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STRUCTURAL DEVELOPMENT STUDIES OF PYRAZOLOKETONE-DERIVED ACETYL-CoA CARBOXYLASE INHIBITORS

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Abstract – Acetyl-CoA carboxylase (ACC) plays a key role in fatty acid homeostasis in humans, and inhibitors of ACC are expected to inhibit fatty acid biosynthesis and to activate fatty acid β-oxidation. Therefore, they are considered to be candidates for treatment of metabolic syndrome and related diseases. In this context, an upstream kinase of ACC, adenosine monophosphate-activated protein kinase (AMPK), has also recently emerged as a potential therapeutic target, because it phosphorylates and inactivates ACC. Here, we designed a fused molecule consisting of a pyrazoloketone-type ACC inhibitor and a recently discovered AMPK activator, aiming to develop a novel combined ACC inhibitor/AMPK activator to regulate fatty acid levels. The designed compound was prepared through a convergent synthetic route. This compound and its methyl ester analogue showed potent ACC2-inhibitory activity with IC_{50} values of 8.8 and 1.3 μM, respectively. Exomethylene derivatives, obtained from an unexpected side reaction during deprotection, also exhibited ACC2-inhibitory activity.

Acetyl-CoA carboxylase (ACC) catalyzes the ATP-dependent conversion of acetyl-CoA to malonyl-CoA, which is the rate-limiting step of fatty acid biosynthesis.[1–3] ACC also plays an important role in fatty acid metabolism because its enzymatic product, malonyl-CoA, is a potent endogenous inhibitor of carnitine palmitoyltransferase 1 (CPT1), a mitochondrial membrane protein that transports long-chain fatty acyl-CoAs into mitochondria for subsequent β-oxidation. In humans, there are two closely related isoforms of ACC, termed ACC1 and ACC2. ACC1 is a cytosolic enzyme that is involved in fatty acid biosynthesis, while ACC2 is embedded in the mitochondrial membrane and is mainly associated with

β-oxidation. Pharmacological inhibition of ACC1 and ACC2 is expected to suppress fatty acid biosynthesis and promote fatty acid oxidation, leading to lower ectopic lipid levels. Thus, ACC inhibitors may have utility for the treatment of obesity, type 2 diabetes (T2D) and other diseases associated with metabolic syndrome.[2–5] For the past decade, numerous efforts have been directed towards the development of novel ACC inhibitors.[6] A group at Pfizer discovered CP-640186 (**1**) as an ACC1/2 dual inhibitor (Figure 1),[7] and subsequent structural optimization led to the identification of pyrazoloketone-type compound **4** (known as PF-05175157) [IC_{50} = 27 nM for human ACC1, IC_{50} = 33 nM for human ACC2] as a clinical candidate for the treatment of T2D.[8–10] Another group at Takeda reported a series of 2-ureidobenzothiophenes as ACC1/2 dual inhibitors and demonstrated that oral administration of **5** in rats decreased the respiratory quotient values, which suggested that fatty acid oxidation was stimulated.[11–13]

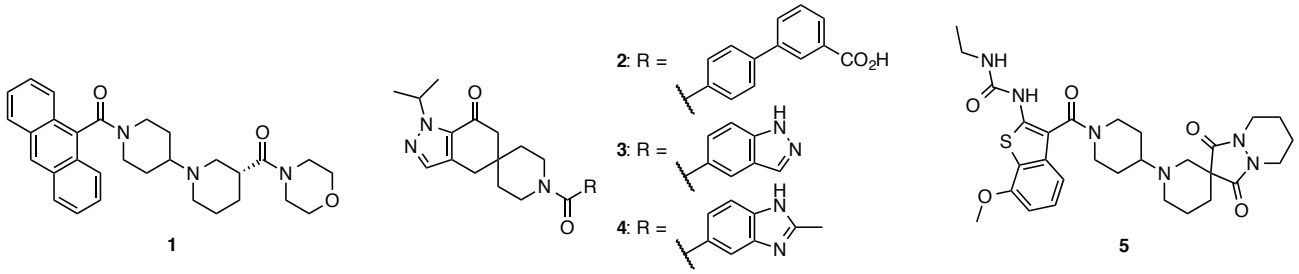


Figure 1. Chemical structures of known ACC1/ACC2 dual inhibitors

In this context, an upstream kinase of ACC, adenosine monophosphate-activated protein kinase (AMPK), has also emerged as a potential therapeutic target.[14–17] AMPK is a heterotrimeric serine/threonine-protein kinase that regulates cellular energy homeostasis. In response to an increase in AMP levels, AMPK is allosterically activated and phosphorylates its downstream substrates, ACC and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), leading to an adaption of cellular metabolism to an adenosine triphosphate (ATP)-conserving state. The phosphorylation and resulting inhibition of ACC and HMGCR suppress fatty acid and cholesterol biosyntheses. Therefore, small molecules that activate AMPK may have potential as novel therapeutics for metabolic diseases.[14,15] In recent years, several AMPK direct activators, such as A-769662 (**6**),[18] 991 (**7**)[19] and **8**,[20] have been developed (Figure 2).

