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Design, synthesis, and structure–activity relationships of novel spiro-piperidines as acetyl-CoA carboxylase inhibitors

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ABSTRACT

Spiro-lactone (*S*)-**1** is a potent acetyl-CoA carboxylase (ACC) inhibitor and was found to be metabolically liable in human hepatic microsomes. To remove one of the risk factors in human study by improving the metabolic stability, we focused on modifying the spiro-lactone ring and the benzothiophene portion of the molecule. Spiro-imide derivative **8c** containing a 6-methylthieno[2,3-*b*]pyridine core exhibited potent ACC inhibitory activity and favorable pharmacokinetic profiles in rats.

Obesity has become a serious health problem worldwide over the past few decades, due in part to increased food intake and reduced physical activity.[1] Moreover, obesity is associated with a variety of serious human diseases, notably type 2 diabetes, cardiovascular diseases, depression, and cancer. Acetyl-CoA carboxylase (ACC) catalyzes the biotin-dependent carboxylation of acetyl-CoA to form malonyl-CoA,[2] which is a known substrate for fatty acid synthase in de novo lipogenesis and is also an allosteric inhibitor of carnitine palmitoyltransferase 1.[3] Therefore, ACC inhibition may lower malonyl-CoA levels, resulting in reduced fatty acid synthesis, accelerated fatty acid oxidation, and consequently improved insulin sensitivity. In rodents and humans, two ACC isoforms, ACC1, which is primarily expressed in lipogenic tissues, and ACC2, which is expressed in oxidative tissues, have been reported.[4] Genetic studies have demonstrated that knocking out ACC1 in mice is embryonically lethal,[5] whereas ACC2 homozygous knockout mice are healthy, fertile, and have a higher fatty acid oxidation rate and reduced fat accumulation compared to wild-type mice.[6]

We previously reported novel spiro-lactone (*S*)-**1** containing a ureidobenzothiophenyl group as a potent ACC inhibitor.[7] The X-ray co-crystal structure data of the human ACC2 carboxyl transferase domain in complex with (*S*)-**1** indicated that not only the two carbonyl groups of amide and lactone are promptly bound to the two backbones of Gly^{2162} and Glu^{2230} but also the ureido moiety interacts with the side chain of Glu^{2230} (Fig. 1). However, (*S*)-**1** showed poor human metabolic stability, which may cause unfavorable pharmokinetic (PK) profiles, including variations in blood exposure level or high elimination clearance in clinical trials. In order to remove one of these risk factors for human study, we examined methods to improve the metabolic stability by lowering the lipophilicity of the spiro-5-membered moiety and benzothiophenyl group as shown in Figure 1. Herein, we describe the design, synthesis, structure–activity relationship (SAR), and the relationship between lipophilicity (log*D*) and metabolic stability.

Synthesis of spiro-lactams **6a–d** and spiro-imides **2a,b** is outlined in Scheme 1. Compound **10**,[7] derived from ester **9**, was converted to piperidinopiperidine derivative **11** by deprotection of the Boc group and subsequent reductive alkylation with 1-Boc-4-piperidone (**18**). Spiro-lactams **13a–c** were obtained by reducing the cyano group with Raney-Ni followed by alkylation of the resultant lactam using NaH and alkyl halide. Finally, desired compounds **6a–d** were prepared by deprotection of the Boc group in **12** and **13a–c**, condensation with benzothiophenecarboxylic acid **19**, and ureide formation using alkyl isocyanate or trichloroacetyl isocyante followed by ammonolysis. Alkylation of ester **9** with benzyl bromoacetate followed by deprotection of the Boc group in **14** and reductive alkylation afforded piperidinopiperidine **15**. After the benzyl group of **15** was removed under reducing conditions, amides **16a,b** were obtained by condensation with primary amines. Cyclization by treatment with NaH proceeded to give imide derivatives **17a,b**. Compound **17a** was converted to the desired compounds **2a,b** using a procedure similar to that used for spiro-lactams **6a–d**. Compounds **13c** and **17b** were asymmetrically separated by chiral HPLC to give

