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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis and in vitro evaluation of some small molecules malonyl CoA decarboxylase inhibitors containing pyrazoline scaffold and study of their binding interactions with malonyl CoA decarboxylase via preliminary docking simulation

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Abstract In the present work series of small molecules (**5a–5m**, **6a–6j**) were schematically designed and synthesized using simple chemical procedures. Their structures were confirmed based upon findings from infrared, 1H nuclear magnetic resonance (NMR), 13C NMR, and mass spectra. The derivatives were evaluated for their in vitro malonyl CoA decarboxylase inhibition activity by using fluorimetric assay. Pyrazol-1-yl-1, 3-thaizol-4(5H)-one derivative (**5a–5m**) showed better activity than pyrazol-1-yl-1-ethanone derivatives (**6a–6j**). Compounds **5e**, **5j**, and 6f showed an excellent in vitro malonyl CoA decarboxylase inhibition activity with IC50 value 0.10, 0.27, and 0.26 µM, respectively. These most active compounds **5e**, **5j**, and **6f** were docked into malonyl-CoA decarboxylase (HsMCD, PDB ID: 2YGW) to study ligand–protein interaction.

Keywords 4, 5-Dihydropyrazole · Malonyl CoA decarboxylase inhibition · Fluorimetric assay · Thiazolone · Ethanone · HsMCD (2YGW)

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Introduction

Cardiovascular disease is one of the leading causes of death in the modern world. Impaired cardiac efficiency is an important contributor to the severity of cardiovascular disease. Impaired cardiac efficiency is caused by an inadequate supply of oxygen to the heart (Ussher and Lopaschuk 2008; Stanley et al. 2005; Frink 2012).

During and after cardiac ischemia, increased levels of fatty acids in circulation result in fatty acid oxidation being the predominant source of energy to the heart. An abnormally high rate of fatty acid oxidation and a low level of glucose oxidation cause uncoupling of glucose oxidation and the glycolysis process. This results in acidosis of heart cells, which further aggravates the ischemic condition (Jaswal et al. 2011; Fillimore and Lopaschuk 2013).

Malonyl-coenzyme A (CoA) regulates fatty acid oxidation by inhibiting the mitochondrial uptake of fatty acids via the inhibition of carnitine palmitoyl transferase I (Dyck et al. 2004; Bandopadhyay et al. 2006; Walters 2012). Malonyl-CoA decarboxylase (MCD) decarboxylates malonyl-CoA to acetyl-CoA. Therefore, the inhibition of MCD increases the level of malonyl-CoA, which further reduces fatty acid oxidation and increases glucose oxidation in the mitochondria (Dyck and Lopaschuk 2002; Cheng et al. 2005a; Clifford and Lopaschuk 2007; Muoio and Newgard 2008). A shift in the mitochondrial metabolism from fatty acid to glucose oxidation increases Adenosine tri phosphate production. Thus, the heart may receive more energy even if the oxygen supply is less. In addition, increased glucose oxidation reduces pyruvate in cellular fluids, improving the pH balance of heart cells (Abel 2007; Yan et al. 2009).

Recently, researchers have synthesized MCD inhibitors based on this novel approach of increasing energy supply

to the heart. In 2004, Arrhenius et al. synthesized thiol-substituted heterocyclic derivatives. Furthermore, Cheng et al. (2005b) worked on hydroxyl-substituted benzofuran derivatives. Cheng et al. (2006a, b) and Arrhenius et al. (2009) synthesized heteroaryl-substituted 1,1,1,3,3,3-hexafluoroisopropanol derivatives. Wallace et al. (2007) synthesized tri-fluoro acetophenone derivatives. These synthesized compounds have exhibited in vitro MCD inhibitory activity. In vivo studies, have revealed that MCD inhibitors increased the levels of malonyl-CoA and the rate of glucose oxidation (Cheng et al. 2006c; Patel and Talele 2007; Tang et al. 2010). They have also been reported to increase level of malonyl-CoA, decrease rate of fatty acid oxidation, increase rate of glucose oxidation, and improve ischemic condition in MCD knock-out mice. (Dyck et al. 2006; Ussher et al. 2009). MCD inhibition could thus be a novel approach in the treatment of ischemia.

Various previously reported MCD inhibitors possess heteroaromatic rings such as 1,2-oxazole; 1,3-thiazole; 1,2-thiazole; and benzothiazole (Fig. 1a). Meanwhile, with a wide range of pharmaceutical activities, 1,2-pyrazolines act as a significant class of heterocyclic biological agents (Jainey and Bhat 2012; Barsoum et al. 2006; Chimenti et al. 2005; Amir et al. 2008). Quantitative structure–activity relationship study reported by Patel and Talele (2007) stated that an aromatic ring bearing an acidic proton with less steric bulk in surrounding is favorable for MCD inhibitory activity.

In view of these facts, it was thought of interest to combine 1,2-pyrazoline ring and acidic phenolic group. Thus, in the present study we describe design and synthesis of twenty three novel 3,5-diaryl-(pyrazol-1-yl)-1,3-thiazole-4(5*H*)-one (**5a–5m**, Fig. 1b) and 3,5-diaryl-(pyrazol-1-yl)-ethanone derivatives (**6a–6j**, Fig. 1c).

As the selected synthesis route was easy, it could be performed under normal laboratory conditions. All synthesized compounds were tested for their in vitro MCD inhibitory activity. Docking simulation studies were performed for the most active compounds on human MCD (**HsMCD**; Protein Data Bank [PDB] ID: **2YGW**) to elucidate their probable mode of action and explore their active binding site and the amino acid residues which interact with MCD inhibitors.

Materials and methods

Experimental

All the solvents and reagents were procured from S D Fine-Chem., Ltd., India. The reagents and solvents used were of analytical grade and were used without further purification. The completion of the reaction was checked by thin layer chromatography on silica gel 60 F_{254} aluminum sheets, obtained from Merck Specialities Pvt. Ltd., India. Purification of synthesized compounds was performed either by recrystallization or flash chromatography. Melting points were determined by open capillary method on a 'Veego' VMP-D melting point apparatus and are uncorrected. Melting points are expressed in °C. The FTIR spectra of synthesized compounds were recorded on a Shimadzu FTIR 8400S spectrophotometer using potassium bromide (KBr) pellets and are expressed in cm^{-1}. Percent purity was determined by using Agilent 1200 series high performance liquid chromatography (HPLC) with C18 column (250 × 4.6 mm i.d.; 5 µm particle size) and EZChrome elite software for data acquisition. The percentage of area under peak which indicates the percent purity was calculated by using area under peak and total area. ^{1}H NMR spectra were recorded on Varian Inc. (300 MHz) NMR spectrometer using tetramethylsilane as an internal standard and DMSO-d_6 or $CDCl_3$ as solvents. The mass spectra were recorded on Varian Inc. MS spectrometer by using Electrospry Ionization technique and ^{13}C NMR spectra were recorded on Bruker Avance II (100 MHz) NMR spectrometer. The ^{1}H NMR, ^{13}C NMR, and Liquid Chromatography-Mass

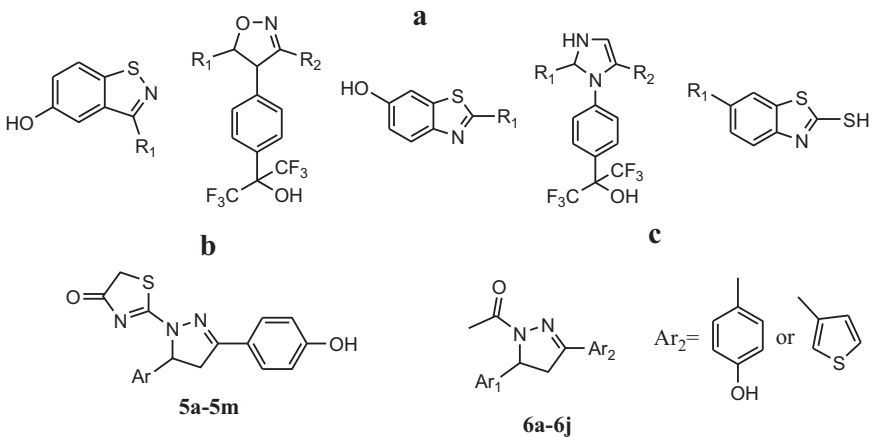
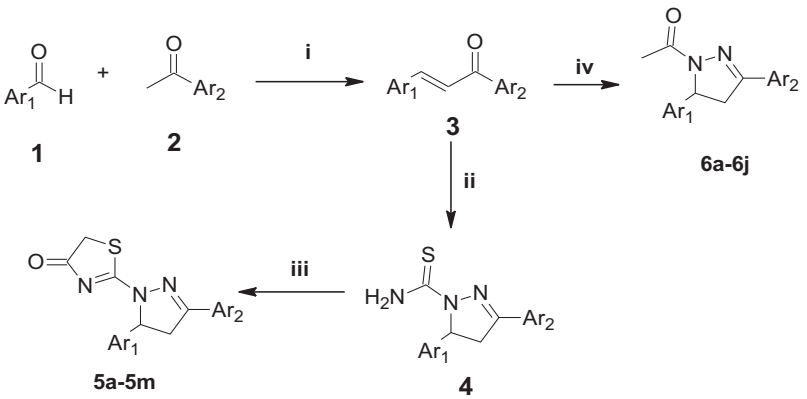


