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Heteroaryl substituted bis-trifluoromethyl carbinols as malonyl-CoA decarboxylase inhibitors

Jie-Fei Cheng,[a,*] Chi Ching Mak,[a] Yujin Huang,[a] Richard Penuliar,[a] Masahiro Nishimoto,[a] Lin Zhang,[a] Mi Chen,[a] David Wallace,[a] Thomas Arrhenius,[a] Donald Chu,[a] Guang Yang,[a] Miguel Barbosa,[a] Rick Barr,[b] Jason R. B. Dyck,[b] Gary D. Lopaschuk[b] and Alex M. Nadzan[a]

[a]*Department of Chemistry and Discovery Biology, Chugai Pharma LLC., 6275 Nancy Ridge Dr., San Diego, CA 92121, USA*
[b]*Metabolic Modulator Research Ltd. (MMRL), 2020 Research Transition Facility, University of Alberta, Edmonton, Alta., Canada*

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Abstract—A series of heteroaryl-substituted bis-trifluoromethyl carbinols were prepared and evaluated as malonyl-CoA decarboxylase (MCD) inhibitors. Some thiazole-based derivatives showed potent in vitro MCD inhibitory activities and significantly increased glucose oxidation rates in isolated working rat hearts.

The enzyme malonyl-CoA decarboxylase[1](MCD, EC 4.1.1.9) catalyzes the degradation of malonyl-CoA to acetyl-CoA and thereby regulates malonyl-CoA levels in the cells. The highest MCD mRNA expression levels in rats were found in muscle and heart tissues, followed by liver, kidney, and pancreas, with detectable amounts found in many other tissues including brain.[2] The human MCD gene is highly homologous to goose and rat genes.[3] Recent studies indicated that MCD exists in cytosolic, mitochondrial, and peroxisomal compartments.[4]

Malonyl-CoA is the key intermediate for fatty acid synthesis and a potent inhibitor of carnitine palmitoyltransferase I (CPT-I), a key enzyme regulating mitochondrial fatty acid oxidation.[5] As a metabolic fuel sensor, malonyl-CoA also regulates nutrition partitioning[6], insulin secretion and sensitivity,[7,8] and food intake.[9] Targeting malonyl-CoA regulation through MCD inhibition is potentially a novel approach to treating certain disorders involving fatty acid/glucose metabolism.

We have recently described the first-generation of small molecule MCD inhibitors.[10] As expected, MCD inhibitors significantly increased malonyl-CoA levels and decreased the fatty acid oxidation rates in ex vivo experiments with isolated working rat hearts. As a result, the glucose oxidation rates were accelerated, consistent with the 'glucose-fatty acid cycle' hypothesis.[11] Previous SAR studies led to the identification of some potent MCD inhibitors (e.g., **1**–**2**, Fig. 1).[10a,b] Herein we described the design, synthesis, and SAR studies of a series of heteroaryl-substituted bis-trifluoromethyl carbinol derivatives **3** as potent MCD inhibitors, which showed powerful stimulation of glucose oxidation in isolated working rat hearts.

Synthesis of pyridine-based MCD inhibitors is illustrated in Scheme 1. Nicotinic acid amide derivatives were prepared by treating 2-bromo-picoline **4** with *n*-BuLi, followed by the reaction of the lithiated picoline intermediate with hexafluoroacetone at −78 °C to afford 1,1,1,3,3,3-hexafluoro-2-(5-methylpyridin-2-yl)propan-2-ol **5** in 48% yield. Oxidation of **5** to its corresponding carboxylic acid **6** was achieved in 87% yield by using $KMnO_4$ under basic conditions. Amide formation under conventional conditions gave rise to the desired amide

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* Corresponding author at present address: Tanabe Research Laboratories USA, Inc. 4540 Towne Centre Court, San Diego, CA 92121, USA. Tel.: +1 858 622 7029; fax: +1 858 558 9383; e-mail: jcheng@trlusa.com

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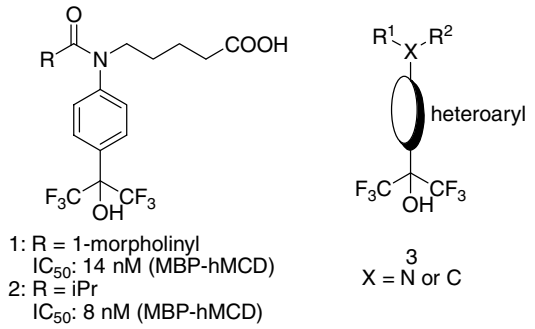
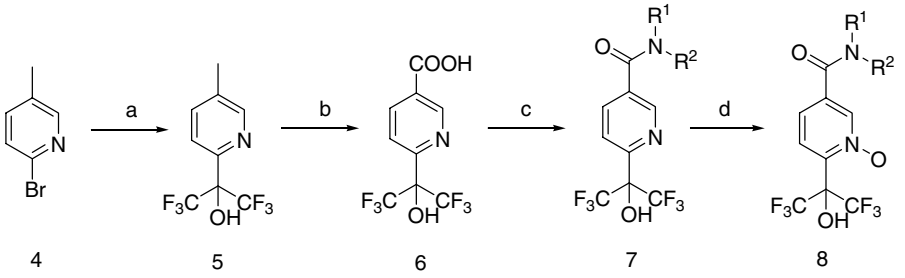


Figure 1. Representative MCD inhibitors.