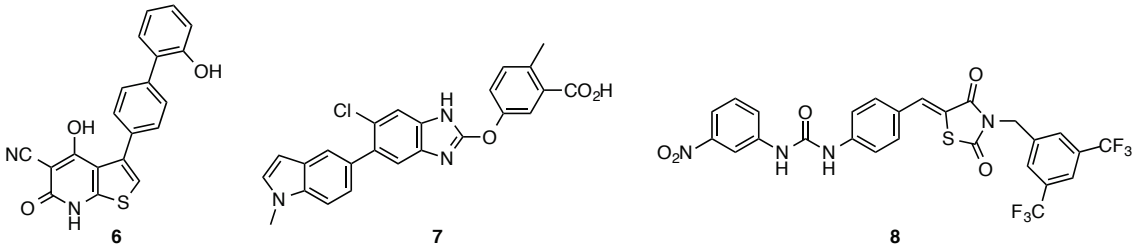


Figure 2. Chemical structures of AMPK direct activators

We have been interested in bioactive molecules with polypharmacological profiles,[21–23] and we recently developed dual modulators of ACC and peroxisome proliferator-activated receptor (PPAR) based on the molecular similarity between known ACC inhibitors and PPAR agonists.[24] As an extension of that work, we became interested in novel ACC inhibitors with AMPK-activating activity, and we therefore designed fused molecules consisting of pyrazoloketone-type ACC inhibitors **2**–**4** and AMPK activator **7** (Figure 3). According to the reported structure-activity relationships (SARs) of pyrazoloketone-type ACC inhibitors, the left part of **2**–**4** contributes greatly to the ACC-inhibitory activity.[8–10] The X-ray cocrystal structure of **3** bound to human-yeast chimeric ACC showed that pyrazoloketone and amide carbonyls form hydrogen bonds to the protein (Figure 3a).[10] The indazole group also forms a hydrogen bond, but its 2-position faces outside from the ligand-binding pocket. In the case of AMPK activator **7**, the carboxyl group and the benzimidazole NH are important for AMPK binding, while the *N*-methylindole moiety and chlorine atom only occupy an open hydrophobic space (Figure 3b).[19] On the basis of the above information, we designed compound **9** as a potential ACC/AMPK dual modulator (Figure 3c). Here we describe the synthesis of **9** and its analogues, and characterization of their ACC2-inhibitory activity.

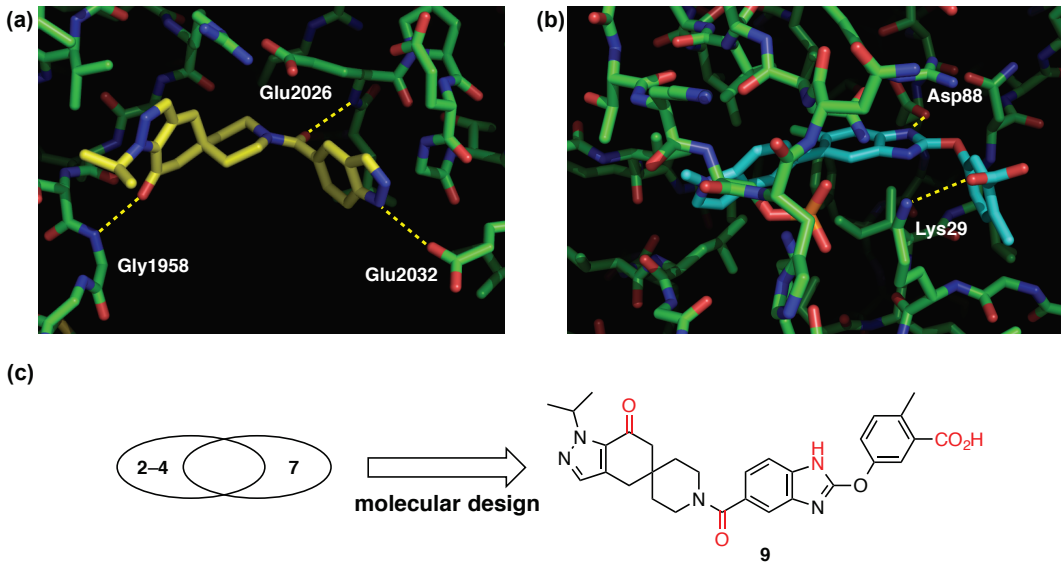
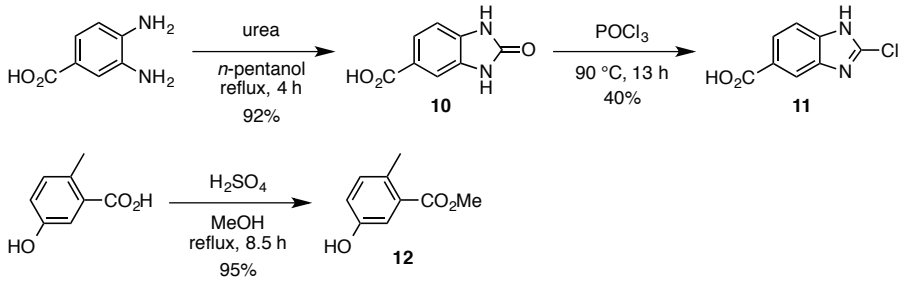