Fig. 1 Designing of small molecule MCD inhibitors: **a** Reported MCD inhibitors, R_1 and R_2 are alkyl or aryl groups; **b** General structure of 3,5-diaryl-(pyrazol-1-yl)-1,3-thiazole-4(5*H*)-one derivatives (**5a–5m**), Ar is aromatic group; **c** General structure of 3,5-diaryl-(pyrazol-1-yl)-ethanone derivatives (**6a–6j**), Ar_1 and Ar_2 are aryl groups

Scheme 1 Synthetic scheme of *N*-substituted novel pyrazolyl derivatives (**5a–5m**, **6a–6j**). *Reagents and conditions:* (i) NaOH, EtOH, stir, 15–25 °C, 4 h; (ii) thiosemicarbazide, EtOH, KOH reflux, 8 h; (iii) ethylbromoacetate, ethylacetate, reflux, 1–2 h; (iv) acetic acid hydrazide, KOH, reflux, 1–2 h



5a; Ar_1= -C_6H_5, Ar_2=4-OHC_6H_4
5b; Ar_1= 4-$OMeC_6H_4$, Ar_2=4-OHC_6H_4
5c;2-Br,4-BrC_6H_3, Ar_2=4-OHC_6H_4
5d;-C_4H_3S, Ar_2=4-OHC_6H_4
5e;-C_4H_3O, Ar_2=3-OMe,4-OMe4-OHC_6H_2
5f;4-BrC_6H_4, Ar_2=4-OHC_6H_4
5g;-C_4H_3O, Ar_2=4-OHC_6H_4
5h;2-NO_2,4-$NO_2C_6H_3$, Ar_2=4-OHC_6H_4
5i;2-NO_2,4-$OMeC_6H_3$, Ar_2=4-OHC_6H_4
5j;3-F,4-FC_6H_3, Ar_2=4-OHC_6H_4
5k;3-OMe,5-$OMeC_6H_3$, Ar_2=4-OHC_6H_4
5l;4-$NO_2C_6H_4$, Ar_2=4-OHC_6H_4
5m; -C_5H_4N, Ar_2=4-OHC_6H_4

6a;2-Cl,4-ClC_6H_3, Ar_2=4-OHC_6H_4
6b;2-F,4-FC_6H_3, Ar_2=4-OHC_6H_4
6c;4-OHC_6H_4, Ar_2=-C_4H_3S
6d;2-Br,4-BrC_6H_3, Ar_2=4-OHC_6H_4
6e;-C_4H_3S, Ar_2=4-OHC_6H_4
6f;3-Br,4-BrC_6H_3, Ar_2=4-OHC_6H_4
6g;-C_4H_3O, Ar_2=4-OHC_6H_4
6h;2-OMe,4-$OMeC_6H_3$, Ar_2=4-OHC_6H_4
6i;4-ClC_6H_4, Ar_2=4-OHC_6H_4
6j;3-Cl,4-ClC_6H_3, Ar_2=4-OHC_6H_4

Spectroscopy spectra were recorded at Sophisticated Analytical Instrumental Facility of IIT, Mumbai or University of Punjab, India.

General procedure for synthesis of pyrazol-1-yl-1, 3-thaizol-4(5*H*)-one derivatives (5a–5m)

A suspension of aromatic aldehyde **1** (40 mmol) and aromatic ketone **2** (40 mmol) in mixture of 10 ml saturated NaOH solution in water and 10 ml ethanol was stirred for 4 h at 20 °C to obtain pure chalcone **3** (Ozdemir et al. 2007; Chimenti et al. 2010). Further, mixture of chalcone **3** (10 mmol) and thiosemicarbazide (20 mmol) was refluxed under stirring with KOH (20 mmol) in 70 mL EtOH for approximately 6 h to get *N*-thiocarbamoyl pyrazole derivative **4** (Scheme 1). This *N*-thiocarbamoyl pyrazole derivative **4** (10 mmol) was added in appropriate quantity of EtOAc and was refluxed with ethyl bromoacetate (30 mmol) for 1.5 h (El-Sabbagh et al. 2009; Seebacher et al. 2003). After completion of reaction, the reaction mixture was mixed with $CHCl_3$ and allowed to evaporate to obtain pyrazol-1-yl-1,3-thaizol-4(5*H*)-one derivative (**5a–5m**, Fig. 2). The refined compound was obtained by subsequent purification with recrystallization by using ethanol-acetone (1:1).

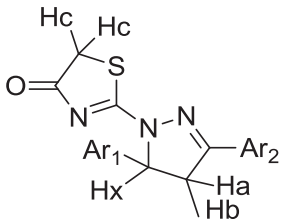


Fig. 2 General structure of substituted (pyrazole-1-yl)-1, 3-thiazol-4 (5*H*)-one derivatives (**5a–5m**)

*2-[3-(4-Hydroxyphenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**5a**)*

Brown powder; yield, 53%; purity, 94.29% (HPLC, methanol:water, 80:20); mp 220–222 °C; IR, ν_{max} (KBr)/cm^{-1}: 3311, 3068, 1685, 1618, 1375, 729. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.73 (s, 1H, OH), 7.23–7.46 (m, 7H, ArH), 7.11 (dd, 1H, ArH), 6.97 (m, 1H, ArH), 5.78 (dd, *J* 3.9, 11.2, 1H, H_x), 4.06 (dd, *J* 11.2, 17.7, 1H, H_b), 3.89 (s, 2H, H_c), 3.51 (dd, *J* 3.9, 17.7, 1H, H_a). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 186.7, 177.1, 175.8, 160.9, 157.2, 156.5, 142.9, 140.5, 132.9, 129.9, 128.9, 126.9, 125.5,

63.0, 61.9, 45.9, 44.1. MS *m/z*: 337.08 ($M^{+\bullet}$, 100%), 310.99 ($M^{+\bullet}$ + 2–28, 55%).

*2-[3-(4-Hydroxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**5b**)*

Pale yellow powder; yield, 46%; purity, 95.40% (HPLC, methanol: water, 80:20); mp 200–202 °C. IR, ν_{max} (KBr)/ cm^{-1} 3317, 3064, 2960, 1739, 1504, 1321, 719. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.73 (s, 1H, OH), 7.41–7.47 (m, 1H, ArH), 7.10–7.31 (m, 3H, AHr), 6.93 (m, 2H, ArH), 6.86 (dd, 2H, ArH), 5.75 (dd, *J* 3.9, 11.1, H_x), 3.96 (dd, *J* 11.1, 17.7, H_b), 3.89 (s, 2H, H_c), 3.78 (s, 3H, OCH_3), 3.53 (dd, *J* 4.2, 18, H_a). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 186.7, 177.1, 175.8, 160.9, 157.2, 156.5, 156.3, 142.9, 140.5, 132.9, 132.1, 129.2, 128.5, 126.8, 63.0, 61.9, 45.9, 44.1. MS *m/z* 388.99 ($M^{+\bullet}$ + Na, 100%), 366.99 ($M^{+\bullet}$, 97%).

*2-[5-(2,4-Dibromophenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**5c**)*

Pale brown powder; yield, 57%; purity, 93.06% (HPLC, methanol:water, 80:20); mp 234–236 °C. IR, ν_{max} (KBr)/ cm^{-1} 3302, 3066, 1695, 1502, 1327, 1004, 725, 534. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.13 (s, 1H, OH), 7.11–7.63 (m, 5H, ArH), 6.95 (dd, 1H, ArH), 6.58 (dd, 1H, ArH), 5.73 (dd, *J* 3.8, 11.2, 1H, H_x), 3.91 (dd, *J* 11.4, 18.6, 1H, H_b), 3.86 (s, 2H, H_c), 3.28 (dd, *J* 3.8, 18, 1H, H_a). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 189.7, 173.0, 172.9, 165.7, 161.9, 159.4, 158.5, 131.8, 131.4, 129.1, 128.7, 128.3, 127.5, 118.4, 117.0, 62.3, 55.9, 35.6. MS *m/z* 493.00 ($M^{+\bullet}$, 19%), 472.29 ($M^{+\bullet}$-17, 100%).