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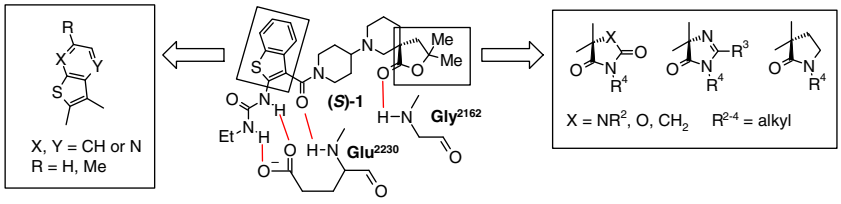
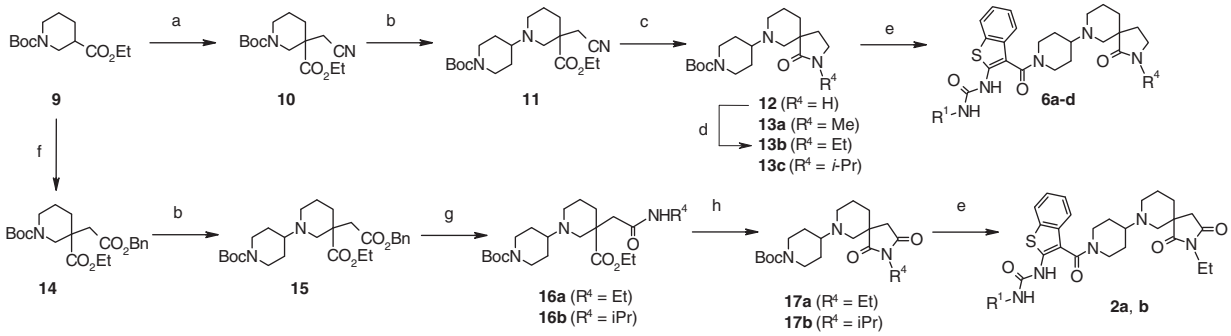
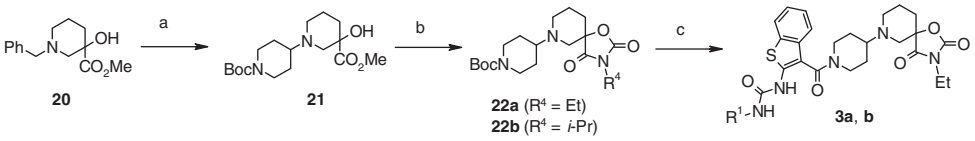


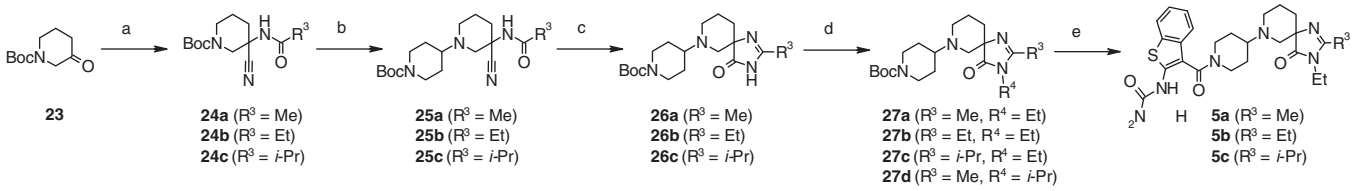
Figure 1. Synthetic strategy for spiro-piperidine derivatives.