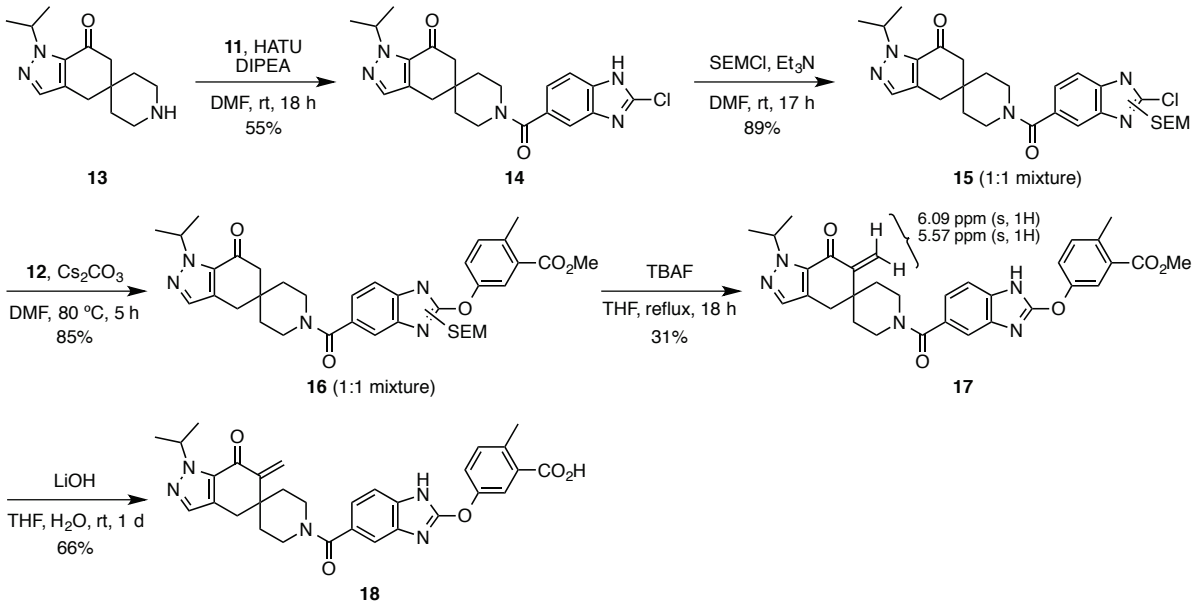
Figure 3. (a) The X-ray cocrystal structure of **3** bound to human-yeast chimeric ACC carboxyl transferase domain (PDB ID: 4WZ8). (b) The X-ray cocrystal structure of **7** bound to human AMPKα2/β1/γ1 (PDB ID: 4CFE). (c) Structure of the designed compound **9**.

We first prepared 5-carboxy-2-chlorobenzimidazole (**11**),[25] corresponding to the middle part of **9**, from 3,4-diaminobenzoic acid (Scheme 1). Refluxing of a solution of 3,4-diaminobenzoic acid and urea in *n*-pentanol afforded benzimidazolinone **10**, which was treated with $POCl_3$ to provide **11**. Methyl 5-hydroxy-2-methylbenzoate (**12**),[26] corresponding to the benzoic acid moiety of **9**, was readily prepared from commercially available 5-hydroxy-2-methylbenzoic acid.

