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Design and synthesis of a new class of malonyl-CoA decarboxylase inhibitors with anti-obesity and anti-diabetic activities

Haifeng Tang *, Yan Yan, Zhe Feng, Reynalda K. de Jesus, Lihu Yang, Dorothy A. Levorse, Karen A. Owens, Taro E. Akiyama, Raynald Bergeron, Gino A. Castriota, Thomas W. Doebber, Kenneth P. Ellsworth, Michael E. Lassman, Cai Li, Margaret S. Wu, Bei B. Zhang, Kevin T. Chapman, Sander G. Mills, Joel P. Berger, Alexander Pasternak

Department of Medicinal Chemistry, Metabolic Disorders, Drug Metabolism and Pharmacology, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA

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ABSTRACT

A new series of thiazole-substituted 1,1,1,3,3,3-hexafluoro-2-propanols were prepared and evaluated as malonyl-CoA decarboxylase (MCD) inhibitors. Key analogs caused dose-dependent decreases in food intake and body weight in obese mice. Acute treatment with these compounds also led to a drop in elevated blood glucose in a murine model of type II diabetes.

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Obesity has become a worldwide pandemic over the last several decades. In the United States alone, about 33% of all adults are obese, and over 66% are regarded as overweight according to a recent CDC survey.[1] This condition is associated with increased risk of type II diabetes, cardiovascular and cerebrovascular diseases, and increased mortality.[2] The severity of this health problem and recent setbacks with several weight-loss drugs emphasize the need for new and effective approaches to anti-obesity therapy.[3]

It is now generally believed that a disruption of the balance between energy intake and energy expenditure is the major cause of obesity.[4] Recently, research in this area has identified the fatty acid intermediate malonyl-CoA as a key fuel sensor in the brain.[5] Malonyl-CoA is synthesized by acetyl-CoA carboxylase (ACC) from acetyl-CoA (Fig. 1). Malonyl-CoA is metabolized by fatty acid synthase (FAS), which catalyzes its incorporation into long-chain fatty acids, and by malonyl-CoA decarboxylase (MCD), which catalyzes its decarboxylation back to acetyl-CoA. In the brain, malonyl-CoA is involved in satiety regulation.[6] Loftus et al. reported that administering the FAS inhibitor C75 to mice provoked profound inhibition of feeding, presumably through increased accumulation of malonyl-CoA, thereby demonstrating a potential role for this metabolite in signaling satiety.[7]

As a potent endogenous inhibitor of carnitine palmitoyltransferase-I (CPT-I), malonyl-CoA is a key regulator of fatty acid

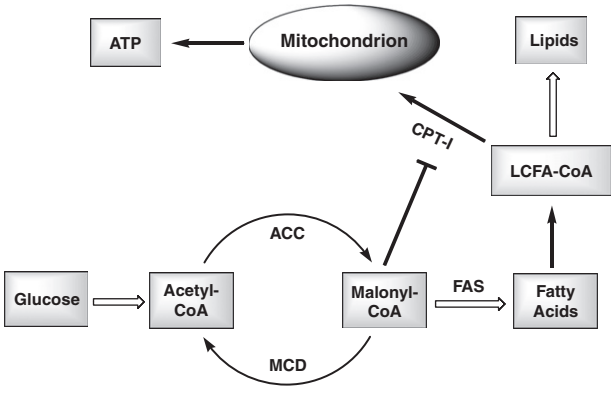

Figure 1. Enzymatic pathways involved in the regulation of malonyl-CoA levels and its role in fatty acid oxidation.

* Corresponding author. Tel.: +1 732 5942932; fax: +1 732 5949556.
E-mail address: haifeng_tang@merck.com (H. Tang).

