Discovery of Potent and Orally Available Malonyl-CoA Decarboxylase Inhibitors as Cardioprotective Agents

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Abstract: Discovery of 5-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-4,5-dihydroisoxazole-3-carboxamides as a new class of malonyl-coenzyme A decarboxylase (MCD) inhibitors is described. *tert*-Butyl 3-(5-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-4,5-dihydroisoxazole-3-carboxamido)butanoate (**5**, CBM-301940) exhibited excellent potency and in vivo PK/ADME properties. It is the most powerful stimulant of glucose oxidation reported to date in isolated working rat hearts. Compound **5** improved the cardiac efficiency and function in a rat heart global ischemia/reperfusion model, suggesting MCD inhibitors may be useful for the treatment of ischemic heart diseases.

A growing body of evidence points to the therapeutic value of reducing fatty acid metabolism for ischemic heart diseases.[1] Fatty acid oxidation was dominated or enhanced in the hearts of human patients and in animal models of diabetes (type I or II) and ischemic heart disease (e.g., angina pectoris).[2] As a consequence of the interdependence of fatty acid oxidation and glucose oxidation (Randle cycle[3]), enhancing fatty acid oxidation would reduce glucose utilization and further complicate the symptoms or worsen the disease. Reducing fatty acid utilization, on the other hand, would promote the shift of energy production from fatty acid to glucose and lead to the improvement of cardiac efficacy and function. In the cellular system, oxidation of long-chain fatty acids is controlled by carnitine palmitoyltransferases[4] (CPT-I and CPT-II). Inhibitors of CPT-I or β-oxidation enzymes are expected to decrease fatty acid oxidation rates and accelerate glucose oxidation. Indeed, a diverse group of antianginal drugs such as perhexiline (2-(2,2-dicyclohexylethyl)piperidine) and amiodarone (((2-butylbenzofuran-3-yl)-[4-(2-diethylaminoethoxy)-3,5-diiodophenyl]methanone) are believed to act as CPT-I inhibitors.[5] The partial fatty acid oxidation inhibitor, ranolazine (*N*-(2,6-dimethylphenyl)-2-[4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]piperazin-1-yl]acetamide), has been approved recently by FDA as a new class of antianginal drug.[6] Another drug, trimetazidine (1-[(2,3,4-trimethoxyphenyl)methyl]piperazine), used for chronic angina pectoris, is a potent inhibitor of 3-ketoacyl coenzyme A thiolase, a key enzyme in the β-oxidation spiral.[7]

MCA[a] is a potent endogenous inhibitor of CPT-I.[8] It is generated from acetyl-CoA by ACCs. MCA is utilized by fatty acid synthase as a substrate for fatty acid synthesis or is degraded by the enzyme malonyl-CoA decarboxylase[9,10] (EC 4.1.1.9). We recently reported the identification and activity of the first-generation, small-molecule MCD inhibitors.[11] As expected, pharmacological inhibition of MCD significantly increased malonyl-CoA levels and shifted the energy production from fatty acid oxidation to glucose oxidation in ex vivo experiments with isolated working rat hearts and in vivo experiments in a pig demand induced ischemia model.[12] On the basis of these first-generation MCD inhibitors,[11] we have successfully developed a novel class of potent MCD inhibitors with excellent PK and ADME properties. Compound **5** (CBM-301940) potently stimulates glucose oxidation in isolated working rat hearts and improved cardiac efficiency and function in a severe global ischemic/reperfusion rat heart model.

The synthesis of isoxazoline-based MCD inhibitors is depicted in Scheme 1. Hexafluoro-2-propanol-containing dipolarophile, 1,1,1-trifluoro-2-trifluoromethylbut-3-en-2-ol, was prepared from hexafluoroacetone (gas) and Grignard reagents according to the literature procedure.[13] Cycloaddition of commercially available chlorooximidate **1** with this dipolarophile, in the presence of triethylamine, afforded the key isoxazoline intermediate (**2**). The reaction was performed by slow addition of triethylamine in THF, using a syringe pump, to a solution of chlorooximidoacetate and 1,1,1-trifluoro-2-trifluoromethylbut-3-en-2-ol in THF at room temperature. Saponification of the isoxazoline ester **2**, followed by coupling with appropriate amines, afforded the desired isoxazoline derivatives **4** in moderate to good yields.