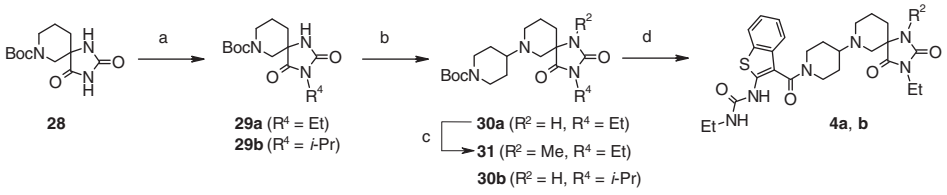
Scheme 1. (a) $BrCH_2CN$, LHMDS, THF, −78 °C, 26%; (b) (i) 4 M HCl–AcOEt; (ii) 1-Boc-4-piperidone (**18**), Et_3N, $NaBH(OAc)_3$, THF, 67% (**11**), 70% (**15**); (c) Raney Ni, H_2 (0.5 MPa), NH_3, EtOH, 88%; (d) R^4-I, NaH, DMF, 75% (**13a**), 68% (**13b**), 60% (**13c**); (e) (i) 4 M HCl, dioxane; (ii) 2-amino-3-benzothiophenecarboxylic acid (**19**), WSC, HOBt, Et_3N, DMF, 46% (for **6a**), 89% (for **6b**), 91% (for **6c**), 50% (for **6d**), 67% (for **2a**,**b**); (iii) $Cl_3CCONCO$, THF then 7 M NH_3–MeOH, 82% (**2a**) or R^1NCO, pyridine, 80 °C, 30% (**6a**), 52% (**6b**), 40% (**6c**), 54% (**6d**), 76% (**2b**); (f) $BrCH_2CO_2Bn$, LHMDS, THF, −78 °C, 78%; (g) (i) H_2, Pd/C, EtOH, 100%; (ii) R^4NH_2, WSC, HOBt, DMF, 80% (**16a**), 100% (**16b**); (h) NaH, DMF, 75% (**17a**), 69% (**17b**).



Scheme 2. (a) (i) H_2, $Pd(OH)_2$/C, EtOH; (ii) **18**, $NaBH(OAc)_3$, THF, 52% (two steps); b) R^4NCO, NaH, THF, 91% (**22a**), 75% (**22b**); (c) 4 M HCl–dioxane, 100%; (ii) **19**, WSC, HOBt, Et_3N, DMF, 87%; iii) $Cl_3CCONCO$, THF then 7 M NH_3–MeOH, 78% (**3a**) or EtNCO, pyridine, 80 °C, 83% (**3b**).



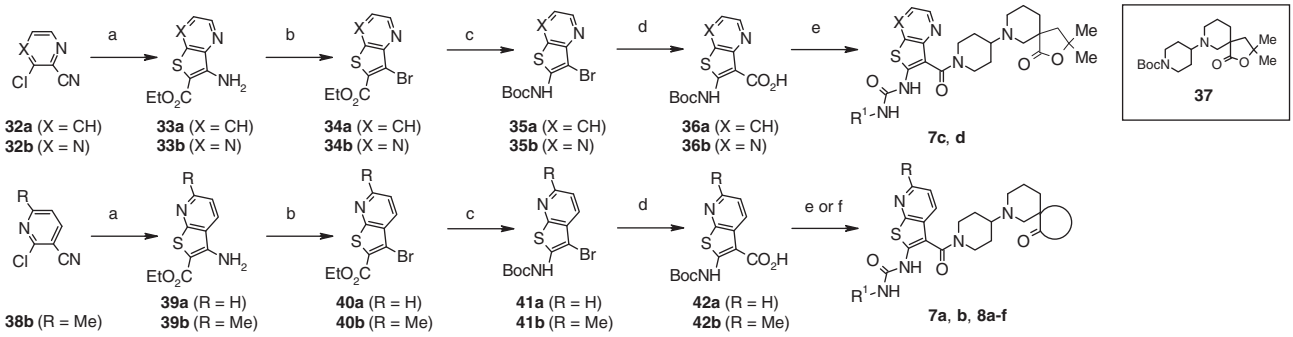
Scheme 3. (a) (i) NH_4Cl, KCN, *i*-PrOH, aq NH_3; (ii) R^3-COCl, Et_3N, THF, 78% (**24a**), 100% (**24b**), 84% (**24c**); (b) (i) 4 M HCl–AcOEt; (ii) **18**, $NaBH(OAc)_3$, THF, 43% (**25a**), 80% (**25b**), 64% (**25c**); (c) aq NaOH, H_2O_2, EtOH, 60 °C, 72% (**26a**), 50% (**26b**), 55% (**26c**); d) R^2-I, NaH, DMF, 85% (**27a**), 71% (**27b**), 30% (**27c**), 33% (**27d**); (e) 4 M HCl–dioxane; (ii) **19**, WSC, HOBt, Et_3N, DMF, 87% (for **5a**), 43% (for **5b**), 80% (for **5c**); (iii) $Cl_3CCONCO$, THF then 7 M NH_3–MeOH, 100% (**5a**), 77% (**5b**), 14% (**5c**).