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catabolism. CPT-I is the rate-limiting enzyme in fatty acid oxidation (FAO) that controls long chain fatty acid transport from the cytosol into the mitochondria. High levels of malonyl-CoA lead to inhibition of CPT-I, which prevents FAO and favors fatty acid synthesis. Conversely, low malonyl-CoA levels favor FAO by allowing the movement of long chain fatty acids into the mitochondria. Based on the reciprocal nature of glucose versus fatty acid oxidation (Randle cycle),[8] it is possible to increase cellular glucose oxidation by increasing malonyl-CoA levels and thereby inhibiting CPT-I activity. In fact, a number of clinical and experimental studies have demonstrated that small molecule MCD inhibitors can shift energy metabolism toward glucose oxidation as an effective approach to treating myocardial ischemia.[9]

Recent data support the proposition that MCD can be targeted for the treatment of metabolic diseases. Lopaschuk and co-workers has demonstrated that MCD knock out mice are healthy, despite elevated malonyl-CoA levels. Importantly, these mice are resistant to diet-induced weight gain and blood glucose elevation.[9] These results indicate that it may be possible to develop therapies for obesity and diabetes by increasing malonyl-CoA levels with small molecule MCD inhibitors.

Several classes of small molecule MCD inhibitors have been reported in the literature by researchers at Chugai Pharmaceutical Company.[10] In order to develop our own small molecule MCD inhibitors, we screened our internal compound collection, and identified a large number of potent MCD inhibitors. Most of the screen hits possess a 1,1,1,3,3,3-hexafluoro-propanol moiety, which is linked directly to an aromatic ring. The structures of the two most potent hits are shown in Figure 2. In this communication, we will outline the medicinal chemistry efforts leading to the discovery of a novel class of MCD inhibitors containing the 1,1,1,3,3,3-hexafluoro-propanol moiety.

While retaining the pivotal left hand 2-aryl-1,1,1,3,3,3-hexafluoro-propanol moiety, we first attempted to simplify the lead structure by truncating the right hand portion of the molecule. To our satisfaction, a variety of substituents can be tolerated at this position. Compound **3**, where the right hand side chain was replaced by a simple *para*-toluyl group, is a fairly potent MCD inhibitor (Scheme 1).[11] Compared to **3**, the 2-phenyl-1,1,1,3,3,3-hexafluoro-propanol analog **4** is much less potent. The sulfur linkage of **3** can be further replaced with a range of other functionalities such as ether (**5**), sulfone (**6**), and piperazine (**7**). Among them, piperazine **7** emerged as a novel and versatile lead structure for small molecule MCD inhibitors (Table 1, **7a**, R = H, IC_{50} = 20 nM).

The synthesis of **7** is relatively straightforward. Starting from 2-amino-thioazole **11**, condensation with hexafluoroacetone hydrate **12** gave rise to **13**. Subsequent treatment of **13** under Sandmeyer conditions furnished bromide **14**. If 1-aryl-piperazine **15** is commercially available, the target compound can be obtained by direct nucleophilic substitution of **15** to bromide **14**. Otherwise, **7** can be accessed by transition metal catalyzed coupling of **18** to piperazine **17**, which in turn was prepared by condensation of **16** with hexafluoroacetone hydrate **12** (Scheme 2).

In addition to **7**, related piperidine analogs **8**, **9**, and **10** were also explored. Unless specified, the compounds were prepared via the general methods described in Scheme 3. The synthesis of 1-aryl-4-[2-[5-(1,1,1,3,3,3-hexafluoro-propan-2-ol)thiazolyl]]-piperidine series **8** started with nucleophilic addition of 2-thioazole anion, generated from 2-bromothioazole **19** by *trans*-metallation with *n*-BuLi, to *N*-Boc-4-piperidone **20**. The resulting alcoxide was intercepted with methanesulfonyl chloride to give the mesylate, which upon treatment with triethylamine eliminated in situ to give olefin **21**. Olefin **21** was then reduced by hydrogenation to afford **22**, which was alkylated with hexafluoroacetone to

