**Supporting Information**

# Enhancing allosteric inhibition of DHDPS through the design and synthesis of novel dimeric compounds

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## Table of Contents

## 1.1 General chemistry methods

All materials were reagent grade purchased from commercial sources. Purification of solvents and reagents, when required, was carried out by the procedures described by Armarego and Chai.[1] All reactions using anhydrous solvents were carried out using oven dried glassware (120 ˚C). Melting points were recorded on a Reichert "Thermopan" microscope hot stage apparatus and are uncorrected.

Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker Avance-400 spectrometer ($^{1}$H at 400.13 MHz and $^{13}$C at 100.62 MHz) or Bruker Avance-500 spectrometer ($^{1}$H at 500.03 MHz and $^{13}$C at 125.75 MHz). Proton chemical shifts are reported in ppm from an internal standard of residual chloroform (7.26 ppm), dimethyl sulfoxide (2.50 ppm) or methanol (3.31 ppm). Each resonance was assigned according to the following convention; chemical shift (δ) (multiplicity, coupling constant(s) in Hz, integration). Carbon chemical shifts are reported in parts per million (ppm) using an internal standard of residual chloroform (77.16 ppm), dimethyl sulfoxide (39.52 ppm) or methanol (49.00 ppm). Chemical shifts were reported as δ values in parts per million (ppm). The following abbreviations have been used upon reporting spectral data: s, singlet; d, doublet; t, triplet; q, quartet; sext, sextet; m, multiplet; app, apparent; and br, broad.

Low-resolution electrospray ionisation (ESI) mass spectrometry was carried out using a Bruker Daltronics (Germany) Esquire 6000 ion trap mass spectrometer or a Bruker Daltonics (Germany) HCT ultra ion trap spectrum in positive or negative mode. The samples were analysed using a flow rate of 4 μL/min, a mass range of 100 – 1000 m/z and a scan rate of 5500 m/z/second for the Esquire 6000 and samples were analysed using a flow rate of 4 μL/min, with a mass range of 50-700 m/z and a scan rate of 8100 m/z/seconds (Standard-Enhanced mode) for the HCT ultra ion trap. High-resolution electrospray ionisation (ESI) mass spectrometry was carried out using an Agilent Technologies Accurate Mass Q-TOF LC-MS 6530 using Autosampler 1260 Infinity II in positive mode. The samples were analysed using a flow rate of 1 mL/min, a mass range of 100 – 1,000 m/z and a scan rate of 10,000 m/z/second.

Flash column chromatography was performed on Davisil® silica gel LC60A (40-63 micron). Thin layer chromatography (TLC) was carried out using Merck Kieselgel 60 F254 aluminium backed plates and visualised by UV light at 254 nm.

Analytical reverse phase high performance liquid chromatography (HPLC) was performed on a Shimadzu Prominence HPLC system fitted with a Phenomenex® Jupiter C18 300 Å column (250 mm × 4.60 mm, 10 µm), using a buffered binary system; solvent A: 0.1% trifluoroacetic acid; solvent B: acetonitrile, monitored at 254 nm. Semi-preparative reverse phase HPLC was performed using the previously described system, fitted with a Phenomenex® Jupiter C18 300 Å column (250 mm × 10.0 mm, 10 µm) monitored at 254 nm.

Single crystals for X-ray crystallography were selected under *n*-paratone oil, mounted on nylon loops and placed into a cold stream (172 K) of $N_2$ on an Oxford CCD diffractometer using Cu Kα radiation. Structure solution and refinement were performed using the SHELXTL suite of software.[2] Images were generated by using Olex2.[3]

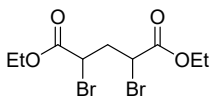
## 1.2 Chemistry experimental procedures and compound characterisation

**General Procedure A: Knoevenagel condensation.**

Knoevenagel condensation was adapted from the methods described previously.[4, 5] To a solution of appropriate thiazolidinedione precursor (1 eq) and 4-methoxybenzaldehyde (2 eq) in toluene (5 mL), three drops piperidine and two drops acetic acid were added. The reaction was heated under reflux for 18 hours then concentrated *in vacuo* to afford the crude product.