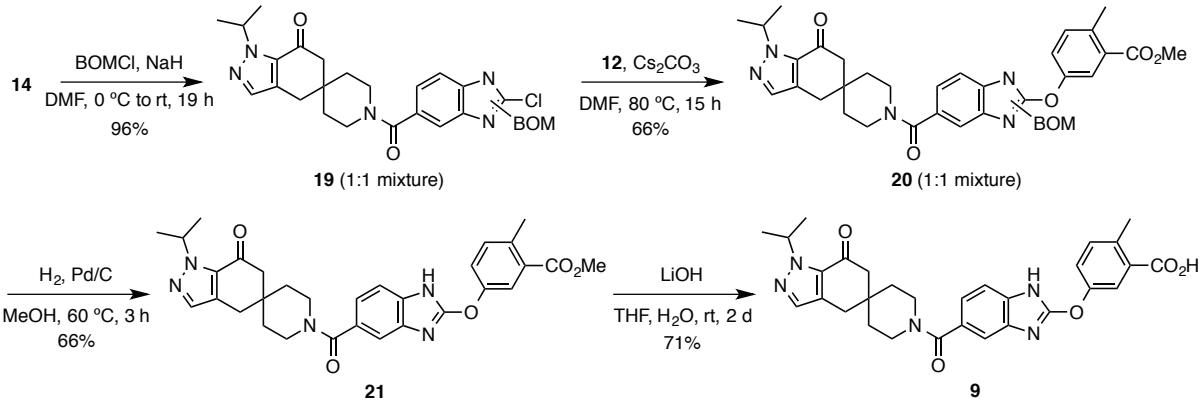


Scheme 1. Synthesis of benzimidazole **11** and methyl benzoate **12**

Then, compound **13**, prepared according to the reported procedure,[27] was condensed with **11** (Scheme 2). This reaction afforded compound **14**, which was protected using 2-(trimethylsilyl)ethoxymethyl chloride (SEMCl) to give **15** as a 1:1 regioisomeric mixture (1-*N*-SEM and 3-*N*-SEM). The coupling reaction of **15** with phenol **12** in the presence of Cs_2CO_3 provided **16**, a precursor of **9**. However, the SEM-deprotection reaction proceeded very slowly and unexpectedly afforded exomethylene compound **17** as a major product. The structure of **17** was confirmed by the ^{1}H-NMR spectrum, in which exomethylene proton signals appeared at 6.09 and 5.57 ppm as two singlets. A plausible reaction mechanism involves reaction of formaldehyde generated during SEM-deprotection with the α-carbon of the pyrazoloketone moiety, due to the basicity of TBAF. This problem was readily resolved by replacing the SEM group with a benzyloxymethyl (BOM) group (Scheme 3). First, compound **14** was protected using BOMCl, and the product **19** was then coupled with phenol **12** to afford **20** as a 1:1 regioisomeric mixture. The BOM group was then easily removed under general hydrogenation conditions (H_2, Pd/C, MeOH), except for heating at 60 ºC. Finally, the designed compound **9** was obtained by the ester hydrolysis of **21**. Compound **17** was similarly converted into **18** for biological evaluation (Scheme 2).



Scheme 2. Attempted preparation of the synthetic intermediate of **9**. Deprotection of the SEM group of **16** gave unique exomethylene product **17**.

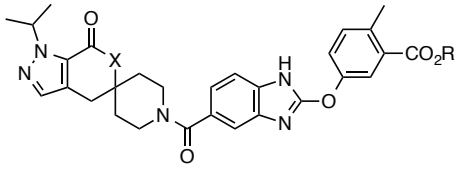


Scheme 3. Synthesis of the designed compound **9** *via* BOM-protected compound **19**

Compound **9** and related analogues (**21**, **18** and **17**) were then evaluated for ACC-inhibitory and AMPK-activating activities. AMPK-activating activity was evaluated using recombinant human AMPKα1/β1/γ1 and a kinase activity assay kit (see experimental). However, none of the prepared compounds, including **9** and **18**, showed activity toward AMPK at <10 μM (data not shown). We suspected that the relatively large spiropiperidine moiety, compared to the *N*-methylimidazole moiety of **7**, unfavorably influenced the AMPK-activating activity. For evaluation of ACC-inhibitory activity, we used recombinant human ACC2 and performed ATP consumption assay (see experimental).[24,28] As a result, the designed compound **9** and its methyl ester analogue **21** were found to exhibit ACC2-inhibitory activity with IC_{50} values of 8.8 and 1.3 μM, respectively (Table 1). It is interesting that exomethylene analogues **18** and **17** retain ACC2-inhibitory activity. Although the ketone group of pyrazoloketone-type ACC inhibitor **3** makes a key hydrogen-bond contact with the protein (Figure 3a), our results demonstrated that inclusion of the relatively small exomethylene structure at the α-position of ketone only minimally affects the activity. Also, methyl esters **21** and **17** show higher activity than the corresponding carboxyl acids **9** and **18**. These SAR data are expected to be useful for further development of pyrazoloketone-derived ACC inhibitors.

We designed compound **9** as a candidate ACC/AMPK dual modulator, and synthesized it *via* a convergent synthetic route. Compound **9** exhibited ACC2-inhibitory activity at low micromolar concentrations [IC_{50} = 8.8 μM], but showed no activity toward AMPKα1/β1/γ1 at <10 μM. In addition, exomethylene compounds **18** and **17**, which were formed in side reactions during deprotection, retained ACC2-inhibitory activity. Among the compounds synthesized, methyl ester **21** was the most potent ACC2 inhibitor [IC_{50} = 1.3 μM].

Table 1. Human ACC2-inhibitory activity[a]



Compound	X	R	ACC2 [IC_{50} (μM)]
9	CH_2	H	8.8
21	CH_2	Me	1.3
18	$C=CH_2$	H	17
17	$C=CH_2$	Me	13
2			0.65

[a]Inhibitory activity towards recombinant human ACC2. Evaluations were performed in triplicate, and the IC_{50} values were determined from the dose-response curves.

EXPERIMENTAL

All chemical reagents and solvents were purchased from Sigma-Aldrich, Kanto Chemical, Tokyo Chemical Industry and Wako Pure Chemical Industries, and were used without further purification. Moisture-sensitive reactions were performed under an atmosphere of argon, and monitored by thin-layer chromatography (TLC, Merck silica gel 60 F254 plate). Bands were visualized using UV light or by application of appropriate reagents followed by heating. Flash chromatography was carried out with silica gel (Silica gel 60N, 40–50 μm particle size) purchased from Kanto Chemical. NMR spectra were recorded on a JEOL JNM-ECA500 (500 MHz) spectrometer, operating at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR. Proton and carbon chemical shifts are expressed in δ values (ppm) relative to internal tetramethylsilane (0.00 ppm), residual $CHCl_3$ (7.26 ppm) or C_2HD_5SO (2.49 ppm) for ^{1}H NMR, and internal tetramethylsilane (0.00 ppm) or dimethyl sulfoxide-d_6 (39.50 ppm) for ^{13}C NMR. Data are reported as follows: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; sep, septet; m, multiplet; br, broad), coupling constants (Hz), integration. High-resolution mass spectra were recorded using a Bruker micrOTOF II mass spectrometer.