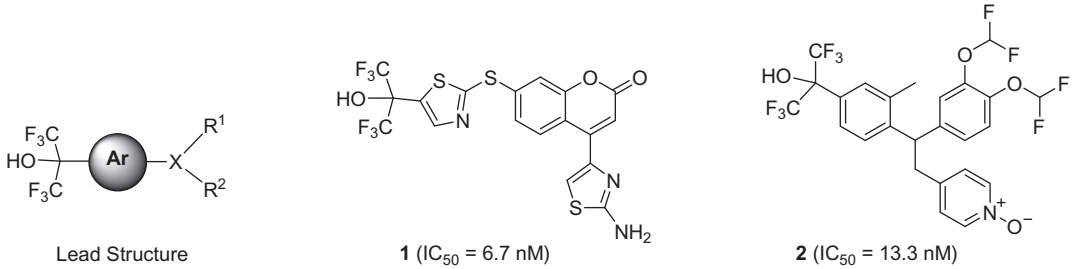
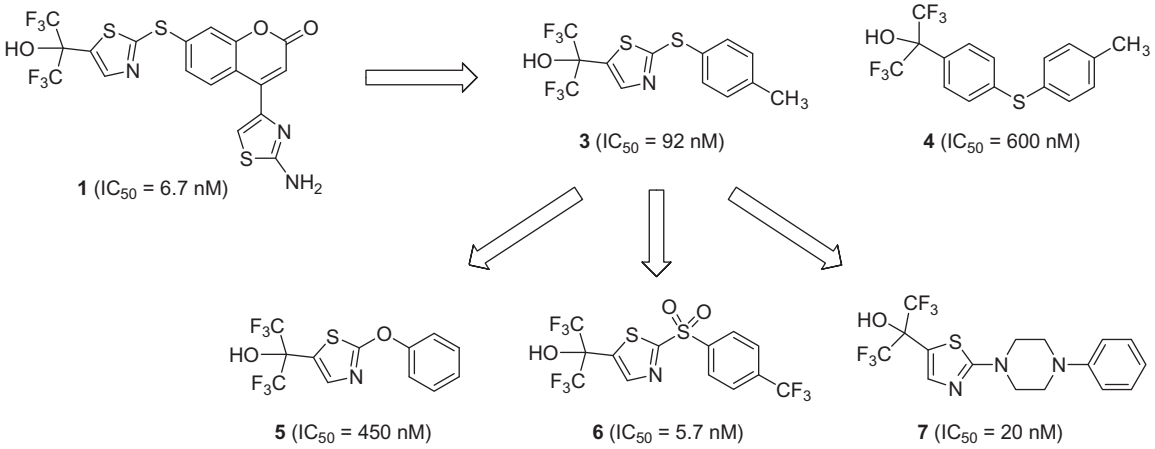


Figure 2. MCD inhibitors identified from internal screen.



Scheme 1. Evolution of the lead strcuture.

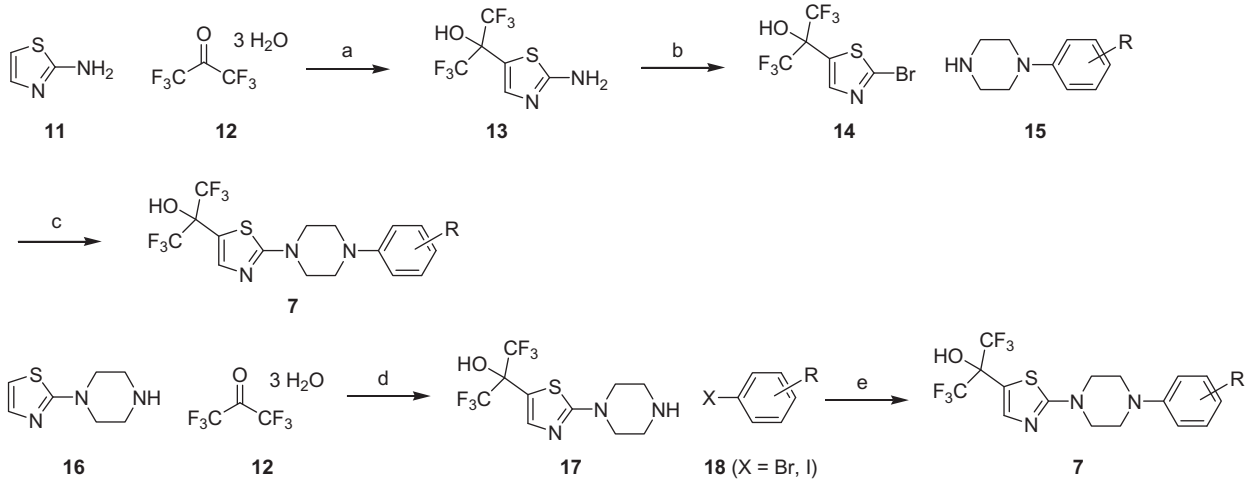
Table 1
In vitro MCD inhibition assay results for compounds **7**–**10**