The synthesis of optically pure diasteromers of **5** (**5c**−**f**) is outlined in Scheme 2. Starting from the diastereoisomeric **5a**, which was prepared from optically pure (*S*)-*tert*-butyl β-aminobutarate and the racemic 3-isoxazoline carboxylic acid **2**, two diastereoisomeric Mosher esters (**6a** and **6b**) were obtained by reacting **5a** with (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride. The two diastereomeric Mosher esters could be separated by column chromatography and exhibited distinct chemical shifts and/or coupling constants in ^{1}H, ^{19}F, and ^{13}C NMR spectra in the isoxazoline and ester regions. Hydrolysis of these two compounds provided optically pure diastereomers **5c** and **5d**. Similarly, two other optically pure diastereoisomers (**5e** and **5f**) were obtained via sequential reactions starting from **5b**, which in turn was prepared from optically pure (*R*)-*tert*-butyl β-aminobutyrate.

The absolute configuration of **5c** was assigned through a combination of chemical reaction and X-ray crystallographic analysis of intermediate Mosher ester (**7a**, Scheme 2 and Figure 1).[14] Mosher ester **7a** was prepared according to Scheme 1 and subsequently converted into **5c**, which is identical in all aspects of spectroscopic data, including optical rotation, to the compound prepared from **5a**. Therefore, the absolute configuration of **5c** was determined as *S*,*R*. The diasteroisomer **5d** was then assigned to have *S* configuration at the C5 position of the isoxazoline ring. Accordingly, the absolute stereochemistry of two remaining diasteroisomers prepared from the *R* amino acid

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[a] Abbreviations: MCA, malonyl-coenzyme A; CoA, coenzyme A; MCD, malonyl-coenzyme A decarboxylase; PK, pharmacokinetic; ADME, absorption, distribution, metabolism, and excretion; CPT, carnitine palmitoyltransferase; ACC, acetyl-coenzyme A carboxylase; GOX, glucose oxidation.

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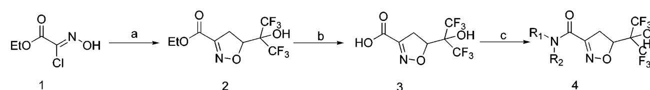
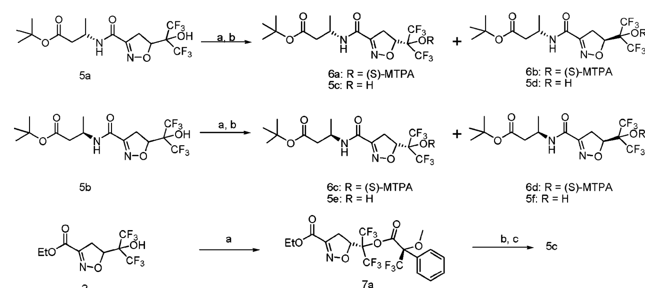
Scheme 1. Synthesis of 5-(1,1,1,3,3,3-Hexafluoro-2-hydroxypropan-2-yl)-4,5-dihydroisoxazole-3-carboxamides[a]



[a] Reagents and conditions: (a) Et_3N (1.1 equiv), 1,1,1-trifluoro-2-trifluoromethylbut-3-en-2-ol; (b) NaOH aq, ethanol; (c) BOP, R_1R_2NH, 4-*N*-methylmorpholine, DMF.

Scheme 2. Preparation of Stereoisomers of Compound **5**[a]



[a] Reagents and conditions: (a) (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride [(*S*)-MTPA-Cl], Et_3N, DMAP, THF; (b) NaOH aq, ethanol; (c) (*S*)-*tert*-butyl β-aminobutyrate, EDAC, DMAP, DCM, room temp.

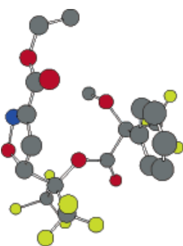


Figure 1. ORTEP representation of the X-ray crystal structure of **7a**.

ester was assigned by comparing the optical rotations and characteristic NMR chemical shifts of Mosher ester intermediates.

MCD inhibitors were tested for their ability to inhibit a soluble maltose binding protein fused human malonyl-coenzyme A decarboxylase as described previously.[11,15] The IC_{50} values of the synthesized compounds are tabulated in Tables 1 and 2. The most potent compounds were further profiled in terms of in vitro pharmaceutical properties (solubility, Caco-2 permeability, rat and human metabolic stability, data not shown) and in vivo PK/ADME properties. Selected compounds were examined for their ability to stimulate glucose oxidation in isolated working rat hearts. These results are summarized in Table 3.

