhibitory activity against human breast cancer cell lines. By optimizing the lead compound (4a) from our library, 2-[(1'-[(4-fluorophenyl)methyl]-2-methyl-1',2',3',6'-tetrahydro[3,4'-bipyridin]-6-yl)oxy)methyl]-3,5-dimethylpyridin-4(1H)-one (25) was found to have potent AMPK activating activity. Compound 25 also showed good aqueous solubility while maintaining the unique selectivity in cell growth inhibitory activity.

Key words AMP-activated protein kinase activator; precision medicine; aqueous solubility; dihedral angle; hydrophobicity; human breast cancer

Introduction

AMP-activated protein kinase (AMPK) is a master regulator of cellular metabolism in response to sources of metabolic stress, such as nutrient starvation, hypoxia and muscle contraction.^{1,2)} AMPK is switched on by a rise in the cellular AMP/ATP ratio. Binding of AMP to the allosteric pocket of AMPK appears to lead to activation, with binding to the AMPK γ subunit in particular serving as an important regulatory feature of the conformational switch.³⁾ AMPK activation stimulates fatty acid oxidation and glucose uptake, inhibition of cholesterol synthesis, triglyceride synthesis and lipogenesis.⁴⁾

Recent studies have suggested that AMPK activation *via* several signalling pathways may be a feasible therapeutic strategy for the treatment of various cancers,⁵⁾ such as 1) enhancement of the stability of p53, a known tumor suppressor protein⁶⁾; 2) inhibition of the expression level of cyclin D1, an established cell cycle regulator that is overexpressed in human cancers⁷⁾; and 3) inhibition of the AKT/mammalian target of rapamycin (mTOR) signalling pathway, which is dysregulated in some human cancer cells.⁸⁾

A number of direct and indirect AMPK activators have been reported to date.^{3,9,10)} Some of the known direct activators are shown in Fig. 1. 5-Aminoimidazole-4-carboxamide riboside (AICAR) was the first direct AMPK activator to be

discovered. This compound is an adenosine analog that is taken up into cells and phosphorylated to generate AICAR monophosphate, which mimics the effects of AMP.^{11,12)}

Additionally, some of the reported indirect activators are shown in Fig. 2. Among these, metformin, a biguanide-type compound, is used as a first line antidiabetic agent. This compound inhibits mitochondrial complex I, leading to an increase in the AMP/ATP ratio.^{13,14)}

To acquire a novel series of AMPK activators, we performed whole cell enzyme-linked immunosorbent assay (ELISA) to measure the Ser79 phosphorylation of acetyl-CoA carboxylase (ACC), a substrate of AMPK, using the human breast cancer cell line MDA-MB-453. By screening our compound library, we discovered that compound **4a**, a 3,5-dimethylpyridin-4(1H)-one-type compound, was a moderate AMPK activator (Fig. 3).

Next, we evaluated AICAR and compound **4a** for cell growth inhibitory activity against human breast cancer cell lines MDA-MB-453 and SK-BR-3. The results are shown in Table 1. We found that, unlike the direct AMPK activator AICAR, which inhibited cell growth in both cell lines, **4a** exhibited growth inhibitory activity against only MDA-MB-453 cells. This data suggests that **4a** indirectly, rather than directly, activates AMPK *via* another target molecule.

We hypothesized that this unique selectivity in cell growth

Fig. 1. AMPK Direct Activators

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Fig. 2. AMPK Indirect Activators

AMPK activation (MDA-MB-453 cell) EC₅₀: 0.89 μM

Fig. 3. Chemical Structure of Lead Compound **4a** and Its AMPK Activation ActivityTable 1. *In Vitro* Antiproliferative Activity of AICAR and **4a**

Compound	MDA-MB-453 growth inhibition IC ₅₀ (μM) ^{a)}	SK-BR-3 growth inhibition IC ₅₀ (μM) ^{a)}
AICAR	1700	180
4a	0.39	29% inh. at 1 μM

a) The IC₅₀ values were determined in triplicate in one experiment.

Reagents and conditions: (a) 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine hydrochloride, Ag₂CO₃, toluene, 110°C, in dark, 39–94%; (b) (4-fluorophenyl)boronic acid, PdCl₂(PPh₃)₂, Na₂CO₃, 1,4-dioxane, H₂O, 90°C, 100%; (c) AlCl₃, CH₂Cl₂, r.t., 45–74%.

Chart 1.

Reagents and conditions: (a) ArB(OH)₂, Pd(OAc)₂, SPhos, K₃PO₄, toluene, H₂O, 110°C, 77–100%; (b) **4d**, **4e**, **4i**: AlCl₃, CH₂Cl₂, r.t., 18–72%; **4f**–**4h**: methionine, MsOH, 90°C, 53–55%.

Chart 2.

inhibitory activity may lead to the identification of interesting and unique candidates for anti-cancer precision medicine compared to currently available direct AMPK activators. Herein, we present the synthesis and structure activity relationship (SAR) of novel AMPK activators with the unique 3,5-dimethylpyridin-4(1*H*)-one scaffold. We dramatically improved *in vitro* AMPK activation activity and cell growth inhibitory

activity in MDA-MB-453 cells, and successfully obtained a compound with both potent cellular activity and high aqueous solubility.

Chemistry

Chart 1 illustrates the synthesis of analogs **4a**–**4c**. Alkylation of commercially available hydroxypyridine derivatives

Reagents and conditions: (a) B(O-*i*Pr)₃, *n*-BuLi (1.7 mol/L in hexane), THF, -62 to 0°C, 71%; (b) ArBr or ArCl, Pd(PPh₃)₄, Na₂CO₃, 1,4-dioxane, H₂O, 100°C, 72–85%; (c) dodecane-1-thiol, *t*-BuOK, DMF, r.t., to 60°C, 16–66%.

Chart 3.

Reagents and conditions: (a) *n*-BuLi (1.7 mol/L in hexane), THF, -64°C then DMF, -63°C, 45%; (b) P(OEt)₃, 150°C, 92%; (c) NaH, DME, 0°C to r.t., 58%; (d) methionine, MsOH, 50°C, 48–56%; (e) 10% Pd/C (wetted with approx. 50%), H₂ (1.0 kgf/cm²), THF, EtOH, r.t., 83%.

Chart 4.

1a–1c with 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine and silver carbonate yielded **2a–2c**, respectively. The *O*-alkylated compounds were then converted to corresponding biaryl compounds **3a–3c** by palladium-catalyzed Suzuki–Miyaura cross coupling with (4-fluorophenyl)boronic acid using sodium carbonate as a base. Finally, analogs **4a–4c** were obtained by AlCl₃ demethylation of the corresponding methyl ethers in dichloromethane solvent.

The synthetic route for **4d–4i** is described in Chart 2. Suzuki–Miyaura cross coupling of **2c** was performed with the requisite commercially available aryl boronic acid derivatives using Pd(OAc)₂ and SPhos in the presence of tripotassium phosphate base to give the intermediates **3d–3i**. As with **4a–4c**, the corresponding methyl ethers were dealkylated using AlCl₃ or methionine to give analogs **4d–4i**.

The synthesis of **4j–4m** was accomplished following slight modifications to the synthetic route shown in Chart 2. As outlined in Chart 3, key intermediate **5** was synthesized by metal-halogen exchange of **2c** and addition to triisopropyl borate. Subsequent Suzuki–Miyaura cross coupling reaction of **5** with the requisite commercially available heteroaryl halides

using Pd(PPh₃)₄ in the presence of aqueous sodium carbonate base in 1,4-dioxane followed by deprotection of the methyl ether group yielded **4j–4m**.

Analog **10** and **12** were generated using the synthetic route outlined in Chart 4. Lithiation of **2c** with *n*-BuLi, followed by trapping with DMF gave aldehyde **6**. Treatment of commercially available **7** with triethyl phosphite at 150°C gave phosphonate **8**. With intermediates **6** and **8** in hand, aldehyde **6** was subjected to the Horner–Wadsworth–Emmons reaction with phosphonate **8** using NaH as a base to give compound **9**. Deprotection of the methyl group in compound **9** using methionine in MsOH as a solvent gave compound **10**. For **12**, palladium-catalyzed hydrogenation of **9** was performed to obtain **11**, and a subsequent deprotection reaction yielded **12**.

The synthesis of analogs **19a–19c** and **21** required an alternative synthetic strategy, as our initial attempt to generate these analogs by hydrogenation of the corresponding 3,6-dihydropyridine-1(2*H*)-carboxylate derivative after introduction of the 4-methoxy-3,5-dimethylpyridine moiety was unsuccessful. As shown in Chart 5, our synthesis began with benzylation of commercially available **1c** using silver carbonate to give

Reagents and conditions: (a) *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate, $\text{Pd}(\text{PPh}_3)_4$, Na_2CO_3 , 1,4-dioxane, H_2O , 100°C , 100%; (b) 20% $\text{Pd}(\text{OH})_2/\text{C}$ (wetted with approx. 50%), H_2 (2.5 kgf/cm²), THF, MeOH, r.t., 92%; (c) 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine hydrochloride, Ag_2CO_3 , toluene, 110°C , 98%; (d) TFA, CH_2Cl_2 , r.t., 100%; (e) 18a: 1*H*-benzotriazol-1-ylmethanol, $\text{NaBH}(\text{OAc})_3$, THF, EtOH, r.t., 73%; 18b, 18c: ArCHO , $\text{NaBH}(\text{OAc})_3$, AcOH, CH_2Cl_2 , r.t., 69–84%; (f) methionine, MsOH , 60°C , 14–46%; (g) 1-bromo-4-fluorobenzene, $\text{Pd}_2(\text{dba})_3$, BINAP, *t*-BuONa, toluene, reflux, 47%.

Chart 5.

Reagents and conditions: (a) *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate, $\text{Pd}(\text{PPh}_3)_4$, Na_2CO_3 , 1,4-dioxane, H_2O , 100°C , 76%; (b) TFA, CH_2Cl_2 , r.t., 87%; (c) 4-fluorobenzaldehyde, $\text{NaBH}(\text{OAc})_3$, AcOH, CH_2Cl_2 , r.t., 84%; (d) AlCl_3 , CH_2Cl_2 , r.t., 25%.

Chart 6.

13.¹⁵⁾ Suzuki–Miyaura cross coupling reaction of **13** with commercially available 3,6-dihydropyridine-1(2*H*)-carboxylate boronate yielded intermediate **14**. Subsequent hydrogenation reaction using Pearlman's catalyst under high hydrogen pressure gave compound **15**. With intermediate **15** in hand, the hydroxypyridine was subjected to alkylation with commercially available benzyl chloride using silver carbonate to provide the key intermediate **17** after TFA deprotection of the Boc group. For the synthesis of **19a–19c**, the amine was alkylated with various aldehydes by reductive amination reaction using sodium triacetoxyborohydride followed by deprotection of the methyl ether group. For **21**, 1-bromo-4-fluorobenzene was subjected to Buchwald–Hartwig amination with intermediate **17** to give **20**. As with the analogs described above, the corresponding methyl ether was dealkylated using methionine to give **21**.