5-Carboxy-2-benzimidazolinone (10)

A solution of 3,4-diaminobenzoic acid (3.04 g, 20.0 mmol) and urea (1.44 g, 24.0 mmol) in *n*-pentanol (10 mL) was refluxed for 4 h, and then cooled to room temperature. Water and aq. HCl were added. The resulting suspension was filtered, and the residue was washed with water and MeOH to afford **10** (3.26 g, 92%) as a white solid.

^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.59 (d, J = 8.0 Hz, 1H), 7.46 (s, 1H), 6.94 (d, J = 8.0 Hz, 1H). The NH and CO_2H proton signals were not observed.; ^{13}C-NMR (125 MHz, DMSO-d_6) δ 168.1, 155.4, 132.9, 129.3, 125.4, 122.7, 109.3, 107.7; ESI-TOF-HRMS calcd for $C_8H_5N_2O_3$ (*m/z*) $[M–H]^-$ 177.0295, found 177.0313.

5-Carboxy-2-chlorobenzimidazole (11)

A solution of **10** (2.50 g, 14.0 mmol) in $POCl_3$ (20 mL, 215 mmol) was stirred at 90 °C for 13 h. The reaction was then quenched by addition of water and saturated aq. $NaHCO_3$ at 0 °C. The resulting suspension was filtered. The residue was washed with AcOEt and purified by silica gel column chromatography ($CHCl_3$/MeOH = 4/1) to afford **11** (1.11 g, 40%) as a white solid.

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.07 (s, 1H), 7.84 (d, J = 8.6 Hz, 1H), 7.57 (d, J = 8.6 Hz, 1H). The NH and CO_2H proton signals were not observed.; ESI-TOF-HRMS calcd for $C_8H_4ClN_2O_2$ (*m/z*) $[M–H]^-$ 194.9956, found 194.9965.

Methyl 5-hydroxy-2-methylbenzoate (12)

To a solution of 5-hydroxy-2-methylbenzoic acid (500 mg, 3.29 mmol) in MeOH (10 mL) was added conc. H_2SO_4 (200 μL, 3.75 mmol). The mixture was refluxed for 8.5 h, and then cooled to room temperature. Saturated aq. $NaHCO_3$ was added to it, and the whole was extracted with AcOEt. The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt = 2/1) to afford **12** (520 mg, 95%) as a white solid.

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.42 (d, J = 2.9 Hz, 1H), 7.11 (d, J = 8.6 Hz, 1H), 6.92 (dd, J = 8.6, 2.9 Hz, 1H), 5.42 (br s, 1H), 3.89 (s, 3H), 2.50 (s, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 168.1, 153.4, 132.9, 132.2, 130.1, 119.3, 117.1, 52.0, 20.8; ESI-TOF-HRMS calcd for $C_9H_{10}O_3Na$ (*m/z*) $[M+Na]^+$ 189.0522, found 189.0517.

1'-(2-Chlorobenzimidazole-6-carbonyl)-1-isopropyl-1,4-dihydrospiro[indazole-5,4'-piperidin]-7-one (14)

To a solution of **13** (945 mg, 3.33 mmol), **11** (789 mg, 4.00 mmol) and HATU (1.52 g, 4.00 mmol) in anhydrous DMF (33.3 mL) was added DIPEA (2.9 mL, 16.7 mmol). The mixture was stirred overnight, diluted with AcOEt, washed with saturated aq. $NaHCO_3$, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/acetone = 3/4) to afford **14** (780 mg, 55%) as a white solid.

^{1}H-NMR (500 MHz, DMSO-d_6, 80 ºC) δ 7.56-7.50 (m, 2H), 7.42 (s, 1H), 7.24 (dd, J = 8.6, 1.7 Hz, 1H), 5.26 (sep, J = 6.6 Hz, 1H), 3.59-3.52 (m, 2H), 3.52-3.43 (m, 2H), 2.81 (s, 2H), 2.60 (s, 2H), 1.51 (br s, 4H), 1.36 (d, J = 6.6 Hz, 6H). The NH proton signal was not observed.; ESI-TOF-HRMS calcd for $C_{22}H_{24}ClN_5O_2Na$ (*m/z*) $[M+Na]^+$ 448.1511, found 448.1504.

1'-(2-Chloro-1-{[2-(trimethylsilyl)ethoxy]methyl}benzimidazole-5-carbonyl)-1-isopropyl-1,4-dihydrospiro[indazole-5,4'-piperidin]-7-one and 1'-(2-chloro-1-{[2-(trimethylsilyl)ethoxy]methyl}-benzimidazole-6-carbonyl)-1-isopropyl-1,4-dihydrospiro[indazole-5,4'-piperidin]-7-one (15)

To a solution of **14** (233 mg, 0.55 mmol) and Et_3N (385 μL, 2.74 mmol) in anhydrous DMF (2.5 mL) was added 2-(chloromethoxy)ethyltrimethylsilane (290 μL, 1.64 mmol), and the mixture was stirred overnight. The reaction was quenched by addition of water, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with 2 N aq. HCl and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 10/1) to afford **15** (270 mg, 89%, 1:1 regioisomeric mixture) as a white solid.