**General Procedure B: Acid hydrolysis of esters.**

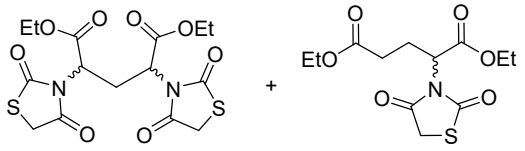
Esters were hydrolysed according to the methods described previously.[4, 5] A mixture of the appropriate ester in glacial acetic acid (6 mL) and concentrated hydrochloric acid (3 mL) was refluxed until successful hydrolysis as determined via TLC, approximately two hours. The reaction was concentrated *in vacuo* and the product thoroughly washed with water and collected via vacuum filtration to afford the desired compound unless otherwise specified.




**Diethyl 2,4-dibromopentanedioate (1)**

Glutaryl dichloride was prepared via the methods described by Das *et al*.[6] A mixture of glutaric acid (1.00 g, 7.57 mmol) and thionyl chloride (4 mL) was heated at 60-70 °C for 4 hours. Excess thionyl chloride was removed via distillation to afford the desired compound as an oil in quantitative yield. $\delta_H$ (400 MHz, $CDCl_3$) 3.02 (t, *J* 8.0, 4H, $CH_2$ x2), 2.08 (quin, *J* 8.0, 2H, $CH_2$). $\delta_C$ (100 MHz, $CDCl_3$) 173.1, 45.1, 20.3.

Glutaryl dichloride was used to synthesise diethyl 2,4-dibromopentanedioate via methods reported by Guanti and Riva.[7] The previously prepared glutaryl dichloride (0.900 g, 5.32 mmol) was placed in a 3 neck flask fitted with a reflux condenser and a scrubber system consisting of 1M NaOH (500 mL) and 10% aq $Na_2S_2O_3$ (50 mL) and warmed to 85 ˚C. Neat bromine (0.7 ml) was added dropwise and then a 150 W sun lamp was placed in front of the reaction with continued to heat at 85 ˚C for 4.5 hours. Once the bromination was complete, the reaction was put under nitrogen and transferred via cannula to anhydrous ethanol at 0 ˚C. Extra ethanol (3 x 5 mL) was used to wash the reaction vessel to ensure the dichloride was transferred quantitatively. The reaction was subsequently stirred at 0 ˚C for 15 mins then allowed to stir at room temperature overnight. The next day, the reaction was cooled to 0 ˚C then solid $NaHCO_3$ was added slowly until $CO_2$ evolution ceased. The reaction was then partitioned between water (20 mL) and ether (20 mL) and separated and the remaining aqueous phase washed with ether (2 x 20 mL). The combined organic phases were washed with 10% $Na_2S_2O_3$ solution followed by brine then dried ($MgSO_4$) and concentrated *in vacuo* to afford a dark red oil. The crude product was purified via fractional distillation to afford a 1:1 mixture of dibrominated stereoisomers with a small amount of diethyl 2-bromopentanedioate as a minor product observed by NMR spectroscopy (6.34 g). $\delta_H$ (400 MHz, $CDCl_3$) 4.51 (dd, *J* 8.0, 6.8, 2H, CH x2), 4.40 (t, *J* 8.0, 2H, CH x2), 4.28 (q, *J* 16.0, 8.0, 8H, $CH_2$ x4), 2.93-2.86 (dt, *J* 14.0, 8.0, 1H, $CH_2$), 2.68 (dd, *J* 7.8, 6.5, 2H, $CH_2$), 2.65 (dt, *J* 14.0, 8.0, 1H, $CH_2$), 1.34 (t, *J* 8.0, 12H, $CH_3$ x4). $\delta_C$ (100 MHz, $CDCl_3$) 168.7, 168.6, 62.47, 62.45, 43.6, 41.7, 39.1, 38.5, 13.9. LRMS (ESI): *m/z* 366.9 (M[$^{79}$Br,$^{79}$Br]+Na)$^+$, 368.9 (M[$^{79}$Br,$^{81}$Br]+Na)$^+$ and 370.9 (M[$^{81}$Br,$^{81}$Br]+Na)$^+$. Diethyl 2-bromopentanedioate observed, LRMS (ESI): *m/z* 289.0 (M[$^{79}$Br]+Na)$^+$ and 291.0 (M[$^{81}$Br]+Na)$^+$.

