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# Synthesis of spiro[chroman-2,4′-piperidin]-4-one derivatives as acetyl-CoA carboxylase inhibitors

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ABSTRACT

Various spiro[chroman-2,4′-piperidin]-4-one derivatives (**38a**–**m** and **43a**–**j**) have been designed, synthesized and evaluated for in vitro acetyl-CoA carboxylase (ACC) inhibitory activity. Several compounds have shown ACC inhibitory activity in low nanomolar range. Compound **38j** reduced the respiratory quotient (RQ) in C57BL/6J mice indicating increase in whole body fat oxidation even in the presence of high carbohydrate diet. Structure–activity relationship (SAR) has been discussed.



Acetyl-CoA carboxylase (ACC), a biotin-dependent enzyme, catalyzes the ATP dependent carboxylation of acetyl-CoA to form malonyl-CoA. ACC plays an important role in energy balance by controlling malonyl-CoA synthesis.[1–4] Malonyl-CoA participates in two opposing pathways: a substrate for fatty acid synthesis and a regulator of fatty acid oxidation. ACCs are involved in controlling the switch between carbohydrate and fatty acid utilization in liver and skeletal muscle and also regulating insulin sensitivity in the liver, skeletal muscle and adipose tissue.[2,3] Inhibition of ACCs, with its resultant inhibition of fatty acid synthesis and stimulation of fatty acid oxidation, has the potential to favorably affect the multitude of risk factors associated with metabolic syndrome, obesity, and type-2 diabetes.[1]

Bispiperidylcarboxamide derivative **I** (CP-640186, Fig. 1), having a tricyclic hydrophobic core, showed inhibition of both ACC1 and ACC2 with $IC_{50}$ of ~55 nM. Compound **I** has shown to increase fatty acid oxidation in vitro in C2C12 cells and in ob/ob mice. It also reduced fatty acid synthesis in HepG2 cells and in SD rats.[1] Recently, several spirochromanone derivatives **II** and **III** (Fig. 1), containing a bicyclic ring hydrophobic core, reported as potent ACC inhibitors. In vivo experiment of spirochromanones resulted in reduced body weight gain, fat mass gain and hepatic triglyceride content in C57BL/6J mice whereas reduced plasma glucose levels in KKAy mice. It is also reported that in humans with a BMI ⩾ 30, effective compounds resulted in loss of body weight or an improvement in insulin levels[5] (structures are not disclosed).

The crystal structure of the carboxytransferase (CT) domain of yeast ACC in complex with compound **I** has been described by Zhang et al.[6] Compound **I** is bound at the active site of the CT domain, at the interface between the two monomers of the CT dimer. The structure suggests that the active site of CT domain is mostly hydrophobic in nature with few hydrogen bonding potentials. From the structure, it appears that a big hydrophobic pocket is formed by the side chains of Ile2033 and Lys1764 and can accommodate a bulkier lipophilic group like tricyclic ring systems or teraryl ring systems. There can be a presence of hydrogen bond acceptor like carbonyl oxygen next to the bulkier hydrophobic group that can make hydrogen bonding with the main chain of Glu2026. There is another hydrophobic groove on the surface of the active site and hydrogen bond forming site formed by the main chain amide of Gly1958. Based on this structural information, we designed and synthesized several spirochromanones linked to various mono-, bi-, and tricyclic hydrophobes and evaluated for ACC inhibitory activity. Herein, we report the synthesis, in vitro ACC inhibitory activity and SAR of various amide (**38a**–**m**) and urea (**43a**–**j**) derivatives of spiro[chroman-2,4′-piperidin]-4-one. The in vivo efficacy profile of one of the potent derivative (**38j**) has also been discussed.