Scheme 4. (a) R^4-I, K_2CO_3, DMF, 32% (**29a**), 41% (**29b**); (b) (i) 4 M HCl–AcOEt; (ii) **18**, $NaBH(OAc)_3$, Et_3N, CH_2Cl_2, 74% (**30a**), 50% (**30b**); (c) NaH, MeI, DMF, 100%; (d) (i) 4 M HCl–AcOEt, 100% (for **4a**), 94% (for **4b**); (ii) **19**, WSC, HOBt, Et_3N, DMF, 100% (for **4a**), 88% (for **4b**); (iii) EtNCO, pyridine, 80 °C, 60% (**4a**), 70% (**4b**).

(*S*)-**13c** and (*S*)-**17b** in 49% and 44% yield, respectively. As shown in Scheme 5, these chiral intermediates were used to prepare **8b** and **8c**, stereochemistry of which was determined using single X-ray crystallographic analysis.[8]

Spiro-oxazolidinediones **3a,b** were prepared using the method shown in Scheme 2. Hydroxy ester **20**, which was prepared from commercially available 1-benzyl-3-piperidone via a known procedure,[9] was converted to piperidinopiperidine derivative **21** by



Scheme 5. (a) $HSCH_2CO_2Et$, NaOEt, DMF, 70 °C, 86% (**33a**), 72% (**33b**), 97% (**39b**); (b) *t*-BuONO, $CuBr_2$, MeCN, 55% (**34a**), 42% (**34b**), 66% (**40a**); (c) (i) aq NaOH, THF–MeOH; (ii) DPPA, Et_3N, *t*-BuOH, 41% (**35a**), 25% (**35b**), 74% (**41a**), 82% (**41b**, two steps); (d) *n*-BuLi, THF, −78 °C; CO_2, 60% (**36a**), 79% (**42a**), 80% (**42b**); (e) (i) **37**, WSC, HOBt, Et_3N, DMF, 99% (for **7a**), 60% (for **7b**, two steps), 70% (for **7c**), 46% (for **7d**); (ii) TFA then R^1NCO, pyridine, 80 °C, 54% (**7a**), 76% (**7b**), 80% (**7c**), 67% (**7d**); (f) (i) (*S*)-**13c**, (*S*)-**17b**, (*S*/*R*)-**22b**, (*S*/*R*)-**27d**, (*S*)-**37**, or **30b** treated with 4 M HCl–AcOEt then WSC, HOBt, Et_3N, DMF, 80% (for **8a**), 86% (for **8b**), 58% (for **8c**), 52% (for **8d**), 70% (for **8e**), 67% (for **8f**); (ii) TFA then R^1NCO, pyridine, 80 °C, 53% (**8a**), 67% (**8b**), 76% (**8c**), 81% (**8d**), 23% (8e), 51% (**8f**).

deprotection of the benzyl group and condensation with **18**. Construction of the oxazolidinedione ring was performed by treatment with alkyl isocyanates and NaH to give **22a**,**b**. Finally, compounds **3a**,**b** were obtained from **22a** in a similar manner to that shown in Scheme 1. Compound **22b** was asymmetrically separated by chiral HPLC to give chiral (*S*/*R*)-**22b** in 42% yield. As shown in Scheme 5, this chiral intermediate was used to prepare **8d**, stereochemistry of which was not determined.