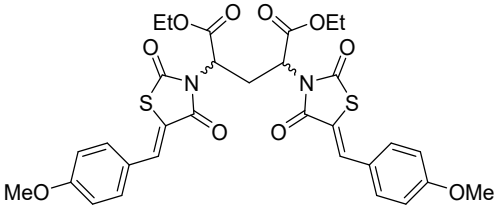



Major product Minor product

**Diethyl 2,4-bis(2,4-dioxothiazolidin-3-yl)pentanedioate (2) as the major disubstituted product with diethyl 2-(2,4-dioxothiazolidin-3-yl)pentanedioate (3) as the minor monosubstituted product**

A stirring suspension of compound **1** (0.500 g, 1.45 mmol), 2,4-thiazolidinedione (0.355 g, 3.03 mmol) and potassium carbonate in dry acetonitrile (60 mL) was set to reflux under nitrogen. After 4 hours, the reaction was concentrated *in vacuo* and partitioned between water

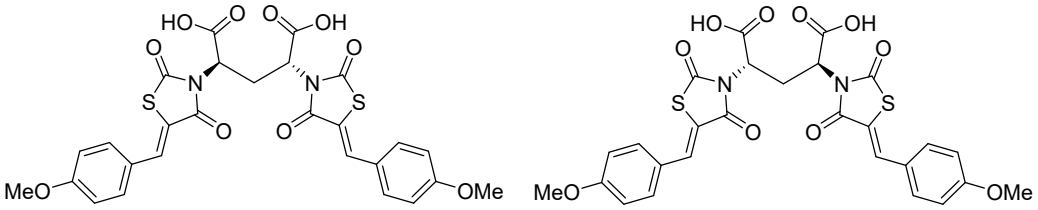
and ethyl acetate then extracted with ethyl acetate (x3). The organic phase was dried ($MgSO_4$) and concentrated to afford a crude dark red oil. The crude product was subjected to column chromatography (silica; 30:70 increasing to 80:20 ethyl acetate/hexanes elution). A small amount of monosubstituted compound **3** eluted first (0.030 g) followed by the major disubstituted product as the desired compound **2** (0.215 g). It was observed that the *meso* isomer could be further isolated from **2** by trituration with diethyl ether but that this was unnecessary for the planned synthesis. Compound **3** as the minor monosubstituted product: $\delta_H$ (400 MHz, $CDCl_3$) 4.85 (dd, *J* 16.0, 8.0, 1H, CH), 4.21 (t, *J* 8.0, 2H, $CH_2$), 4.12 (q, *J* 16.0, 4.0, 2H, $CH_2$), 3.99 (d, *J* 2.0, 2H, $CH_2$), 2.58-2.49 (m, 1H, $CH_2$), 2.41-2.31 (m, 3H, $CH_2$), 1.25 (t, *J* 8.0, 6H, $CH_3$). $\delta_C$ (100 MHz, $CDCl_3$) 172.2, 171.1, 170.8, 167.7, 62.2, 60.8, 54.6, 33.5, 30.8, 23.2, 14.2, 14.1. LRMS (ESI): *m/z* 326.22 $(M + Na)^+$. Compound **2** as the disubstituted major product: $\delta_H$ (400 MHz, $CDCl_3$) 4.83 (dd, *J* 8.0, 4.0, 2H, CH x2), 4.60 (dd, *J* 9.6, 8.0, 2H, CH x2), 4.29-4.16 (m, 4H, $CH_2$ x2), 4.18-4.09 (m, 4H, $CH_2$ x2), 4.01 (s, 4H, $CH_2$ x2), 3.99 (s, 4H, $CH_2$ x2), 3.32 (dt, *J* 12.0, 4.0, 1H, $CH_2$), 3.15 (t, *J* 8.0, 2H, $CH_2$), 2.55 (dt, *J* 16.0, 8.0, 1H, $CH_2$), 1.26 (t, *J* 8.0, 6H, $CH_3$ x2), 1.25 (t, *J* 8.0, 6H, $CH_3$ x2). $\delta_C$ (100 MHz, $CDCl_3$) 171.6, 171.3, 171.2, 170.7, 167.0, 62.58, 62.56, 52.6, 51.6, 33.82, 33.75, 28.0, 25.9, 14.03, 14.01. LRMS (ESI): *m/z* 440.66 $(M + Na)^+$. HRMS (ESI): $(M + H)^+$ calcd for $C_{15}H_{18}N_2O_8S_2^+$, 419.0583; found, 419.0581.




