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Symmetrical approach of spiro-pyrazolidinediones as acetyl-CoA carboxylase inhibitors

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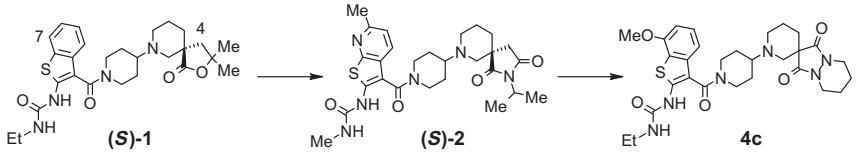
ABSTRACT

Spiro-pyrazolidinedione derivatives without quaternary chiral center were discovered by structure-based drug design and characterized as potent acetyl-CoA carboxylase (ACC) inhibitors. The high metabolic stability of the spiro-pyrazolo[1,2-*a*]pyridazine scaffold and enhancement of the activity by incorporation of a 7-methoxy group on the benzothiophene core successfully led to the identification of compound **4c** as an orally bioavailable and highly potent ACC inhibitor. Oral administration of **4c** significantly decreased the values of the respiratory quotient in rats, indicating the stimulation of fatty acid oxidation.

Acetyl-CoA carboxylase (ACC) catalyzes the biotin-dependent carboxylation of acetyl-CoA to form malonyl-CoA,[1] which is a known substrate for fatty acid synthase in de novo lipogenesis as well as an allosteric inhibitor of carnitine palmitoyltransferase 1 (CPT-1).[2] ACC inhibitors, which could reduce fatty acid synthesis and accelerate fatty acid oxidation by lowering malonyl-CoA levels, are widely known to be promising therapeutic targets for the treatment of obesity.[3] We have previously reported on spiro series as novel ACC inhibitors.[4,5] In particular, spiro-lactone (*S*)-**1** containing a ureidobenzothiophenyl group was designed by structure-based drug design, and its potent inhibitory activity has been demonstrated.[4] Moreover, we successfully identified highly potent and orally bioavailable inhibitor (*S*)-**2** through optimization of the benzothiophene and spiro-piperidine groups by using the relationship between the log*D* value and metabolic stability.[5] This time we made an attempt to adopt a symmetrical approach for spiro structures as one of the solutions. Herein, we describe the synthesis and structure-activity relationship (SAR) of spiro-pyrazolidinediones derivatives and the excellent metabolic stability of the spiro-pyrazolo[1,2-*a*]pyridazine scaffold in human hepatic microsomes.



Spiro-pyrazolidinediones **3a**, **b** were synthesized following the route illustrated in Scheme 1. Carboxylation of ester **5** with benzyl chloroformate gave diester **6**. After the benzyl group of **6** was deprotected under reductive conditions, amide **8** was obtained by condensation with **7** and *Z*-hydrazine. Removal of the Z group in **8** by catalytic hydrogenation followed by cyclization under basic and heat conditions afforded spiro-pyrazolidinedione **9**. Dialkylation of **9** afforded *N,N*-dialkyl derivatives **10a**, **b** with moderate yield, which were easily separated from byproduct *N,O*-dialkyl compounds **11a**, **b** by using silica gel column chromatography. Deprotection of the Boc groups in **10a**, **b** followed by reductive

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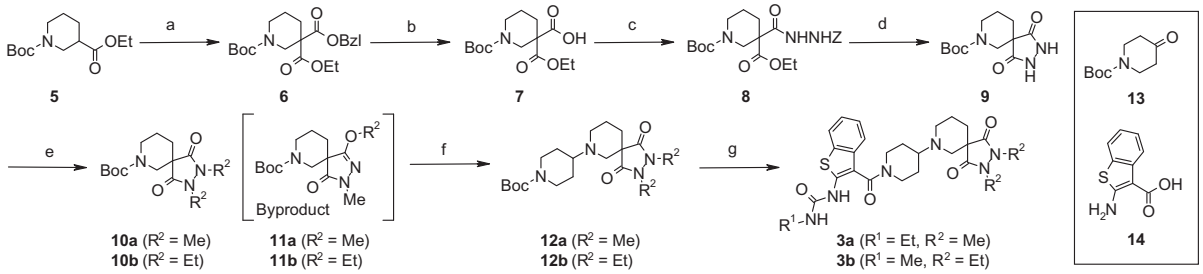
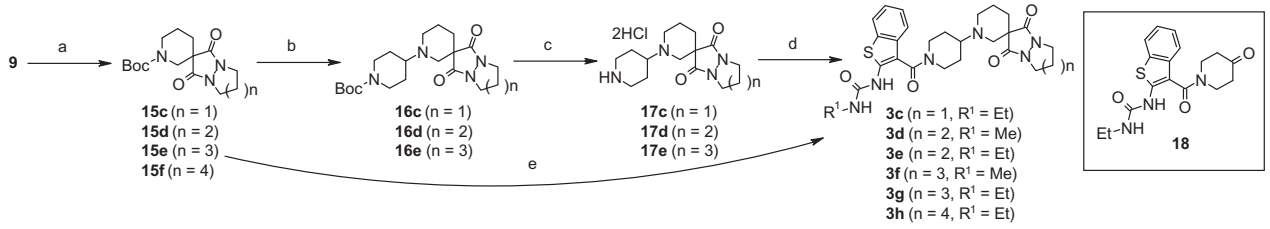