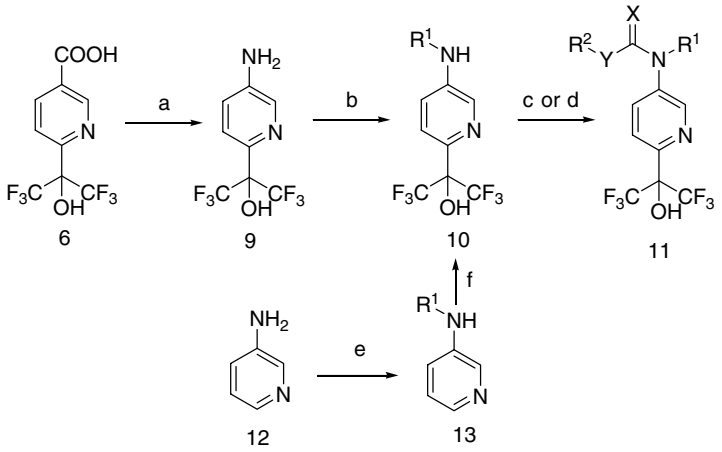
derivatives **7** in good yield. Oxidation of pyridine ring with mCPBA afforded the corresponding *N*-oxide derivatives **8**.

Nicotinic acid derivative **6** could be converted into the corresponding aminopyridine compound **9** via a Curtius rearrangement using diphenyl phosphorazidate (Scheme 2). Reductive alkylation of the aminopyridine **9** provided the key N-alkylated intermediate **10** in moderate yield. Alternatively, intermediate **10** could be prepared from 3-*N*-alkylaminopyridine compound **13**, which in turn was synthesized from 3-aminopyridine through alkylation in the presence of lithium 2,2,6,6-tetramethylpiperidide (LiTMP) at low temperature (−107 °C).[12] Amide derivatives were prepared through the reaction of **10** with acid chlorides or with carboxylic acids in the presence of coupling reagents. Urea formation was performed using two different routes depending on the availability of intermediates. In the first route compound 10 was treated with a substituted isocyanate or thioisocyanate to provide derivatives **11**. Alternatively compound **10** was first treated with triphosgene or thiophosgene to provide the carbamoyl chloride or thiocarbamoyl chloride intermediate, which without further purification was reacted with a primary or secondary amine to afford the desired urea or thiourea derivative **11**.

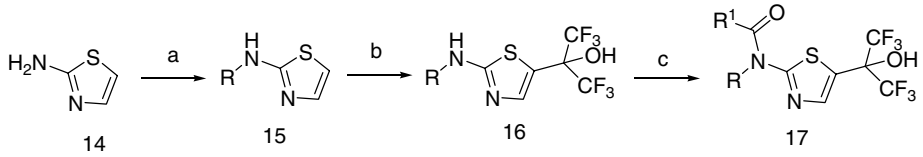
Thiazole derivatives were prepared according to Scheme 3. Reductive alkylation of 2-aminothiazole **14** with aldehydes in the presence of $NaBH_4$ afforded the desired N-alkylated 2-aminothiazole intermediates **15** in excellent yields. Introduction of hexafluoroisopropanol was achieved by reacting the N-alkylated 2-aminothiazole intermediates with hexafluoroacetone at elevated temperature. The condensation reaction between aminothiazole and hexafluoroacetone went smoothly in the presence of molecular sieves and afforded only one product, which was assigned the C-5 hexafluoroisopropanol structure **16**, based on NMR spectroscopic



Scheme 1. Reagents and conditions: (a) *n*-BuLi, CF_3COCF_3(g), −78 °C to rt, 48%; (b) $KMnO_4$, NaOH, 55 °C; 87%; (c) HBTU, DIPEA, R^1R^2NH, CH_2Cl_2, >90%; (d) mCPBA, DCM (∼20%).



Scheme 2. Reagents and conditions: (a) DPPA, Et_3N, then LiOH, 25%; (b) RCHO, $NaBH(OAc)_3$, AcOH, ∼60%; (c) R^2COCl, pyridine, rt or R^2NCO or R^2NCS, toluene, rt to 80 °C; (d) triphosgene or $CSCl_2$, DIPEA, then R^2YNH_2, 27–48%; (e) R^1I (R^1Br), NaH, DMSO, rt, >90% (f) CF_3COCF_3(gas), −78 °C, THF, then LiTMP, THF, ether, −107 °C, 1 h, −78 °C to rt, ∼45%.