*2-[3-(4-Hydroxyphenyl)-5-(thiophen-3-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**5d**)*

Pale yellow powder; yield, 62%; purity, 91.01% (HPLC, methanol:water, 80:20); mp 284–286 °C. IR, ν_{max} (KBr)/ cm^{-1} 3333, 3068, 1693, 1500, 1271, 705. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 10.27 (s, 1H, OH), 7.74 (dd, 1H, ArH), 7.42–7.47 (m, 2H, ArH), 7.11 (d, 1H, ArH), 6.93–7.00 (m, 3H, ArH), 6.05 (dd, *J* 3.3, 10.9, 1H, H_x), 4.16 (dd, *J* 10.8, 18.7, 1H, H_b), 3.96 (s, 2H, H_c), 3.67 (dd, *J* 3.3, 18.9, 1H, H_a). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 188.7, 174.0, 164.7, 162.9, 159.4, 158.5, 131.8, 131.4, 129.1, 128.5, 127.6, 118.4, 117.3, 62.3, 62.3, 55.91, 35.6. MS *m/z* 343.09 ($M^{+\bullet}$, 100%).

*2-[5-(Furan-3-yl)-3-(4-hydroxy-3,5-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**5e**)*

Brown powder; yield, 65%; purity, 96.33% (HPLC, methanol: water, 80:20); mp 247–249 °C. IR, ν_{max} (KBr)/ cm^{-1} 3333, 3068, 1693, 1500, 1271, 705. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.43 (s, 1H, OH), 6.94 (d, 1H, ArH), 6.77–6.82 (m, 3H, ArH), 6.57 (dd, 1H, ArH), 5.72 (dd, *J* 3.6, 12Hz, 1H, H_x), 3.88 (dd, *J* 11.4, 17.4Hz, 1H, H_b), 3.86 (s, 2H, H_c), 3.84 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.32 (dd, *J* 3.6, 18 Hz, 1H, H_a), ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 187.0, 177.1, 175.8, 161.9, 157.2, 156.5, 142.9, 132.9, 129.8, 128.9, 127.8, 126.8, 125.5, 73.7, 63.0, 61.8, 45.9, 44.1. MS *m/z* 388.79 ($M^{+\bullet}$ + 1, 12%), 325.19 ($M^{+\bullet}$-63, 88%).

*2-[5-(4-Bromophenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**5f**)*

Brown powder; yield 62%; purity, 91.19% (HPLC, methanol: water, 80:20); mp 219–221 °C. IR, ν_{max} (KBr)/ cm^{-1} 3333, 3068, 1693, 1518, 1139, 746, 551. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.39 (s, 1H, OH), 7.63 (d, 1H, ArH), 7.22–7.37 (m, 5H, ArH), 6.94 (d, 1H, ArH), 6.57 (dd, 1H, ArH), 5.77 (dd, *J* 3.6, 11.1, 1H, H_x), 3.90 (dd, *J* 10.9, 17.5, 1H, H_b), 3.86 (s, 2H, H_c), 3.32 (dd, *J* 3.6, 17.7, 1H, H_a), ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 186.8, 176.7, 161.2, 157.1, 150.0, 132.9, 129.2, 127.7, 126.6, 119.7, 116.9, 115.9, 112.4, 63.5, 59.5, 45.6. MS *m/z* 414.99 ($M^{+\bullet}$, 25%), 332.99 ($M^{+\bullet}$-81, 100%).

*2-[5-(Furan-3-yl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**5g**)*

Pale yellow powder; yield, 68%; purity, 97.31% (HPLC, methanol:water, 80:20); mp 210–212 °C. IR, ν_{max} (KBr)/ cm^{-1} 3344, 3053, 1730, 1518, 1268, 705. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.66 (s, 1H, OH), 7.32–7.48 (m, 3H, ArH), 6.98–7.12 (m, 2H, ArH), 6.59 (d, 1H, ArH), 6.34 (dd, 1H, ArH), 5.87 (dd, *J* 4.8, 10.5 Hz, 1H, H_x), 3.92 (s, 2H, H_c), 3.83 (dd, *J* 5.1, 9.0 Hz, 1H, H_b), 3.74 (dd, *J* 4.8, 15.9 Hz, 1H, H_a), ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 186.6, 177.1, 160.8, 157.2, 150.5, 142.8, 132.8, 129.0, 119.5, 116.8, 115.6, 110.5, 108.6, 65.5, 56.5, 41.7. MS *m/z* 327.99 ($M^{+\bullet}$, 87%), ($M^{+\bullet}$-116, 100%).

*2-[5-(2,4-Dinitrophenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**5h**)*

Light brown powder; yield, 57%; purity, 96.78% (HPLC, methanol:water, 80:20); mp 265–268 °C. IR, ν_{max} (KBr)/ cm^{-1} 3315, 3049, 1687, 1695, 1497, 1259, 759. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.63 (s, 1H, OH), 8.04–8.21 (m, 3H, ArH), 7.67–7.72 (m, 2H, ArH), 7.17 (d, 1H, ArH), 6.73 (dd, 1H, ArH), 5.96 (dd, *J* 4.2, 12, 1H, H_x), 4.08 (dd, *J* 11.5, 18.0, 1H, H_b), 3.96 (s, 2H, H_c), 3.44 (dd, *J* 4.8, 18.0, 1H, H_a). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 186.4, 176.8, 162.0, 160.4, 159.5, 157.4, 157.2, 132.7, 130.4, 130.3,

129.0, 119.5, 116.9, 116.8, 115.37, 70.7, 62.0, 35.0. MS *m/z* 427.09 ($M^{+\bullet}$, 7%), 355.99 ($M^{+\bullet}$-71, 100%).

*2-[3-(4-Hydroxyphenyl)-5-(4-methoxy-2-nitrophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**5i**)*

Yellow powder; yield, 55%; purity, 95.72% (HPLC, methanol: water, 80:20); mp 262–264 °C. IR, ν_{max} (KBr)/cm^{-1} 3317, 3017, 1680, 1500, 1327, 719. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.19 (s, 1H, OH), 7.62 (d, 1H, ArH), 7.19 (d, 2H, ArH), 6.94 (d, 1H, ArH), 6.83 (d, 2H, ArH), 6.56 (dd, 1H, ArH), 5.75 (dd, *J* 3.9, 11.1, 1H, H_x), 3.84 (s, 2H, H_c), 3.84 (dd, *J* 11.4, 18.3, 1H, H_b), 3.76 (s, 3H, OCH_3), 3.31 (dd, *J* 4.2, 18.3, 1H, H_a). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 186.6, 177.2, 173.6, 163.0, 158.9, 150.9, 146.7, 145.1, 132.0, 127.0, 116.4, 114.1, 112.5, 82.7, 64.7, 55.0, 43.0. MS *m/z* 412.08 ($M^{+\bullet}$, 8%), 366.89 ($M^{+\bullet}$-46, 100%).

*2-[5-(3,4-Difluorophenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**5j**)*

Yellow powder; yield, 61%; purity, 92.56% (HPLC, methanol: water, 80:20); mp 234–236 °C. IR, ν_{max} (KBr)/cm^{-1} 3319, 2962, 1732, 1518, 1268, 1020, 686. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.36 (s, 1H, OH), 7.63 (d, 1H, ArH), 7.21–7.30 (m, 2H, ArH), 6.95–7.05 (m, 3H, ArH), 6.58 (dd, 1H, ArH), 5.76 (dd, *J* 3.9, 11.1, 1H, H_x), 3.91 (dd, *J* 11.4, 18.3, 1H, H_b), 3.86 (s, 2H, H_c), 3.30 (dd, *J* 4.2, 18.3, 1H, H_a). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 187.7, 177.1, 160.8, 157.2, 150.5, 143.8, 132.8, 130.0, 129.5, 116.8, 115.6, 110.5, 108.6, 65.5, 56.5, 41.7. MS *m/z* 373.09 ($M^{+\bullet}$, 100%), 328.99 ($M^{+\bullet}$-44, 33%).

*2-[5-(3,5-Dimethoxyphenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**5k**)*

Pale yellow powder; yield, 65%; purity, 93.75% (HPLC, methanol:water, 80:20); mp 280–282 °C. IR, ν_{max} (KBr)/cm^{-1} 3266, 3088, 1696, 1492, 1100, 712. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.79 (s, 1H, OH), 7.41–7.46 (m, 1H, ArH), 7.30 (dd, 1H, ArH), 7.10–7.17 (m, 2H, ArH), 6.90–7.01 (m, 1H, ArH), 6.65 (d, 2H, ArH), 5.68 (dd, *J* 3.9, 11.1 Hz, 1H, H_x), 3.97 (dd, *J* 11.4, 18.3 Hz, 1H, H_b), 3.87 (s, 2H, H_c), 3.54 (dd, *J* 4.2, 18 Hz, 1H, H_a), 2.93 (s, 6H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 186.8, 176.7, 161.2, 157.1, 150.0, 132.9, 129.2, 127.6, 126.6, 119.7, 116.9, 115.9, 112.4, 99.5, 94.6, 62.8, 59.5, 45.6. MS *m/z* 397.79 ($M^{+\bullet}$, 89%), 340.99 ($M^{+\bullet}$ + 2–59, 100%).