Analog **25** was synthesized as shown in Chart 6. Suzuki–Miyaura cross coupling reaction of intermediate **2c** with commercially available 3,6-dihydropyridine-1(2*H*)-carboxylate boronate gave compound **22**, which upon deprotection with TFA in dichloromethane followed by reductive amination reaction with 4-fluorobenzaldehyde gave **24**. Finally, removal of the methyl group of intermediate **24** with AlCl_3 in dichloromethane as shown in Charts 1 and 2 yielded the desired compound **25**.

Results and Discussion

The AMPK activation activity of the synthesized compounds was measured by phospho-ACC whole cell ELISA using the MDA-MB-453 human breast cancer cell line. Selected compounds were also evaluated for aqueous solubility in the Japanese Pharmacopoeia 2nd fluid for disintegration test

Fig. 4. Initial SAR of Derivatives of **4a**

Compound	Dihedral angle (°)
4a'	52.6
4b'	81.4
4c'	81.2

^a Global minimum energy conformations and dihedral angles were calculated using MOE (ver. 2016.0802).¹⁶⁾

Fig. 5. Global Minimum Energy Conformations and Dihedral Angles of **4a'** (Cyan), **4b'** (Yellow) and **4c'** (White)^a

Table 2. SARs of Methyl Group-substituted Derivatives

Compound	R	AMPK activation EC ₅₀ (μM) ^{a)}
4a	3-Me	0.89
4b	4-Me	0.28
4c	6-Me	0.074

^{a)} The EC₅₀ values were examined by phospho-ACC whole cell ELISA using the MDA-MB-453 cell line, and determined in triplicate in one experiment.

(JP2; pH = 6.8) and their hydrophobicity was measured at pH 7.4 (Log *D*_{7.4}).

The initial SAR of derivatives of lead compound **4a** is illustrated in Fig. 4 (data not shown). No AMPK activation effect was observed for the 4-methoxypyridine intermediate. Additionally, shifting the central pyridine nitrogen led to reduced cellular potency. These early modifications demonstrated that the 3,5-dimethylpyridin-4(1*H*)-one nucleus and position of the nitrogen atom in the central heteroaromatic ring were key elements of the pharmacophore. These elements were readily incorporated using efficient chemistry methodologies, allowing us to optimize the position of the methyl group of the central aromatic ring and substitution of the terminal aromatic moiety for cellular potency.

First, we explored the importance of the position of a substituted methyl group on the central aromatic ring *via* preparation of compounds **4b** and **4c**. The SAR of analogs **4b** and **4c** (Table 2) revealed that substitution of a methyl group at the 6-position was key to improving AMPK activation activity. Compound **4b** showed 3-fold more potent cellular activity than compound **4a**. Furthermore, 6-methyl analog **4c** displayed 10-fold more potent AMPK activation activity than 3-methyl analog **4a**.

In the comparison of the 3-methyl derivative (**4a**) with the 4-methyl (**4b**) or the 6-methyl (**4c**) derivative, we focused on the dihedral angle in the biphenyl moiety of these derivatives. To simplify the calculation of the global minimum conformation, we generated the virtual compounds **4a'**–**4c'**, which do not possess common 3,5-dimethylpyridin-4(1*H*)-one moieties in **4a**–**4c**. Figure 5 shows the dihedral angles of the 2-methoxypyridine analogs **4a'**, **4b'** and **4c'** calculated using Molecular Operating Environment software (MOE).¹⁶⁾ This calculation indicated that **4b'** and **4c'** had larger dihedral angles than **4a'** (Fig. 5; **4a'**: 52.6°, **4b'**: 81.4°, **4c'**: 81.2°). Based on this result, we concluded that a perpendicular conformation was important for potent cellular activity. On the other hand, 6-methyl derivative **4c** showed 4-fold more potent AMPK activation and a similar dihedral angle to that of 4-methyl derivative **4b**. As mentioned above, these compounds likely do not activate AMPK directly. However, because the molecule to which these compounds directly bind is unclear, we reasoned the difference between the potency of **4b** and **4c** based on their cellular activity and chemical structure. There are some potential reasons that explain why we observed different ac-

Table 3. SAR of 5-Aryl-Type Compounds

Compound	R	AMPK activation EC_{50} (μM) ^{a)}
4c	4'-F	0.074
4d	4'-CN	0.089
4e	4'-Me	0.018
4f	4'-CF ₃	0.010
4g	3'-CF ₃	0.19
4h	2'-CF ₃	0.30

a) The EC_{50} values were examined by phospho-ACC whole cell ELISA using the MDA-MB-453 cell line, and determined in triplicate in one experiment.

tivities among these compounds, as one of them, we speculate that the 6-methyl group may occupy a hydrophobic pocket in its target molecule.

Further optimization efforts focused on the substituent on the terminal aromatic moiety to obtain more potent compounds (Table 3). Replacement of the fluoro group with a cyano group (**4d**) produced a compound with similar potency (EC_{50} = 0.089 μM) to **4c**. However, replacement with a methyl group (**4e**) resulted in a significant improvement in potency (EC_{50} = 0.018 μM). Further improvement in cellular activity was observed when the fluoro group was replaced with a CF₃ group (**4f**), with the resulting compound showing an EC_{50} of 0.010 μM . There was a clear preference for substitution in the 4'-position, as exemplified by the difference in potency between the 3'-CF₃ compound (**4g**) and 2'-CF₃ compound (**4h**) (EC_{50} = 0.19 and 0.30 μM , respectively), compared to **4f**.

While compound **4f** showed potent cellular activity, its aqueous solubility at pH 6.8 was low (< 1 μM) because of its high hydrophobicity ($\log D_{7.4}$ = 4.6). It is well acknowledged that compounds with poor aqueous solubility in gastric pH are often associated with poor oral absorption.¹⁷⁾ To investigate the potential anti-tumor effects of this series of compounds *in vivo*, we decided to improve the aqueous solubility of compound **4f** while maintaining its cellular potency.

In general, hydrophobicity is the most often encountered cause of poor solubility.¹⁸⁾ Therefore, strategies for decreasing hydrophobicity by chemical modification, such as introducing hetero atoms into compounds, is widely used to increase aqueous solubility.¹⁹⁾ Accordingly, we initially focused on introducing nitrogen atoms into the terminal phenyl moiety to improve the aqueous solubility of **4f**. Table 4 shows the AMPK activation EC_{50} values, aqueous solubility at pH 6.8 and measured $\log D$ values at pH 7.4 for compounds **4i–4m**. The hydrophobicity of pyridine analog **4i** and **4j** ($\log D_{7.4}$ = 3.2 and 3.3, respectively) was reduced compared to that of **4f**, while their aqueous solubility was slightly improved (7.8 and 9.6 μM , respectively). However, their cellular activity was reduced by 3–5 fold (EC_{50} = 0.034 and 0.042 μM , respectively) compared to that of **4f**. While the measured $\log D_{7.4}$ values of the pyrimidine analog (**4k**) and pyrazine analog (**4l**) were lower ($\log D_{7.4}$ = 3.0 and 2.8, respectively) than those of **4i**

Table 4. SAR of 5-Heteroaryl-Type Compounds

Compound	R	AMPK activation EC_{50} (μM) ^{a)}	JP2 (μM) ^{b)}	Measured $\log D_{7.4}$
4f		0.010	< 1	4.6
4i		0.034	7.8	3.2
4j		0.042	9.6	3.3
4k		0.10	6.3	3.0
4l		0.098	3.8	2.8
4m		1.1	22.8	2.1

a) The EC_{50} values were examined by phospho-ACC whole cell ELISA using the MDA-MB-453 cell line, and determined in triplicate in one experiment. b) Aqueous solubility in the Japanese Pharmacopoeia 2nd fluid for disintegration test (JP2; pH = 6.8).

and **4j**, the aqueous solubility was similar (6.3 and 3.8 μM , respectively). Additionally, the AMPK activation activity was further reduced (EC_{50} = 0.10 and 0.098 μM , respectively). Recently, Walker reported that pyridazine was the most water soluble among 2-nitrogen atom-containing, 6-membered ring heterocycles due to its higher dipole moment, pK_{HBX} , pK_a , and lower hydrophobicity compared to corresponding pyrimidines and pyrazines.²⁰⁾ Likewise, our pyridazine analog (**4m**) had the lowest hydrophobicity ($\log D_{7.4}$ = 2.1) of these compounds and its aqueous solubility was markedly improved (22.8 μM). Unfortunately, the cellular activity of compound **4m** was more than 100-fold lower (EC_{50} = 1.1 μM) than that of **4f**. Therefore, we concluded that it was difficult to achieve a significant improvement in aqueous solubility through decreasing hydrophobicity while maintaining potency.

A recent study by Brown *et al.* reported that removal of the biaryl structure led to improved aqueous solubility.^{21,22)} Therefore, we next focused on a strategy for removing the biaryl substituent to improve aqueous solubility. We did this by inserting a linker group between the central pyridine moiety and the terminal aromatic group²¹⁾ (Table 5) or by aromatic group saturation²²⁾ (Table 6).

The SAR of compounds **10** and **12**, which contain a carbon

Table 5. SAR of Ethylene or Alkene Spacer Compounds

Compound	R	AMPK activation EC ₅₀ (μM) ^a	JP2 (μM) ^b	Measured logD _{7.4}
4i		0.034	7.8	3.2
10		0.13	< 1	3.9
12		0.13	2.7	3.2

^a The EC₅₀ values were examined by phospho-ACC whole cell ELISA using the MDA-MB-453 cell line, and determined in triplicate in one experiment. ^b Aqueous solubility in the Japanese Pharmacopoeia 2nd fluid for disintegration test (JP2; pH = 6.8).

Table 6. SAR of Piperidine or Tetrahydropyridine-Type Compounds

Compound	R	AMPK activation EC ₅₀ (μM) ^a	JP2 (μM) ^b	Measured logD _{7.4}
4f		0.010	< 1	4.6
19a		> 3	≥ 100	NT ^c
21		0.074	1.7	3.8
19b		0.14	≥ 100	2.8
19c		0.095	2.6	4.2
25		0.016	≥ 100	3.2

^a The EC₅₀ values were examined by phospho-ACC whole cell ELISA using the MDA-MB-453 cell line, and determined in triplicate in one experiment. ^b Aqueous solubility in the Japanese Pharmacopoeia 2nd fluid for disintegration test (JP2; pH = 6.8). ^c NT: not tested.

spacer between two aromatic moieties, is shown in Table 5. The aqueous solubility of ethynylene linker analog **10** was undetectable (< 1 μM), probably due to its high lipophilicity (log D_{7.4} = 3.9). The hydrophobicity of ethylene linker analog **12** was equal (log D_{7.4} = 3.2) to that of biaryl compound **4i**, but its aqueous solubility was comparable (2.7 μM) to that of **4i**. Therefore, we concluded that it also was difficult to improve

the aqueous solubility through insertion of a two-carbon atom linker chain.