^{1}H-NMR (500 MHz, DMSO-d_6, 60 ºC) δ 7.72 (s, 1/2H), 7.70 (d, J = 9.0 Hz, 1/2H), 7.64 (d, J = 8.0 Hz, 1/2H), 7.63 (s, 1/2H), 7.44 (s, 1H), 7.37 (dd, J = 8.6, 1.7 Hz, 1/2H), 7.30 (dd, J = 8.6, 1.7 Hz, 1/2H), 5.66 (s, 1H), 5.66 (s, 1H), 5.26 (sep, J = 6.6 Hz, 1H), 3.59 (t, J = 8.0 Hz, 1H), 3.57 (t, J = 7.5 Hz, 1H), 3.55-3.35 (m, 4H), 2.81 (s, 2H), 2.61 (s, 2H), 1.52 (br s, 4H), 1.37 (d, J = 6.3 Hz, 6H), 0.84 (t, J = 7.4 Hz, 1H), 0.83 (t, J = 7.4 Hz, 1H), -0.10 (s, 9/2H), -0.12 (s, 9/2H); ESI-TOF-HRMS calcd for $C_{28}H_{38}ClN_5O_3SiNa$ (*m/z*) $[M+Na]^+$ 578.2325, found 578.2315.

Methyl 5-{[5-(1-isopropyl-7-oxo-1,4,6,7-tetrahydrospiro[indazole-5,4'-piperidine]-1'-carbonyl)-1-{[2-(trimethylsilyl)ethoxy]methyl}benzimidazol-2-yl]oxy}-2-methylbenzoate and methyl 5-{[6-(1-isopropyl-7-oxo-1,4,6,7-tetrahydrospiro[indazole-5,4'-piperidine]-1'-carbonyl)-1-{[2-(trimethylsilyl)ethoxy]methyl}benzimidazol-2-yl]oxy}-2-methylbenzoate (16)

To a solution of **15** (262 mg, 0.47 mmol) and **12** (118 mg, 0.71 mmol) in anhydrous DMF (2.0 mL) was added Cs_2CO_3 (461 mg, 1.42 mmol), and the mixture was stirred at 80 °C for 5 h. The reaction was quenched by addition of water, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt = 4/1) to afford **16** (274 mg, 85%, 1:1 regioisomeric mixture) as a white solid.

^{1}H-NMR (500 MHz, DMSO-d_6, 80 ºC) δ 7.82 (s, 1H), 7.59-7.53 (m, 2H), 7.47-7.40 (m, 3H), 7.24 (d, J = 8.3 Hz, 1/2H), 7.20 (d, J = 8.3 Hz, 1/2H), 5.61 (s, 1H), 5.60 (s, 1H), 5.22-5.30 (m, 1H), 3.85 (s, 3H), 3.69-3.63 (m, 2H), 3.60-3.40 (m, 4H), 2.82 (s, 1H), 2.81 (s, 1H), 2.61 (s, 1H), 2.60 (s, 1H), 2.56 (s, 3H), 1.56-1.47 (m, 4H), 1.38 (d, J = 6.3 Hz, 6H), 0.88 (t, J = 6.8 Hz, 1H), 0.87 (t, J = 7.3 Hz, 1H), -0.08 (s, 9/2H), -0.10 (s, 9/2H); ESI-TOF-HRMS calcd for $C_{37}H_{47}N_5O_6SiNa$ (*m/z*) $[M+Na]^+$ 708.3188, found 708.3201.

Methyl 5-{[6-(1-isopropyl-6-methylene-7-oxo-1,4,6,7-tetrahydrospiro[indazole-5,4'-piperidine]-1'-carbonyl)-benzimidazol-2-yl]oxy}-2-methylbenzoate (17)

To a solution of **16** (270 mg, 0.394 mmol) in THF (2.7 mL) was added TBAF (1 M in THF, 1.18 mL,

1.18 mmol), and the mixture was stirred at 80 °C for 12 h. Then, TBAF (1 M in THF, 1.18 mL, 1.18 mmol) was added, and stirring was continued at 80 °C for 6 h. The mixture was then cooled to room temperature, diluted with AcOEt and washed with water. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/acetone = 4/1) to afford **17** (67.9 mg, 31%) as a white solid.

^{1}H-NMR (500 MHz, DMSO-d_6, 80 ºC) δ 12.34 (br s, 1H), 7.77 (d, J = 2.9 Hz, 1H), 7.51 (dd, J = 8.6, 2.9 Hz, 1H), 7.47 (s, 1H), 7.42-7.36 (m, 3H), 7.15 (dd, J = 8.3, 1.4 Hz, 1H), 6.09 (s, 1H), 5.57 (s, 1H), 5.35 (sep, J = 6.6 Hz, 1H), 3.84 (s, 3H), 3.60-3.56 (m, 2H), 3.49-3.45 (m, 2H), 2.90 (s, 2H), 2.54 (s, 3H), 1.78-1.70 (m, 2H), 1.70-1.61 (m, 2H), 1.39 (d, J = 6.3 Hz, 6H); ESI-TOF-HRMS calcd for $C_{32}H_{33}N_5O_5Na$ (*m/z*) $[M+Na]^+$ 590.2374, found 590.2346.

