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Design and synthesis of disubstituted (4-piperidinyl)-piperazine derivatives as potent acetyl-CoA carboxylase inhibitors

Tomomichi Chonan [a,*], Hiroaki Tanaka [a], Daisuke Yamamoto [b], Miyoko Yashiro [c], Takahiro Oi [a], Daisuke Wakasugi [a], Ayumi Ohoka-Sugita [b], Fusayo Io [b], Hiroko Koretsune [b], Akira Hiratate [a]

[a] Medicinal Chemistry Laboratories, Taisho Pharmaceutical Co., Ltd, 1-403 Yoshino-cho, Kita-ku, Saitama-shi, Saitama 331-9530, Japan
[b] Molecular Function and Pharmacology Laboratories, Taisho Pharmaceutical Co., Ltd, 1-403 Yoshino-cho, Kita-ku, Saitama-shi, Saitama 331-9530, Japan
[c] Research Computer System, Taisho Pharmaceutical Co., Ltd, 1-403 Yoshino-cho, Kita-ku, Saitama-shi, Saitama 331-9530, Japan

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ABSTRACT

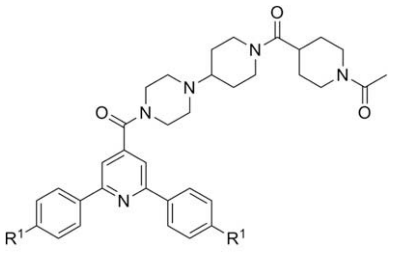
Acetyl-CoA carboxylases (ACCs), the rate limiting enzymes in de novo lipid synthesis, play important roles in modulating energy metabolism. The inhibition of ACC has demonstrated promising therapeutic potential for treating obesity and type 2 diabetes mellitus in transgenic mice and preclinical animal models. We describe herein the structure-based design and synthesis of a novel series of disubstituted (4-piperidinyl)-piperazine derivatives as ACC inhibitors. Our structure-based approach led to the discovery of the indole derivatives **13i** and **13j**, which exhibited potent in vitro ACC inhibitory activity.

Acetyl-CoA carboxylase (ACC) is a biotin-dependent homo oligomeric protein composed of a carboxyltransferase (CT), a biotin carboxyl carrier protein and biotin carboxylase (BC) domains. ACC is involved in the synthesis of malonyl-CoA from acetyl-CoA in an ATP-dependent manner. Malonyl-CoA works not only as a substrate for de novo fatty acid synthesis, but also as an allosteric inhibitor of carnitine palmitoyl transferase (CPT-1), a key enzyme that positively regulates mitochondrial β-oxidation. Therefore, inhibition of ACC is expected to reduce de novo fatty acid synthesis (FAS) and to enhance fatty acid β-oxidation (FAO) through disinhibition of CPT-1, which might benefit treatment of metabolic disorders such as obesity and diabetes.[1]

Two ACC isoforms, ACC1 and ACC2, have been cloned in rodents and humans. ACC1 is predominantly expressed in lipogenic tissues such as liver and adipose tissue, while ACC2 is predominantly expressed in oxidative tissues such as liver, skeletal muscle and heart.[2] Recent studies reported by Harada et al. indicate that hepatic ACC2 could partially cover ACC1 function as a backup system.[3] Consequently, reduction of malonyl-CoA levels in these tissues by ACC1/2 non-selective inhibitors is expected to reduce de novo fatty acid synthesis and triglyceride (TG)-rich lipoprotein secretion in liver, while increasing fatty acid β-oxidation in liver and skeletal muscle. Therefore, an ACC1/2 non-selective inhibitor might provide a novel therapeutic approach for treating various metabolic disorders.

We previously reported preliminary structure–activity relationships (SAR) of disubstituted (4-piperidinyl)-piperazine derivatives and the discovery of compounds **1** and **2** as potent ACC1/2 non-selective inhibitors (Fig. 1).[4] In this Letter, further optimization of compound **1** by modification of the 2,6-diarylpyridine portion is reported.

To obtain clues for further optimization, the previously constructed ACC-compound **1** docking model was reexamined.[5] The sites in which the 2,6-diaryl groups on the left-hand pyridine group fit were found to be quite different. One site provided a tight lipophilic space, while the other site provided a large space, which was connected to the acetyl-CoA binding site. Thus, we hypothesized that appropriate modification of one of the 2,6-diphenyl



1, R^1 = H : IC_{50} 126 nM
2, R^1 = Me : IC_{50} 76 nM

Figure 1. Structure and ACC activity of compounds **1** and **2**.