Finally, we investigated saturation of the terminal aromatic moiety to improve aqueous solubility (Table 6). Based on the calculated hydrophobicity, a simple dearomatization of the terminal aromatic group was predicted to increase hydrophobicity (data not shown). Therefore, we designed and introduced a piperidine linker, a saturated cyclic moiety with an additional polar amino group, into our compound. Replacement of the terminal phenyl moiety with methyl piperidine (**19a**) dramatically improved the aqueous solubility (≥ 100 μM). Although the potency of compound **19a** was undetectable (EC₅₀ > 3 μM), this result encouraged us to synthesize piperidine derivatives for further SAR analysis. Replacement of the methyl group of **19a** with a 4-fluorophenyl group enhanced the AMPK activation activity (**21**; EC₅₀ = 0.074 μM), while the aqueous solubility remained low (1.7 μM). Interestingly, 4-fluorobenzylamine analog **19b** showed moderate cellular activity (EC₅₀ = 0.14 μM) and high aqueous solubility (≥ 100 μM). Encouraged by this finding, we sought to further optimize **19b** to improve potency. Based on the above SAR studies (Table 3), trifluoromethyl analog **19c** was synthesized and evaluated. While the potency of **19c** was slightly improved (EC₅₀ = 0.095 μM), the modification reduced aqueous solubility (2.6 μM), probably due to an increase in hydrophobicity (log D_{7.4} = 4.2). Therefore, we decided to improve the potency of **19b** through subtle structural modifications without increasing hydrophobicity. We replaced the fully saturated piperidine moiety with a partially saturated tetrahydropyridine moiety, which we predicted may mimic the perpendicular conformation of biaryl-type compound **4c**. As expected, there was a significant increase in potency following addition of an *sp*² carbon into the piperidine moiety (**25**; EC₅₀ = 0.016 μM, which was comparable to that of **4f**). Furthermore, the hydrophobicity of tetrahydropyridine analog **25** was lower (log D_{7.4} = 3.2) than that of **19c** but comparable to that of **19b**, indicating that **25** was a potent compound with favorable aqueous solubility (≥ 100 μM).

To better understand the increased potency of compound **25**, we compared the global minimum energy conformation of **25** with that of **4c** and **19b**. For the same purpose as the experiments shown in Fig. 5, we generated virtual compounds **19b'** and **25'**. Figure 6 shows the superimposition of the global minimum energy conformations of **4c'**, **19b'** and **25'** calculated using MOE.¹⁶⁾ As expected, tetrahydropyridine analog **25'** adopted a conformation closer to that of biaryl analog **4c'** than that of piperidine analog **19b'**. We hypothesize that addition of two *sp*² carbons into the piperidine moiety allowed the compound to adopt the preferred perpendicular conformation shown in Fig. 5. As described above, hydrophobic substituents were preferred at the 4'-position of the phenyl moiety (Table 3; **4c** vs. **4f**, EC₅₀ = 0.074 vs. 0.010 μM), indicating the presence of a hydrophobic pocket around the 4'-position of the terminal phenyl moiety. Because the hydrophobic 4-fluorobenzyl group in **25'** is oriented in the same direction as the fluoro group in **4c'**, these groups may occupy the same hydrophobic pocket in the target molecule. We hypothesize that the hydrophobic interaction is another important factor for improving the potency of compound **25**.

Given the promising solubility and good potency, we further examined compound **25** in a pharmacological study. Compound **25** was evaluated for its antiproliferative activity

Fig. 6. Global Minimum Energy Conformations of **4c'** (White), **19b'** (Purple) and **25'** (Orange)^a

Table 7. *In Vitro* Antiproliferative Activity of **25** Compared with AICAR and **4a**

Compound	AMPK activation EC ₅₀ (μM) ^{a)}	MDA-MB-453 growth inhibition IC ₅₀ (μM) ^{b)}	SK-BR-3 growth inhibition IC ₅₀ (μM) ^{b)}
AICAR	NT ^{c)}	1700	180
4a	0.89	0.39	29% inh. at 1 μM
25	0.016	0.0058	13% inh. at 3 μM

^{a)} The EC₅₀ values were examined by phospho-ACC whole cell ELISA using the MDA-MB-453 cell line, and determined in triplicate in one experiment. ^{b)} The IC₅₀ values were determined in triplicate in one experiment. ^{c)} NT: not tested.

against MDA-MB-453 and SK-BR-3 human breast cancer cell lines. As shown in Table 7, compound **25** showed almost 70-fold more potent cell growth inhibitory activity against MDA-MB-453 than compound **4a**. Furthermore, the unique cellular selectivity of compound **4a** was maintained in **25**, which showed quite weak antiproliferative activity against SK-BR-3, similar to **4a**. The AMPK activation activity and antiproliferative activity of **25** was relatively potent compared to reported AMPK activators, suggesting that compound **25** may be a novel AMPK activating anti-cancer option in precision medicine.

Conclusion

The present study describes a SAR study of novel 3,5-dimethylpyridin-4(1*H*)-one compounds and the unique biological profiles of a new class of indirect AMPK activators. Compound **4a** from our library showed moderate AMPK activation activity and unique cancer cell selectivity. Our structural optimization of the central pyridine ring and terminal aromatic group led to the discovery of trifluoromethyl substituted compound **4f** with potent AMPK activation activity. Due to the poor aqueous solubility of **4f**, we further optimized the compound using molecular conformation calculations to acquire a compound with both potent activity and high aqueous solubility. As a result, we discovered tetrahydropyridine-type **25**, a potent and highly water-soluble compound. Further studies on these unique AMPK activating compounds will be reported in due course. In this report, we will show the results of the target identification study and discuss the background of the cancer cell growth selectivity.

Experimental

Chemistry ¹H-NMR spectra were recorded on a Varian VNS-400, Varian 400-MR, JEOL JNM Lambda-300, JEOL JNM Lambda-400 or Bruker AVANCE III-HD500 spectrometer. Chemical shifts were expressed in δ values (ppm)

using tetramethylsilane as the internal standard (s = singlet, d = doublet, t = triplet, m = multiplet and br = broad peak). MS were recorded on a JEOL GC Mate II, Waters SQD, Waters ZQ-2000, Thermo Fisher LCQ Advantage or Thermo Fisher Exactive Plus Orbitrap. All reactions were performed using commercially available reagents and solvents without further purification. The following abbreviations are used: BINAP, 2,2'-bis(diphenylphosphino)-1,1'-binaphthalene; BuLi, butyllithium; *t*-BuOK, potassium *tert*-butoxide; *t*-BuONa, sodium *tert*-butoxide; DME, 1,2-dimethoxyethane; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; ESI, electrospray ionization; EtOAc, ethyl acetate; EtOH, ethanol; IPE, diisopropyl ether; MeOH, methanol; MsOH, methanesulfonic acid; NaBH(OAc)₃, sodium triacetoxyborohydride; PdCl₂(PPh₃)₂, bis(triphenylphosphine)-palladium(II) dichloride; Pd₂(dba)₃, tris(dibenzylideneacetone)-dipalladium(0); Pd(OAc)₂, palladium(II) acetate; Pd(PPh₃)₄, tetrakis(triphenylphosphine)palladium(0); SPhos, 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

2-[(5-Bromo-3-methylpyridin-2-yl)oxy]methyl-4-methoxy-3,5-dimethylpyridine (2a) To a mixture of 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine hydrochloride (600 mg, 2.7 mmol) and 5-bromo-2-hydroxy-3-methylpyridine (**1a**; 510 mg, 2.7 mmol) in toluene (6.0 mL) was added Ag₂CO₃ (1.9 g, 6.8 mmol). The mixture was stirred at 110°C in the dark for 6 h. After cooling to room temperature, the mixture was filtered through a Celite pad. The filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (hexane/EtOAc) to give the product (360 mg, 39%). ¹H-NMR (DMSO-*d*₆) δ: 2.11 (3H, s), 2.21 (3H, s), 2.23 (3H, s), 3.74 (3H, s), 5.36 (2H, s), 7.78–7.83 (1H, m), 8.09–8.13 (1H, m), 8.19 (1H, s); MS *m/z*: 337 (M + H)⁺.

2-[(5-Bromo-4-methylpyridin-2-yl)oxy]methyl-4-methoxy-3,5-dimethylpyridine (2b) Compound **2b** was prepared from 5-bromo-2-hydroxy-4-methylpyridine (**1b**) in

94% yield using a similar approach to that described for **2a**. ¹H-NMR (DMSO-*d*₆) δ: 2.22 (6H, s), 2.31 (3H, s), 3.74 (3H, s), 5.33 (2H, s), 6.92 (1H, s), 8.19 (1H, s), 8.26 (1H, s); MS *m/z*: 337 (M + H)⁺.

2-[(5-Bromo-6-methylpyridin-2-yl)oxy]methyl-4-methoxy-3,5-dimethylpyridine (2c) Compound **2c** was prepared from 3-bromo-6-hydroxy-2-methylpyridine (**1c**) in 92% yield using a similar approach to that described for **2a**. ¹H-NMR (DMSO-*d*₆) δ: 2.22 (3H, s), 2.24 (3H, s), 2.49 (3H, s), 3.74 (3H, s), 5.33 (2H, s), 6.67 (1H, d, *J* = 8.8 Hz), 7.86 (1H, d, *J* = 8.8 Hz), 8.20 (1H, s); MS *m/z*: 337 (M + H)⁺.

2-[(5-(4-Fluorophenyl)-3-methylpyridin-2-yl)oxy]methyl-4-methoxy-3,5-dimethylpyridine (3a) To a mixture of 2-[(5-bromo-3-methylpyridin-2-yl)oxy]methyl-4-methoxy-3,5-dimethylpyridine (**2a**; 100 mg, 0.30 mmol) and 1,4-dioxane (3.0 mL) were added (4-fluorophenyl)boronic acid (83 mg, 0.59 mmol), 2.0 mol/L Na₂CO₃ aqueous solution (0.74 mL, 1.5 mmol) and PdCl₂(PPh₃)₂ (10 mg, 0.015 mmol). The mixture was stirred at 90°C for 15 h. After cooling to room temperature, the mixture was diluted with H₂O and extracted with EtOAc. The organic layer was washed with saturated NaHCO₃ aqueous solution and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (hexane/EtOAc) to give the product (100 mg, 100%). ¹H-NMR (DMSO-*d*₆) δ: 2.19 (3H, s), 2.23 (3H, s), 2.26 (3H, s), 3.76 (3H, s), 5.43 (2H, s), 7.24–7.34 (2H, m), 7.66–7.75 (2H, m), 7.86–7.92 (1H, m), 8.21 (1H, s), 8.26–8.33 (1H, m); MS *m/z*: 353 (M + H)⁺.