**Diethyl *rac*-2,4-bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)pentanedioate (4a); *meso*-Diethyl 2,4-bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)pentanedioate (4b)**

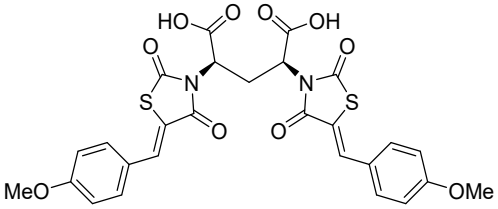
Compound **2** (0.115 g, 0.275 mmol) was coupled with 4-methoxybenzaldehyde (0.067 mL, 0.28 mmol) according to General Procedure A. After 24 hours at reflux, the reaction was concentrated *in vacuo* and the crude residue subject to column chromatography (silica; 30:70 ethyl acetate/hexanes elution). The *rac* compound **4a** eluted first (0.053 g, 29%), mp 168-172 ˚C, followed by the *meso* compound **4b** (0.064 g, 35%), mp 50-52 ˚C. Compound **4a**: $\delta_H$ (400 MHz, $CDCl_3$) 7.83 (s, 2H, CH x2), 7.47 (d, *J* 8.0, 4H, ArH), 6.99 (d, *J* 8.0, 4H, ArH), 4.93 (t, *J* 8.0, 2H, CH x2), 4.26-4.19 (m, 4H, $CH_2$ x2), 3.88 (s, 6H, $CH_3$ x2), 3.28 (t, *J* 8.0, 2H, $CH_2$),

1.26 (t, *J* 8.0, 6H, $CH_3$ x2). $\delta_C$ (100 MHz, $CDCl_3$) 167.9, 167.4, 165.9, 161.6, 134.6, 132.5, 125.8, 117.6, 114.8, 62.5, 55.5, 51.8, 26.8, 14.1. LRMS (ESI): *m/z* 677.2 $(M + Na)^+$. HRMS (ESI): $(M + H)^+$ calcd for $C_{31}H_{31}N_2O_{10}S_2^+$, 655.1420; found, 655.1421. Compound **4b**: $\delta_H$ (400 MHz, $CDCl_3$) 7.77 (s, 2H, CH x2), 7.31 (d, *J* 8.0, 4H, ArH), 6.88 (d, *J* 8.0, 4H, ArH), 5.08 (dd, 12.0, 8.0, 2H, CH x2), 4.31- 4.18 (m, 4H, $CH_2$ x2), 3.85 (s, 6H, $CH_3$ x2), 3.37 (dt, *J* 12.0, 4.0, 1H, $CH_2$), 2.88 (dt, *J* 16.0, 8.0, 1H, $CH_2$), 1.26 (t, *J* 8.0, 6H, $CH_3$). $\delta_C$ (100 MHz, $CDCl_3$) 167.3, 165.6, 161.5, 134.6, 132.3, 125.6, 117.5, 114.7, 62.5, 55.4, 52.9, 27.3, 14.1. LRMS (ESI): *m/z* 677.2 $(M + Na)^+$. HRMS (ESI): $(M + H)^+$ calcd for $C_{31}H_{31}N_2O_{10}S_2^+$, 655.1420; found, 655.1422.