Synthesis of amide derivatives of various unsubstituted, 6-substituted, 7-substituted, and 6,7-disubstituted spiro[chroman-

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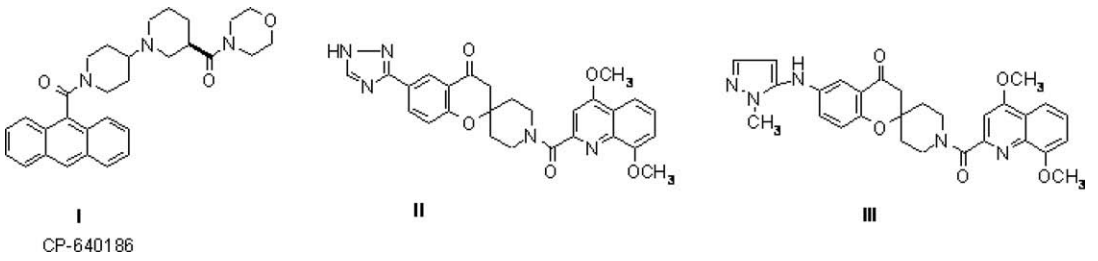
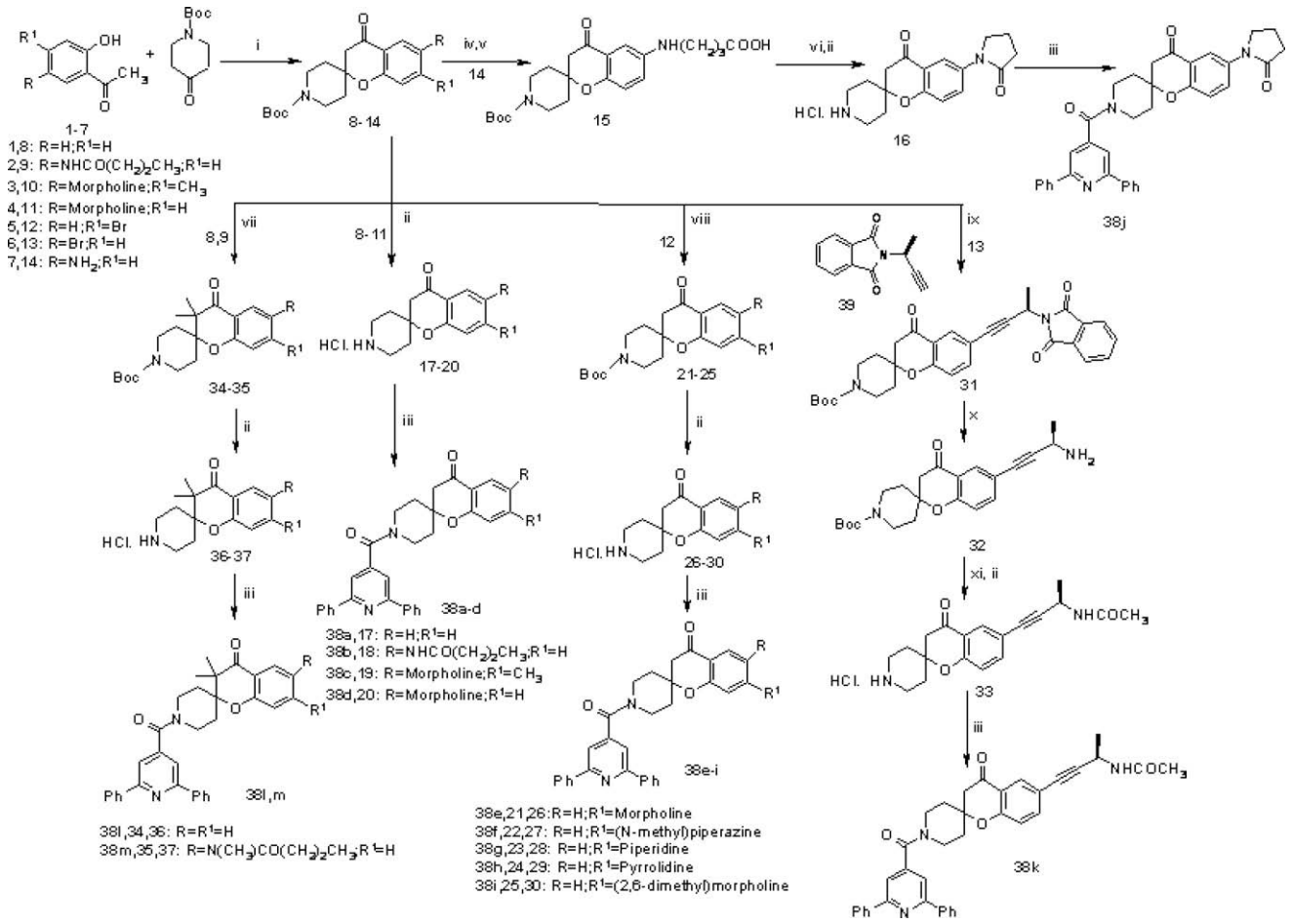
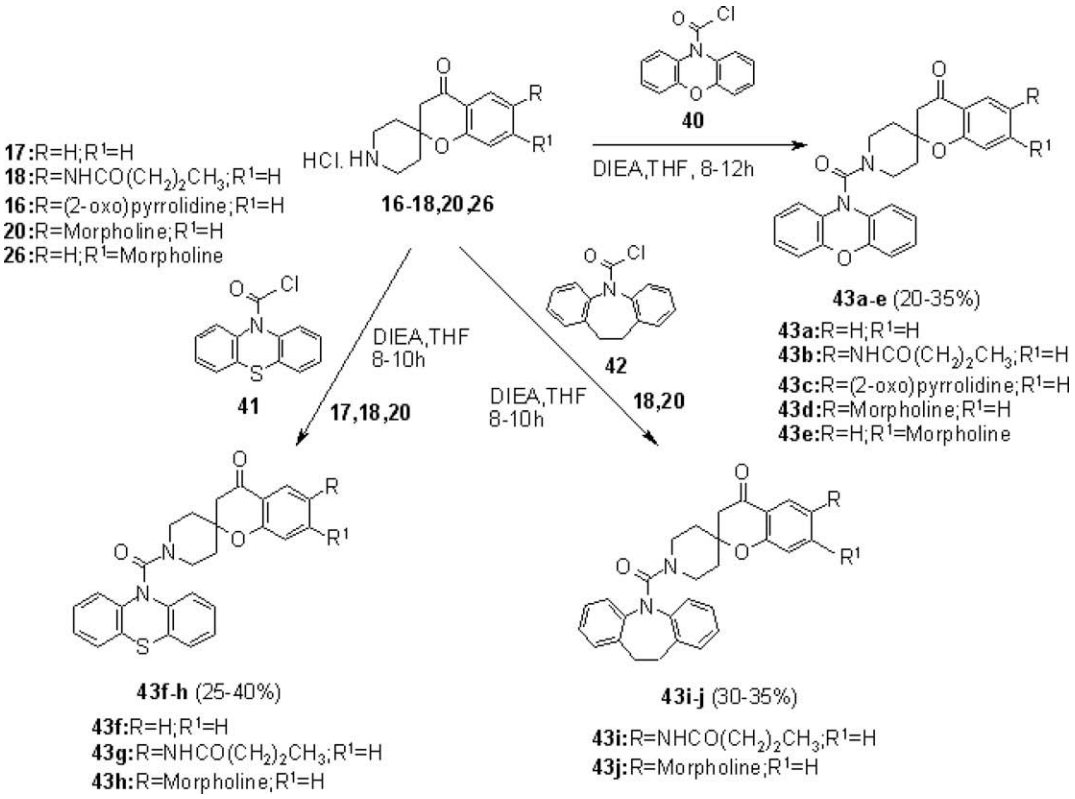