2-[(5-(4-Fluorophenyl)-4-methylpyridin-2-yl)oxy]methyl-4-methoxy-3,5-dimethylpyridine (3b) Compound **3b** was prepared from **2b** in 100% yield using a similar approach to that described for **3a**. ¹H-NMR (CDCl₃) δ: 2.20 (3H, s), 2.28 (3H, s), 2.34 (3H, s), 3.79 (3H, s), 5.47 (2H, s), 6.73 (1H, s), 7.07–7.16 (2H, m), 7.21–7.30 (2H, m), 7.97 (1H, s), 8.28 (1H, s); MS *m/z*: 353 (M + H)⁺.

2-[(5-(4-Fluorophenyl)-6-methylpyridin-2-yl)oxy]methyl-4-methoxy-3,5-dimethylpyridine (3c) Compound **3c** was prepared from **2c** in 100% yield using a similar approach to that described for **3a**. ¹H-NMR (DMSO-*d*₆) δ: 2.23 (3H, s), 2.28 (3H, s), 2.36 (3H, s), 3.75 (3H, s), 5.37 (2H, s), 6.75 (1H, d, *J* = 8.3 Hz), 7.22–7.33 (2H, m), 7.36–7.47 (2H, m), 7.55 (1H, d, *J* = 8.3 Hz), 8.22 (1H, s); MS *m/z*: 353 (M + H)⁺.

2-[(5-(4-Fluorophenyl)-3-methylpyridin-2-yl)oxy]methyl-3,5-dimethylpyridin-4(1H)-one (4a) To a solution of 2-[(5-(4-fluorophenyl)-3-methylpyridin-2-yl)oxy]methyl-4-methoxy-3,5-dimethylpyridine (**3a**; 100 mg, 0.30 mmol) in CH₂Cl₂ (3.1 mL) was added AlCl₃ (120 mg, 0.89 mmol). After being stirred at room temperature for 1 h, to the mixture was slowly added H₂O. The mixture was extracted with CHCl₃. The organic layer was washed with saturated NaHCO₃ aqueous solution and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CHCl₃/MeOH) to give the product (53 mg, 53%). ¹H-NMR (DMSO-*d*₆) δ: 1.87 (3H, s), 1.96 (3H, s), 2.24 (3H, s), 5.32 (2H, s), 7.25–7.32 (2H, m), 7.52 (1H, d, *J* = 5.7 Hz), 7.66–7.75 (2H, m), 7.88–7.93 (1H, m), 8.27–8.31 (1H, m), 11.11–11.27 (1H, m); MS *m/z*: 339 (M + H)⁺; ESI-MS *m/z*: 339.1503 (M + H)⁺ (Calcd for C₂₀H₂₀O₂N₂F: 339.1503).

2-[(5-(4-Fluorophenyl)-4-methylpyridin-2-yl)oxy]methyl-3,5-dimethylpyridin-4(1H)-one (4b) Compound **4b** was prepared from **3b** in 74% yield using a similar approach to

that described for **4a**. ¹H-NMR (DMSO-*d*₆) δ: 1.87 (3H, s), 1.95 (3H, s), 2.22 (3H, s), 5.28 (2H, s), 6.88 (1H, s), 7.23–7.34 (2H, m), 7.36–7.46 (2H, m), 7.46–7.58 (1H, m), 7.98 (1H, s), 11.26 (1H, brs); MS *m/z*: 339 (M + H)⁺; ESI-MS *m/z*: 339.1504 (M + H)⁺ (Calcd for C₂₀H₂₀O₂N₂F: 339.1503).

2-[(5-(4-Fluorophenyl)-6-methylpyridin-2-yl)oxy]methyl-3,5-dimethylpyridin-4(1H)-one (4c) Compound **4c** was prepared from **3c** in 45% yield using a similar approach to that described for **4a**. ¹H-NMR (DMSO-*d*₆) δ: 1.86 (3H, s), 1.98 (3H, s), 2.36 (3H, s), 5.29 (2H, s), 6.80 (1H, d, *J* = 8.4 Hz), 7.23–7.32 (2H, m), 7.36–7.44 (2H, m), 7.48–7.55 (1H, m), 7.58 (1H, d, *J* = 8.3 Hz), 11.25 (1H, brs); MS *m/z*: 339 (M + H)⁺; ESI-MS *m/z*: 339.1504 (M + H)⁺ (Calcd for C₂₀H₂₀O₂N₂F: 339.1503).

4-{6-[(4-Methoxy-3,5-dimethylpyridin-2-yl)methoxy]-2-methylpyridin-3-yl}benzonitrile (3d) To a mixture of 2-[(5-bromo-6-methylpyridin-2-yl)oxy]methyl-4-methoxy-3,5-dimethylpyridine (**2c**; 400 mg, 1.2 mmol), toluene (7.0 mL) and H₂O (1.0 mL) were added (4-cyanophenyl)boronic acid (350 mg, 2.4 mmol), K₃PO₄ (650 mg, 3.1 mmol), SPhos (60 mg, 0.15 mmol) and Pd(OAc)₂ (30 mg, 0.13 mmol) under an argon atmosphere. The mixture was stirred at 110°C overnight. After cooling to room temperature, the mixture was diluted with H₂O and extracted with EtOAc. The organic layer was washed with brine, diluted with CHCl₃, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CHCl₃/MeOH) to give the product (410 mg, 96%). ¹H-NMR (DMSO-*d*₆) δ: 2.23 (3H, s), 2.28 (3H, s), 2.38 (3H, s), 3.75 (3H, s), 5.39 (2H, s), 6.75–6.83 (1H, m), 7.57–7.65 (3H, m), 7.88–7.96 (2H, m), 8.21 (1H, s); MS *m/z*: 360 (M + H)⁺.

4-Methoxy-3,5-dimethyl-2-[(6-methyl-5-(4-methylphenyl)pyridin-2-yl)oxy]methylpyridine (3e) Compound **3e** was prepared from **2c** and (4-methylphenyl)boronic acid in 92% yield using a similar approach to that described for **3d**. ¹H-NMR (DMSO-*d*₆) δ: 2.23 (3H, s), 2.28 (3H, s), 2.35 (3H, s), 2.36 (3H, s), 3.75 (3H, s), 5.37 (2H, s), 6.73 (1H, d, *J* = 8.4 Hz), 7.21–7.27 (4H, m), 7.52 (1H, d, *J* = 8.4 Hz), 8.21 (1H, s); MS *m/z*: 349 (M + H)⁺.

4-Methoxy-3,5-dimethyl-2-[(6-methyl-5-[4-(trifluoromethyl)phenyl]pyridin-2-yl)oxy]methylpyridine (3f) Compound **3f** was prepared from **2c** and [4-(trifluoromethyl)phenyl]boronic acid in 77% yield using a similar approach to that described for **3d**. ¹H-NMR (DMSO-*d*₆) δ: 2.23 (3H, s), 2.28 (3H, s), 2.38 (3H, s), 3.75 (3H, s), 5.39 (2H, s), 6.80 (1H, d, *J* = 8.4 Hz), 7.59–7.65 (3H, m), 7.81 (2H, d, *J* = 8.2 Hz), 8.22 (1H, s); MS *m/z*: 403 (M + H)⁺.

4-Methoxy-3,5-dimethyl-2-[(6-methyl-5-[3-(trifluoromethyl)phenyl]pyridin-2-yl)oxy]methylpyridine (3g) Compound **3g** was prepared from **2c** and [3-(trifluoromethyl)phenyl]boronic acid in 82% yield using a similar approach to that described for **3d**. ¹H-NMR (DMSO-*d*₆) δ: 2.23 (3H, s), 2.28 (3H, s), 2.37 (3H, s), 3.75 (3H, s), 5.39 (2H, s), 6.78 (1H, d, *J* = 8.4 Hz), 7.63 (1H, d, *J* = 8.4 Hz), 7.66–7.78 (4H, m), 8.22 (1H, s); MS *m/z*: 403 (M + H)⁺.

4-Methoxy-3,5-dimethyl-2-[(6-methyl-5-[2-(trifluoromethyl)phenyl]pyridin-2-yl)oxy]methylpyridine (3h) Compound **3h** was prepared from **2c** and [2-(trifluoromethyl)phenyl]boronic acid in quantitative yield using a similar approach to that described for **3d**. ¹H-NMR (DMSO-*d*₆) δ: 2.10 (3H, s), 2.23 (3H, s), 2.29 (3H, s), 3.75

(3H, s), 5.27–5.48 (2H, m), 6.70–6.77 (1H, m), 7.40 (1H, d, $J = 7.5$ Hz), 7.45 (1H, d, $J = 8.6$ Hz), 7.60–7.68 (1H, m), 7.71–7.78 (1H, m), 7.86 (1H, d, $J = 7.5$ Hz), 8.23 (1H, s); MS m/z : 403 ($M + H$)⁺.

6-[(4-Methoxy-3,5-dimethylpyridin-2-yl)methoxy]-2-methyl-6'-(trifluoromethyl)-3,3'-bipyridine (3i) Compound **3i** was prepared from **2c** and [6-(trifluoromethyl)pyridin-3-yl]boronic acid in 88% yield using a similar approach to that described for **3d**. ¹H-NMR (DMSO- d_6) δ : 2.23 (3H, s), 2.28 (3H, s), 2.40 (3H, s), 3.75 (3H, s), 5.41 (2H, s), 6.84 (1H, d, $J = 8.4$ Hz), 7.71 (1H, d, $J = 8.4$ Hz), 7.98 (1H, d, $J = 8.2$ Hz), 8.11–8.17 (1H, m), 8.22 (1H, s), 8.78–8.86 (1H, m); MS m/z : 404 ($M + H$)⁺.

4-{6-[(3,5-Dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methoxy]-2-methylpyridin-3-yl}benzonitrile (4d) Compound **4d** was prepared from **3d** in 72% yield using a similar approach to that described for **4a**. ¹H-NMR (DMSO- d_6) δ : 1.86 (3H, s), 1.97 (3H, s), 2.38 (3H, s), 5.30 (2H, s), 6.85 (1H, d, $J = 8.4$ Hz), 7.48–7.56 (1H, m), 7.57–7.62 (2H, m), 7.65 (1H, d, $J = 8.4$ Hz), 7.89–7.97 (2H, m), 11.26 (1H, brs); MS m/z : 346 ($M + H$)⁺; ESI-MS m/z : 346.1545 ($M + H$)⁺ (Calcd for C₂₁H₂₀O₂N₃: 346.1550).

3,5-Dimethyl-2-[(6-methyl-5-(4-methylphenyl)pyridin-2-yl)oxy]methylpyridin-4(1H)-one (4e) Compound **4e** was prepared from **3e** in 63% yield using a similar approach to that described for **4a**. ¹H-NMR (DMSO- d_6) δ : 1.86 (3H, s), 1.97 (3H, s), 2.35 (3H, s), 2.36 (3H, s), 5.28 (2H, s), 6.79 (1H, d, $J = 8.4$ Hz), 7.20–7.30 (4H, m), 7.46–7.60 (1H, m), 7.55 (1H, d, $J = 8.4$ Hz), 11.24 (1H, brs); MS m/z : 335 ($M + H$)⁺; ESI-MS m/z : 335.1747 ($M + H$)⁺ (Calcd for C₂₁H₂₃O₂N₂: 335.1754).