***rac*-2,4-Bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)pentanedioic acid (5a)**

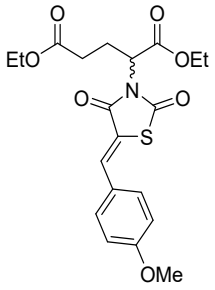
Ester **4a** (0.400 g, 0.061 mmol) was hydrolysed according to General Procedure B to afford the desired product (0.025 g, 68%). mp 240-242 ˚C. $\delta_H$ (400 MHz, DMSO) 7.91 (s, 2H, CH x2), 7.63 (d, *J* 8.0, 4H, ArH), 7.14 (d, *J* 8.0, 4H, ArH), 4.85-4.78 (m, 2H, CH x2), 3.86 (s, 6H, $OCH_3$ x2), 3.03 (t, *J* 8.0, 2H, $CH_2$). $\delta_C$ (100 MHz, $CDCl_3$) 169.4, 167.6, 165.7, 161.9, 134.5, 133.0, 125.7, 117.4, 115.5, 56.0, 52.2, 26.4. LRMS (ESI): *m/z* 637.1 $(M + K)^+$. HRMS (ESI): $(M + H)^+$ calcd for $C_{27}H_{23}N_2O_{10}S_2^+$, 599.0794; found 599.0793.




***meso*-2,4-Bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)pentanedioic acid (5b)**

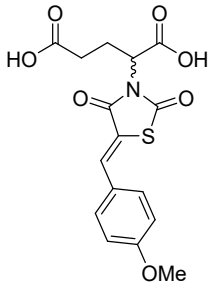
Ester **4b** (0.600 g, 0.092 mmol) was hydrolysed according to General Procedure B to afford the desired product (0.041 g, 74%), mp 238-242 ˚C. $\delta_H$ (400 MHz, DMSO) 7.75 (s, 2H, CH x2), 7.31 (d, *J* 10.0, 4H, ArH), 6.89 (d, *J* 10.0, 4H, ArH), 5.13-5.11 (m, 2H, CH x2), 3.79 (s, 6H, $OCH_3$ x2), 3.07-3.02 (m, 1H, $CH_2$), 2.98-2.91 (m, 1H, $CH_2$). $\delta_C$ (125 MHz, DMSO) 169.3,

167.1, 165.4, 161.7, 134.8, 132.8, 125.4, 116.7, 115.2, 55.9, 53.8, 25.1. LRMS (ESI): *m/z* 621.1 $(M + Na)^+$. HRMS (ESI): $(M + H)^+$ calcd for $C_{27}H_{23}N_2O_{10}S_2^+$, 599.0794; found 599.0796.




**Diethyl (*Z*)-2-(5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)pentanedioate (6)**

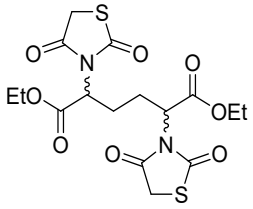
Compound **3** (130 mg, 0.311 mmol) was coupled with 4-methoxybenzaldehyde (0.038 mL, 0.311 mmol) according to General Procedure A. The crude residue subject to column chromatography (silica; 30:70 ethyl acetate/hexanes elution) to afford the desired compound as an oil (0.138 g, 68%). $\delta_H$ (400 MHz, $CDCl_3$) 7.86 (s, 1H, CH), 7.47 (d, *J* 8.0, 2H, ArH), 6.99 (d, *J* 8.0, 2H, ArH), 5.00 (dd, *J* 8.0, 4.0, 1H, CH), 4.23 (t, *J* 8.0, 2H, $CH_2$), 4.12 (q, *J* 16.0, 8.0, 2H, $CH_2$), 3.87 (s, 3H, $CH_3$), 2.65-2.55 (m, 1H, $CH_2$), 2.48-2.36 (m, 3H, $CH_2$), 1.25 (t, *J* 8.0, 3H, $CH_3$), 1.24 (t, *J* 8.0, 3H, $CH_3$). $\delta_C$ (100 MHz, $CDCl_3$) 172.1, 167.9, 167.5, 165.8, 161.6, 134.5, 132.4, 125.8, 117.6, 114.8, 62.1, 60.8, 55.5, 54.4, 30.8, 23.6, 14.2, 14.1. LRMS (ESI): *m/z* 444.1 $(M + Na)^+$. HRMS (ESI): $(M + H)^+$ calcd for $C_{20}H_{24}NO_7S^+$, 422.1273; found 422.1273.