Scheme 1. Reagents and conditions: (a) 1.1 M LHMDS, ZCl, cyclopentylmethyl ether, −78 °C, quant.; (b) H_2/Pd-C, THF, 90%; (c) $ZNHNH_2$, WSC, HOBt, DMF, quant.; (d) (i) H_2/Pd-C, THF, 90%; (ii) pyridine, 100 °C, 91%; (e) MeI or EtI, NaH, DMF, 53% (**10a**), 50% (**10b**); (f) (i) 4 M HCl–EtOAc, 94% (R^2 = Me), quant. (R^2 = Et); (ii) **13**, $NaBH(OAc)_3$, THF–AcOH, 30% (**12a**), 55% (**12b**); (g) (i) 4 M HCl–EtOAc, 92% (R^2 = Me), quant. (R^2 = Et); (ii) **14**, WSC, HOBt, Et_3N, DMF, 72% (R^2 = Me), 85% (R^2 = Et); (iii) R^1NCO, pyridine, 80 °C, 77% (**3a**), 66% (**3b**).



Scheme 2. Reagents and conditions: (a) $Br(CH_2)_nBr$, NaH, DMF, 48% (**15c**), 70% (**15d**), 70% (**15e**), 14% (**15f**); (b) (i) 4 M HCl–EtOAc, 87% (n = 1), 87% (n = 2); (ii) **13**, $NaBH(OAc)_3$, THF or CH_2Cl_2, 30% (**16a**), 55% (**16b**), 71% (2 steps, **16c**); (c) 4 M HCl–EtOAc, 92% (n = 1), quant. (n = 2), 90% (n = 3); (d) (i) **14**, WSC, HOBt, Et_3N, DMF, 65% (n = 1), 32% (n = 2); (ii) R^1NCO, pyridine, 70 °C, 71% (**3c**), 34% (**3d**), 83% (**3e**), 29% (2 steps, **3f**), (e) (i) 4 M HCl–EtOAc; (ii) **18**, $NaBH(OAc)_3$, Et_3N, CH_2Cl_2, 51% (2 steps, **3g**), 37% (2 steps, **3h**).

alkylation with Boc-4-piperidone (**13**) provided compounds **12a**, **b**, which were converted to desired compounds **3a**, **b** by deprotection of the Boc groups, acylation with 2-amino-3-benzothiophenecarboxylic acid (**14**), and formation of an ureido group using alkyl isocyanate.

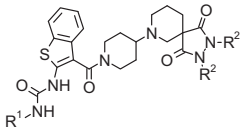
Other spiro-pyrazolidinediones (**3c–f**) were also easily synthesized (Scheme 2), in a manner similar to the one shown in Scheme 1. Only **3g**, **h** were directly prepared by reductive alkylation of deprotected **15e**, **f** with 1-ethyl-3-(3-[(4-oxopiperidin-1-yl)carbonyl]-1-benzothiophen-2-yl)urea (**18**).[6]

Synthesis of **4a–c** with 7-methoxybenzothiophenyl derivatives is shown in Scheme 3. Starting from commercially available thiophene **19**, α-keto bromide **20** was prepared according to the known protocol.[7] Successive aromatization and alkylation of phenol **21** afforded **22**, which was hydrolyzed to give carboxylic acid **23**. Compound **23** was converted to **4a–c** by condensation with **17d** and subsequent formation of an ureido group.