*2-[5-(4-Nitrophenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**5l**)*

Yellow powder; yield, 64%; purity, 96.73% (HPLC, methanol:water, 80:20); mp 247–249 °C. IR, ν_{max} (KBr)/cm^{-1} 3288, 3072, 1695, 1589, 1438, 721. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.68 (s, 1H, OH), 7.30–7.47 (m, 1H, ArH), 7.12–7.29 (m, 4H, ArH), 6.96–7.10 (m, 3H, ArH), 6.18 (dd, *J* 3.9, 11.1 Hz, 1H, H_x), 4.05 (dd, *J* 11.4, 17.8 Hz, 1H, H_b), 3.89 (s, 2H, H_c), 3.57 (dd, *J* 13.8 Hz, 1H, H_a). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 186.4, 176.8, 162.0, 160.4, 159.4, 157.4, 157.2, 132.7, 130.4, 130.3, 129.0, 119.5, 116.9, 116.8, 115.4, 57.5, 55.6, 35.7. MS *m/z* 379.99 ($M^{+\bullet}$, 100%), 263.99 ($M^{+\bullet}$-16, 37%).

*2-[5-(Pyridin-2-yl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**5m**)*

Yellow powder; yield, 59%; purity, 98.87% (HPLC, methanol:water, 80:20); mp 256–258 °C. IR, ν_{max} (KBr)/cm^{-1} 3395, 3101, 1647, 1510, 1334, 843. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.66 (s, 1H, OH), 7.41–7.48 (m, 3H, ArH), 7.27–7.28 (m, 1H, ArH), 7.12–7.18 (m, 3H, ArH), 6.95–7.09 (m, 1H, ArH), 5.73 (dd, *J* 2.7, 11.1, 1H, H_x), 4.08 (dd, *J* 11.4, 17.8, 1H, H_b), 3.90 (s, 2H, H_c), 3.50 (dd, *J* 3.0, 17.8, 1H, H_a). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 186.2, 177.7, 174.3, 163.0, 158.9, 150.9, 146.7, 145.1, 132.7, 127.0, 116,4, 114.6, 65.0, 55.0, 42.6. MS *m/z* 338.09 ($M^{+\bullet}$, 50%), ($M^{+\bullet}$-92, 100%).

General procedure for synthesis of pyrazol-1-yl-1-ethanone derivatives (6a–6j)

Aromatic chalcone **3** was synthesized as discussed in 2.2.1. (Scheme 1) and 10 mmol was refluxed with acetic acid hydrazide (20 mmol) in presence of 20 mL of saturated ethanolic KOH for 1.5 h. The reaction mixture was poured in water to get precipitate of pyrazol-1-yl-1-ethanone derivative (**6a–6j**, Fig. 3) which was recrystallized by using ethanol-acetone (1:1).

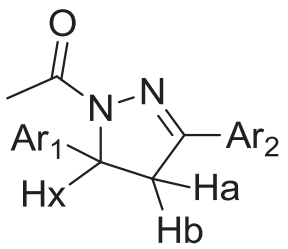


Fig. 3 General structure of substituted (pyrazol-1-yl) ethanone derivatives (**6a–6j**)

*1-[5-(2,4-Dichlorophenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**6a**)*

Pale white powder; yield, 69%; purity, 91.17% (HPLC, methanol: water, 80:20); mp 211–213 °C. IR, ν_{max} (KBr)/cm^{-1} 3311, 3068, 1685, 1375, 846. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.81 (s, 1H, OH), 7.55–7.57 (m, 1H, ArH), 7.29–7.35 (m, 3H, ArH), 7.09–7.16 (m, 3H, ArH), 6.04 (dd, *J* 3.6, 11.4, 1H, H_x), 3.88 (dd, *J* 11.4, 17.4, 1H, H_b), 3.19 (dd, *J* 4.2, 17.7, 1H, H_a), 2.65 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 168.2, 153.2, 140.6, 139.6, 132.9, 132.0, 131.9, 131.0, 129.9, 128.6, 128.1, 127.8, 127.4, 63.4, 42.6, 24.4. MS *m/z* 350.09 ($M^{+\bullet}$ + 1, 50%), 175.99 ($M^{+\bullet}$-174, 100%).

*1-[5-(2,4-Difluorophenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone (**6b**)*

Yellow powder; yield, 67%; purity, 89.23% (HPLC, methanol:water, 80:20); mp 190–192 °C. IR, ν_{max} (KBr)/cm^{-1} 3304, 2962, 1689, 1377, 1197. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.81 (s, 1H, OH), 7.33–7.57 (m, 1H, ArH), 6.99–7.21 (m, 6H, ArH), 6.06 (dd, *J* 3.6, 11.3, 1H, H_x), 3.87 (dd, *J* 11.4, 17.7, 1H, H_b), 3.19 (dd, *J* 3.6, 17.8, 1H, H_a), 2.64 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 168.1, 162.5, 160.1, 153.3, 136.8, 133.0, 132.1, 131.1, 128.2, 127.9, 127.8, 115.3, 115.2, 63.4, 42.7, 24.3. MS *m/z* 318.09 ($M^{+\bullet}$ + 2, 61%), 300.09 ($M^{+\bullet}$-15, 44%).

*1-[5-(4-Hydroxyphenyl)-3-(thiophen-3-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone (**6c**)*

Brownish white powder; yield, 58%; purity, 91.80% (HPLC, methanol: water, 80:20); mp 224–226 °C IR, ν_{max} (KBr)/cm^{-1} 3335, 2956, 1732, 1514, 1292, 759. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.84 (s, 1H, OH), 7.53- 7.55 (m, 1H, ArH), 7.27–7.37 (m, 5H, ArH), 7.09–7.21 (m, 1H, ArH), 6.07 (dd, *J* 3.6, 11.1, H_x), 3.87 (dd, *J* 3.9, 17.6, 1H, H_b), 3.22 (dd, *J* 3.6, 17.6, H_a), 2.65 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 168.1, 153.4, 140.8, 132.2, 131.1, 128.6, 128.2, 127.2, 125.6, 64.1, 42.9, 24.3. MS *m/z* 286.69 ($M^{+\bullet}$, 14%), 242.99 ($M^{+\bullet}$-43, 100%).

*1-[5-(2,4-Dibromophenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone (**6d**)*

Yellow powder; yield, 60%; purity, 92.88% (HPLC, methanol:water, 80:20); mp 245–247 °C. IR, ν_{max} (KBr)/cm^{-1} 3329, 2931, 1734, 1514, 1288, 516. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.81 (s, 1H, OH), 7.56 (dd, 1H, ArH), 7.45–7.51 (m, 2H, ArH), 7.34 (dd, 1H, ArH), 7.07–7.15 (m, 3H, ArH), 6.02 (dd, *J* 3.7, 11.3, 1H, H_x), 3.87 (dd, *J* 11.3, 17.7, 1H, H_b), 3.19 (dd, *J* 3.7, 17.7, 1H, H_a), 2.65 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 168.2, 157.4, 153.2, 148.6, 141.1, 140.1, 138.1, 133.6, 132.2, 131.5, 130.6, 128.4, 127.9, 120.5, 43.5, 63.5, 25.4. MS *m/z* 438.19 ($M^{+\bullet}$, 100%), 249.99 ($M^{+\bullet}$ + 1–190, 69%).

*1-[3-(4-Hydroxyphenyl)-5-(thiophen-3-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone (**6e**)*

Pale yellow powder; yield, 61%; purity, 98.36% (HPLC, methanol:water, 80:20); mp 223–225 °C. IR, ν_{max} (KBr)/cm^{-1} 3335, 2929, 1720, 1647, 1288, 715. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.75 (s, 1H, OH), 7.56 (dd, 1H, ArH), 7.38 (dd, 1H, ArH), 6.92–7.25 (m, 5H, ArH), 6.41 (dd, *J* 3.0, 10.8, 1H, H_x), 3.83 (dd, *J* 10.5, 17.6, 1H, H_b), 3.39 (dd, *J* 3.0, 17.4, 1H, H_a), 2.65 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 168.3, 153.7, 142.6, 132.9, 132.3, 131.3, 128.3, 126.5, 125.3, 125.1, 59.7, 42.6, 24.5. MS *m/z* 286.69 ($M^{+\bullet}$ + 3, 11%), 147 ($M^{+\bullet}$-136, 28%).