* Corresponding author.
E-mail address: tomomichi.chonan@po.rd.taisho.co.jp (T. Chonan).

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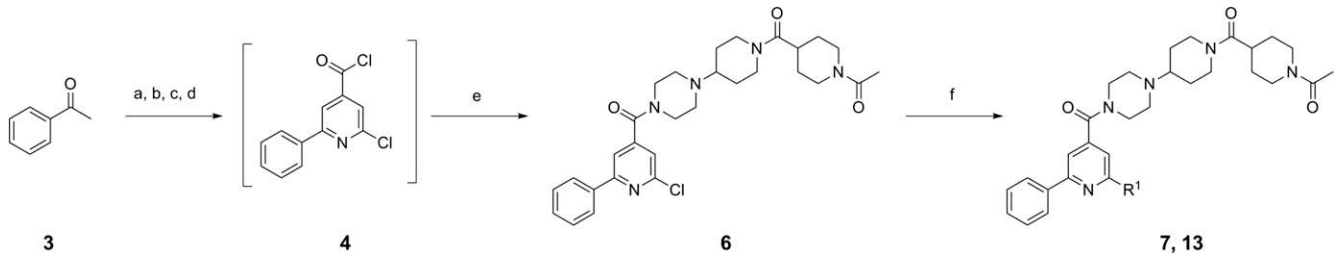


Scheme 1. Reagents and conditions: (a) $(COOEt)_2$, Na, EtOH, rt, 97%; (b) 2-cyanoacetamide, K_2CO_3, acetone, 60 °C, 57%; (c) concd H_2SO_4, H_2O, 160 °C, 63%; (d) $POCl_3$, PCl_5, 110 °C, obtained as a mixture with 2-oxo-6-phenyl-1,2-dihydropyridine-4-carbonyl chloride (**4′**) (**4**:**4′** = 3.5:1); (e) 1-{1-[(1-acetylpiperidin-4-yl)carbonyl]piperidin-4-yl}piperazine (**5**), Et_3N, $CHCl_3$, 0 °C, 48%; (f) arylboronic acid or arylboronic acid pinacol ester, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, CH_3CN/H_2O, 90 °C.

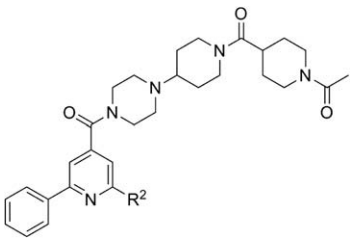
groups of compound **1** would influence the acetyl-CoA binding site, resulting in interference of acetyl-CoA binding and thereby improvement of ACC inhibitory activity.

The synthetic approaches employed to prepare various substituted aryl derivatives are outlined in Schemes 1 and 2. Scheme 1 illustrates the synthesis of compounds **7a**–**m** and **13a**–**j**. The synthesis of the starting 2-chloro-6-phenyl isonicotinoyl chloride (**4**) was reported by Isler et al.[6] Acid chloride **4** was treated with 1-{1-[(1-acetylpiperidin-4-yl)carbonyl]piperidin-4-yl}piperazine (**5**)[4] to give amide **6**, which was coupled with the desired aryl boronic acid or aryl boronic acid pinacol ester[7] to afford target compounds **7a**–**m** and **13a**–**j**. Preparation of the indole derivatives **12a**–**k** is illustrated in Scheme 2. Commercially available 2,6-dichloroisonicotinoic acid (**8**) was coupled with 5-indole boronic acid pinacol ester (**9**) followed by condensation with **5** to give **11**. Compound **11** was coupled with the desired arylboronic acid or arylboronic acid pinacol ester to furnish the target compounds **12a**–**k**.