3,5-Dimethyl-2-[(6-methyl-5-[4-(trifluoromethyl)phenyl]pyridin-2-yl)oxy]methylpyridin-4(1H)-one (4f) To a mixture of 4-methoxy-3,5-dimethyl-2-[(6-methyl-5-[4-(trifluoromethyl)phenyl]pyridin-2-yl)oxy]methylpyridine (**3f**; 300 mg, 0.75 mmol) and MsOH (2.0 mL) was added methionine (350 mg, 2.3 mmol). The mixture was stirred at 90°C overnight. After cooling to room temperature, the mixture was poured into a saturated NaHCO₃ aqueous solution. The mixture was diluted with 10% citric acid aqueous solution and extracted with CHCl₃. The organic layer was dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CHCl₃/MeOH) to give the product (160 mg, 55%). ¹H-NMR (DMSO- d_6) δ : 1.86 (3H, s), 1.98 (3H, s), 2.38 (3H, s), 5.30 (2H, s), 6.85 (1H, d, $J = 8.4$ Hz), 7.49–7.56 (1H, m), 7.61 (2H, d, $J = 8.2$ Hz), 7.65 (1H, d, $J = 8.4$ Hz), 7.81 (2H, d, $J = 8.2$ Hz), 11.26 (1H, brs); MS m/z : 389 ($M + H$)⁺; ESI-MS m/z : 389.1472 ($M + H$)⁺ (Calcd for C₂₁H₂₀O₂N₂F₃: 389.1471).

3,5-Dimethyl-2-[(6-methyl-5-[3-(trifluoromethyl)phenyl]pyridin-2-yl)oxy]methylpyridin-4(1H)-one (4g) Compound **4g** was prepared from **3g** in 53% yield using a similar approach to that described for **4f**. ¹H-NMR (DMSO- d_6) δ : 1.86 (3H, s), 1.98 (3H, s), 2.37 (3H, s), 5.30 (2H, s), 6.84 (1H, d, $J = 8.4$ Hz), 7.48–7.56 (1H, m), 7.60–7.80 (4H, m), 7.67 (1H, d, $J = 8.4$ Hz), 11.26 (1H, brs); MS m/z : 389 ($M + H$)⁺; ESI-MS m/z : 389.1470 ($M + H$)⁺ (Calcd for C₂₁H₂₀O₂N₂F₃: 389.1471).

3,5-Dimethyl-2-[(6-methyl-5-[2-(trifluoromethyl)phenyl]pyridin-2-yl)oxy]methylpyridin-4(1H)-one (4h) Compound **4h** was prepared from **3h** in 53% yield using a similar approach to that described for **4f**. ¹H-NMR (DMSO- d_6) δ : 1.87 (3H, s), 1.98 (3H, s), 2.10 (3H, s), 5.19–5.38 (2H, m), 6.79 (1H,

d, $J = 8.4$ Hz), 7.39 (1H, d, $J = 7.5$ Hz), 7.49 (1H, d, $J = 8.4$ Hz), 7.53 (1H, s), 7.60–7.69 (1H, m), 7.70–7.79 (1H, m), 7.86 (1H, d, $J = 7.7$ Hz), 11.26 (1H, brs); MS m/z : 389 ($M + H$)⁺; ESI-MS m/z : 389.1469 ($M + H$)⁺ (Calcd for C₂₁H₂₀O₂N₂F₃: 389.1471).

3,5-Dimethyl-2-[(2-methyl-6'-(trifluoromethyl)[3,3'-bipyridin]-6-yl)oxy]methylpyridin-4(1H)-one (4i) Compound **4i** was prepared from **3i** in 18% yield using a similar approach to that described for **4a**. ¹H-NMR (DMSO- d_6) δ : 1.86 (3H, s), 1.98 (3H, s), 2.41 (3H, s), 5.31 (2H, s), 6.89 (1H, d, $J = 8.4$ Hz), 7.52 (1H, s), 7.74 (1H, d, $J = 8.4$ Hz), 7.99 (1H, d, $J = 8.2$ Hz), 8.11–8.19 (1H, m), 8.78–8.83 (1H, m), 11.27 (1H, brs); MS m/z : 390 ($M + H$)⁺; ESI-MS m/z : 390.1419 ($M + H$)⁺ (Calcd for C₂₀H₁₉O₂N₃F₃: 390.1424).

6-[(4-Methoxy-3,5-dimethylpyridin-2-yl)methoxy]-2-methylpyridin-3-ylboronic Acid (5) To a solution of 2-[(5-bromo-6-methylpyridin-2-yl)oxy]methyl-4-methoxy-3,5-dimethylpyridine (**2c**; 1.0 g, 3.0 mmol) and triisopropyl borate (1.1 mL, 4.8 mmol) in THF (15 mL) was added *n*-BuLi (2.8 mL, 4.6 mmol, 1.7 mol/L in hexane) at –62°C. After stirring at –60°C for 1 h, the mixture was stirred at 0°C for an additional 1 h. To the mixture were added H₂O and EtOAc, then the mixture was stirred at room temperature for 30 min. The mixture was neutralized with 1.0 mol/L HCl aqueous solution and extracted with EtOAc. The organic layer was washed with brine and dried over Na₂SO₄. The filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CHCl₃/MeOH). To the residue were added IPE and hexane, and then the precipitated solid was collected by filtration to obtain the product (640 mg, 71%). ¹H-NMR (DMSO- d_6) δ : 2.21 (3H, s), 2.24 (3H, s), 2.52 (3H, s), 3.74 (3H, s), 5.33 (2H, s), 6.58 (1H, d, $J = 8.2$ Hz), 7.77 (1H, d, $J = 8.2$ Hz), 7.99 (2H, s), 8.19 (1H, s); MS m/z : 303 ($M + H$)⁺.

6'-[(4-Methoxy-3,5-dimethylpyridin-2-yl)methoxy]-2'-methyl-5-(trifluoromethyl)-2,3'-bipyridine (3j) Compound **3j** was prepared from **5**, 2-bromo-5-(trifluoromethyl)pyridine and Pd(PPh₃)₄ in 85% yield using a similar approach to that described for **3a**. ¹H-NMR (CDCl₃) δ : 2.28 (3H, s), 2.36 (3H, s), 2.55 (3H, s), 3.79 (3H, s), 5.51 (2H, s), 6.78 (1H, d, $J = 8.4$ Hz), 7.54 (1H, d, $J = 8.2$ Hz), 7.70 (1H, d, $J = 8.4$ Hz), 7.91–8.06 (1H, m), 8.27 (1H, s), 8.89–8.99 (1H, m); MS m/z : 404 ($M + H$)⁺.

2-{6-[(4-Methoxy-3,5-dimethylpyridin-2-yl)methoxy]-2-methylpyridin-3-yl}-5-(trifluoromethyl)pyrimidine (3k) Compound **3k** was prepared from **5**, 2-chloro-5-(trifluoromethyl)pyrimidine and Pd(PPh₃)₄ in 82% yield using a similar approach to that described for **3a**. ¹H-NMR (CDCl₃) δ : 2.27 (3H, s), 2.36 (3H, s), 2.82 (3H, s), 3.79 (3H, s), 5.54 (2H, s), 6.79 (1H, d, $J = 8.6$ Hz), 8.27 (1H, s), 8.33 (1H, d, $J = 8.6$ Hz), 8.98–9.06 (2H, m); MS m/z : 405 ($M + H$)⁺.

2-{6-[(4-Methoxy-3,5-dimethylpyridin-2-yl)methoxy]-2-methylpyridin-3-yl}-5-(trifluoromethyl)pyrazine (3l) Compound **3l** was prepared from **5**, 2-chloro-5-(trifluoromethyl)pyrazine and Pd(PPh₃)₄ in 80% yield using a similar approach to that described for **3a**. ¹H-NMR (CDCl₃) δ : 2.28 (3H, s), 2.37 (3H, s), 2.61 (3H, s), 3.80 (3H, s), 5.52 (2H, s), 6.82 (1H, d, $J = 8.6$ Hz), 7.76 (1H, d, $J = 8.6$ Hz), 8.28 (1H, s), 8.76–8.87 (1H, m), 8.95–9.05 (1H, m); MS m/z : 405 ($M + H$)⁺.

3-{6-[(4-Methoxy-3,5-dimethylpyridin-2-yl)methoxy]-2-methylpyridin-3-yl}-6-(trifluoromethyl)pyridazine (3m) Compound **3m** was prepared from **5**, 3-chloro-6-

(trifluoromethyl)pyridazine and $\text{Pd}(\text{PPh}_3)_4$ in 72% yield using a similar approach to that described for **3a**. $^1\text{H-NMR}$ (CDCl_3) δ : 2.28 (3H, s), 2.37 (3H, s), 2.60 (3H, s), 3.80 (3H, s), 5.53 (2H, s), 6.83 (1H, d, $J=8.6$ Hz), 7.73–7.80 (2H, m), 7.87 (1H, d, $J=8.8$ Hz), 8.28 (1H, s); MS m/z : 405 ($\text{M} + \text{H}$) $^+$.

3,5-Dimethyl-2-([2'-methyl-5-(trifluoromethyl)]2,3'-bipyridin]-6'-yl]oxy)methylpyridin-4(1H)-one (4j) To a solution of *t*-BuOK (360 mg, 3.2 mmol) in DMF (3.5 mL) was added the solution of dodecane-1-thiol (0.79 mL) in DMF (6.0 mL). The mixture was stirred at room temperature for 30 min. To the mixture was added the solution of 6'-[(4-methoxy-3,5-dimethylpyridin-2-yl)methoxy]-2'-methyl-5-(trifluoromethyl)-2,3'-bipyridine (**3j**; 260 mg, 0.65 mmol) in DMF (2.5 mL). The mixture was stirred at 60°C for 2 h. After cooling to room temperature, to the mixture were added H_2O , 1.0 mol/L HCl aqueous solution and brine. The mixture was extracted with $\text{CHCl}_3/\text{MeOH}$ (5:1). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel ($\text{CHCl}_3/\text{MeOH}$). The residue was purified by column chromatography on amino functionalized silica gel (EtOAc/MeOH) to give the product (170 mg, 66%). $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 1.87 (3H, s), 1.98 (3H, s), 2.52 (3H, s), 5.33 (2H, s), 6.88 (1H, d, $J=8.6$ Hz), 7.53 (1H, s), 7.84 (1H, d, $J=8.2$ Hz), 7.90 (1H, d, $J=8.6$ Hz), 8.25–8.34 (1H, m), 9.02–9.09 (1H, m), 11.26 (1H, brs); MS m/z : 390 ($\text{M} + \text{H}$) $^+$; ESI-MS m/z : 390.1416 ($\text{M} + \text{H}$) $^+$ (Calcd for $\text{C}_{20}\text{H}_{19}\text{O}_2\text{N}_3\text{F}_3$: 390.1424).