**(*Z*)-2-(5-(4-Methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)pentanedioic acid (7)**

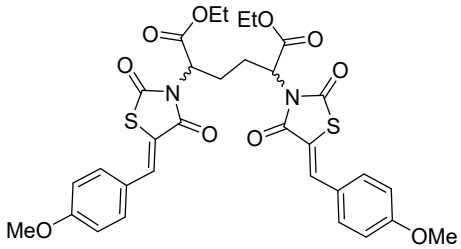
Ester **6** (0.080 g, 0.190 mmol) was hydrolysed according to General Procedure B. The reaction was concentrated *in vacuo* and partitioned between water and ethyl acetate and separated. The aqueous layer was extracted with ethyl acetate (x3) then the combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo* to afford the desired product as an oil which solidifies under vacuum (0.062 g, 78%), mp 56-58 ˚C. $\delta_H$ (400 MHz, $CDCl_3$) 7.88 (s, 1H, CH), 7.48 (d, *J* 8.0, 2H, ArH), 7.01 (d, *J* 8.0, 2H, ArH), 5.12-5.09 (m, 1H, CH), 3.88 (s, 3H, $OCH_3$), 2.64-

2.58 (m, 1H, $CH_2$), 2.48-2.45 (m, 3H, $CH_2$). $\delta_C$ (100 MHz, $CDCl_3$) 177.6, 172.9, 167.5, 165.7, 161.7, 135.1, 132.4, 125.7, 117.3, 114.9, 55.5, 53.8, 30.3, 23.2. LRMS (ESI): *m/z* 388.1 $(M + Na)^+$. HRMS (ESI): $(M + Na)^+$ calcd for $C_{16}H_{15}NO_7SNa^+$, 388.0467; found 388.0466.



**Diethyl 2,5-bis(2,4-dioxothiazolidin-3-yl)hexanedioate (9)**

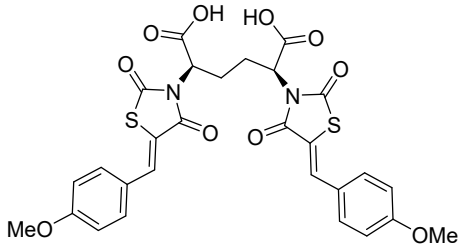
A suspension of diethyl *meso*-2,5,-dibromoadipate **8** (0.250 g, 0.694 mmol), 2,4-thiazolidinedione (0.171 g, 1.46 mmol) and potassium carbonate (0.384 g, 2.78 mmol) in dry acetonitrile (60 mL) was set to heat under reflux for two hours. The reaction was concentrated *in vacuo* and partitioned between water and ethyl acetate and separated. The aqueous layer was extracted with ethyl acetate (x3) then the combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. The crude product can be further purified by triturating with small amounts of ethyl acetate to afford a white solid as a mixture of an approximate 1:1 mixture of stereoisomers (0.117 g, 39%), mp 138-140 ˚C. $\delta_H$ (400 MHz, $CDCl_3$) 4.83 (d, *J* 8.0, 2H, CH x2), 4.69 (t, *J* 8.0, 2H, CH x2), 4.24-4.12 (m, 8H, $CH_2$ x4), 4.01 (s, 4H, $CH_2$ x2), 4.00 (s, 4H, $CH_2$ x2), 2.20-2.03 (m, 8H, $CH_2$ x4), 1.24 (t, *J* 16.0, 6H, $CH_3$ x2), 1.23 (t, *J* 16.0, 6H, $CH_3$ x2). $\delta_C$ (100 MHz, $CDCl_3$) 171.4, 171.2, 171.0, 170.8, 167.58, 167.57, 62.3, 54.8, 54.4, 33.6, 33.5, 25.2, 23.7, 14.0. LRMS (ESI): *m/z* 455.01 $(M + Na)^+$. HRMS (ESI): $(M + H)^+$ calcd for $C_{16}H_{21}N_2O_8S_2^+$, 433.0739; found, 433.0739.