As summarized below, several key modifications provided insight into the general SAR of this series. Although disubstituted (tertiary) amides with small aliphatic moieties such as **4b** (IC_{50} = 676 nM) and **4c** (IC_{50} = 137 nM) are well tolerated, the more potent MCD inhibitors proved to be appropriately monosubstituted amides (e.g., **4e** and **4f**). In general, increasing hydrophobicity at the terminus of the side chain increased the in vitro enzyme inhibitory potency. For example, *tert*-butyl ester **4k** (IC_{50} = 31 nM), diisopropylamide **4l** (IC_{50} = 17 nM) and bulkier ester compounds **4m** and **4n** (IC_{50} = 7 and 9 nM) exhibited higher potency than the corresponding ethyl ester compounds **4i** (IC_{50} = 463 nM). The compound possessing a terminal *tert*-butyl ether group (**4g**, IC_{50} = 28 nM) was as potent as the corresponding *tert*-butyl ester **4k**. However, **4k** exhibited a better PK profile than the ether **4g** (data not shown), perhaps owing, in part, to the improved solubility. Removal of the *tert*-butyl function led to the less potent carboxylic acid (data not shown), indicating that a hydrophobic group is indeed required for the higher activity.

Introduction of a small alkyl group near the amide nitrogen atom is well tolerated and beneficial. An isobutyl group (e.g., **4p** and **4q**) is apparently better than a benzyl function (**4o**). Introduction of a methyl group on the amine side chain led to the discovery of **5**, which contains a *tert*-butyl ester at the

Table 1. Isoxazoline-Based MCD Inhibitors (I)

compd	R_1	R_2	IC_{50} (nM)[a]
4a	^{i}Bu−	H	801
4b	^{i}Bu−	Me	676
4c	^{i}Bu−	^{i}Bu	137
4d	$tBuCH_2CH_2$−	H	489
4e	2-hepatyl−	H	60
4f	6-methyl-2-heptyl−	H	28
4g	$tBuOCH_2CH_2CH_2$−	H	28
4h	EtO_2CCH_2−	H	1767
4i	$EtO_2CCH_2CH_2$−	H	463
4j	$EtO_2CCH_2CH_2CH_2$−	H	421
4k	$tBuO_2CCH_2CH_2$−	H	31
4l	$^iPr_2NCOCH_2CH_2$−	H	17
4m	$CH_3(Et)_2CO_2CCH_2CH_2$−	H	7
4n	$CH_3(^iBu)_2CO_2CCH_2CH_2$−	H	9
4o	*S*-$tBuO_2CCH(Bn)$−	H	113
4p	*S*-$tBuO_2CCH_2CH(^iBu)$−	H	15
4q	(±)-$tBuO_2CCH_2CH(^iBu)$−	H	30
4r	*R*-$tBuO_2CCH_2CH(^iBu)$−	H	45
4s	(±)-$Me(^iBu)_2CO_2CCH_2CH(Me)$−	H	37
4t	*S*-$CH_3(Et)_2CO_2CCH_2CH(Me)$−	H	5

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

Table 2. Isoxazoline-Based MCD Inhibitors (II)

compd	A	B	IC_{50} (nM)[a]
5	*RS*	*RS*	23
5a	*S*	*RS*	7
5b	*R*	*RS*	10
5c	*S*	*S*	136
5d	*S*	*R*	5
5e	*R*	*R*	8
5f	*R*	*S*	168

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

Table 3. MCD Inhibitors and Glucose Oxidation Rates

compd	IC_{50} (nM)	GOX rate (%)[a]
DMSO		1
4c	137	2.11
4k	31	4.54
4e	60	0.80
4f	28	1.26
5a	7	5.01
4p	15	3.24
4g	28	5.42
4q	30	4.28
5	23	7.32, 3.07[b]
ranolazine HCl[c]		1.77
trimetazidine[d]		2.01

[a] GOX rates were calculated as a ratio of test compound (10 μM) to DMSO (0.05%) control (n = 8 hearts). [b] At 1 μM test concentration. [c] See ref 6a. [d] See ref 7.