The derivatives were screened against partially purified human liver ACC enzymes. Among the tested compounds, the selected potent compounds were further evaluated for their ability to decrease FAS and increase FAO in HepG2 cells. FAS inhibition was assessed by measuring the decrease in [^{14}C] acetate incorporation into cellular lipids,[1a] and the effects on FAO were evaluated by measuring the generation of T_2O in the culture media.[8]

The unsymmetric substituted derivatives **7a**–**m** were initially evaluated (Table 1). Substitution of the 3- or 4-position of the phenyl ring with polar functional groups such as hydroxyl, carbamoyl, or carboxylic acid groups as in **7a**–**h** led to a three to fourfold improvement in potency. Of these, the 4-hydroxyethylphenyl derivative **7e** displayed the most potent activity (IC_{50} = 27 nM). However, compound **7e** exhibited considerably reduced activity in the cell based FAS assay (IC_{50} = 1622 nM). The discrepancy between the enzyme and cell activities was considered to be due to

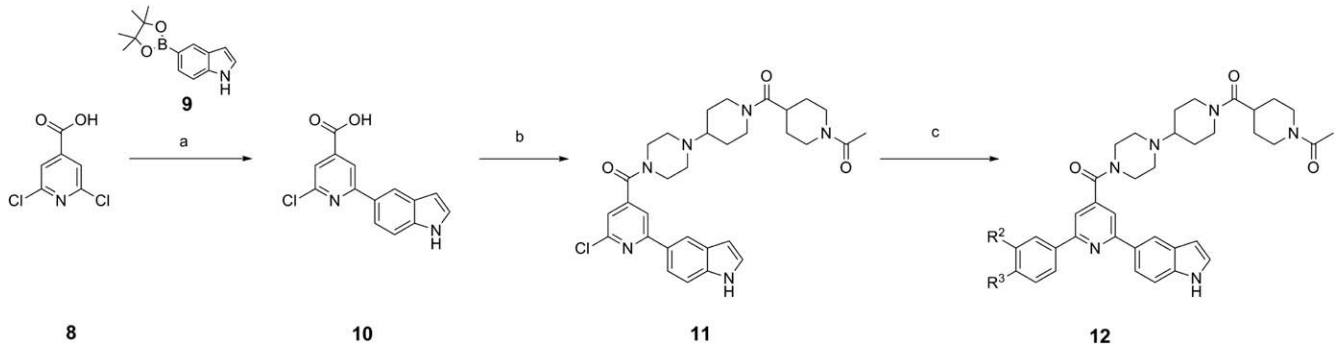
Table 1
In vitro activities of ACC inhibitors **1**, **7a**–**7m**



Compd	R^2	ACC IC_{50}[a] (nM)
1	Phenyl	126
7a	4-HO-phenyl	47
7b	3-$HOCH_2$-phenyl	47
7c	4-$HOCH_2$-phenyl	42
7d	4-H_2NCO-phenyl	80
7e	4-$HOCH_2CH_2$-phenyl	27
7f	4-$HOOCCH_2$-phenyl	75
7g	4-$HOCH_2CH_2O$-phenyl	47
7h	4-$HOCH_2CH_2CH_2$-phenyl	70
7i	1*H*-Indole-5-yl	55
7j	1*H*-Indole-6-yl	47
7k	1*H*-Indazole-5-yl	42
7l	1*H*-Indazole-6-yl	57
7m	Indolin-2-one-5-yl	54

[a] Inhibitory activity of compounds on the malonyl-CoA synthesis of human ACC1/2. The IC_{50} value represents the mean from at least two independent experiments.

the limited cell permeability of **7e**. Indeed, **7e** was found to have poor membrane permeability: PAMPA permeability of **7e** was 6.7×10^{-6} cm/s at pH 6.2. In contrast, compound **1** exhibited high permeability in the PAMPA assay (61.5×10^{-6} cm/s at pH 6.2) and



Scheme 2. Reagents and conditions: (a) **8**, $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O/EtOH = 7/3/2, 150 °C/20 min (microwave); (b) 1-{1-[(1-acetylpiperidin-4-yl)carbonyl]piperidin-4-yl}piperazine (**5**), WSC, HOBt, $CHCl_3$, rt, 62% (from **8**); (c) arylboronic acid or arylboronic acid pinacol ester, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, CH_3CN/H_2O, 150 °C/20 min (microwave), 73–86%.