Compds	R	R′	IC_{50} (nM)
7a	H		20
7b	3-CF_3		240
7c	3-OCF_3		320
7d	4-CF_3		110
7e	4-CH_3		59
7f	4-OCH_3		76
7g	3-OCH_3		46
7h	3-Cl		79
7i	3-F		8.7
7j	4-F		8.1
7k	3,4-F		11
7l	3-SO_2Me		24
7m	4-SO_2Me		44
7n	3-NO_2		12
7o	4-NO_2		8.9
7p	3-CN		16
7q	4-CN		42
7r	3-NH_2		3.2
7s	4-NH_2		2.7
7t	3-COOH		1.3
7u	2-COOH		5.1
7v	4-COOH		19
7w	3-$CONH_2$		2.2
7x	3-(2-Tetrazole)		1.6
7y	3-$CONHCH_3$		2.3
8a	H		37
8b	3-F		36
8c	4-F		19
8d	3-$CONH_2$		14
9a	H		13
9b	3-F		11
9c	2-COOH		1.7
9d	3-COOH		0.9
9e	4-COOH		4.0
9f	3-$CONH_2$		1.8
9g	3-$CONHCH_3$		3.1
10a	H	OH	4.5
10b	H	CN	11
10c	H	COOH	1.1
10d	H	$CONH_2$	4.9
10e	H	$CONHCH_3$	4.7
10f	4-F	COOH	1.4
10g	2-F	2-Tetrazole	1.6
10h	3-COOH	$CONH_2$	0.5
10i	3-COOH	COOH	0.6
10j	H	$CONHSO_2CH_3$	0.9
10k	H	$CONHCH_2CF_3$	3.3