**Figure 1.** Structures of previously reported compounds as ACC inhibitors.




**Scheme 1.** Synthesis of compounds (**38a**–**m**). Reagents and conditions: (i) pyrrolidine, EtOH, reflux, 24–36 h; (ii) MeCN–HCl, 1–2 h; (iii) 2,6-diphenylisonicotinic acid, EDCI, HOBt, DIEA, THF, 8–12 h, 10–88%; (iv) Br-$(CH_2)_3$-COOEt, NaOAc, $H_2O$, 12 h; (v) NaOH, MeOH–$H_2O$, 18 h; (vi) EDCI, HOBt, DMAP, THF, 8–12 h, 82%; (vii) MeI, NaH, THF, 4–6 h, 68–70%; (viii) $R^1$-H, reflux; (ix) $Pd(Ph_3)_2Cl_2$, CuI, $Et_3N$, THF, 18 h; (x) hydrazine hydrate, EtOH, reflux, 4 h; (xi) $CH_3COCl$, $Et_3N$, THF, 3 h.

2,4′-piperidin]-4-ones (**38a**–**m**) are described in Scheme 1. Appropriate 2-hydroxyacetophenones **1**–**7** were treated, separately, with *N*-BOC-4-piperidone to provide respective spiro[chroman-2,4′-piperidin]-4-one derivatives **8**–**14** using the similar method as reported in the literature.[7] The removal of Boc group in **8**–**11**, through acidic hydrolysis yielded respective amine derivatives as their hydrochloride salts **17**–**20**. The coupling reaction of compounds **17**–**20** with 2,6-diphenylisonicotinic acid in the presence of diisopropylethylamine (DIEA), 3-ethyl-1-(*N*,*N*-dimethyl) aminopropylcarbodiimide (EDCI), and hydroxybenzotriazole (HOBt) to yield corresponding amide derivatives **38a**–**d**. The N-alkylation of 6-amino spirochroman **14** with ethyl 4-bromobutanoate in the presence of sodium acetate followed by its basic hydrolysis with sodium hydroxide yielded compound **15**. Intramolecular cyclization of **15** was carried out in the presence of EDCI and HOBt to give its pyrrolidinone derivative and further acidic hydrolysis resulted into compound **16**. Compound **38j** was prepared by the reaction of **16** with 2,6-diphenylisonicotinic acid using the same coupling condition as described for **38a**. Introduction of *gem*-dimethyl in compounds **8** and **9** were carried out in the presence of methyl iodide using sodium hydride as a base and THF as a solvent to afford compounds **34** and **35**, respectively. Deprotection of compounds **34** and **35** using acetonitrile-hydrogen chloride followed by coupling with 2,6-diphenylisonicotinic acid, using the same condition as described for compounds **38a**–**c**, yielded compounds **38l** and **38m**, respectively. Sonogashira coupling[8] of **13** with (*S*)-2-(1-methyl-prop-2-ynyl)-isoindole-1,3-dione (**39**)[9] yielded compound **31**, which was then refluxed in the mixture of hydrazine hydrate and




**Scheme 2.** Synthesis of compounds (**43a–j**).

ethanol to yield amino derivative **32**. N-acetylation of **32** followed by Boc deprotection yielded compound **33**. Compound **38k** was prepared by coupling of **33** with 2,6-diphenylisonicotinic acid using the same amide coupling reagent as described earlier.

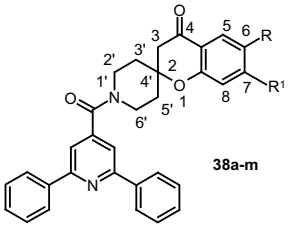
The 7-bromo group in **12** was replaced, by reacting various secondary cyclic amines such as morpholine, *N*-methylpiperazine, piperidine, pyrrolidine, and 2,6-dimethylmorpholine resulting to corresponding 7-amino spirochroman derivatives **21–25**. The acidic hydrolysis of **21–25** yielded compounds **26–30**. As described earlier for amide formation, compounds **26–30** were coupled, separately, with 2,6-diphenylisonicotinic acid to yield corresponding 7-substituted spiro[chroman-2,4′-piperidin]-4-one derivatives **38e–38i**.