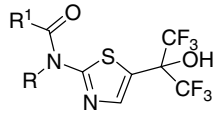
Scheme 3. Reagents and conditions: (a) R^2CHO, toluene; $NaBH(AcO)_3$ or $NaBH_4$, MeOH; (b) CF_3COCF_3, toluene, 100 °C, 24–86% in two steps; (c) $(R^1CO)_2O$, dioxane, 100 °C, 21–75%.

data and by analogy to the other Friedel–Crafts type of reactions of 2-aminothiazoles.[13] Finally, acylation of the intermediates **16** using symmetric or mixed carboxylic acid anhydrides provided the desired amide compounds **17**. Interestingly, it was found that acylation of 2-alkylaminothiazole intermediates using acid chlorides provided a mixture of 2-N′ and N-3 acylation products.

MCD inhibitors were evaluated for their ability to inhibit a soluble maltose binding protein, fused human malonyl-CoA decarboxylase (MBP-hMCD) as described previously.[10a,14] The IC_{50} values for some selected compounds are tabulated in Tables 1 and 2. Representative compounds were subsequently chosen for glucose oxidation assessment in isolated working rat hearts.

Intermediary compounds **5**, **6**, **9**, and 2-(5-(ethylamino)pyridin-2-yl)-1,1,1,3,3,3-hexafluoropropan-2-ol (**10**, R^1 = Et) were all inactive, consistent with previous observations that an extra hydrophobic moiety possessing a hydrogen-bond acceptor is required for good potency, in addition to the required bis-trifluoromethyl carbinol function.[10a,b] Tertiary nicotinamides containing small aliphatic groups (**7a** and **7b**) showed good MCD inhibitory activities. Amides, ureas or thioureas in aminopyridine series (**11**) all showed reasonably good activities. However, little potency improvement was seen for pyridine derivatives over the corresponding phenyl analogs.[10a] Oxidation of the pyridine ring to the corresponding *N*-oxide derivative resulted in decreased activity (IC_{50}: **7a**: 47 nM vs **8**: 235 nM).

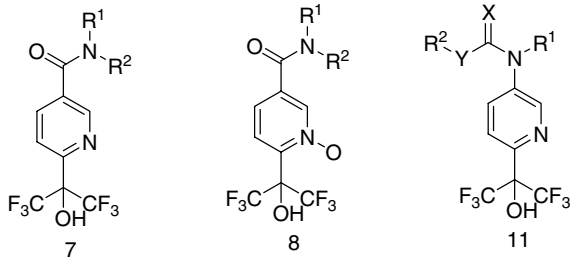
Table 2. Thiazole-based MCD inhibitors



Compound	R^1	R	IC_{50}[a] (nM)
17a	*i*-Pr	Et	794
17b	*i*-Pr	*n*-Bu	984
17c	*i*-Pr	Ph	1711
17d	*i*-Pr	Bn	689
17e	*i*-Pr	4-CNBn	20
17f	*i*-Pr	4-MeO_2CBn–	54
17g	*i*-Pr	4-ClBn–	148
17h	*i*-Pr	3,4-Dichlorobenzyl	438
17i	*i*-Pr	4-TetrazolylBn–	1280
17j	*i*-Pr	4-MeOBn–	353
17k	*i*-Pr	2-Furanylmethyl	1576
17l	*i*-Pr	4-Pyridinylmethyl	42
17m	*i*-Pr	2-Pyridinylmethyl	187
17n	*i*-Pr	1-Methyl-2-imidazolylmethyl	356
17o	Me	*n*-Bu	177
17p	*n*-Pr	*n*-Bu	526
17q	Ph	*n*-Bu	3332
17r	*p*-Pyridinyl	*n*-Bu	111
17s	4-Pyridinyl	4-Pyridinylmethyl	22
17t	3,5-Dichlorophenyl	4-Carboxylbenzyl	11
17u	4-Pyridinyl	4-CNBn	9

[a] Data are reported as means of $n \geqslant 3$ determinations. SD was generally ±20% of the average.

Table 1. Pyridine-based MCD inhibitors



Compound	R^1	R^2	X	Y	IC_{50}[a] (nM)
7a	*i*-Bu	*i*-Bu	—	—	47
7b	*n*-Pr	Cyclopropylmethyl	—	—	117
8	*i*-Bu	*i*-Bu	—	—	235
11a	3-Tetrahydrofuranylmethyl	–($CH_2CH_2OCH_2CH_2$)–	S	N	84
11b	3-Tetrahydrofuranylmethyl	–CH_2CH_2CN	S	NEt	24
11c	Et	*i*-Pr	O	bond	22

[a] Data are reported as means of $n \geqslant 3$ determinations. SD was generally ±20% of the average.