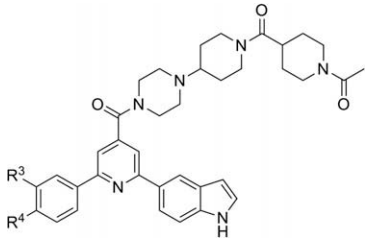
exhibited a relatively small discrepancy between the enzyme and cell assays (IC_{50} = 540 nM). Subsequently, fused heterocycle derivatives were investigated. The 1*H*-indole, 1*H*-indazole and indoline-2-one derivatives **7i**–**m** were found to be two to three fold more potent than compound **1**. Herein, we focus on the 1*H*-indole-5-yl derivative **7i** and describe further SAR studies for the substituent effects on the 6-phenyl and 2-indolyl groups. Membrane permeability of **7i** was good (PAMPA, Pe 46.5 $\times$ 10^{-6} cm/s at pH 6.2) and the cell activity of **7i** exhibited an IC_{50} value of 467 nM.

Substituent effects on the 6-phenyl group of **7i** were evaluated (Table 2). The introduction of fluorine or methyl groups to the phenyl ring at the 3- or 4-position are essentially equipotent to **7i**. The 3- or 4-methoxy derivatives **12e** and **12f** provides slight increased in potency. The trifluoromethyl derivatives **12g** and **12h** were less potent than the parent **7i**. The 3,4-disubstituted derivatives **12i**, **12j** and **12k** were as well or less potent than the mono-substituted derivatives **12a**, **12d**, **12e** and **12f**. No significant substitution effects were observed with this modification. Most likely, the 6-phenyl group is placed in the tight lipophilic pocket while the 2-indolyl group is in the large space connected to the acetyl-CoA binding site.

Finally, substituent effects on the 2-indolyl group of **7i** were examined (Table 3). The mono-methyl derivatives **13a–c** were equipotent to the parent **7i**. Among the dimethyl derivatives **13d–f**, the 2,3-dimethyl derivative **13f** showed the most potent activity. The trimethyl derivative **13g** was less potent than **13f**. Hence, we focused on 2,3-disubstituted derivatives. The 3-ethyl derivative **13h** was substantially less potent than the corresponding dimethyl derivative **13f**. However, the 2-methyl-3-cyano derivative **13i** and 2-*N*,*N*-dimethylaminomethyl-3-methyl derivative **13j** had significantly potent activity.

Molecular modeling studies of compound **13i** (in yellow) are shown in Figure 2 along with overlays of CP-640186 (in purple) and acetyl-CoA (in brown).[5,9] The proposed binding mode of compound **13i** and hACC2 indicates that two hydrogen bonds are formed with the acetyl-Tyr1974 and amide-Glu2230′.[4] The 2-methyl and 3-cyano substituents on the indole group of **13i** are in close proximity to acetyl-CoA in the acetyl-CoA binding site where the nitrogen atom of the cyano group of compound **13i** and the oxygen atom of the phosphate group of acetyl-CoA are close enough to cause steric repulsion (3.19 Å). Consequently,

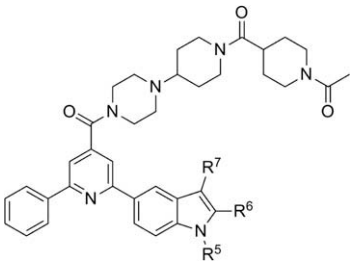
Table 2
In vitro activities and PAMPA permeability of ACC inhibitors **7i**, **12a–12k**



Compd	R^3	R^4	ACC IC_{50}[a] (nM)	PAMPA Pe[b] ($\times 10^{-6}$ cm/s)
7i	H	H	55	46.5
12a	F	H	33	19.9
12b	H	F	48	44.3
12c	Me	H	37	28.1
12d	H	Me	30	39.8
12e	MeO	H	32	ND[c]
12f	H	MeO	21	21.5
12g	CF_3	H	100	ND[c]
12h	H	CF_3	72	ND[c]
12i	F	Me	49	ND[c]
12j	F	MeO	61	ND[c]
12k	MeO	MeO	30	ND[c]

[a] Inhibitory activity of compounds on the malonyl-CoA synthesis of human ACC1/2. The IC_{50} value represents the mean from at least two independent experiments.
[b] Effective permeability at pH 6.2.
[c] No data.