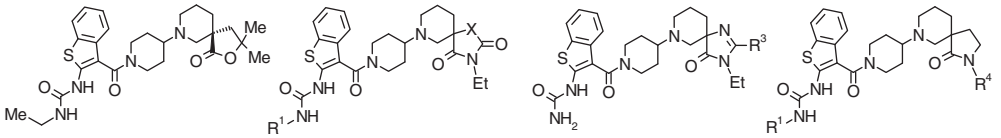
The synthetic route for spiro-imidazolones **5a–c** is summarized in Scheme 3. The Strecker reaction of **23** followed by acylation afforded 3-acylamino-3-cyanopoperidines **24a–c**. Successive deprotection of the Boc group and reductive alkylation with **18** provided compounds **25a–c**. Cyclization of **25a–c** proceeded smoothly under basic conditions to give imidazolone derivatives **26a–c**. After introduction of an alkyl group to **26a–c** using NaH and alkyl halide, **5a–c** was obtained from **27a–c** using the same method shown in Scheme 1. Optically active (*S*/*R*)-**27d**, which was obtained by chiral resolution of **25a** and subsequent cyclization and N-alkylation, was used to synthesize **8e** as shown in Scheme 5. The stereochemistry of **8e** was also not determined.

Preparation of spiro-imidazolidinediones **4a,b** was carried out using the method described in Scheme 4. Starting with spiro-imidazolidinedione **28**, selective N-alkylation of the ring in **29a**,**b** and introduction of a 1-Boc-piperidine moiety afforded **30a**,**b**. Compound **30a** was methylated on the remaining nitrogen atom of the ring to give **31**. Compounds **30a** and **31** were converted to compounds **4a**,**b** using the similar method to that shown in Scheme 1.

Compounds **7c**,**d** were prepared according to the synthetic route shown in Scheme 5. Commercially available pyridine or pyrazine derivatives **32a**,**b** were converted into thienopyridine or thienopyrazine **33a**,**b**, which were subjected to the Sandmeyer reaction to afford **34a**,**b**.[10] Successive hydrolysis and the Curtius rearrangement reaction gave **35a**,**b**, followed by introduction of carboxylic acid at the 3-position to obtain **36a**,**b**. Condensation with deprotected spiro-lactone **37**[7] and conversion of the Boc group to a ureide moiety afforded desired compounds **7c**,**d**. Compounds **7a**,**b** and **8a–f** were also prepared using the same method as was used for the synthesis of **7c**,**d**.

The spiro compounds prepared in this study were evaluated for their ability to inhibit human ACC1 and ACC2 using the malachite

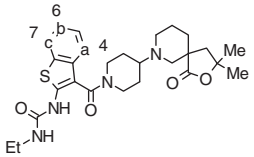
Table 1
ACC inhibitory activity, human metabolic stability, and log*D* of spiro-piperidines **2–6**[a]



	Substituents					IC_{50} (nM)		hMS[b]	log*D*[c]
	X	R^1	R^2	R^3	R^4	ACC1	ACC2		
(*S*)-**1**	—	—	—	—	—	32	5.4	230	3.01
1						63	13	180	3.01
2a	CH_2	H	—	—	—	350	23	64	2.20
2b	CH_2	Et	—	—	—	230	32	130	2.82
3a	O	H	—	—	—	290	49	41	1.85
3b	O	Et	—	—	—	190	40	98	2.47
4a	N	Et	H	—	—	280	22	110	2.44
4b	N	Et	Me	—	—	360	51	190	2.79
5a	—	—	—	Me	—	300	75	60	1.77
5b	—	—	—	Et	—	410	43	150	2.09
5c	—	—	—	*i*-Pr	—	80	17	230	2.43
6a	—	Et	—	—	H	650	79	35	2.06
6b	—	Et	—	—	Me	510	64	45	2.23
6c	—	Et	—	—	Et	350	33	71	2.62
6d	—	Me	–	——	*i*-Pr	120	18	66	2.61

[a] Racemate.
[b] In vitro metabolic clearance in human hepatic microsomes (μL/min/mg protein).
[c] log*D* was measured using HPLC (pH 7.4).