5-{[6-(1-Isopropyl-6-methylene-7-oxo-1,4,6,7-tetrahydrospiro[indazole-5,4'-piperidine]-1'-carbonyl)-benzimidazol-2-yl]oxy}-2-methylbenzoic acid (18)

To a solution of **17** (19.2 mg, 0.034 mmol) in THF (340 μL) was added 1 M aq. LiOH (68 μL, 0.068 mmol). The mixture was stirred at room temperature for 1 d, and then 2 N aq. HCl and brine were added. The resulting mixture was extracted with AcOEt. The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by PTLC ($CHCl_3$/MeOH = 10/1) to afford **18** (12.5 mg, 66%) as a white solid.

^{1}H-NMR (500 MHz, DMSO-d_6, 80 ºC) δ 7.63 (s, 1H), 7.48 (s, 1H), 7.38-7.35 (m, 2H), 7.31-7.23 (m, 2H), 7.15 (d, J = 8.0 Hz, 1H), 6.09 (s, 1H), 5.59 (s, 1H), 5.38-5.35 (m, 1H), 3.69-3.60 (m, 2H), 3.50-3.41 (m, 2H), 2.91 (s, 2H), 2.50 (s, 3H), 1.76-1.70 (m, 2H), 1.70-1.64 (m, 2H), 1.41 (d, J = 6.5 Hz, 6H), The NH and CO_2H proton signals were not observed.; ESI-TOF-HRMS calcd for $C_{31}H_{30}N_5O_5$ (*m/z*) $[M–H]^-$ 552.2241, found 552.2258.

1'-(1-Benzyloxymethyl-2-chlorobenzimidazole-5-carbonyl)-1-isopropyl-1,4-dihydrospiro[indazole-5,4'-piperidin]-7-one and 1'-(1-benzyloxymethyl-2-chlorobenzimidazole-6-carbonyl)-1-isopropyl-1,4-dihydrospiro[indazole-5,4'-piperidin]-7-one (19)

To a solution of **14** (150.7 mg, 0.354 mmol) in anhydrous DMF (4.0 mL) was added NaH (60% in oil, 16.4 mg, 0.407 mmol) at 0 °C, and the mixture was stirred for 20 min. Benzyl chloromethyl ether (53 μL, 0.389 mmol) was added, and stirring was continued overnight at room temperature. The organic layer was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 20/1) to afford **19** (184.9 mg, 96%, 1:1 regioisomeric mixture) as a yellow paste.

^{1}H-NMR (500 MHz, DMSO-d_6, 80 ºC) δ 7.69-7.62 (m, 2H), 7.43 (s, 1H), 7.37-7.20 (m, 6H), 5.77 (s, 1H), 5.75 (s, 1H), 5.27 (sep, J = 6.6 Hz, 1H), 4.61 (s, 1H), 4.60 (s, 1H), 3.58-3.43 (m, 4H), 2.81 (s, 2H), 2.60 (s, 2H), 1.56-1.48 (m, 4H), 1.37 (d, J = 6.3 Hz, 6H); ESI-TOF-HRMS calcd for $C_{30}H_{32}ClN_5O_3Na$ (*m/z*)

$[M+Na]^+$ 568.2086, found 568.2085.

Methyl 5-{[1-benzyloxymethyl-5-(1-isopropyl-7-oxo-1,4,6,7-tetrahydrospiro[indazole-5,4'-piperidine]-1'-carbonyl)-benzimidazol-2-yl]oxy}-2-methylbenzoate and methyl 5-{[1-benzyloxymethyl-6-(1-isopropyl-7-oxo-1,4,6,7-tetrahydrospiro[indazole-5,4'-piperidine]-1'-carbonyl)-benzimidazol-2-yl]oxy}-2-methylbenzoate (20)

To a solution of **19** (185 mg, 0.339 mmol) and **12** (84.4 mg, 0.508 mmol) in anhydrous DMF (2.0 mL) was added Cs_2CO_3 (332 mg, 1.02 mmol), and the mixture was stirred at 80 °C for 15 h. The reaction was quenched by addition of water, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/AcOEt = 1/2) to afford **20** (150 mg, 66%, 1:1 regioisomeric mixture) as a white solid.

^{1}H-NMR (500 MHz, DMSO-d_6, 80 ºC) δ 7.81 (s, 1H), 7.59-7.54 (m, 2H), 7.46-7.42 (m, 3H), 7.28-7.18 (m, 6H), 5.72 (s, 1H), 5.70 (s, 1H), 5.30-5.22 (m, 1H), 4.67 (s, 1H), 4.65 (s, 1H), 3.85 (s, 3H), 3.60-3.42 (m, 4H), 2.81 (s, 1H), 2.80 (s, 1H), 2.60 (s, 1H), 2.59 (s, 1H), 2.54 (s, 3H), 1.51 (br s, 4H), 1.39-1.35 (m, 6H); ESI-TOF-HRMS calcd for $C_{39}H_{41}N_5O_6Na$ (*m/z*) $[M+Na]^+$ 698.2949, found 698.2972.