Table 4. Pharmacokinetic Parameters for MCD Inhibitor **5**[a]

compd	$t_{1/2}$, iv (h)	CL (mL h^{-1} kg^{-1})	T_{max}, po (h)	C_{max}, po (ng mL^{-1}, po)	V_{ss} (mL kg^{-1})	*F* (%)
5	2.3	915	0.5	2683	2193	74

[a] After iv or oral dose of 10 mg/kg in Sprague-Dawley rats. Bioavailability (*F*) was calculated as the ratio of dose-normalized po to iv AUCs for the same time frame. Clearance (CL) is the rate at which a given amount of plasma is totally cleared of unbound compound. Volume of distribution (V_{ss}) is an approximation of the volume that the compound would occupy at steady state. Oral dosing vehicle was 80% PG/20% EtOH.

terminus (Table 2). Compound **5** is as potent as the parent **4k** or the isobutyl substituted analogue **4q**. The chirality of the side chain portion does not seem to be a determinant. The *R* and *S* diastereoisomers **5a** and **5b** exhibited only a 2- to 3-fold increase in MCD inhibitory activitiy compared to the racemic parent **5** (Table 2). However, a more profound influence of chirality on activity was evidenced by a greater than 20-fold increase in activity of the isoxazolines with *R* configuration at the C5 position of the isoxazoline ring vs their *S* counterparts (**5c** vs **5d**, **5e** vs **5f**).

As described previously, MCD inhibitors cause an elevation of malonyl-CoA levels, an increase of glucose oxidation rate, and a decrease in fatty acid oxidation rates in isolated working rat hearts.[11,12] A number of the isoxazoline-based MCD inhibitors were selected on the basis of their favorable in vitro properties, especially in vitro potency, solubility, and Caco-2 permeability, and tested for their ability to stimulate glucose oxidation (Table 3).[16] A clear correlation of in vitro potency with glucose oxidation rate cannot be established probably because of the differences in solubility and permeability among the compounds tested. In general, however, the more potent compounds exhibited a greater enhancement in glucose oxidation rate. Compounds **4k**, **5a**, and the *tert*-butyl ether **4g** caused a 4- to 5-fold increase in glucose oxidation at 10 μM vs the DMSO control. Compound **5** was found to be the most potent metabolic modulator reported to date, demonstrating a 732% increase in glucose oxidation rate when tested at 10 μM. Even at a concentration as low as 1 μM, **5** accelerated the glucose oxidation rate by more than 3-fold. The stimulation of glucose oxidation by **5** is significantly more effective than known metabolic modulators such as ranolazine[6] and trimetazidine[7] and the other reported inhibitors from our own laboratories.[11]

Compound **5** showed excellent PK properties (Table 4). A single oral dose of **5** in rats at 10 mg/kg gave an average C_{max} of 2.68 μg/mL at 30 min, corresponding to 6.3 μM, which is well above the concentration (1 μM) that caused a 3-fold increase in glucose oxidation in isolated working rat hearts. The compound also has greater than 70% oral bioavailability and a terminal $t_{1/2}$ of ~2.3 h.

Compound **5** was tested in a severe no-flow global ischemic reperfusion rat heart model. Hearts were aerobically perfused for 30 min, followed by 30 min of global no-flow ischemia and 60 min of aerobic reperfusion. Compound **5** (1 μM) was added to the perfusate before the ischemia or at the onset of reperfusion. No significant alterations in the mechanical function on hearts were seen with **5** before ischemia. Cardiac function on aerobic reperfusion after ischemia was significantly improved in hearts treated with **5**, independent of whether it was added before or at the onset of the ischemia Compound **5** also significantly improved rate pressure product, cardiac output, aortic flow, coronary flow, and cardiac power on aerobic reperfusion of ischemic hearts (data not shown).[12a]

In conclusion, a novel series of potent, isoxazoline-based, small-molecule MCD inhibitors were designed and synthesized. Through a process of structural optimization, an isoxazoline-hexafluoro-2-propanol analogue, **5**, was identified as a lead MCD inhibitor. Compound **5** demonstrated low nanomolar MCD inhibitory activity and a 7-fold increase at 10 μM or a 3-fold increase at 1 μM in glucose oxidation rates in the isolated working hearts. Compound **5** also demonstrated cardioprotective activity in a global ischemia-reperfusion rat model. In rats, **5** showed a greater than 70% oral bioavailability and an excellent terminal half-life. The combination of high inhibitory potency, excellent PK profile, potent glucose oxidation stimulating activity, and in vivo efficacy in an animal model of disease may render **5** as a valuable pharmacological tool in elucidating the roles of MCD in cell signaling pathways and the potential for usage in the treatment of ischemic heart disease and other diseases that can be ameliorated by modulation of glucose and fatty acid metabolism.

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Supporting Information Available: Experimental procedures and characterization of compounds, HRMS data for listed compounds, and X-ray crystallographic data for **7a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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