Synthesis of urea derivatives of various spiro[chroman-2,4′-piperidin]-4-ones **43a–j** have been described in Scheme 2. Compounds **17**, **18**, **16**, **20**, **26** were treated with phenoxazine-10-carbonyl chloride (**40**) in the presence of DIEA in solvent THF to give **43a**, **43b**, **43c**, **43d**, and **43e**, respectively. Similarly, reaction of phenothiazine-10-carbonyl chloride (**41**) with **17**, **18**, and **20** yielded corresponding urea derivatives **43f–h** and treatment of 10,11-dihydro-dibenzo[*b*,*f*]azepine-5-carbonyl chloride (**42**) with **18** and **20** furnished **43i** and **43j**, respectively.

All the synthesized spiro[chroman-2,4′-piperidin]-4-one derivatives **38a–m** and **43a–j** were screened for their in vitro inhibitory activity against rat skeletal muscle ACC enzyme. Compounds showing >90% ACC inhibition at 10 μM concentration, were subjected for $IC_{50}$ evaluation.[10] The data are summarized in Tables 1 and 2. A number of synthesized compounds showed ACC activity in nanomolar range and structure–activity relationship is described below.

Amongst amide derivatives **38a–m**, the unsubstituted spiro[chroman-2,4′-piperidin]-4-one derivative **38a** was initially found active ($IC_{50}$ 1200 nM) against ACC enzyme (Table 1). ACC inhibitory activity was at least 19-fold improved upon introducing

**Table 1**
In vitro ACC activity of amide derivatives of various spiro[chroman-2,4′-piperidin]-4-ones **38a–m**

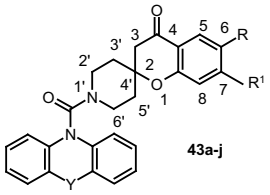



| Compound | R | $R^1$ | $IC_{50}$ (nM) |
|---|---|---|---|
| **38a** | H | H | 1200 |
| **38b** | $NHCO(CH_2)_2CH_3$ | H | 6 |
| **38c** | Morpholine | Methyl | 2 |
| **38d** | Morpholine | H | 6 |
| **38e** | H | Morpholine | 26 |
| **38f** | H | (*N*-Methyl)piperazine | 27 |
| **38g** | H | Piperidine | 36 |
| **38h** | H | Pyrrolidine | 61 |
| **38i** | H | (2,6-Dimethyl)morpholine | 59 |
| **38j** | (2-oxo)pyrrolidine | H | 14 |
| **38k** | $C{\equiv}CCH(CH_3)NHCOCH_3$ | H | 61 |
| **38l** | H | H | 44%[a] |
| **38m** | $N(CH_3)CO(CH_2)_2CH_3$ | H | 83%[a] |
| **I**[1,6] | — | — | 55 |
| **II**[5] | — | — | ~100%[b] |
| **III**[5] | — | — | ~100%[b] |

[a] Inhibition at 10 μM.
[b] Inhibition at 1 μM/L; —, not applicable.

substituent at either position-6 or -7 in spiro[chroman-2,4′-piperidin]-4-one except in compound **38l**. We found that butanamide (**38b**) and morpholine (**38d**) substituent at position-6 in

**Table 2**
In vitro ACC activity of urea derivatives of various spiro[chroman-2,4′-piperidin]-4-ones **43a–j**




| Compound | R | $R^1$ | Y | $IC_{50}$ (nM) |
|---|---|---|---|---|
| **43a** | H | H | O | 51%[b] |
| **43b** | $NHCO(CH_2)_2CH_3$ | H | O | 101 |
| **43c** | (2-Oxo)pyrrolidine | H | O | 236 |
| **43d** | Morpholine | H | O | 99 |
| **43e** | H | Morpholine | O | 208 |
| **43f** | H | H | S | 16%[a] |
| **43g** | $NHCO(CH_2)_2CH_3$ | H | S | 48%[a] |
| **43h** | Morpholine | H | S | 64%[a] |
| **43i** | $NHCO(CH_2)_2CH_3$ | H | $(CH_2)_2$ | 4%[a] |
| **43j** | Morpholine | H | $(CH_2)_2$ | 7%[a] |
| **I**[1,6] | — | — | | 55 |
| **II**[5] | — | — | | ~100%[c] |
| **III**[5] | — | — | | ~100%[c] |