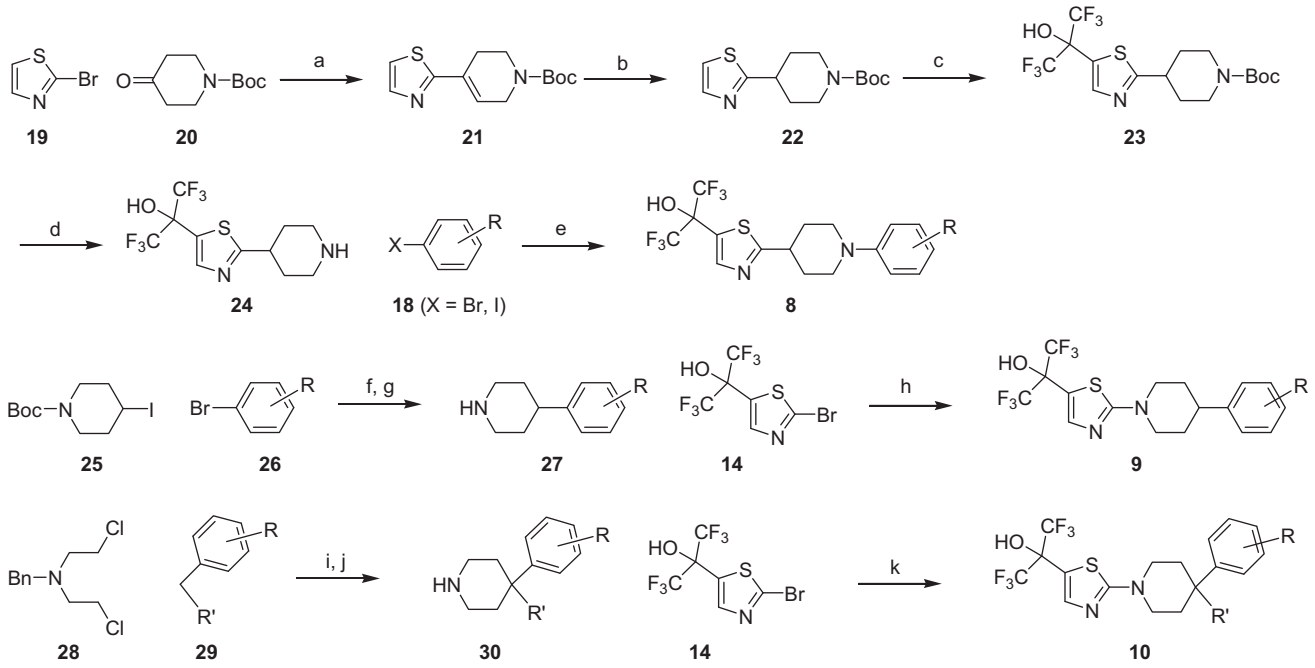
furnish **23**. Subsequent removal of the Boc group gave rise to **24**. Final coupling of the aryl tail piece **18** with **24** via transition metal catalysis led to **8**. The 1-[2-[5-(1,1,1,3,3,3-hexafluoro-propan-2-ol)thiazolyl]]-4-aryl-piperidine series **9** was prepared from nucleophilic substitution of 4-arylpiperidine **27** with bromide **14**. The 4-arylpiperidine **27** in turn was prepared from Negishi coupling of zinc reagent derived from iodide **25** and aryl bromide **26**, followed by removal of the Boc group. The syntheses of 4,4-disubstituted piperidine series **10** started from cyclization of **29** with dichloride **28**. The benzyl protected piperidine was then treated under hydrogenation conditions to furnish **30**. Piperidine **30** was finally coupled to bromide **14** in the presence of base, following routine functional group manipulations, to give rise to **10**.

Once prepared, all compounds were assayed in vitro for their ability to inhibit human MCD activity.[12] SAR studies of the phenyl ring revealed that substitution with hydrophobic groups led to a decrease in potency (Table 1, **7b**–**7g**).[13] While fluoride is tolerated, substitution with chloride leads to a decrease in potency. Introduction of hydrogen bond acceptors did not result an increase in potency, but they are generally tolerated (**7l**–**7q**). A significant increase in potency was achieved when hydrogen bond donors were incorporated into the phenyl ring (**7r** and **7s**). The most potent compound in this series was achieved when carboxylic acid was introduced at the 3-position (**7t**). Analogs with carboxylic acid at 2- or 4-position are significantly less potent (**7u** and **7v**). SAR of piperidine series **8** and **9** is largely parallel to that of **7**. In general, analogs of series **8** are less potent than those of **7**; while analogs of series **9** are slightly more potent than those of **7**. The most potent compounds were prepared from the 4,4-disubstituted piperidine series **10**. It was found that hydrogen bond donors at the 4-position of the piperidine ring significant boost inhibition potency (**10a**).[14] A further boost of potency was achieved when a second hydrogen bond donor group was incorporated into the 4-phenyl ring (**10h** and **10i**). It is also worth noting that the aryl group can be replaced with an alkyl group in this series, leading to potent MCD inhibitors such as **10l** and **10m** (Fig. 3).

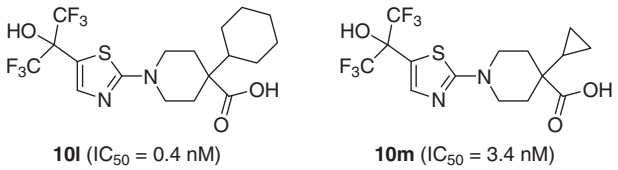
Based on their enzyme inhibitory potency and structural diversity, a selected set of compounds were further evaluated in a db/db mouse hepatocyte whole cell assay (Table 2).[15] In contrast to their high potency in the in vitro assay, these compounds were much less active in the whole cell assay. In general, polar functional groups such as carboxylic acid and carboxamide are essential for whole cell activity. Without polar groups, the compounds are



Scheme 2. Reagents and conditions: (a) 4 Å molecular sieves, toluene, 120 °C, 2 h, 89%; (b) $NaNO_2$, CuBr, water, 48% HBr, 65%; (c) Et_3N, DMSO, 100 °C, 20–90%; (d) 4 Å molecular sieves, toluene, 120 °C, 2 h, 60%; (e) $Pd(OAc)_2$, X-Phos, NaO*t*-Bu, toluene, 100 °C, 10–80%.