*1-[5-(3,4-Dibromophenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone (**6f**)*

Yellowish orange powder; yield, 68%; purity, 91.15% (HPLC, methanol:water, 80:20); mp 243–245 °C. IR, ν_{max} (KBr)/cm^{-1} 3344, 3053, 1730, 1518, 1325, 569. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.82 (s, 1H, OH), 7.56 (dd, 1H, ArH), 7.41 (dd, 1H, ArH), 7.32–7.33 (m, 2H, ArH), 7.20–7.24 (m, 1H, ArH), 7.11–7.15 (m, 2H, ArH), 6.02 (dd, *J* 3.7, 11.4, 1H, H_x), 3.88 (dd, *J* 11.4, 17.4, 1H, H_b), 3.21 (dd, *J* 3.8, 17.7, 1H, H_a), 2.65 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 168.0, 153.4, 143.4, 132.9, 132.3, 131.2, 130.7, 130.1, 128.4, 128.2, 124.8, 121.9, 63.6, 42.6, 24.2. MS *m/z* 438.89 ($M^{+\bullet}$ + 1, 100%), 249.99 ($M^{+\bullet}$ + 1–190, 65%).

*1-[5-(Furan-3-yl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone (**6g**)*

Yellowish white powder; yield, 63%; purity, 97.90% (HPLC, methanol:water, 80:20); mp 235–236 °C. IR, ν_{max} (KBr)/cm^{-1} 3308, 2962, 1701, 1683, 1323. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.74 (s, 1H, OH), 7.55–7.57 (m, 1H, ArH), 7.31–7.42 (m, 3H, ArH), 7.12–7.14 (m, 1H, ArH), 6.33–6.43 (m, 2H, ArH), 6.20 (dd, *J* 3.3, 10.8, 1H, H_x), 3.72 (dd, *J* 11.1, 17.7, 1H, H_b), 3.48 (dd, *J* 3.3, 17.5, 1H, H_a), 2.64 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 168.4, 153.6, 152.7, 151.1, 150.9, 142.4, 141.9, 133.7, 132.2, 131.2, 130.16, 110.3, 57.6, 56.6, 24.5. MS *m/z* 272.99 ($M^{+\bullet}$ + 2, 14%), 255.09 ($M^{+\bullet}$-15, 100%).

*1-[5-(2,4-Dimethoxyphenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-l]ethanone (**6h**)*

Yellow powder; yield, 64%; purity, 95.04% (HPLC, methanol:water, 80:20); mp 215–217 °C. IR, ν_{max} (KBr)/cm^{-1} 3356, 2962, 1716, 1606, 1280. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.82 (s, 1H, OH), 7.55 (dd, 1H, ArH), 7.33 (dd, 1H, ArH), 7.10 (dd, 1H, ArH), 6.71–6.83 (m, 4H, ArH), 6.00 (dd, *J* 3.6, 11.1, 1H, H_x), 3.87 (dd, *J* 8.4, 11.8, 1H, H_b), 3.85 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.23 (dd, *J* 3.6, 7.8, 1H, H_a), 2.65 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 167.9, 153.3, 148.8, 147.9, 133.2, 133.1, 131.9, 130.9, 128.1, 117.7, 111.6, 109.6, 63.9, 55.4, 24.1. MS *m/z* 340.99 ($M^{+\bullet}$ + 1, 100%), 325.99 ($M^{+\bullet}$-15, 45%).

*1-[5-(4-Chlorophenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone (**6i**)*

Yellow powder; yield, 67%; purity, 90.33% (HPLC, methanol:water, 80:20); mp 235–237 °C. IR, ν_{max} (KBr)/cm^{-1} 3331, 3028, 1728, 1589, 1288, 825. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.83 (s, H, OH), 7.55 (dd, 1H, ArH), 7.32 (dd, 1H, ArH), 7.24–7.28 (m, 3H, ArH), 7.17 (d, 1H, ArH), 7.08–7.12 (m, 2H, ArH), 6.03 (dd, *J* 3.7, 11.3, 1H, H_x), 3.92 (dd, *J* 11.4, 17.7, 1H, H_b), 3.21 (dd, *J* 3.7, 17.8, 1H, H_a), 2.65 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 167.9, 143.2, 133.3, 132.9, 131.3, 130.6, 128.3, 127.3, 125.6, 124.5, 63.6, 54.5, 24.1 MS *m/z* 314.09 ($M^{+\bullet}$, 64%), 300.09 ($M^{+\bullet}$ + 1–15, 18%).

*1-[5-(3,4-Dichlorophenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone (**6j**)*

Yellowish white powder; yield, 58%; purity, 91.29% (HPLC, methanol:water, 80:20); mp 212–214 °C. IR, ν_{max} (KBr)/cm^{-1} 3317, 2958, 1689, 1319, 844. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 9.84 (s, 1H, OH), 7.55 (dd, 2H, ArH), 7.10–7.44 (m, 4H, ArH), 6.94 (d, 1H, ArH), 6.31 (dd, *J* 3.9, 1.4, H, H_x), 3.94 (dd, *J* 11.4, 17.8, 1H, H_b), 3.11 (dd, *J* 3.9, 18, 1H, H_a), 2.66 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 168.1, 153.2, 143.3, 132.9, 132.1, 131.1, 130.7, 130.1, 128.4, 128.1, 124.7, 121.9, 63.5, 42.6, 24.3. MS *m/z* 350.99 ($M^{+\bullet}$ + 2, 100%), 177.00 ($M^{+\bullet}$ + 1–173, 20%).

In vitro MCD inhibition analysis

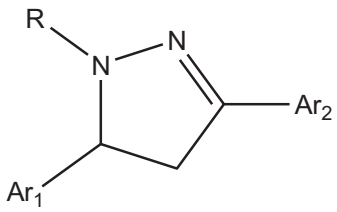
Albino Wistar rats (300–350 g) were anesthetised with pentobarbital sodium and their hearts were quickly excised and separated from their atrias. The ventricles were used for preparation of MCD (Dyck et al. 1998). Ventricles were cut into 1-mm cubes and rinsed with ice-cold mannitol-sucrose-EGTA (MSE: 225 mmol mannitol, 75 mmol sucrose and 1 mmol EGTA, pH 7.5) buffer. Tissue was homogenized for 20 s taking care that low temperature should be maintained by intermittent cooling. Homogenate was diluted to 10 ml/g of wet tissue with MSE buffer and centrifuged at 480×*g* for 5 min. The supernatant was filtered through cheese cloth and then centrifuged at 8000×*g* for 10 min. The obtained pellet was gently rinsed with MSE buffer and resuspended in MSE buffer and centrifuged. The obtained pellet was then suspended in 10 mmol sodium phosphate buffer (50 ml/g of wet tissue, pH 7.6), containing 0.5 mmol dithiothreitol. The suspension was frozen for 10 min and thawed in a stirring ice bath for 2 h. This mixture was centrifuged at 24,000×*g* for 10 min and pellet was resuspended in sodium phosphate buffer. This mixture was stirred for 2 h and was centrifuged at 24,000 for 10 min. Both the supernatants were combined and saturated to 40% with powdered ammonium sulphate and stirred for 1 h on ice. The precipitated protein was separated by centrifugation at 24,000×*g* for 10 min. The solution was saturated to 55% with powdered ammonium sulphate, and stirred for 1 h on ice, and centrifuged at 24,000×*g* for 10 min. The pellet was resuspended in sodium phosphate buffer and used for MCD inhibitory assay.

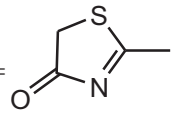
A fluorimetric assay which monitors the formation of acetyl CoA from malonyl CoA in a coupled assay using citrate synthase and malate dehydrogenase was used to measure MCD activity. Reaction mixture of 2 ml contained 0.1 M Tris/HCl, pH 8.0, 1 mM dithioerythritol, 10 mM malic acid, 0.17 mM NAD^+, 0.136 mM malonyl CoA, 14 units of malate dehydrogenase and 0.53 units of citrate synthase. The reaction was initiated by 20 µl of MCD. Synthesized compounds of various dilutions (0.01–50 µM) were added in above solution. After 2 min, formation of NADH from NAD^+ was measured at an excitation wavelength of 340 nm and emission wavelength of 460 nm (Dyck et al. 2000). IC_{50} value was calculated.