3,5-Dimethyl-2-([6-methyl-5-[5-(trifluoromethyl)pyrimidin-2-yl]pyridin-2-yl]oxy)methylpyridin-4(1H)-one (4k) Compound **4k** was prepared from **3k** in 16% yield using a similar approach to that described for **4j**. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 1.80–1.90 (3H, m), 1.98 (3H, s), 2.75 (3H, s), 5.35 (2H, s), 6.93 (1H, d, $J=8.6$ Hz), 7.40–7.64 (1H, m), 8.36 (1H, d, $J=8.6$ Hz), 9.28–9.44 (2H, m), 11.26 (1H, brs); MS m/z : 391 ($\text{M} + \text{H}$) $^+$; ESI-MS m/z : 391.1378 ($\text{M} + \text{H}$) $^+$ (Calcd for $\text{C}_{19}\text{H}_{18}\text{O}_2\text{N}_4\text{F}_3$: 391.1376).

3,5-Dimethyl-2-([6-methyl-5-[5-(trifluoromethyl)pyrazin-2-yl]pyridin-2-yl]oxy)methylpyridin-4(1H)-one (4l) Compound **4l** was prepared from **3l** in 41% yield using a similar approach to that described for **4j**. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 1.87 (3H, s), 1.99 (3H, s), 2.57 (3H, s), 5.34 (2H, s), 6.94 (1H, d, $J=8.6$ Hz), 7.49–7.57 (1H, m), 8.02 (1H, d, $J=8.6$ Hz), 9.09–9.13 (1H, m), 9.25–9.28 (1H, m), 11.27 (1H, brs); MS m/z : 391 ($\text{M} + \text{H}$) $^+$; ESI-MS m/z : 391.1374 ($\text{M} + \text{H}$) $^+$ (Calcd for $\text{C}_{19}\text{H}_{18}\text{O}_2\text{N}_4\text{F}_3$: 391.1376).

3,5-Dimethyl-2-([6-methyl-5-[6-(trifluoromethyl)pyridazin-3-yl]pyridin-2-yl]oxy)methylpyridin-4(1H)-one (4m) Compound **4m** was prepared from **3m** in 23% yield using a similar approach to that described for **4j**. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 1.87 (3H, s), 1.99 (3H, s), 2.52 (3H, s), 5.35 (2H, s), 6.95 (1H, d, $J=8.4$ Hz), 7.47–7.60 (1H, m), 7.98 (1H, d, $J=8.4$ Hz), 8.25 (1H, d, $J=8.6$ Hz), 8.36 (1H, d, $J=8.6$ Hz), 11.29 (1H, brs); MS m/z : 391 ($\text{M} + \text{H}$) $^+$; ESI-MS m/z : 391.1371 ($\text{M} + \text{H}$) $^+$ (Calcd for $\text{C}_{19}\text{H}_{18}\text{O}_2\text{N}_4\text{F}_3$: 391.1376).

6-[(4-Methoxy-3,5-dimethylpyridin-2-yl)methoxy]-2-methylpyridine-3-carbaldehyde (6) To a mixture of 2-[[5-bromo-6-methylpyridin-2-yl]oxy]methyl]-4-methoxy-3,5-dimethylpyridine (**2c**; 1.4 g, 4.0 mmol) in THF (20 mL) was added dropwise *n*-BuLi (2.4 mL, 4.0 mmol, 1.7 mol/L in hexane) at -64°C . To the mixture was added dropwise DMF (0.65 mL, 8.4 mmol) at -63°C , then stirred at the same temperature for

30 min. To the mixture was added saturated NH_4Cl aqueous solution. After warming to room temperature, the mixture was diluted with H_2O and extracted with EtOAc . The organic layer was washed with brine, dried over MgSO_4 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (hexane/ EtOAc) to give the product (520 mg, 45%). $^1\text{H-NMR}$ (CDCl_3) δ : 2.27 (3H, s), 2.34 (3H, s), 2.78 (3H, s), 3.79 (3H, s), 5.53 (2H, s), 6.75 (1H, d, $J=8.6$ Hz), 8.00 (1H, d, $J=8.6$ Hz), 8.26 (1H, s), 10.21 (1H, s); MS m/z : 287 ($\text{M} + \text{H}$) $^+$.

Diethyl {[6-(trifluoromethyl)pyridin-3-yl]methyl}phosphonate (8) A mixture of 5-(chloromethyl)-2-(trifluoromethyl)pyridine (**7**; 480 mg, 2.5 mmol) in triethyl phosphite (3.0 mL, 17 mmol) was stirred at 150°C for 4 h. After cooling to room temperature, the mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (hexane/ $\text{CHCl}_3/\text{MeOH}$) to give the product (670 mg, 92%). $^1\text{H-NMR}$ (CDCl_3) δ : 1.28 (6H, t, $J=7.1$ Hz), 3.20 (2H, d, $J=21.8$ Hz), 4.03–4.13 (4H, m), 7.65 (1H, d, $J=8.2$ Hz), 7.82–7.90 (1H, m), 8.62 (1H, s); MS m/z : 298 ($\text{M} + \text{H}$) $^+$.

4-Methoxy-3,5-dimethyl-2-[(6-methyl-5-{(E)-2-[6-(trifluoromethyl)pyridin-3-yl]ethenyl}pyridin-2-yl]oxy]methylpyridine (9) To a mixture of NaH (55% in oil, 37 mg, 0.85 mmol) in DME (1.0 mL) was added dropwise the solution of diethyl {[6-(trifluoromethyl)pyridin-3-yl]methyl}phosphonate (**8**; 240 mg, 0.79 mmol) in DME (2.0 mL) at 0°C . The mixture was stirred at the same temperature for 30 min. To the mixture was added dropwise the solution of 6-[(4-methoxy-3,5-dimethylpyridin-2-yl)methoxy]-2-methylpyridine-3-carbaldehyde (**6**; 200 mg, 0.70 mmol) in DME (2.0 mL). The mixture was stirred at the same temperature for 1 h, and then stirred at room temperature for 2 h. The mixture was diluted with H_2O and extracted with EtOAc . The organic layer was washed with brine, dried over MgSO_4 and then concentrated *in vacuo*. The residue was purified by column chromatography on amino functionalized silica gel (hexane/ EtOAc) to give the product (180 mg, 58%). $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 2.22 (3H, s), 2.27 (3H, s), 2.59 (3H, s), 3.75 (3H, s), 5.38 (2H, s), 6.77 (1H, d, $J=8.6$ Hz), 7.22 (1H, d, $J=16.3$ Hz), 7.66 (1H, d, $J=16.3$ Hz), 7.89 (1H, d, $J=8.4$ Hz), 8.08 (1H, d, $J=8.6$ Hz), 8.21 (1H, s), 8.29–8.39 (1H, m), 8.93–9.00 (1H, m); MS m/z : 430 ($\text{M} + \text{H}$) $^+$.

3,5-Dimethyl-2-[(6-methyl-5-{(E)-2-[6-(trifluoromethyl)pyridin-3-yl]ethenyl}pyridin-2-yl]oxy]methylpyridin-4(1H)-one (10) Compound **10** was prepared from **9** in 48% yield using a similar approach to that described for **4f**. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 1.86 (3H, s), 1.97 (3H, s), 2.59 (3H, s), 5.28 (2H, s), 6.82 (1H, d, $J=8.6$ Hz), 7.24 (1H, d, $J=16.3$ Hz), 7.45–7.57 (1H, m), 7.66 (1H, d, $J=16.3$ Hz), 7.90 (1H, d, $J=8.4$ Hz), 8.11 (1H, d, $J=8.6$ Hz), 8.30–8.41 (1H, m), 8.89–9.07 (1H, m), 11.22 (1H, brs); MS m/z : 416 ($\text{M} + \text{H}$) $^+$; ESI-MS m/z : 416.1578 ($\text{M} + \text{H}$) $^+$ (Calcd for $\text{C}_{22}\text{H}_{21}\text{O}_2\text{N}_3\text{F}_3$: 416.1580).

4-Methoxy-3,5-dimethyl-2-[(6-methyl-5-{2-[6-(trifluoromethyl)pyridin-3-yl]ethyl}pyridin-2-yl]oxy]methylpyridine (11) To a solution of 4-methoxy-3,5-dimethyl-2-[(6-methyl-5-{(E)-2-[6-(trifluoromethyl)pyridin-3-yl]ethenyl}pyridin-2-yl]oxy]methylpyridine (**9**; 95 mg, 0.22 mmol) in THF (1.5 mL)/ EtOH (1.5 mL) was added 10% Pd/C (wetted with approx. 50% water, 20 mg). After stirring under a hydrogen atmosphere at room temperature for 4 h, the mixture was filtered through a Celite pad and the filtrate was concentrated

in vacuo. The residue was purified by column chromatography on silica gel (hexane/EtOAc) to give the product (79 mg, 83%). ¹H-NMR (CDCl₃) δ: 2.26 (3H, s), 2.34 (3H, s), 2.39 (3H, s), 2.82–2.97 (4H, m), 3.78 (3H, s), 5.41 (2H, s), 6.59 (1H, d, *J* = 8.4 Hz), 7.21 (1H, d, *J* = 8.4 Hz), 7.55–7.60 (2H, m), 8.26 (1H, s), 8.54 (1H, s); MS *m/z*: 432 (M + H)⁺.

3,5-Dimethyl-2-((6-methyl-5-(2-((6-(trifluoromethyl)pyridin-3-yl)ethyl)pyridin-2-yl)oxy)methyl)pyridin-4(1H)-one (12) Compound **12** was prepared from **11** in 56% yield using a similar approach to that described for **4f**. ¹H-NMR (DMSO-*d*₆) δ: 1.85 (3H, s), 1.94 (3H, s), 2.37 (3H, s), 2.78–3.02 (4H, m), 5.21 (2H, s), 6.65 (1H, d, *J* = 8.2 Hz), 7.43–7.54 (2H, m), 7.82 (1H, d, *J* = 8.2 Hz), 7.90–7.99 (1H, m), 8.59–8.67 (1H, m), 11.18 (1H, brs); MS *m/z*: 418 (M + H)⁺; ESI-MS *m/z*: 418.1731 (M + H)⁺ (Calcd for C₂₂H₂₃O₂N₃F₃: 418.1737).