Scheme 3. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, 1 h, 1.2 equiv MsCl, 2.0 equiv Et_3N, 0 °C, 60%; (b) H_2, 10% Pd/C, MeOH, 95%; (c) LDA, THF, CF_3COCF_3, −78 °C, 75%, (d) TFA, 25 °C, 2 h, 98%; (e) 10 mol % $Pd(OAc)_2$, 15 mol % X-Phos, NaO*t*-Bu, toluene, 100 °C, 20–60%; (f) Zn powder, 5 mol % $Pd(dppf)Cl_2$, 10 mol % CuI, DMA, 85 °C, 50–90%, (g) TFA, 25 °C, 2 h, 98%; (h) Et_3N, DMSO, 100 °C, 20–90%; (i) 3.0 equiv NaH, THF, 80 °C, 20–80%; (j) Pd/C, H_2, EtOH, 95%; (k) Et_3N, DMSO, 100 °C, 20–90%.



10l (IC_{50} = 0.4 nM) **10m** (IC_{50} = 3.4 nM)

Figure 3. 4-Alkyl substituted piperidine analogs **10l** and **10m**.

generally inactive (**7k, 8c** >20,000 nM). However, polar groups usually lead to undesirable pharmacokinetics and brain penetration (PK/BP) properties. Since energy balance is regulated by the hypothalamus, adequate brain penetration is believe to be crucial for compounds to demonstrate efficacy in proof of concept studies. Gratifyingly, we were able to improve PK/BP properties while maintaining whole cell potency by converting the primary carboxamide (**7w**) into the corresponding secondary carboxamide (**7y**).[16] It is also worth noting that the 4,4-disubstituted series **10** was shown to have better PK/BP properties than the other series. Compound **10d** has good PK/BP properties despite the primary carboxamide group.[17]

Based on their superior hepatocyte assay potency and desirable PK/BP properties, compounds **7y** and **10d** were scaled up for a 14-day chronic efficacy studies in obese C57B6N mice; **7w**, a less brain penetrant compound, was synthesized to serve as a negative control. The inhibitors were dosed at 30 and 100 mpk/day po for 2 weeks. At the end of the studies, hypothalami were collected for quantitative malonyl-CoA analysis.[18] The two brain penetrant compounds, **7y** and **10d** reduced food intake (FI) and body weight (BW) at 100 mpk, while no significant effect was observed with **7w**. These results correlated well with hypothalamic malonyl-CoA levels (Table 3). Mice treated with the brain penetrant compound **7y** showed a dose-dependent response; 30 and 100 mpk treatments resulted in 65% and 186% increases in hypothalamic malonyl-CoA levels, respectively, versus the vehicle control. The other brain penetrant compound, **10d** provided a 70% increase in malonyl-CoA at 100 mpk treatment, but no significant elevation at 30 mpk treatment. No significant increase of hypothalamic malonyl-CoA levels was obtained with the less brain penetrant compound, **7w**.

Table 2
Hepatocyte whole cell assay for selected compounds **7–10**

Compds	IC_{50} (nM)	EC_{50}[a] (nM)
7k	11.4	>20,000
7w	2.2	1400
7y	2.3	630
8c	19	>20,000
8d	13.8	2000
9d	0.9	230
9f	1.8	950
10d	4.9	1100
10e	4.7	640
10h	0.5	150
10j	0.9	500

[a] $n = 3$.