[a] Inhibition at 10 μM.
[b] Inhibition at 5 μM.
[c] Inhibition at 1 μM/L; —, not applicable.

spiro[chroman-2,4′-piperidin]-4-one ($IC_{50}$ 6 nM) elicited 200-fold better activity in comparison to parent compound **38a**. The spiro[chroman-2,4′-piperidin]-4-one derivative having pyrrolidinone substitutent (**38j**) was found 85-fold more potent ($IC_{50}$ 14 nM) as compared to parent compound **38a**. Compound **38k** possessing alkyne substituent linked to spiro[chroman-2,4′-piperidin]-4-one through C–C bond, elicited relatively lesser activity ($IC_{50}$ 61 nM) than compounds **38b**, **38d**, and **38j**. However, compound **38k** was still 28-fold more potent than parent compound **38a**. These findings suggested that morpholine and butanamide at position-6 in spiro[chroman-2,4′-piperidin]-4-one were better substituents.

Introduction of morpholine substituent (**38e**) at position-7 in spiro[chroman-2,4′-piperidin]-4-one, activity ($IC_{50}$ 26 nM) was 4-fold lowered in comparison to its positional isomer **38d**. No major change in the activity was observed upon replacement of morpholine with other six-membered rings such as *N*-methylpiperazine (**38f**) or piperidine (**38g**). Replacement of morpholine with five-membered rings such as pyrrolidine (**38h**), inhibitory activity ($IC_{50}$ 61 nM) was further 2-fold reduced when compared to compound **38e**. After introducing dimethyl group in morpholine ring in compound **38e**, resulting compound **38i** showed 2-fold less activity than parent compound **38e**. The 7-substituted spiro[chroman-2,4′-piperidin]-4-one derivatives **38e–i** exhibited lesser activity than 6-substuited derivatives **38b**, **38d**, **38j**, and **38k** but still found to be at least 19-fold more active than unsubstituted spirochromanone **38a**. The ACC inhibitory activity did not improve even after introducing *gem*-dimethyl group in unsubstituted or 6-substituted spiro[chroman-2,4′-piperidin]-4-one derivatives, such as compounds **38l** and **38m**, which showed 44% and 82% ACC inhibition at 10 μM concentration, respectively. It was interesting to note that the 6,7-disubstituted derivative such as 7-methyl-6-morpholino-spiro[chroman-2,4′-piperidin]-4-one derivative **38c** was found most potent ($IC_{50}$ 2 nM) compound. Compound **38c** has shown 3-fold more activity than its structurally closer congener **38d**.

Amongst urea derivatives of spiro[chroman-2,4′-piperidin]-4-one derivatives **43a–j**, only compounds containing phenoxazines