***tert*-Butyl 6-(Benzzyloxy)-2-methyl-3',6'-dihydro[3,4'-bipyridine]-1'(2'H)-carboxylate (14)** Compound **14** was prepared from **13**,¹⁵⁾ *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate and Pd(PPh₃)₄ in 100% yield using a similar approach to that described for **3a**. ¹H-NMR (CDCl₃) δ: 1.50 (9H, s), 2.25–2.37 (2H, m), 2.41 (3H, s), 3.61 (2H, t, *J* = 5.6 Hz), 3.96–4.10 (2H, m), 5.36 (2H, s), 5.48–5.63 (1H, m), 6.55–6.61 (1H, m), 7.25–7.40 (4H, m), 7.44–7.50 (2H, m); MS *m/z*: 381 (M + H)⁺.

***tert*-Butyl 4-(6-Hydroxy-2-methylpyridin-3-yl)piperidine-1-carboxylate (15)** To a solution of *tert*-butyl 6-(benzyloxy)-2-methyl-3',6'-dihydro[3,4'-bipyridine]-1'(2'H)-carboxylate (**14**; 2.2 g, 5.7 mmol) in THF (20 mL)/MeOH (20 mL) was added 20% Pd(OH)₂/C (wetted with approx. 50% water, 400 mg). After stirring under a hydrogen atmosphere (2.5 kgf/cm²) at room temperature for 6 h, the mixture was filtered through a Celite pad. The filtrate was concentrated *in vacuo*. To the residue was added IPE and the precipitated solid was collected by filtration to obtain the product (1.5 g, 92%). ¹H-NMR (DMSO-*d*₆) δ: 1.34–1.47 (2H, m), 1.41 (9H, s), 1.47–1.63 (2H, m), 2.20 (3H, s), 2.54–2.92 (3H, m), 3.91–4.14 (2H, m), 6.11 (1H, d, *J* = 9.3 Hz), 7.33 (1H, d, *J* = 9.3 Hz), 11.39 (1H, brs); MS *m/z*: 293 (M + H)⁺.

***tert*-Butyl 4-{6-[(4-Methoxy-3,5-dimethylpyridin-2-yl)-methoxyl]-2-methylpyridin-3-yl}piperidine-1-carboxylate (16)** Compound **16** was prepared from 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine hydrochloride and **15** in 98% yield using a similar approach to that described for **2a**. ¹H-NMR (CDCl₃) δ: 1.44–1.58 (2H, m), 1.48 (9H, s), 1.66–1.77 (2H, m), 2.26 (3H, s), 2.34 (3H, s), 2.48 (3H, s), 2.67–2.96 (3H, m), 3.78 (3H, s), 4.15–4.36 (2H, m), 5.41 (2H, s), 6.63 (1H, d, *J* = 8.4 Hz), 7.35 (1H, d, *J* = 8.4 Hz), 8.25 (1H, s); MS *m/z*: 442 (M + H)⁺.

4-Methoxy-3,5-dimethyl-2-((6-methyl-5-(piperidin-4-yl)pyridin-2-yl)oxy)methylpyridine (17) To a solution of *tert*-butyl 4-{6-[(4-methoxy-3,5-dimethylpyridin-2-yl)-methoxyl]-2-methylpyridin-3-yl}piperidine-1-carboxylate (**16**; 2.3 g, 5.1 mmol) in CH₂Cl₂ (20 mL) was added TFA (5.0 mL, 65 mmol). After stirring at room temperature for 6 h, the mixture was neutralized with saturated NaHCO₃ aqueous solution while cooling on ice. The mixture was extracted with CHCl₃/MeOH (5:1). The organic layer was dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on amino functionalized silica gel (CHCl₃/MeOH) to give the product (1.8 g, 100%). ¹H-NMR (DMSO-*d*₆) δ: 1.31–1.66 (4H, m), 2.21 (3H, s), 2.24 (3H, s), 2.42 (3H, s),

2.52–2.80 (3H, m), 2.91–3.10 (2H, m), 3.74 (3H, s), 5.29 (2H, s), 6.63 (1H, d, *J* = 8.4 Hz), 7.51 (1H, d, *J* = 8.4 Hz), 8.19 (1H, s); MS *m/z*: 342 (M + H)⁺.

4-Methoxy-3,5-dimethyl-2-((6-methyl-5-(1-methylpiperidin-4-yl)pyridin-2-yl)oxy)methylpyridine (18a) To a solution of 4-methoxy-3,5-dimethyl-2-((6-methyl-5-(piperidin-4-yl)pyridin-2-yl)oxy)methylpyridine (**17**; 200 mg, 0.59 mmol) in THF (3.0 mL)/EtOH (3.0 mL) was added 1H-benzotriazol-1-methanol (150 mg, 0.97 mmol). After stirring at room temperature for 10 min, to the mixture was added NaBH(OAc)₃ (290 mg, 1.4 mmol). After stirring at room temperature for 18 h, to the mixture was added saturated NaHCO₃ aqueous solution. The mixture was extracted with CHCl₃. The organic layer was dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CHCl₃/MeOH) to give the product (150 mg, 73%). ¹H-NMR (CDCl₃) δ: 1.58–1.82 (4H, m), 1.99–2.15 (2H, m), 2.26 (3H, s), 2.33 (6H, s), 2.47 (3H, s), 2.53–2.70 (1H, m), 2.91–3.05 (2H, m), 3.77 (3H, s), 5.40 (2H, s), 6.63 (1H, d, *J* = 8.6 Hz), 7.41 (1H, d, *J* = 8.6 Hz), 8.25 (1H, s); MS *m/z*: 356 (M + H)⁺.

2-((5-((1-((4-Fluorophenyl)methyl)piperidin-4-yl)-6-methylpyridin-2-yl)oxy)methyl)-4-methoxy-3,5-dimethylpyridine (18b) To a solution of 4-methoxy-3,5-dimethyl-2-((6-methyl-5-(piperidin-4-yl)pyridin-2-yl)oxy)methylpyridine (**17**; 200 mg, 0.59 mmol) in CH₂Cl₂ (6.0 mL) were added 4-fluorobenzaldehyde (77 μL, 0.73 mmol) and acetic acid (67 μL, 1.2 mmol). After stirring at room temperature for 1 h, to the mixture was added NaBH(OAc)₃ (250 mg, 1.2 mmol). After stirring at room temperature for 18 h, to the mixture was added saturated NaHCO₃ aqueous solution. The mixture was extracted with CHCl₃. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CHCl₃/MeOH) to give the product (220 mg, 84%). ¹H-NMR (CDCl₃) δ: 1.63–1.77 (4H, m), 2.01–2.15 (2H, m), 2.25 (3H, s), 2.33 (3H, s), 2.46 (3H, s), 2.55–2.72 (1H, m), 2.91–3.06 (2H, m), 3.51 (2H, s), 3.77 (3H, s), 5.40 (2H, s), 6.62 (1H, d, *J* = 8.4 Hz), 6.93–7.10 (2H, m), 7.27–7.34 (2H, m), 7.42 (1H, d, *J* = 8.4 Hz), 8.25 (1H, s); MS *m/z*: 450 (M + H)⁺.

4-Methoxy-3,5-dimethyl-2-((6-methyl-5-(1-((4-(trifluoromethyl)phenyl)methyl)piperidin-4-yl)pyridin-2-yl)oxy)methylpyridine (18c) Compound **18c** was prepared from **17** and 4-(trifluoromethyl)benzaldehyde in 69% yield using a similar approach to that described for **18b**. ¹H-NMR (DMSO-*d*₆) δ: 1.53–1.71 (4H, m), 2.05–2.18 (2H, m), 2.21 (3H, s), 2.24 (3H, s), 2.41 (3H, s), 2.56–2.73 (1H, m), 2.84–2.96 (2H, m), 3.60 (2H, s), 3.73 (3H, s), 5.28 (2H, s), 6.62 (1H, d, *J* = 8.4 Hz), 7.49–7.61 (3H, m), 7.69 (2H, d, *J* = 7.9 Hz), 8.19 (1H, s); MS *m/z*: 500 (M + H)⁺.

3,5-Dimethyl-2-((6-methyl-5-(1-methylpiperidin-4-yl)pyridin-2-yl)oxy)methylpyridin-4(1H)-one (19a) Compound **19a** was prepared from **18a** in 14% yield using a similar approach to that described for **4f**. ¹H-NMR (DMSO-*d*₆) δ: 1.53–1.67 (4H, m), 1.85 (3H, s), 1.89–2.04 (2H, m), 1.95 (3H, s), 2.19 (3H, s), 2.41 (3H, s), 2.52–2.63 (1H, m), 2.79–2.91 (2H, m), 5.21 (2H, s), 6.68 (1H, d, *J* = 8.4 Hz), 7.45–7.52 (1H, m), 7.56 (1H, d, *J* = 8.4 Hz), 11.17 (1H, brs); MS *m/z*: 342 (M + H)⁺; ESI-MS *m/z*: 342.2175 (M + H)⁺ (Calcd for C₂₀H₂₈O₂N₃: 342.2176).

2-((5-((1-((4-Fluorophenyl)methyl)piperidin-4-yl)-6-methylpyridin-2-yl)oxy)methyl)-3,5-dimethylpyridin-

4(1H)-one (19b) Compound **19b** was prepared from **18b** in 39% yield using a similar approach to that described for **4f**. ¹H-NMR (DMSO-*d*₆) δ : 1.54–1.69 (4H, m), 1.85 (3H, s), 1.94 (3H, s), 2.00–2.13 (2H, m), 2.41 (3H, s), 2.55–2.70 (1H, m), 2.84–2.96 (2H, m), 3.48 (2H, s), 5.20 (2H, s), 6.67 (1H, d, J = 8.4 Hz), 7.07–7.20 (2H, m), 7.27–7.40 (2H, m), 7.44–7.53 (1H, m), 7.58 (1H, d, J = 8.4 Hz), 11.16 (1H, brs); MS m/z : 436 (M + H)⁺; ESI-MS m/z : 436.2388 (M + H)⁺ (Calcd for C₂₆H₃₁O₂N₃F: 436.2395).

3,5-Dimethyl-2-([6-methyl-5-(1-[4-(trifluoromethyl)phenyl]methyl)piperidin-4-yl]pyridin-2-yl)oxy)methylpyridin-4(1H)-one (19c) Compound **19c** was prepared from **18c** in 46% yield using a similar approach to that described for **4f**. ¹H-NMR (DMSO-*d*₆) δ : 1.55–1.71 (4H, m), 1.84 (3H, s), 1.94 (3H, s), 2.04–2.20 (2H, m), 2.42 (3H, s), 2.55–2.73 (1H, m), 2.82–2.97 (2H, m), 3.60 (2H, s), 5.20 (2H, s), 6.68 (1H, d, J = 8.4 Hz), 7.45–7.52 (1H, m), 7.56 (2H, d, J = 8.2 Hz), 7.59 (1H, d, J = 8.4 Hz), 7.69 (2H, d, J = 8.2 Hz), 11.02–11.30 (1H, m); MS m/z : 486 (M + H)⁺; ESI-MS m/z : 486.2354 (M + H)⁺ (Calcd for C₂₇H₃₁O₂N₃F₃: 486.2363).