Methyl 5-{[6-(1-isopropyl-7-oxo-1,4,6,7-tetrahydrospiro[indazole-5,4'-piperidine]-1'-carbonyl)-benzimidazol-2-yl]oxy}-2-methylbenzoate (21)

To a solution of **20** (67.8 mg, 0.100 mmol) in MeOH (2.0 mL) was added 10% Pd/C (20.4 mg), and the mixture was stirred at 60 ºC under a hydrogen atmosphere. Stirring was continued for 3 h, then the mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The residue was purified by PTLC ($CHCl_3$/MeOH = 10/1) to afford **21** (36.7 mg, 66%) as a white solid.

^{1}H-NMR (500 MHz, DMSO-d_6, 80 ºC) δ 12.34 (br s, 1H), 7.77 (d, *J* = 2.9 Hz, 1H), 7.51 (dd, *J* = 8.6, 2.9 Hz, 1H), 7.42-7.36 (m, 4H), 7.15 (dd, *J* = 8.3, 1.4 Hz, 1H), 5.26 (sep, *J* = 6.6 Hz, 1H), 3.84 (s, 3H), 3.58-3.43 (m, 4H), 2.81 (s, 2H), 2.60 (s, 2H), 2.54 (s, 3H), 1.53-1.47 (m, 4H), 1.37 (d, *J* = 6.9 Hz, 6H); ESI-TOF-HRMS calcd for $C_{31}H_{33}N_5O_5Na$ (*m/z*) $[M+Na]^+$ 578.2374, found 578.2366.

5-{[6-(1-Isopropyl-7-oxo-1,4,6,7-tetrahydrospiro[indazole-5,4'-piperidine]-1'-carbonyl)-benzimidazol-2-yl]oxy}-2-methylbenzoic acid (9)

To a solution of **21** (21.4 mg, 0.039 mmol) in THF (390 μL) was added 1 M aq. LiOH (78 μL, 0.078 mmol). The mixture was stirred for 2 d, and then 2 N aq. HCl and brine were added. The resulting mixture was extracted with AcOEt. The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by PTLC ($CHCl_3$/MeOH = 5/1) to afford **9** (14.9 mg, 71%) as a white solid.

^{1}H-NMR (500 MHz, DMSO-d_6, 80 ºC) δ 7.46-7.40 (m, 2H), 7.36-7.32 (m, 2H), 7.14-7.08 (m, 3H), 5.27 (sep, *J* = 6.5 Hz, 1H), 3.58-3.43 (m, 4H), 2.80 (s, 2H), 2.59 (s, 2H), 2.46 (s, 3H), 1.53-1.48 (m, 4H), 1.37

(d, J = 7.0 Hz, 6H), The NH and CO_2H proton signals were not observed.; ESI-TOF-HRMS calcd for $C_{30}H_{30}N_5O_5$ (*m/z*) $[M–H]^-$ 540.2241, found 540.2247.

ACC-inhibitory activity assay

ACC2 activity was determined by means of an ATP consumption assay. The assay was performed using 30 mM HEPES buffer (pH 7.5), 18 mM $NaHCO_3$, 1.0 mg/mL BSA, 0.5 μM ATP, 1 mM DTT, 4 mM $MgCl_2$, 2 mM sodium citrate, 0.5 mM acetyl-CoA, 1% DMSO, and 50 ng of enzyme (human ACC2, recombinant, C-terminal His-tag, BPS Bioscience, catalog number 50201) per well (96-well half plate). ACC inhibitor was added at concentrations between 0 and 100 μM. The reaction was started by addition of enzyme (50 ng/well) followed by incubation at 37 ºC for 90 min. Unconverted ATP was determined using ATP monitoring reagent (Lonza, ViaLight plus kit) according to the manufacturer's instructions. Evaluations were performed in triplicate, and the IC_{50} value was calculated from the dose-response curve.

AMPK-activation activity assay

Recombinant human AMPKα1/β1/γ1 (Thermo Fisher Scientific, catalog number PV4672) was used in a FRET assay format (Invitrogen, Z-Lyte kinase assay kit). Assay conditions were as follows: 100 μM ATP, 2 μM peptide substrate (Invitrogen, catalog number PV4645), 1% DMSO, and 0.6 ng of enzyme in Z-Lyte kinase buffer. The reaction was started by addition of enzyme followed by incubation at 30 °C for 1 h. The mixture was further incubated at 30 °C for 1 h, after addition of development reagent (Invitrogen, catalog number PV3295). The FRET signals were then measured and converted to "% peptide phosphorylation" according to the manufacturer's instructions. Evaluations were performed in triplicate, and the EC_{200} value (compound concentration that lead to two-fold increase of AMPK activity) was calculated from the dose-response curves. The EC_{200} value of A-769662 was 4.9 nM.

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