The spiro compounds prepared in this study were evaluated for their inhibitory activities toward human ACC1/2 by using the malachite green method.[8] The results of ACC inhibition, human metabolic stability,[9] and lipophilicity (log$D$) are summarized in Tables 1–3. Because the 50% inhibitory concentration (IC_{50}) ratios of ACC2 and ACC1 inhibitory activities were nearly the same, we primarily discuss the SAR for ACC2 inhibition in the present report.

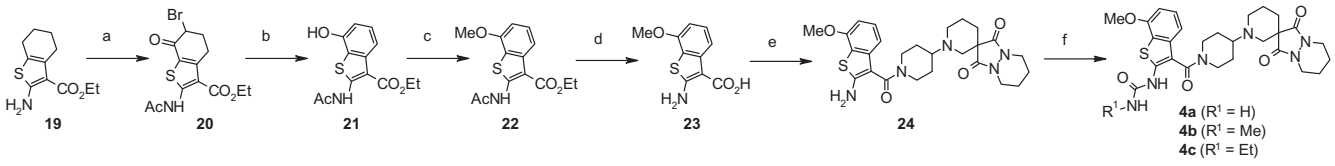
As previously reported,[4] the co-crystal data of human ACC2 and (*S*)-**1** revealed that the carbonyl group of the spiro-lactone ring interacts with the backbone of Gly2162. In fact, the chiral resolution of **1** demonstrated that (*S*)-**1** (ACC2: IC_{50} = 5.4 nM) was more potent than (*R*)-**1** (ACC2: IC_{50} = 1,600 nM). Moreover, a wide space filled with water at the periphery of the 4-position (methylene) in spiro-lactone (*S*)-**1** was also observed (Fig. 1). The result suggests

Table 1
Acetyl-CoA carboxylase (ACC) inhibitory activity, human metabolic stability (hMS), and log$D$ values of spiro-pyrazolidinediones **3a–h**



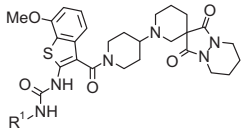
	Substituents		IC_{50} (nM)		hMS[a]	log$D$[b]
	R^1	R^2	ACC1	ACC2		
3a	Et	Me	120	20	12	2.03
3b	Me	Et	41	8.1	120	2.33
3c	Et	$-(CH_2)_3-$	170	33	25	1.93
3d	Me	$-(CH_2)_4-$	200	36	12	2.14
3e	Et	$-(CH_2)_4-$	130	23	18	2.45
3f	Me	$-(CH_2)_5-$	48	8.7	71	2.14
3g	Et	$-(CH_2)_5-$	43	7.5	100	2.48
3h	Et	$-(CH_2)_6-$	22	5.1	200	2.73

[a] In vitro metabolic clearance in human hepatic microsomes (μL/min/mg protein).
[b] Log$D$ values were obtained using high-performance liquid chromatography (HPLC) at pH 7. IC_{50} = 50% inhibitory concentration.



Scheme 3. Reagents and conditions: (a) (i) Ac_2O, AcOH; (ii) $Ce(SO_4)_2$, AcOH, $CHCl_3$, 45%; (iii) Br_2, $CHCl_3$, reflux, 89%; (b) Li_2CO_3, DMF, 120 °C, 41%; (c) MeI, K_2CO_3, DMF, 59%; (d) 8 M NaOH, EtOH, reflux, 68%; (e) **17d**, WSC, HOBt, Et_3N, DMF, 81%; (f) $Cl_3CCONCO$, THF then 7 M NH_3 in MeOH, 77% (**4a**) or R^1NCO, pyridine, 70 °C, 85% (**4b**), 51% (**4c**).

Table 2
Acetyl-CoA carboxylase (ACC) inhibitory activity, human metabolic stability (hMS), and log*D* values of spiro-pyrazolo[1,2-*a*]pyridazines **4a–c**



	R^1	IC_{50} (nM)		hMS[a]	log*D*[b]
		ACC1	ACC2		
4a	H	22	5.9	15	1.76
4b	Me	25	6.1	6	2.08
4c	Et	21	4.9	35	2.33

[a] In vitro metabolic clearance in human hepatic microsomes (μL/min/mg protein).
[b] Log*D* values were obtained by using high-performance liquid chromatography (HPLC) at pH 7. IC_{50} = 50% inhibitory concentration.