Table 2
ACC inhibitory activity, human metabolic stabilities, and log*D* of spiro-Lactones **7**[a]



	Substituents			IC_{50} (nM)		hMS[b]	log*D*[c]
	a	b	c	ACC1	ACC2		
7a	C–H	C–H	N	110	16	110	2.08
7b	C–H	C–Me	N	78	14	79	2.49
7c	N	C–H	C–H	150	27	120	2.45
7d	N	C–H	N	150	37	110	2.32

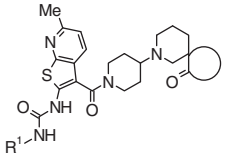
[a] Racemate.
[b] In vitro metabolic clearance in human hepatic microsomes (μL/min/mg protein).
[c] log*D* was measured using HPLC (pH 7.4).

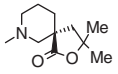
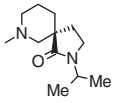
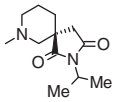
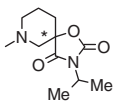
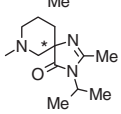
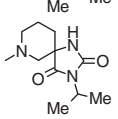
green method.[11] The results of ACC1/2 inhibition studies, human metabolic stability,[12] and lipophilicity (log*D*) are summarized in Tables 1–3. Because the IC_{50} ratios of the ACC2 and ACC1 inhibitory activities were nearly the same, we primarily discuss only the SAR for ACC2 inhibition in the present report.

First, we converted the spiro-lactone ring into various spiro-5-membered rings to conduct a detailed investigation of SAR and human metabolic stability (Table 1). Since designed spiro structures contain a key carbonyl group that can interact with the backbone of Gly^{2162}, all compounds (imide **2**, oxazolidinedione **3**, imidazolidinedione **4**, imidazolone **5**, and lactam **6**) predictably displayed 10^{-8} M order potency. As a result of modification of the spiro ring, installation of an oxo group (**2b**) to the spiro-lactam ring (**6c**), and replacement of hetero atoms (**3b**, **4a**) to the spiro-imide ring (**2b**), activity was retained. Methylation (R^2 groups) of the imidazolidinedione core resulted in a threefold decrease in potency (**4a** vs **4b**). We next examined the substitution effect of R^3 and R^4 groups in the spiro ring. Incorporation of a dimethyl group at the 3-position of the spiro-lactone ring significantly enhanced activity;[7] introduction of additional bulky substituent at the R^3 position of the imidazolone ring led to more potent inhibitory activity (**5a** vs **5b** vs **5c**). Furthermore, the X-ray co-crystal structure data of ACC2 and (*S*)-**1** indicates that a wide lipophilic cavity exists around the substituent R^4 and that alkyl substitution on the nitrogen of the lactam ring potentiates ACC2 inhibitory activity. The inhibitory activities were predictably increased in the order of an unsubstituted (**6a**), methyl (**6b**), ethyl (**6c**), and isopropyl group (**6d**) in the lactam set. Converting the substituent on the ureide group (R^1) did not affect ACC2 inhibition (**2a** vs **2b**, **3a** vs **3b**), as we previously reported.[7] Regarding human metabolic stability, all spiro derivatives exhibit an inverse correlation with their log*D* values as shown in Figure 2. Among these, the imidazolone ring (red diamonds: **5a–c**) was characterized as a metabolically liable core, particularly in the case of **5b,c**. A methyl group (**5a**) introduced as the R^3 in the imidazolone core showed acceptable inhibitory activity and metabolic stability. These results suggest that lipophilic substitution (isopropyl) of the R^4 group of **2–6** and no additional substitution as R^2 (hydrogen) for **4** or R^3 (methyl) group for **5** are preferable for ACC2 inhibitory activity and metabolic stability.