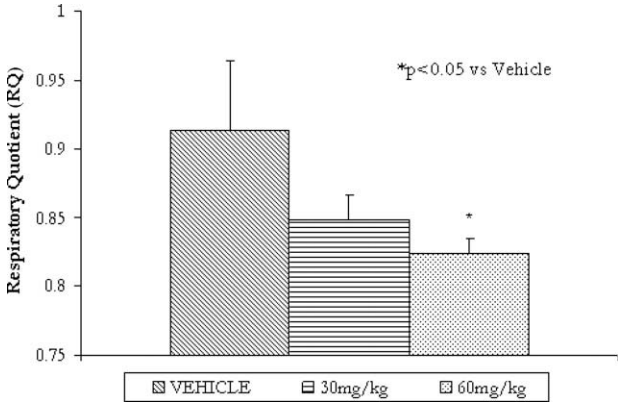



**Figure 2.** Effect of compound **38j** on RQ in C57BL/6J mice through ip route.

have shown ACC inhibitory activity in nanomolar range (Table 2). For example, unsubstituted spiro[chroman-2,4′-piperidin]-4-one derivative **43a** was found active (51% ACC inhibition at 5 μM). The activity was several fold increased upon incorporating substituent at position-6 in spiro[chroman-2,4′-piperidin]-4-one in **43a** such as compounds **43b** and **43d**, having butanamide and morpholine substituent, respectively, have shown $IC_{50} \sim 100$ nM. The activity was, however, >2-fold lowered either upon introducing 2-pyrrolidinone substituent at position-6 (compound **43c**). The compounds **43c** and **43e**, were found still more potent than **43a**. Interestingly, none of the phenothiazine (**43f–h**) and iminodibenzyl (**43i–j**) derivatives showed significant ACC inhibitory activity as they exhibited 4–64% inhibition at 10 μM concentration as shown in Table 2. It appeared that upon replacement of oxygen atom in phenoxazine hydrophobe by sulfur or di-methylene, activity is lowered.

Based on in vitro ACC inhibitory activity and permeability data, compound **38j** was selected for in vivo studies. Although compounds **38b–d** are more potent than **38j**, but all three having low permeability as compared to **38j**. Compound **38j** showed moderate permeability (PAMPA, Pe $26.76 \times 10^{-6}$ cm/s at pH 6.8). As inhibition of ACC would be expected to promote fat oxidation, we examined effect of compound **38j**[11] on respiratory quotient (RQ) in C57BL/6J mice,[12] which reflects contribution of carbohydrate and fat oxidation to total energy expenditure. The RQ is the ratio of $CO_2$ production to $O_2$ consumption. The value of RQ reduced, when there is shift from carbohydrate to fat metabolism. Compound **38j** was administered intraperitoneally (ip) at two doses, that is, 30 mg/kg and 60 mg/kg (Fig. 2). The compound **38j** dose dependently decreased the RQ indicating increase in whole body fat oxidation even in the presence of high carbohydrate diet.

In conclusion, we have found that spirochromanones linked to hydrophobic core through amidic linkage were, in general, superior to those having urea linkage. The spiro[chroman-2,4′-piperidin]-4-one attached to diphenylisonicotinoyl hydrophobic core showed potential for providing potent ACC inhibitory activity. In particular, substitution at position-6 in aromatic ring of spiro[chroman-2,4′-piperidin]-4-one through C–N bond, has played crucial role for eliciting activity. These results suggest that further derivatives at position-6 and -7 of spiro[chroman-2,4′-piperidin]-4-one may yield compounds with better in vivo activity.