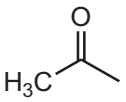
Preliminary docking simulation studies

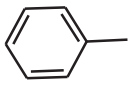
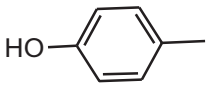
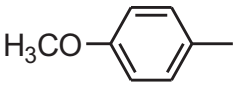
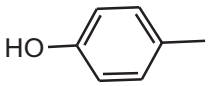
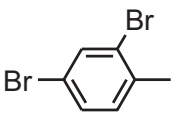
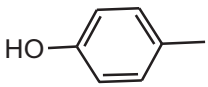
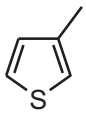
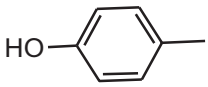
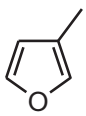
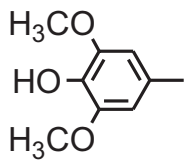
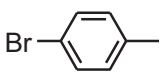
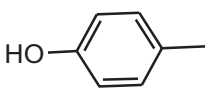
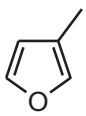
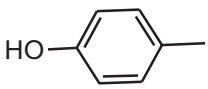
The preliminary docking studies of compounds **5e**, **5j**, and **6f** were performed using Molecular Operating Environment (MOE-Dock) 2009.10 module as computational software, in order to rationalize the binding interactions and obtained biological results (Pal et al. 2014). Crystal structure human MCD (**HsMCD**, **PDB ID 2YGW**, resolution 2.8 Å) was retrieved from the PDB for preliminary docking study. The target protein was taken, ligands were extracted, and hydrogens were added. Protonation was employed with protonating 3D programs. Furthermore, partial charges and force field were employed with MMFF94x. As the co-crystal structure of MCD complex with selective inhibitor is still not available in the PDB data, the site for interaction of the enzyme was determined by referring work done by

Table 1 In vitro MCD inhibition activity of compounds (**5a–5m**, **6a–6j**)



5a-5m, R= 

6a-6j, R= 

Compound	Ar_1	Ar_2	IC50 μM[a,b]
5a			2.03
5b			3.55
5c			7.65
5d			1.60
5e			0.10
5f			1.52
5g			2.23

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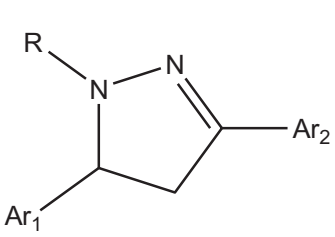
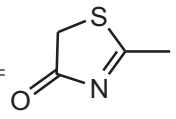
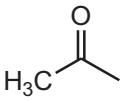


5a-5m, R= 

6a-6j, R= 

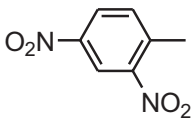
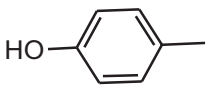
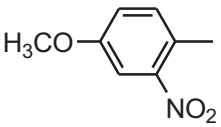
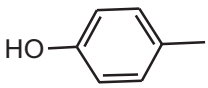
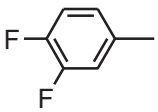
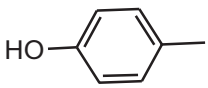
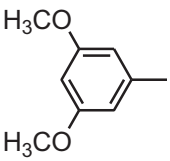
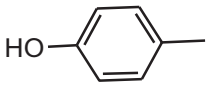
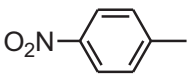
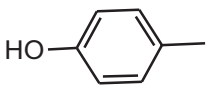
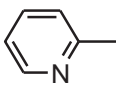
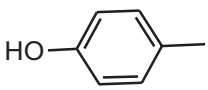
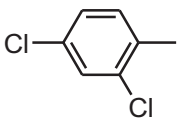
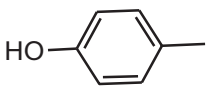
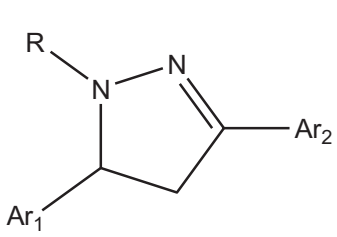
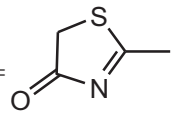
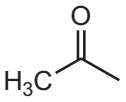
Compound	Ar_1	Ar_2	IC50 μM[a,b]
5h			2.04
5i			2.25
5j			0.27
5k			3.08
5l			1.92
5m			2.34
6a			6.58

Table 1 continued



5a-5m, R= 

6a-6j, R= 

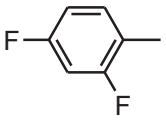
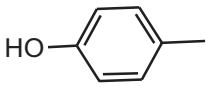
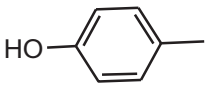
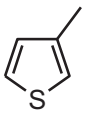
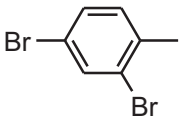
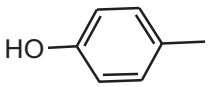
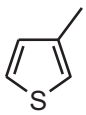
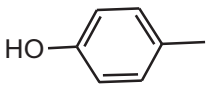
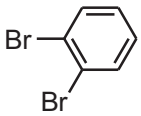
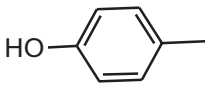
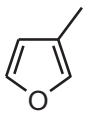
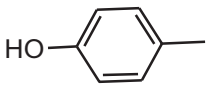
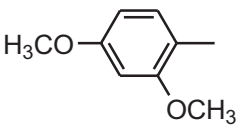
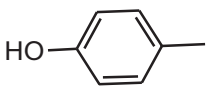
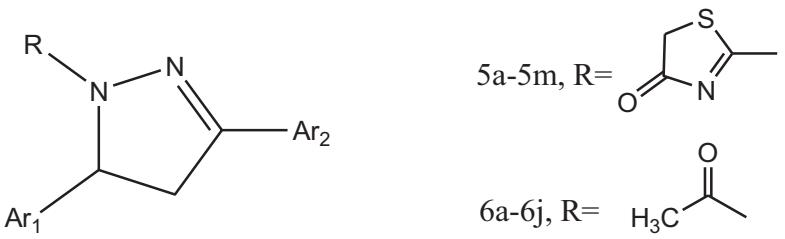
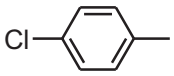
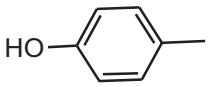
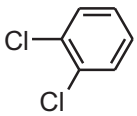
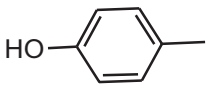
Compound	Ar_1	Ar_2	IC50 μM[a,b]
6b			3.65
6c			>50
6d			5.90
6e			6.33
6f			0.26
6g			6.97
6h			7.68

Table 1 continued



Compound	Ar_1	Ar_2	IC50 μM[a,b]
6i			4.42
6j			3.47

[a] Data are reported as mean of $n = 3$ determinations

[b] SD was generally ±20% of the average

Froese et al. (2013). After extensive efforts, Froese et al. (2013) found out that Ser329 and His423 hold a very important catalytic role in MCD activity and Phe288 may provide a nonpolar environment for the CO_2 group of acetyl CoA or malonyl CoA. After generation of various binding sites of the MCD enzyme by using alpha site finder mode of MOE, the site 4 containing Ser329, His423, and Phe288 was selected for docking simulation study. Ligand molecules were constructed using builder mode and were energy minimized. Docking simulations were performed using Alpha triangle placement method with London dG scoring. The ligands were docked using automated docking program of MOE 2009.10. Hydrogen bonding, arene–arene interactions and *E* scores were obtained from MOE.

Table 2 Comparison among docking results and experimental IC_{50} values (μM)

Compounds	*E* scores	Interacted residues	IC_{50}values (μM)
5e	−12.45	Glu216[HB]	0.10
5j	−12.96	His219[HB], Ser290[HB]	0.27
6f	−12.32	Glu216[HB], His423[HB],[AA]	0.26
Malonyl-CoA[a]	–	Ser329, His423, Phe288	–

[a] The important interactions of malonyl-CoA at the catalytic domain of MCD according to the findings reported by Froese et al. [27]; [HB] H bond; [AA] arene–arene interactions

Results and discussion

Results of in vitro MCD inhibition

We successfully synthesized a series of novel simple pyrazoline derivatives substituted with a thiazolone ring and an acetyl group. On the basis of previous studies and design of compounds, the aromatic hydroxyl group was maintained as a common structural feature in all the compounds. All novel derivatives were confirmed using infra red, proton ^{1}HNMR, Carbon-13 NMR, and mass spectra. In vitro MCD inhibitory activity of the synthesized compounds was determined using a fluorometric assay. The MCD enzyme for this assay was isolated from the hearts of anesthetized rats.