Table 3
Results from the chronic FI/BW studies with C57B6N mice

Compds (dosage at mpk/day)	Body weight change (%)	Total caloric intake (%)	Hypothalamic malonyl-CoA change (%)
7w (30)	−3.3	0.8	50
7w (100)	−2.2	0.8	36
7y (30)	−2.1	−1.3	65[a]
7y (100)	−20.0[a]	−12.4[a]	186[b]
10d (30)	−5.5	−2.4	29
10d (100)	−14.3[a]	−5.7	70[b]

C57B6N mice maintained on D12492 for 16 weeks, $n = 9$ per group.
% Normalized versus control.
[a] $p < 0.05$.
[b] $p < 0.01$.

Table 4
Results from the acute glycolysis assay with db/db mice

Compds	Dosage (mpk)	Blood glucose correction (%)	β-HBA decrease (%)
7w	30	32[a]	78[b]
7y	30	30[a]	65[b]
10d	30	41[a]	60[b]
10j	3	28	−12
10j	10	53[b]	53[b]
10j	30	48[a]	60[b]

Db/db mice 11 weeks old, $n = 5$.
[a] $p < 0.05$.
[b] $p < 0.005$.

In order to explore the hypothesis that small molecule MCD inhibitors can serve as therapy for type II diabetes, the acute metabolic effects of four compounds were studied in a diabetic db/db mice model.[19] Blood glucose levels were determined at the end of the study. In addition, the circulating levels of β-hydroxybutyrate (β-HBA), a ketone emanating from fatty acid oxidation (FAO) in the liver, were also measured at the termination of the study as a biomarker of hepatic FAO activity. To our delight, mice treated with MCD inhibitors displayed decreased β-HBA levels, and most importantly, significant reductions in their elevated blood glucose levels (Table 4).

In conclusion, we have discovered a new class of potent small molecule MCD inhibitors. SAR studies led us to the synthesize a number of brain penetrant compounds with good PK profiles. Subsequently, we used these inhibitors to demonstrate that raising malonyl-CoA in the hypothalamus is associated with a reduction in body weight and food intake, thus validating MCD as a viable target for the treatment of obesity. Moreover, administration of these compounds to diabetic db/db mice ameliorated hyperglycemia by apparently shifting catabolic nutrient metabolism from fatty acid to glucose oxidation, thereby establishing MCD as a potential therapy for type II diabetes.

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11. SAR study of **3** on the phenyl ring led to significant improvement in enzyme inhibition potency. These results will be reported in a separate communication.
12. Truncated human MCD was used in the assay. A set of 12 compounds were assayed with the truncated human MCD and the full length human MCD. The IC_{50} values were within twofolds of each other. For detailed assay protocols, please see: Dyck, J. R. B.; Berthiaume, L. G.; Thomas, P. D.; Kantor, P. F.; Barr, A. J.; Barr, R.; Singh, D.; Hopkins, T. A.; Voilley, N.; Prentki, M.; Lopaschuk, G. D. *Biochem. J.* **2000**, *350*, 599–608.
13. Analogs substituted with other aromatic rings were also synthesized, but are generally less potent than their phenyl analogs.
14. Compound **10a** was prepared from direct nucleophilic substitution of commercially available 4-hydroxy-4-phenylpiperidine to bromide **14**.
15. Compounds of interest were incubated with primary db/db mouse hepatocytes for 30 min. Malonyl-CoA levels were then measured to assess their ability to inhibit whole cell MCD activity.
16. Plasma/brain drug levels at 30 mpk 4 h after po dosing in mice: **7w** plasma 2.38 μM, brain 0.08 μM; **7y** plasma 20.5 μM, brain 0.79 μM.
17. Plasma/brain drug levels for **10d** at 30 mpk 4 h after po dosing in mice: plasma 15.8 μM, brain 5.4 μM.
18. The levels of malonyl-CoA in the collected hypothalami were quantified by HPLC with known internal standard.
19. Assay protocol: db/db mice were orally dosed with compounds or vehicle in 0.5% methylcellulose 5 h after food was removed. Two hours later blood samples were obtained by the tail vein. Plasma glucose and β-HBA levels of mice dosed with compounds and vehicle were assayed. Percent correction of glucose and percent decrease in β-HBA of the compounds were calculated versus vehicle control.