We next assessed the effect of introducing hetero atoms into the benzothiophene ring to lower the lipophilicity of the compounds. The results with prototype spiro-lactone are shown in Table 2. Introduction of a nitrogen atom at the 7-position of the benzothiophene ring (thieno[2,3-*b*]pyridine **7a**) retained activity compared with racemate **1** (ACC2: IC_{50} = 13 nM). Thieno[3,2-*b*]pyridine **7c** and thieno[2,3-*b*]pyrazine **7d** showed slightly less potent activity compared with **7a**. Methyl substitution at the 6-position of **7a** had a minimal effect with regard to ACC2 inhibitory activity, but rendered higher metabolic stability to **7b** despite elevation of the log*D* value (**7b** vs **7a**). This result suggests that blocking the 6-position of thieno[2,3-*b*]pyridine core as a metabolic site may be successful. We then evaluated various spiro-piperidines with the 6-methylthieno[2,3-*b*]pyridine to confirm the potential of improvement in metabolic stability.

Table 3
ACC inhibitory activity, human metabolic stabilities, and log*D* of spiro-piperidines **8**



	R^1	Spiro ring	Configuration	IC_{50} (nM)		hMS[a]	log*D*[b]
				ACC1	ACC2		
8a	Me		*S*	48	4.4	90	2.18
8b	Et		*S*	93	9.0	45	2.43
8c	Me		*S*	160	8.5	33	2.51
8d	Et		ND[c]	160	15	38	2.39
8e	Et		ND[c]	76	15	47	2.27
8f	Et		Racemate	150	17	26	2.39

[a] In vitro metabolic clearance in human hepatic microsomes (μL/min/mg protein).
[b] log*D* was measured using HPLC (pH 7.4).
[c] Absolute configuration has not been determined.

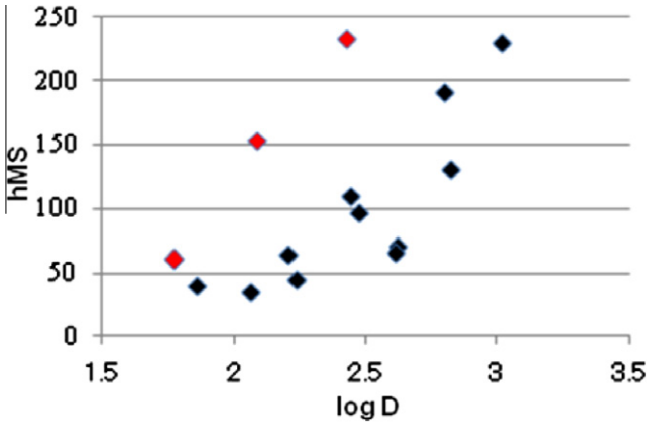


Figure 2. Relationship between log*D* and metabolic stability of various spiro-piperidines. Red diamonds indicate spiro-imidazolone and black diamonds indicate other spiro derivatives.

Table 4
Pharmacokinetic profile of **8c**[a]

Compound	iv (1 mg/kg)			po (3 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] (%)
8c	0.95	1.3	1.3	440	0.5	1400	45

[a] $n = 3$; Crl: CD(SD)(IGS) rats (male, 8W).
[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.

The results of the spiro-compounds possessing the 6-methylthieno[2,3-*b*]pyridine are summarized in Table 3. Lactone **8a**, lactam **8b**, and imides **8c** exhibited 10^{-9} M order potency and oxazolidinedione **8d**, imidazolone **8e**, and imidazolidinedione **8f** also showed 10^{-8} M order potency; in particular, lactone **8a** exhibited the most potent activity. In addition, all enantiomers of **8a–e** predictably gave less potency as we previously reported.[7] The metabolic stability of all compounds, except for lactone **8a**, significantly improved relative to the corresponding benzothiophene derivatives **2–6**. Among these compounds, spiro-imide **8c**, which showed potent ACC inhibitory activity exhibited favorable pharmacokinetic profiles in rats as shown in Table 4.