The results of the MCD inhibition assay are summarized in Table 1. The MCD inhibitory activity of the thiazolone-substituted series (**5a–5m**) was higher than that of the acetyl-substituted derivatives (**6a–6j**). Furthermore, MCD inhibitory activity did not change substantially when the phenyl ring present at the fifth position of the pyrazoline ring was replaced with hetero-aromatic rings such as thiophene (**5d**), furan (**5g**), and pyridine (**5m**). Compounds 2-[5-(Furan-3-yl)-3-(4-hydroxy-3,5-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl]-1,3-thiazol-4(5H)-one (**5e**), 2-[5-(3,4-difluorophenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl]-1,3-thiazol-4

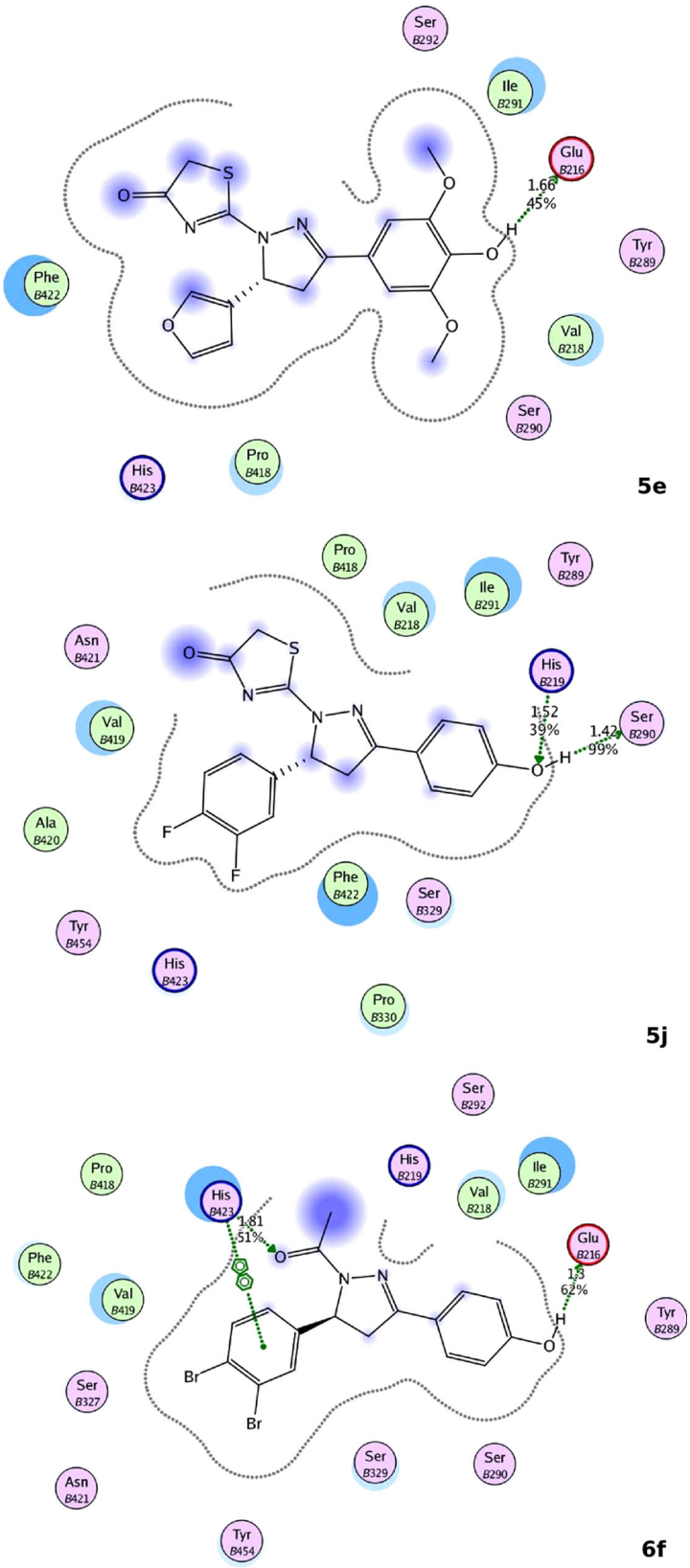


Fig. 4 Binding interactions of **5e**, **5j**, and **6f** in site 4 of HsMCD (**PDB ID:2YGW**) generated by MOE Dock 2009.10. ---------> indicates hydrogen bonding; ⬡ indicates arene–arene interaction

(5H)-one(**5j**), and 1-[5-(3,4-dibromophenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**6f**) showed the highest in vitro MCD inhibitory activity with the IC_{50} values of 0.10, 0.27, and 0.26, respectively. The introduction of electron-withdrawing groups, such as fluoro (**5j**) and bromo (**6f**), at the meta and para positions of the phenyl ring increased in vitro MCD inhibitory activity. However, the introduction of chloro or bromo at the ortho and meta positions of the phenyl ring (**5c**, **6a**, **6b**, and **6d**) reduced in vitro MCD inhibitory activity. Among compounds **5e**, **5j**, and **6f**, compound **5e** exhibited the highest activity. Compound **5e** has electron-donating methoxy groups present adjacent to the phenolic hydroxyl group of the phenyl ring at the third position of the pyrazoline ring. Furthermore, introduction of electron-donating groups (**5b**, **5i**, **5k**, **6c**, and **6h**), instead of electron-withdrawing groups, reduced in vitro MCD inhibitory activity.

Results of the preliminary docking simulation study

On the basis of presence of His423, Ser329, and Phe288 amino acids, site 4 of the MCD enzyme was selected for docking simulation studies. As listed in Table 1, compounds **5e**, **5j**, and **6f** were found to be significantly active as MCD inhibitors. Thus, these compounds were docked into site 4 of human MCD (**HsMCD**; **PDB ID:2YGW**) by using the MOE-Dock software, 2009.10 module, to investigate ligand–protein interactions. The results of the comparison among docking scores (*E* scores) and docking interactions are listed in Table 2. The *E* scores of compounds **5e**, **5j**, and **6f** were −12.45, −12.96, and −12.32, respectively. In vitro studies demonstrated that compounds **5e**, **5j**, and **6f** exhibited MCD inhibitory activity, and docking results were in agreement with the experimental data.

Docking studies (Fig. 4) indicated that the acidic hydroxyl group of compound **5e** showed hydrogen bonding interactions with Glu216 (distance = 1.66 Å). The acidic hydroxyl group of compound **5j** exhibited hydrogen bonding interactions with His219 (distance = 1.52 Å) and Ser290 (distance = 1.42 Å). Furthermore, the acidic hydroxyl group and the acetyl group of compound **6f** showed hydrogen bonding with Glu216 (distance = 1.3 Å) and His423 (distance = 1.81 Å), respectively. The aryl group present at the fifth position of pyrazoline in compound **6f** demonstrated an arene–arene interaction with His423. Thus, docking simulation study confirmed that the presence of an acidic proton is necessary for MCD inhibitory activity.

Conclusion

In the present study, we describe the design and synthesis of small molecule MCD inhibitors in normal laboratory conditions. In addition, we have investigated the in vitro MCD inhibitory activity of all synthesized compounds and conducted docking simulation studies of most active compounds. The results revealed that all synthesized compounds possessed MCD inhibitory activity. In particular, compounds **5e**, **5j**, and **6 f** exhibited highest MCD inhibitory activity.

The most active compounds, **5e**, **5j**, and **6f**, were docked at HsMCD (**PDB ID:2YGW**) to investigate ligand–protein interactions. Froese et al. reported that Ser329, His423, and Phe288 play a crucial role in the decarboxylase activity of MCD. Molecular docking studies demonstrated that compounds **5e**, **5j**, and **6f** interact with the MCD enzyme by binding to Glu216, His219, Ser290, and/or His423. Furthermore, *E* scores obtained from the docking study of compounds **5e**, **5j**, and **6f** correlated with in vitro MCD inhibitory activity.

In conclusion, compounds **5e**, **5j**, and **6f** reveal a novel scaffold as an MCD inhibitor and can be further developed as potent MCD inhibitors based on the pyrazoline scaffold.

The inhibition of MCD inhibition leads to a shift in the mitochondrial metabolism pathway and can be beneficial in the treatment of ischemia. In addition, this shift in metabolism prevents acidosis of heart cells. Increasing research is being conducted in this field, and the results of many studies have indicated that MCD inhibition is a promising route for the treatment of ischemia. Thus, the compounds synthesized in this study can be further modified and therapeutically exploited as anti-ischemic agents.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no competing interests.

Ethical approval Animals studies were in accordance with the ethical standards of the institution where the studies were conducted.