Similarly, 2-(*N*-alkylamino)thiazolylhexafluoroisopropanol intermediates **16** did not exhibit significant MCD inhibitory activity. Additional functionality, such as an acyl or an ureido group, is required for good activity. Generally, small aliphatic acyl groups such as methyl or ethyl are preferred over a phenyl or substituted phenyl group. For example, compounds **17o** and **17p** are 6–20 times more potent than **17q**, a benzamide derivative (Table 2). A similar trend was observed in the urea series (data not shown). 2-Arylaminothiazole derivatives usually exhibited low activity (e.g., **17c**, IC_{50}: 1.71 μM) as compared to the corresponding 2-alkylaminothiazole derivatives. A significant improvement of MCD inhibitory activity was observed for those compounds with an *N*-4-substituted benzyl group or an *N*-pyridinylmethyl group. Pyridinylmethyl and 4-cyanobenzyl groups are among the most preferred substituents at this position.

Two potent thiazole-based MCD inhibitors (**17s** and **17t**) having excellent solubility (data not shown) were selected for testing in the isolated working rat heart model[15] to evaluate their ability to regulate fatty acid/glucose oxidations. Compound **17s** produced almost a 5-fold increase in glucose oxidation rates at 10 μM versus the DMSO control. Compound **17t** was slightly less potent than compound **17s** in stimulation of glucose oxidation, partly due to the lower permeability of the former. As a comparison, known fatty acid oxidation inhibitors ranolazine[15b] and trimetazidine,[15c] which function by inhibiting mitochondrial β-oxidation, only increased glucose oxidation rates by a factor of two or less (see Table 3).

In summary, a series of heteroaryl MCD inhibitors were designed and synthesized. Some thiazole-based bis-trifluoromethyl carbinols showed potent MCD inhibitory activities as well as powerful stimulation of glucose oxidation in isolated working rat hearts. Similar to previous observations in the original series,[10a] the mono-trifluoromethyl analog of compound **7a** showed a 60-fold decrease in the MCD inhibition (IC_{50}: 3.25 μM vs 47 nM). These results indicate that the acidity of the hydroxyl group may play a significant role in the binding to the MCD active site, since the hydroxyl group of the bis-trifluoromethyl carbinol moiety is relatively acidic (pK_a: 8.5 for 2-phenyl-hexafluoro-2-propanol)[16] compared to the hydroxyl function of mono-trifluoromethyl isopropanol or a substituted isopropanol. Compounds similar to the above MCD inhibitors and possessing the bis-trifluoromethyl carbinol pharmacophore have been reported to be LXR agonists[17a] or PDE4 inhibitors.[17b] X-ray crystallographic studies on the LXR inhibitors revealed that the hydroxyl group in bis-trifluoromethyl carbinol moiety forms a hydrogen bond with the imidazole unit of a histidine residue in the LXR ligand binding domain of the protein.[18a,b] The strong hydrogen bond interaction could be partially explained by the pK_a match of bis-trifluoromethyl carbinol with the histidine side chain (pK_a: 6.5–7.0).[18c] A histidine residue in the PDE4 catalytic site may also play an important role mechanistically in the hydrolysis of the cAMP phosphodiester.[17c] Although little is known regarding the mechanism of action of MCD or the nature of the active site[19], crystal structures of other crotonase superfamily members such as methylmalonyl-CoA decarboxylase,[20a] chalcone synthase/fatty acid synthases[20b,c] and carboxymethylproline synthase[20d] have demonstrated the importance of a key histidine residue in the active sites, possibly as part of an oxy-anion hole required for the decarboxylation of malonyl-CoA. It is therefore reasonable to hypothesize that one of 13 histidine residues found in the MCD protein may participate in the active site and facilitate the decarboxylation process. If so, the putative histidine residue may form strong hydrogen bonds with bis-trifluoromethyl carbinol moiety of the MCD inhibitors. The SAR results also suggest the presence of a hydrophobic region in the active site which could interact with the small aliphatic side chains of these MCD inhibitors. The identification of the above potent MCD inhibitors may facilitate crystallization of the MCD enzyme and provide insights into the mechanism of action of the decarboxylation process as well as the design of other novel MCD inhibitors.

Table 3. MCD inhibitors and glucose oxidation rates in isolated working rat hearts

Compound	IC_{50} (nM)	GOX[a]
DMSO	—	1
17s	22	4.92
17t	11	3.35
Ranolazine[b]	—	1.77
Trimetazidine[c]	—	2.01

[a] GOX (glucose oxidation rates) were calculated as a ratio of test compound (10 μM) to DMSO (0.05%) control. $n = 8$.
[b] See Ref. 15b,c.
[c] See Ref. 15c.

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