In conclusion, to improve the metabolic stability of (*S*)-**1**, we modified the spiro-5-membered ring and the benzothiophene ring. All compounds possessing the imide, oxazolidinedione, imidazolidinedione, imidazolone, or lactam scaffold displayed highly-potent ACCs inhibitory activities. The SAR study revealed that installation of an oxo group onto the spiro-lactam ring and replacement of a nitrogen or oxygen atom on the spiro-imide ring preserved the activity of the molecule. Moreover, bulky lipophilic groups, such as an isopropyl group at the R^4 position, was effective for maintaining activity, and an R^3 (methyl) group on imidazolone **5** was preferable for maintaining metabolic stability in humans. Although modification of the spiro group led to lipophilicity-related improvement in metabolic stability, incorporation of a nitrogen atom in the benzothiophene ring followed by methylation at the 6-position improved metabolic stability. We identified a novel, highly potent, and bioavailable spiro-imide derivative **8c** possessing a thieno[2,3-*b*]pyridine core, which displayed high human metabolic stability and favorable rat pharmacokinetic profiles.

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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.2012.04.047.

References and notes

1. (a) Friedman, J. M. *Science* **2003**, *299*, 856; (b) Hill, J. O.; Wyatt, H. R.; Reed, G. W.; Peters, J. C. *Science* **2003**, *299*, 853; (c) Pi-Sunyer, X. *Science* **2003**, *299*, 859.
2. (a) Munday, M. R.; Hemingway, C. J. *Adv. Enzyme Regul.* **1999**, *39*, 205; (b) Ruderman, N. B.; Saha, A. K.; Vavvas, D.; Witters, L. A. *Am. J. Physiol.* **1999**, *276*, E1; (c) Ruderman, N. B.; Saha, A. K.; Kraegen, E. W. *Endocrinology* **2003**, *144*, 5166.
3. Rudermen, N.; Prentki, M. *Nat. Rev. Drug Disc.* **2004**, *3*, 340.
4. (a) Mao, J.; Chirala, S. S.; Wakil, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 7515; (b) Abu-Elheiga, L.; Almarza-Ortega, D. B.; Baldini, A.; Wakil, S. J. *J. Biol. Chem.* **1997**, *272*, 10669.
5. Abu-Elheiga, L.; Matzuk, M. M.; Kordari, P.; Oh, W.; Shaikenov, T.; Gu, Z.; Wakil, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12011.
6. Abu-Elheiga, L.; Matzuk, M. M.; Abo-Hashema, K. A.; Wakil, S. J. *Science* **2001**, *291*, 2613.
7. Yamashita, T.; Kamata, M.; Endo, S.; Yamamoto, M.; Kakegawa, K.; Watanabe, H.; Miwa, K.; Yamano, T.; Funata, M.; Sakamoto, J.; Tani, A.; Mol, C. D.; Zou, H.; Dougan, D. R.; Sang, B.; Snell, G.; Fukatsu, K. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6314.
8. The X-ray data for **8b** and **8c** were deposited to CCDC. **8b**: CCDC No. 873680, **8c**: CCDC No. 873679.
9. Tsukamoto, S.; Ichihara, M.; Wanibuchi, F.; Usada, S.; Hidaka, K.; Harada, M.; Tamura, T. *J. Med. Chem.* **1993**, *36*, 2292.
10. Queiroz, M. J.; Calhelha, R. C.; Vale-Silva, L. A.; Pinto, E.; São-José Nascimento, M. *Eur. J. Med. Chem.* **2009**, *44*, 1893.
11. Bernal, C.; Palacin, C.; Boronat, A.; Imperial, S. *Anal. Biochem.* **2005**, *337*, 55.
12. Koike, T.; Takai, T.; Hoashi, Y.; Nakayama, M.; Kosugi, Y.; Nakashima, M.; Yoshikubo, S.; Hirai, K.; Uchikawa, O. *J. Med. Chem.* **2011**, *54*, 4207.