References

Abel ED (2007) Glucose for the aging heart. Circulation 116:884–887

Amir M, Kumar H, Khan SA (2008) Synthesis and pharmacological evaluation of pyrazoline derivatives as new anti-inflammatory and analgesic agents. Bioorg Med Chem Lett 18:918–922

Arrhenius T, Chen M, Cheng JF, Huang Y, Nadzan A, Penuliar R, Wallace D, Zhnag L (2009) Malonyl-CoA decaroxylase inhibitors useful as metabolic modulators. U.S. Patent 7,279,477, 9 Oct 2007

Bandopadhyay GK, Yu JG, Ofrecio J, Olefsky JM (2006) Increased malonyl CoA levels in muscle from obese and type 2 diabetic subjects lead to decreased fatty acid oxidation and increased lipogenesis; Thiazolidinedione treatment reverses these defects. Diabetes 55:2277–2285

Barsoum FF, Hosni HM, Girgis AS (2006) Study of novel bis (1-acyl-2-pyrazolines) of potential anti-inflammatory and molluscidal properties. Bioorg Med Chem 14:3929–3937

Cheng JF, Liu B, Angeles L, Arrhenius T, Mar D, Wilson ME, Ramona AM, Encinitas CA, Lopaschuk GD, Dyck JR, Park S, Serafimov R (2005a) Cyanoguanidine based azole compounds useful as malonyl-CoA decarboxylase inhibitors. U.S. Patent 7,786,145, 31 August 2010

Cheng JF, Nguyen N, Liu X, Lopaschuk GD, Dyck JR (2005b) Heterocyclic compounds useful as malonyl-CoA decarboxylase inhibitors. U.S. Patent a7,696,365, 13 April 2010

Cheng JF, Mak CC, Huang Y, Penuliar R, Nishimoto M, Zhang L, Wallace D, Arrhenius T, Chu D, Yang G, Barbosa M, Barr R, Dyck JR, Lopaschuk GD, Nadzan AM (2006a) Heterocyclic substituted bis-trifluoromethyl carbinols as malonyl-CoA decarboxylase inhibitors. Bioorg Med Chem Lett 16:3484–3488

Cheng JF, Chen M, Tith S, Haramura M, Liu B, Mak CC, Arrhenius T, Reily S, Brown S, Thorn V, Harmon C, Barr R, Dyck JR, Lopaschuk GD, Nadzan AM (2006b) Synthesis and structure activity relationship of small molecule malonyl coenzyme A decarboxylase inhibitors. J Med Chem 49:1517–1525

Cheng JF, Huang Y, Penuliar R, Nishimoto M, Liu L, Arrheniu sT, Yang G, O'Leary E, Barbosa M, Barr R, Dyck JR, Lopaschuk GD (2006c) Discovery of potent and orally available malonyl-CoA decarboxylase inhibitors as cardioprotective agents. J Med Chem 49:4055–4058

Chimenti F, Maccioni E, Secci D, Bolasco A, Chimenti P, Granese A, Befani O, Turini P, Alcaro S, Ortuso F, Cirilli R, La Torre F, Cardia MC, Distino S (2005) Synthesis, molecular modelling studies and selective inhibitory activity against monoamine oxidase of 1-thiocarbamoyl-3,5-diaryl-4,5-dihydro-(1H)-pyrazol derivatives. J Med Chem 48:7113–7122

Chimenti F, Carradori S, Secci D, Bolasco A, Bizzarri B, Chimenti P, Granese A, Yanez M, Orallo F (2010) Synthesis and inhibitory activity against human monoamine oxidase of N1-thiocarbamoyl-3,5-di(hetero)aryl-4,5-dihydro-(1H)-pyrazole derivatives. Eur J Med Chem 45:800–804

Clifford DL, Lopaschuk GD (2007) Role of malonyl-CoA in heart disease and the hypothalamic control of obesity. Cardiovasc Res 73:278–287

Dyck JRB, Barr AJ, Barr RL, Kolattukudy PE, Lopaschuk GD (1998) Characterization of cardiac malonyl-CoA decarboxylase and its putative role in regulating fatty acid oxidation. Heart Circ Physiol 44:H2122–H2129

Dyck JRB, Berthiaume LG, Panakkezhum DT, Kantor PF, Barr AJ, Barr R, Singh D, Hopkins TA, Violley N, Prentki M, Lopaschuk GD (2000) Characterization of rat liver malonyl-CoA decarboxylase and the study of its role in regulating fatty acid metabolism. Biochem J 350:599–608

Dyck JRB, Lopaschuk GD (2002) Malonyl-CoA control of fatty acid oxidation in the ischemic heart. J Mol Cell Cardiol 34:1099–1109

Dyck JRB, Cheng JF, Stanley WC, Rick B, Chandler MP, Brown S, Wallace D, Arrhenius T, Harmon C, Yang G, Nadzan AM, Lopaschuk GD (2004) Malonyl coenzyme A decarboxylase inhibition protects the ischemic heart by inhibiting fatty acid oxidation and stimulating glucose oxidation. Circ Res 94:e78–e84

Dyck JRB, Hopkins TA, Bonnet S, Michelakins ED, Young ME, Watanabe M, Kawase Y, Jishage KI, Lopaschuk GD (2006) Absence of malonyl coenzyme A decarboxylase in mice increases cardiac glucose oxidation and protects the heart from ischemic injury. Circulation 114:1721–1728

EI-Sabbagh OI, Baraka MM, Ibrahim SM, Pannecouque C, Andrei G, Snoeck R, Balzarini J, Rashad AA (2009) Synthesis and antiviral activity of new pyrazole and thiazole derivatives. Eur J Med Chem 44:3746–3753

Fillimore N, Lopaschuk GD (2013) Targeting mitochondrial oxidative metabolism as an approach to treat heart failure. Biochim Biophys Acta 1833:857–865

Frink RJ (2012) Sudden cardiac death is not caused by ischemia. Int J Cardiovasc Res 1:1–2

Froese DS, Forouhar F, Tran TH, Vollmar M, Kim YS, Lew S, Neely H, Seetharaman J, Shen Y, Xiao R, Action TB, Everett JK, Cannone G, Puranik S, Savitsky P, Krojer T, Pilka ES, Kiyani W, Lee WH, Marsden BD, Delft FV, Allerston CK, Spagnolo L, Gileadi O, Montelione GT, Oppermann U, Yue WW, Tong L (2013) Crystal structures of malonyl-coenzyme A decarboxylase provide insights into its catalytic mechanism and disease causing mutations. Structure 21:1182–1192

Jainey PJ, Bhat IK (2012) Antitumor, analgesic and anti-inflammatory activities of synthesized pyrazolines. J Young Pharm 4:82–87

Jaswal JS, Keung W, Wang W, Ussher JR, Lopaschuk GD (2011) Targeting fatty acid and carbohydrate oxidation—a novel therapeutic intervention in the ischemic and failing heart. Biochim Biophys Acta 1813:1333–1350

Muoio DM, Newgard CB (2008) Fatty acid oxidation and insulin action. Diabetes 57:1455–1456

Ozdemir A, Turan-Zitouni G, Kalplancikli ZA, Revial G, Guven K (2007) Synthesis and antimicrobial activity of 1-(4-aryl-2-thiazolyl)-3-(2-thienyl)-5-aryl—pyrazoline derivatives. Euro J Med Chem 42:403–409

Pal T, Joshi H, Ramaa CS (2014) Design and development of oxazol-5-ones as partial PPAR-γ agonist against cancer cell lines. Anti-Cancer Agents Med Chem 14:872–883

Patel MR, Talele TT (2007) 3D-QSAR studies on malonyl coenzyme A decarboxylase inhibitors. Bioorg Med Chem 15:4470–448

Seebacher W, Belaj F, saf R, Brun R, Weis R (2003) New 1,3-Thiazole and 1,3-Thiazines from Thiocarbamoylpyrazoles. Monatschfte Fur Chemie 134:1623–1628

Stanley WC, Recchia FA, Lopaschuk GD (2005) Myocardial substrate metabolism in the normal and failing heart. Physiol Rev 85:1093–1129

Tang H, Yan Y, Feng Z, Jesus RK, Yang L, Levorse DA, Owens KA, Raynald TE, Bergeron R, Castriota GA, Doebber TW, Ellsworth KP, Lassman ME, Li MS, Wu C, Zhang BB, Chapman KT, Mills SG, Berger JP, Pasternak A (2010) Design and synthesis of a new class of malonyl-CoA decarboxylase inhibitors with anti-obesity and anti-diabetic activities. Bioorg Med Chem Lett 20:6088–6092

Ussher JR, Lopaschuk GD (2008) The malonyl-CoA axis as a potential target for treating ischemic heart disease. Cardiovasc Res 79:259–268

Ussher JR, Koves TR, Jaswal JS, Zhang L, Ilkayeva O, Dyck JRB, Muoio DM, Lopaschuk GD (2009) Insulin stimulated cardiac glucose oxidation is increased in high fat induced obese mice lacking malonyl-CoA decarboxylase. Diabetes 58:1766–1775

Wallace DM, Haramura M, Cheng JF, Arrhenius T, Nadzan AM (2007) Novel trifluoroacetophenone derivatives as malonyl-CoA decarboxylase inhibitors. Bioorg Med Chem Lett 17:1127–1130

Walters AM (2012) Mitochondria as a drug target in ischemic heart disease and cardiomyopathy. Circ Res 111:1222–1236

Yan J, Young ME, Cui L, Lopaschuk GD, Liao R, Tian R (2009) Increased glucose uptake and oxidation in mouse hearts prevent high fatty acid oxidation but cause cardiac dysfunction in diet induced obesity. Circulation 119:2818–2828