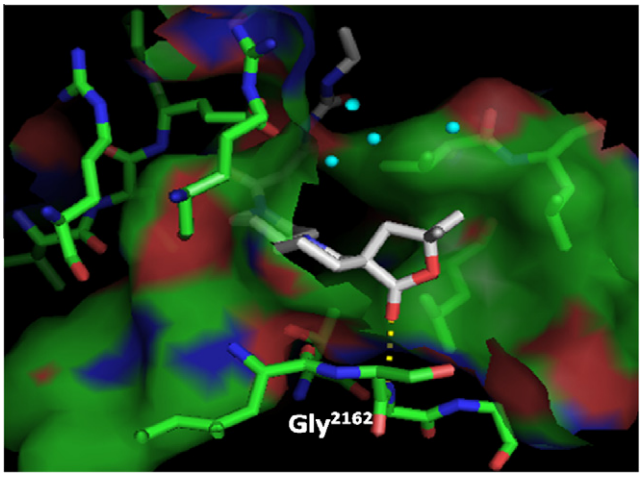


Figure 1. Detailed crystal structure of the spiro-lactone core in (*S*)-**1** with the carboxyl terminal (CT) domain of human acetyl-CoA carboxylase 2 (ACC2).

that incorporation of a polar group, for example, an oxo group at the 4-position in the spiro-lactone, would be favorable for ACC2 inhibition and prompted us to design a symmetrical spiro-pyrazolidinedione scaffold. The results of the in vitro activities of the spiro-pyrazolidinedione derivatives are summarized in Table 1. All synthesized compounds showed activities in the order of 10^{-8} to 10^{-9} M. As introduction of a more lipophilic and bulky substituent on the imidazolone or lactam core potentiated the activity,[5] compounds **3b** and **3f–h** displayed potencies of nanomolar order.

In addition, we could identify a high metabolic property of pyrazolidinediones. Examination of substituents on the spiro-5-membered ring suggested that the log*D* values inversely correlated with metabolic stability in human microsomes except for those of **3d**, **e** (Fig. 2). Interestingly, pyrazolo[1,2-*a*]pyridazine derivatives (red diamonds: **3d**, **e**) exhibited higher metabolic stability, though the log*D* values of **3d**, **e** were the same as those of **3b** and **3f–h**. Therefore, we selected the pyrazolo[1,2-*a*]pyridazine core and modified the benzothiophene moiety to enhance the activity.

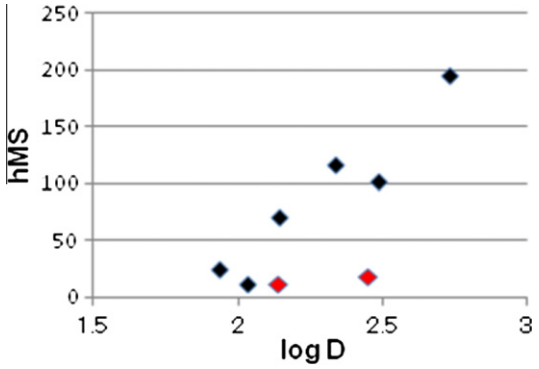


Figure 2. Relationships between log*D* values and metabolic stability of spiro-pyrazolidinediones **3a–h**. Red diamonds correspond to pyrazolo[1,2-*a*]pyridazine derivatives **3d** and **3e** and black diamonds correspond to the compounds. hMS: human metabolic stability.

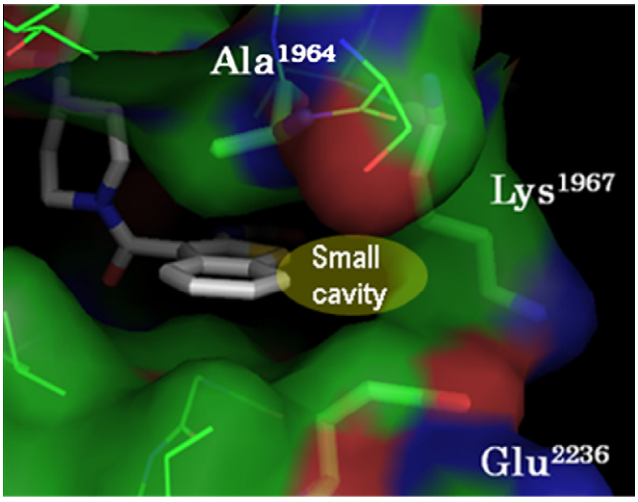


Figure 3. Detailed crystal structure of the benzothiophene part in (*S*)-**1** with the carboxyl terminal (CT) domain of human acetyl-CoA carboxylase 2 (ACC2).