**Diethyl *meso*-2,5-Bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)hexanedioate (10a); *rac*-Diethyl 2,5-bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)hexanedioate (10b)**

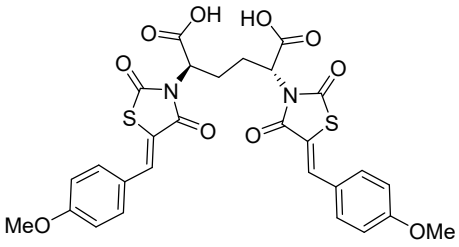
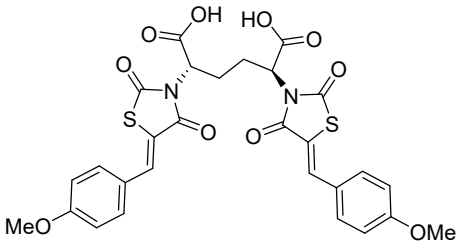
Compound **9** (0.150 g, 0.347 mmol) was coupled with 4-methoxybenzaldehyde (0.094 g, 0.694 mmol) according to General Procedure A. After concentration *in vacuo,* the crude residue was triturated with small amounts of methanol to afford a pale yellow solid which was collected via vacuum filtration to afford the *meso* compound **10a** (0.065 g, 28%), mp 201-203 ˚C.

Crystals suitable for X-ray crystallography studies were grown via slow evaporation from a concentrated solution of the material in a mixture of chloroform and methanol. The filtrate was subsequently recrystallised from a mixture of methanol and chloroform to afford the racemic mixture **10b** (0.056 g, 24%), mp 168-170 ˚C, with minor contaminants of the *meso* product **10a**, to be purified at the next step. Compound **10a**: $\delta_H$ (400 MHz, $CDCl_3$) 7.87 (s, 2H, CH x2), 7.48 (d, *J* 8.0, 4H, ArH), 7.00 (d, *J* 8.0, 4H, ArH), 4.89 (br s, 2H, CH x2), 4.26-4.13 (m, 4H, $CH_2$ x2), 3.88 (s, 6H, $OCH_3$ x2), 2.25-2.23 (m, 4H, $CH_2$ x2), 1.22 (t, *J* 8.0, 6H, $CH_3$ x2). $\delta_C$ (100 MHz, $CDCl_3$) 167.8, 167.5, 165.8, 161.6, 134.6, 132.4, 125.8, 117.7, 114.8, 62.2, 55.5, 54.9, 25.6, 14.0. LRMS (ESI): *m/z* 691.11 $(M + Na)^+$. HRMS (ESI): $(M + H)^+$ calcd for $C_{32}H_{33}N_2O_{10}S_2^+$, 669.1576; found, 669.1574. Compound **10b**: $\delta_H$ (400 MHz, $CDCl_3$) 7.83 (s, 2H, CH x2), 7.45 (d, *J* 8.0, 4H, ArH), 6.98 (d, *J* 8.0, 4H, ArH), 5.02 (br s, 2H, CH x2), 4.27-4.11 (m, 4H, $CH_2$ x2), 3.87 (s, 6H, $OCH_3$ x2), 2.27-2.16 (m, 4H, $CH_2$ x2), 1.22 (t, *J* 8.0, 3H, $CH_3$ x2). $\delta_C$ (100 MHz, $CDCl_3$) 167.8, 167.6, 165.9, 161.6, 134.6, 132.4, 125.8, 117.6, 114.8, 62.1, 55.5, 54.2, 24.3, 14.0. LRMS (ESI): *m/z* 691.2 $(M + Na)^+$. HRMS (ESI): $(M + H)^+$ calcd for $C_{32}H_{33}N_2O_{10}S_2^+$, 669.1576; found, 669.1576.