2-([5-[1-(4-Fluorophenyl)piperidin-4-yl]-6-methylpyridin-2-yl]oxy)methyl-4-methoxy-3,5-dimethylpyridine (20) To a mixture of 4-methoxy-3,5-dimethyl-2-([6-methyl-5-(piperidin-4-yl)pyridin-2-yl]oxy)methylpyridine (**17**; 200 mg, 0.59 mmol) and toluene (6.0 mL) were added 1-bromo-4-fluorobenzene (0.13 mL, 1.2 mmol), *t*-BuONa (110 mg, 1.2 mmol), BINAP (22 mg, 0.035 mmol) and Pd₂(dba)₃ (12 mg, 0.013 mmol) under an argon atmosphere. The mixture was refluxed for 18 h. After cooling to room temperature, the mixture was diluted with EtOAc and filtered through a Celite pad. The filtrate was washed with H₂O and brine, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CHCl₃/MeOH). The residue was purified by column chromatography on amino functionalized silica gel (hexane/CHCl₃) to give the product (120 mg, 47%). ¹H-NMR (CDCl₃) δ : 1.74–1.94 (4H, m), 2.26 (3H, s), 2.34 (3H, s), 2.50 (3H, s), 2.63–2.93 (3H, m), 3.60–3.74 (2H, m), 3.78 (3H, s), 5.42 (2H, s), 6.65 (1H, d, J = 8.4 Hz), 6.87–7.04 (4H, m), 7.43 (1H, d, J = 8.4 Hz), 8.26 (1H, s); MS m/z : 436 (M + H)⁺.

2-([5-[1-(4-Fluorophenyl)piperidin-4-yl]-6-methylpyridin-2-yl]oxy)methyl-3,5-dimethylpyridin-4(1H)-one (21) Compound **21** was prepared from **20** in 33% yield using a similar approach to that described for **4f**. ¹H-NMR (DMSO-*d*₆) δ : 1.64–1.82 (4H, m), 1.85 (3H, s), 1.95 (3H, s), 2.46 (3H, s), 2.63–2.89 (3H, m), 3.62–3.78 (2H, m), 5.22 (2H, s), 6.70 (1H, d, J = 8.4 Hz), 6.90–7.15 (4H, m), 7.41–7.54 (1H, m), 7.60 (1H, d, J = 8.4 Hz), 11.18 (1H, brs); MS m/z : 422 (M + H)⁺; ESI-MS m/z : 422.2235 (M + H)⁺ (Calcd for C₂₅H₂₉O₂N₃F: 422.2238).

tert-Butyl 6-[(4-Methoxy-3,5-dimethylpyridin-2-yl)methoxy]-2-methyl-3',6'-dihydro[3,4'-bipyridine]-1'(2'H)-carboxylate (22) Compound **22** was prepared from **2c**, *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate and Pd(PPh₃)₄ in 76% yield using a similar approach to that described for **3a**. ¹H-NMR (CDCl₃) δ : 1.50 (9H, s), 2.26 (3H, s), 2.27–2.37 (2H, m), 2.34 (3H, s), 2.40 (3H, s), 3.61 (2H, t, J = 5.6 Hz), 3.78 (3H, s), 3.96–4.11 (2H, m), 5.42 (2H, s), 5.50–5.63 (1H, m), 6.61 (1H, d, J = 8.4 Hz), 7.23–7.29 (1H, m), 8.26 (1H, s); MS m/z : 440 (M + H)⁺.

6-[(4-Methoxy-3,5-dimethylpyridin-2-yl)methoxy]-2-methyl-1',2',3',6'-tetrahydro-3,4'-bipyridine (23) Com-

pound **23** was prepared from **22** in 87% yield using a similar approach to that described for **17**. ¹H-NMR (CDCl₃) δ : 2.21–2.28 (2H, m), 2.26 (3H, s), 2.34 (3H, s), 2.43 (3H, s), 3.08 (2H, t, J = 5.6 Hz), 3.44–3.53 (2H, m), 3.78 (3H, s), 5.42 (2H, s), 5.57–5.65 (1H, m), 6.60 (1H, d, J = 8.4 Hz), 7.23–7.31 (1H, m), 8.26 (1H, s); MS m/z : 340 (M + H)⁺.

1'-[(4-Fluorophenyl)methyl]-6-[(4-methoxy-3,5-dimethylpyridin-2-yl)methoxy]-2-methyl-1',2',3',6'-tetrahydro-3,4'-bipyridine (24) Compound **24** was prepared from **23** and 4-fluorobenzaldehyde in 84% yield using a similar approach to that described for **18b**. ¹H-NMR (CDCl₃) δ : 2.26 (3H, s), 2.30–2.37 (2H, m), 2.34 (3H, s), 2.42 (3H, s), 2.67 (2H, t, J = 5.6 Hz), 3.07–3.18 (2H, m), 3.61 (2H, s), 3.77 (3H, s), 5.41 (2H, s), 5.52–5.57 (1H, m), 6.59 (1H, d, J = 8.4 Hz), 6.97–7.08 (2H, m), 7.29 (1H, d, J = 8.4 Hz), 7.31–7.37 (2H, m), 8.25 (1H, s); MS m/z : 448 (M + H)⁺.

2-([1'-[(4-Fluorophenyl)methyl]-2-methyl-1',2',3',6'-tetrahydro[3,4'-bipyridin]-6-yl]oxy)methyl-3,5-dimethylpyridin-4(1H)-one (25) Compound **25** was prepared from **24** in 25% yield using a similar approach to that described for **4a**. ¹H-NMR (DMSO-*d*₆) δ : 1.85 (3H, s), 1.95 (3H, s), 2.22–2.32 (2H, m), 2.36 (3H, s), 2.62 (2H, t, J = 5.5 Hz), 2.98–3.07 (2H, m), 3.58 (2H, s), 5.22 (2H, s), 5.54–5.60 (1H, m), 6.67 (1H, d, J = 8.4 Hz), 7.09–7.21 (2H, m), 7.33–7.41 (2H, m), 7.45 (1H, d, J = 8.4 Hz), 7.47–7.54 (1H, m), 11.20 (1H, brs); MS m/z : 434 (M + H)⁺; ESI-MS m/z : 434.2234 (M + H)⁺ (Calcd for C₂₆H₂₉O₂N₃F: 434.2238).

Biology

Cell Lines The breast cancer cell lines MDA-MB-453 and SK-BR-3 were purchased from American Type Culture Collection (Manassas, VA, U.S.A.). MDA-MB-453 was cultured in Leibovitz's L-15 Medium (Life Technologies, Carlsbad, CA, U.S.A.) under CO₂-free conditions at 37°C. SK-BR-3 was cultured in RPMI 1640 medium (Sigma-Aldrich Co. LLC., St. Louis, MO, U.S.A.) at 37°C in 5% CO₂. All media were supplemented with 10% fetal bovine serum (Sigma-Aldrich Co. LLC.) and 1% penicillin/streptomycin (Cat. No. 15070-063; Life Technologies).

Whole Cell ELISA MDA-MB-453 cells were seeded onto 384-well clear flat plates at 1.5 × 10⁴ cells/well. The following day, the cells were treated with the test compound at final concentrations of 0 (DMSO only), 0.1, 0.3, 1, 3, 10, 30, 100, 300, 1000, and 3000 nmol/L. The final concentration of DMSO in each well was 0.1% (v/v). Two hours after addition of the test compound, the cells were fixed in 40% glyoxal solution (Nakarai Chemical Ltd., Kyoto, Japan) for 30 min at room temperature. For probing, the supernatant was discarded and the cells were permeabilized with 0.1% Triton X-100 in phosphate-buffered saline for 10 min at room temperature. Subsequently, the supernatant was discarded and the cells were blocked with ODYSSEY Blocking solution (Li-Cor Biosciences, Lincoln, NE, U.S.A.) for 1 h at room temperature. After discarding the supernatant, a primary phospho-Acetyl-CoA Carboxylase (Ser79) Antibody (1:500 in ODYSSEY Blocking solution; Cat. No. 3661, Cell Signaling Technologies, Danvers, MA, U.S.A.) was added. After incubating overnight at 4°C, the plates were washed 3 times with Tris-buffered saline containing 0.05% Tween20 (TBS/Tween). Goat anti-rabbit IRDye 800CW (Li-Cor Biosciences; 1:1000 in ODYSSEY Blocking solution) was added, and the plates were incubated

for 1 h at room temperature. After the incubation, the plates were washed 3 times with TBS/Tween and dried for at least 3 h at room temperature. Fluorescence signals were quantified using the Aeries automated infrared imaging system (Li-Cor Biosciences). The assays were performed in triplicate, and the data were analyzed using Prism5 software (GraphPad Software Inc., San Diego, CA, U.S.A.). The EC_{50} value of each test compound was calculated using Sigmoid-Emax model non-linear regression analysis. The average signal was normalized by regarding the signal in the DMSO-treated group as 0% in each experiment.

Cell Growth Inhibition Assay Each breast cancer cells above were seeded onto non-adherent 384-well white plates (Thermo Scientific Nunc, Copenhagen, Denmark) at 500 cells/well. The following day, the test compound was added to each well at final concentrations of 0 (DMSO only), 0.3, 1, 3, 10, 30, 100, 300, 1000, and 3000 nmol/L. The final concentration of DMSO in each well was 0.1% (v/v). Four days after addition of the test compound, cell viability was determined using a CellTiter-Glo[®] Luminescent Cell Viability Assay (Promega, Madison, WI, U.S.A.). CellTiter-Glo[®] Reagent was added to each well, and luminescence was measured using ARVO SX[®] (PerkinElmer, Inc., Waltham, MA, U.S.A.). The assay was performed in triplicate. Cell viability according to the luminescence intensity was normalized by regarding the average luminescence intensity following treatment with DMSO only as 100% and no luminescence intensity as 0%. The IC_{50} value of each test compound was calculated by Sigmoid-Emax non-linear regression analysis using Prism5 software (GraphPad Software Inc.).

Aqueous Solubility The test compounds in 10 mM DMSO solution (13 μ L) were diluted to 130 μ M by adding the Japanese Pharmacopoeia 2nd fluid for disintegration test (pH = 6.8). After incubation at 25°C for 20 h, precipitates were separated by filtration. The filtrate and a standard solution comprising a 100 μ M DMSO solution of the test compound were examined using liquid chromatography. The ratio of the peak area of the sample solution to the peak area of the standard solution was calculated to determine the aqueous solubility.

Measured $\log D_{7.4}$ The $\log D_{7.4}$ value was calculated as the natural logarithm of the compound concentration ratio between 1-octanol and Dulbecco's phosphate buffered salts (PBS) aqueous solution (pH = 7.4). The concentration within each of the two layers was evaluated using LC-MS after 10 mM DMSO stock solution of the compound was dissolved and partitioned at 23°C.

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Conflict of Interest All authors were employees of Astellas Pharma Inc. when the study was conducted and have no further conflicts of interest to declare.

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