The results of the spiro-compounds possessing the pyrazolo[1,2-*a*]pyridazine moiety are summarized in Table 2. As the X-ray co-crystal data of ACC2 with (*S*)-**1** also suggest the presence of a small cavity formed by Glu2236, Lys1967, and Ala1964 at the 7-position of the benzothiophene ring (Fig. 3), introduction of a 7-methoxy group on the ring was examined as to whether it potentiates the

Table 3
Pharmacokinetic profile of **4c**[a]

	i.v. (0.1 mg/kg)			p.o. (1 mg/kg)			
	CL_{total}[b] (L/h/kg)	V_{ss}[c] (L/kg)	MRT[d] (h)	C_{max}[e] (ng/mL)	T_{max}[f] (h)	AUC[g] (ng·h/mL)	F[h] (%)
4c	0.78	0.97	1.3	71	0.25	180	14

[a] Rat cassette dosing (n = 3); nonfasted Crl: CD(SD)(IGS) rats.
[b] Total clearance.
[c] Volume of distribution at steady state.
[d] Mean residence time.
[e] Maximal plasma concentration.
[f] Time of maximal concentration.
[g] Area under the plasma concentration versus time curve.
[h] Bioavailability.

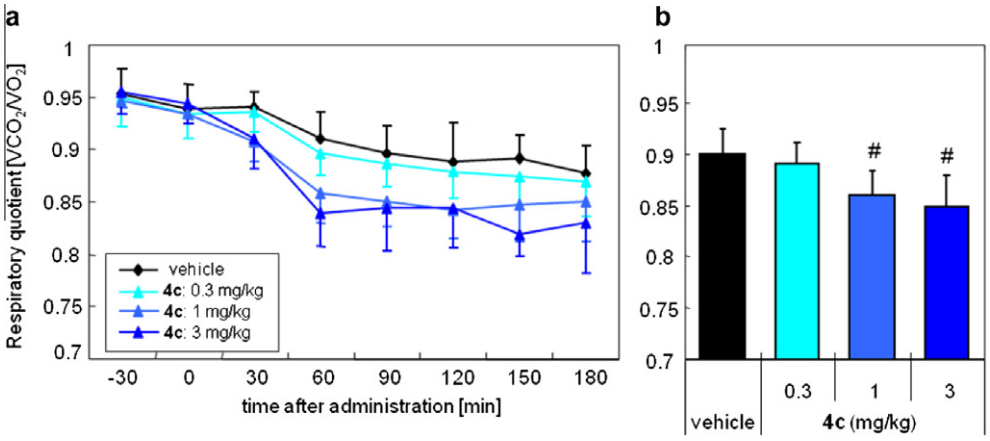


Figure 4. Decrease in the respiratory quotient (RQ) values by **4c**. Wistar fatty rats ($n = 6$, male) were administered with either vehicle or compound **4c** (0.3, 1, 3 mg/kg). (a) Times course of RQ. (b) Average RQ values at 3 h after administration. Values are presented as means ± SEM. $^{\#}p < 0.025$ (Williams test).

ACC2 inhibitory activity. In fact, the 7-methoxybenzothiophene derivatives **4a–c** exhibited potencies of nanomolar order. Moreover, all compounds retained excellent metabolic stability independent of their log*D* values. Among these compounds, **4c** with the highest ACC inhibitory activity had a favorable pharmacokinetic profile in rat cassette dosing [10] as described in Table 3.

The selected compound **4c** also exhibited potent rat ACC inhibitory activity (rACC1: IC_{50} = 28 nM, rACC2: IC_{50} = 13 nM) and was tested at doses of 0.3, 1, and 3 mg/kg in Wistar fatty rats to confirm increased fatty acid oxidation by lowering the respiratory quotient (RQ). The compound **4c**-induced decrease in RQ values was dose-dependent and reached a significant level at a dose of 1 mg/kg, indicating stimulation of fatty acid oxidation (Fig. 4).[11]

In conclusion, we designed and synthesized various spiropyrazolidinedione derivatives without an asymmetric center. The compounds displayed high ACC inhibitory activities and their log*D* values inversely correlated with their metabolic stability in human microsomes. Only pyrazolo[1,2-*a*]pyridazines **3d** and **3e** showed excellent metabolic stability independent of their log*D* values. Moreover, X-ray co-crystal data showed that the introduction of a methoxy group at the 7-position of the benzothiophene core increased the activity. We identified orally bioavailable and highly potent ACC inhibitor **4c**, which stimulated fatty acid oxidation in rats, by combining the pyrazolo[1,2-*a*]pyridazine scaffold and the 7-methoxybenzothiophene moiety.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.062.

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