Table 3
In vitro activities and metabolic stability in human liver microsomes of ACC inhibitors **7i**, **13a–13j**



Compd	R^5	R^6	R^7	ACC IC_{50}[a] (nM)	hMS[b] (%)
7i	H	H	H	55	64
13a	Me	H	H	69	85
13b	H	Me	H	59	ND[c]
13c	H	H	Me	36	72
13d	Me	Me	H	117	ND[c]
13e	Me	H	Me	127	ND[c]
13f	H	Me	Me	33	99
13g	Me	Me	Me	105	ND[c]
13h	H	Me	Et	169	ND[c]
13i	H	Me	CN	9.7	ND[c]
13j	H	CH_2NMe_2	Me	8.4	ND[c]

[a] Inhibitory activity of compounds on the malonyl-CoA synthesis of human ACC1/2. The IC_{50} value represents the mean from at least two independent experiments.
[b] % Remaining after 15-min incubation with human liver microsomes (1 mg protein/mL).
[c] No data.

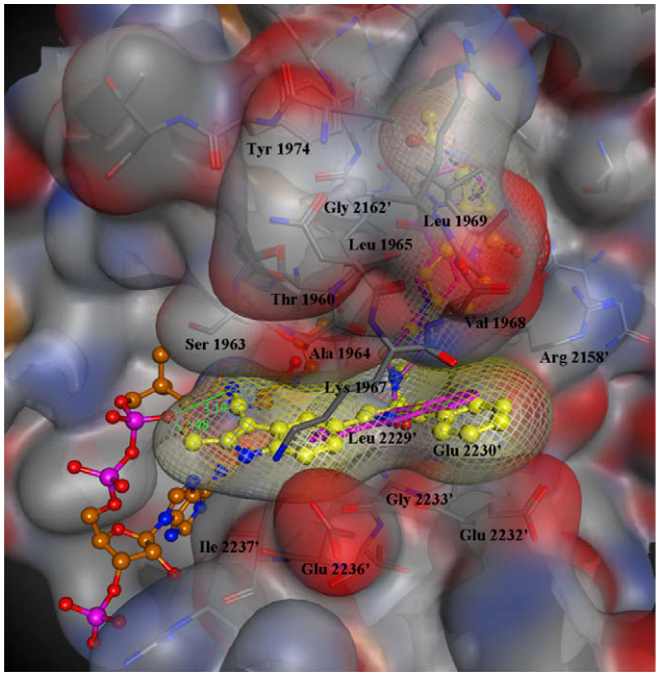


Figure 2. Optimized binding mode of **13i** and acetyl-CoA.

compound **13i** would be predicted to interfere in the binding of acetyl-CoA, leading to the observed improvement in the ACC inhibitory activity of **13i**.

Compound **13f** was metabolically stable in human liver microsomes.[10] In HepG2 cell assays, compound **13f** showed dose-dependent inhibition of FAS with an IC_{50} of 290 nM. In addition, **13f** showed good solubility in water (1116 μg/mL) and good permeability (PAMPA, Pe 32.8×10^{-6} cm/s at pH 6.2).

In summary, the synthesis and evaluation of novel unsymmetric disubstituted pyridines as ACC1/2 inhibitors are reported. Compounds **13i** and **13j** displayed excellent potency as non-selective ACC1/2 inhibitors through our structure-based approach. This work is expected to provide helpful information for the discovery of novel structural ACC inhibitors.

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7. *Typical procedure*. In a glass vial was placed 2-methyl-3-cyano-5-bromoindole (331 mg, 1.41 mmol), bis(pinacolate)diboron (536 mg, 2.11 mmol), Pd(dppf)Cl_2 (115 mg, 0.141 mmol), potassium acetate (414 mg, 4.22 mmol) and DMSO (1.50 mL) under Ar atmosphere. The sealed vial was heated at 90 °C for 24 h and allowed to cool to ambient temperature. The insoluble material was removed by filtration and washed with $CHCl_3$. The filtrate was concentrated in vacuo and the residue was purified by silica gel column chromatography (0–40% AcOEt/*n*-hexane) to afford 2-methyl-3-cyano-5-indole boronic acid pinacol ester (250 mg, 0.89 mmol) as a colorless powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.37 (s, 12H), 2.63 (s, 3H), 7.29–7.37 (m, 1H), 7.64–7.73 (m, 1H), 8.19 (s, 1H), 8.49 (br s, 1H). MS ESI/APCI Dual *m*/*z* 283 $[M+H]^+$.
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