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## References and notes


1. Harwood, H. J., Jr.; Petras, S. F.; Shelly, L. D.; Zaccaro, L. M.; Perry, D. A.; Makowski, M. R.; Hargrove, D. M.; Martin, K. A.; Tracey, W. R.; Chapman, J. G.; Magee, W. P.; Dalvie, D. K.; Soliman, V. F.; Martin, W. H.; Mularski, C. J.; Eisenbeis, S. A. *J. Biol. Chem.* **2003**, *278*, 37099.
2. McGarry, J. D.; Brown, N. F. *Eur. J. Biochem.* **1997**, *244*, 1.
3. Abu-Elheiga, L.; Matzuk, M. M.; Abo-Hashema, K. A.; Wakil, S. J. *Science* **2001**, *291*, 2613.
4. Harwood, H. J., Jr. *Curr. Opin. Investig. Drugs* **2004**, *5*, 283.
5. Takeru, Y.; Hideki J.; Kenji, N.; Koji, Y.; Tomoharu, I.; Mitsuru, O.; Hideaki, I.; Jun S.; Jun, K.; Lihu, Y. WO/2007/011809.
6. Zhang, H.; Tweel, B.; Li, J.; Tong, L. *Structure* **2004**, *12*, 1683.
7. Yang, L.; Morriello, G.; Prendergast, K.; Cheng, K.; Jacks, T.; Chan, W. W.; Schleim, K. D.; Smith, R. G.; Patchett, A. A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 107.
8. Clark, R. F.; Zhang, T.; Wang, X.; Wang, R.; Zhang, X.; Camp, H. S.; Beutel, B. A.; Sham, H. L.; Gu, Y. G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1961.
9. Beutel, B. A.; Camp, H. S.; Clark, R. F.; Gu, Y.-G.; Hansen, T. M.; Keyes, R. F.; Li, Q.; Liu, G.; Sham, H. L.; Wang, X.; Weitzberg, M.; Xin, Z.; Xu, X.; Zhand, T. US Patent 0,178,400, 2006.
10. ACC enzyme was isolated from rat skeletal muscle by streptavidin affinity chromatography. Briefly, ammonium sulfate precipitated skeletal muscle homogenate was dialyzed over-night. Dialyzed sample was passed through streptavidin column for protein binding. Protein was eluted by higher concentration of biotin (5 mM).[1,13] Protein concentration was estimated by Bradford Method. The enzyme activity was measured by the loss of acetyl CoA and/or the appearance of malonyl CoA using HPLC.[9,13,14] Assay buffer contained 50 mM HEPES (pH 7.5), 20 mM $MgCl_2$, 10 mM tripotassium citrate, and 0.075% Bovine Serum Albumin (BSA), 2 mM ATP, 1 mM acetyl-CoA, 17.6 mM $NaHCO_3$ and 0.25 μg ACC enzyme (specific activity of 0.8–1 μmol/mg/min). The reaction was carried out at 37 °C temperature for 30 min in presence of test compound and terminated by addition of 25 μl of 10% perchloric acid. In case of compounds **38a** and **43a**, where assay buffer contained 50 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 10 mM tripotassium citrate, dithiothreitol (DTT) 1 mM and 0.100% BSA, 2 mM ATP, 0.0625 mM acetyl CoA and 7 mM $KHCO_3$. The supernatants analyzed by HPLC. The HPLC method used column packed with octadecyl silane, embedded with polar substituents, on silica support as stationary phase and ammonium phosphate buffer and acetonitrile as mobile phase.
11. **38j**: $R_f$ = 0.6 (ethylacetate/hexane, 75:25); IR (KBr): 3358, 3064, 2956, 1694, 1624, 1546, 1488, 1440, 1223, 972, 692 $cm^{-1}$; $^1H$ NMR (400 MHz,$CDCl_3$): $\delta$ 8.26–8.15 (m, 5H), 7.68–7.65 (m, 3H), 7.55–7.46 (m, 6H), 7.07 (d, 1H, $J$ = 9.2 Hz), 4.62 (d, 1H, $J$ = 13.2 Hz), 3.88 (t, 2H, $J$ = 6.8 Hz), 3.62–3.56 (m, 2H), 3.38–3.32 (m, 1H), 2.79 (s, 2H), 2.63 (t, 2H, $J$ = 8.0 Hz), 2.28–2.15 (m, 3H), 2.09–2.05 (m, 1H) 1.84–1.76 (m, 1H), 1.67–1.66 (m, 1H); ESMS (relative intensity) 558 (M+1) (100%); HPLC purity 98.77%.
12. C57BL/6J mice (7–8 weeks old) fed with normal chaw diet (carbohydrate 64%, fat 7%, and protein 20%) were divided in following three groups: Group I: vehicle, ($n$ = 8); Group II: compound **38j**, 30 mg/kg, ($n$ = 8); and Group III: compound **38j**, 60 mg/kg, ($n$ = 8). The mice were treated with the respective treatment as mentioned above intraperitoneally, once a day for 8 days. On 7th day of treatment the mice were kept in the oxymax cages for 24 h to acclimatize and stabilize. After 24 h acclimatization, the mice were given the respective treatment and then kept in oxymax chamber immediately for recording $O_2$ consumption and $CO_2$ production. Initial 30-min observation was not been considered in the analysis. This is the time taken by mice to stabilize in the oxymax cages after injection. For determining RQ, the average of initial 8 h reading was calculated and then compared statistically using Student's $t$ test.
13. Tipper, J. P.; Witters, L. A. *Biochim. Biophys. Acta* **1982**, *715*, 162.
14. Levert, K. L.; Waldrop, G. L.; Stephens, J. M. *J. Biol. Chem.* **2002**, *277*, 16347.