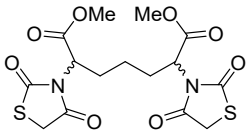

***meso*-2,5-Bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)hexanedioic acid (11a)**

Ester **10a** (0.040 g, 6.0 μmol) was hydrolysed according to General Procedure B to afford the desired compound (0.016 g, 44%), mp 266-268 ˚C. $\delta_H$ (400 MHz, DMSO) 7.95 (s, 2H, CH x2), 7.63 (d, *J* 8.0, 4H, ArH), 7.13 (d, *J* 8.0, 4H, ArH), 4.87 (br s, 2H, CH x2), 3.84 (s, 6H, $OCH_3$ x2), 2.0-1.99 (m, 4H, $CH_2$ x2). $\delta_C$ (125 MHz, DMSO) 169.7, 167.5, 165.7, 161.9, 134.6, 132.9, 125.7, 117.5, 115.5, 56.0, 55.3, 25.3. LRMS (ESI): *m/z* 613.08 $(M + H)^+$. HRMS (ESI): $(M + H)^+$ calcd for $C_{28}H_{25}N_2O_{10}S_2^+$, 613.0950; found, 613.0952.

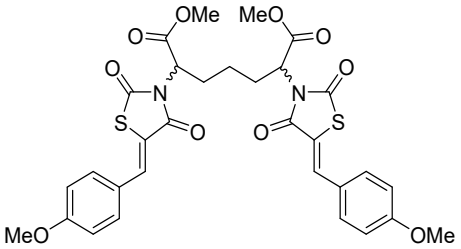
***rac*-2,5-Bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)hexanedioic acid (11b)**

Ester **10b** (0.026 g, 3.9 μmol) was hydrolysed according to General Procedure B. The crude product was purified by semi-preparative RP-HPLC (gradient elution of solvent A to solvent B: 10-40% over 10 mins, 40%-80% over 120 min, 80-90% over 10 mins) to afford the desired compound (0.006 g, 26%), mp > 260 ˚C. $\delta_H$ (400 MHz, DMSO) 7.89 (s, 2H, CH x2), 7.57 (d, *J* 8.0, 4H, ArH), 7.10 (d, *J* 8.0, 4H, ArH), 4.91 (d, *J* 8.0, 2H, CH x2), 3.84 (s, 6H, $OCH_3$ x2), 2.19-2.13 (m, 2H $CH_2$), 1.95-1.86 (m, 2H, $CH_2$). $\delta_C$ (125 MHz, DMSO) 169.7, 167.5, 165.7, 161.9, 134.7, 132.9, 125.7, 117.2, 115.5, 56.0, 54.9, 24.4. LRMS (ESI): *m/z* 635.1 $(M + Na)^+$. HRMS (ESI): $(M + H)^+$ calcd for $C_{28}H_{25}N_2O_{10}S_2^+$, 613.0950; found, 613.0951.



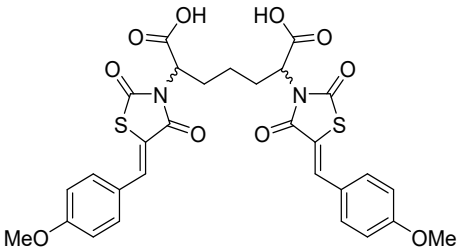
**Dimethyl 2,6-bis(2,4-dioxothiazolidin-3-yl)heptanedioate (13)**

Dimethyl 2,6-dibromoheptandioate **12** (0.250 mL, 1.15 mmol) was added to a stirring suspension of 2,4-thiazolidinedione (0.406 g, mmol, 3.45 mmol) and potassium hydrogen carbonate (0.288 g, 2.86 mmol) in dry DMF (5 mL) under nitrogen. The reaction was allowed to stir at room temperature for three days and then was concentrated *in vacuo*. The residue was partitioned between ethyl acetate and water (15 mL each) and stirred vigorously for one hour. The resulting fine precipitate was collected via vacuum filtration to afford the desired product (0.267 g, 51%), mp 156-158 ˚C. $\delta_H$ (400 MHz, $CDCl_3$) 4.77 (dd, J 12.0, 4.0, 2H, CH x2), 4.04 (s, 4H, $CH_2$ x2), 3.75 (s, 6H, $CH_3$ x2), 2.29-2.14 (m, 2H, $CH_2$), 2.11-2.01 (m, 2H, $CH_2$), 1.43-1.31 (m, 1H, $CH_2$), 1.31-120 (m, 1H, $CH_2$). $\delta_C$ (100 MHz, $CDCl_3$) 171.2, 170.9, 168.4, 54.8, 53.0, 33.6, 27.2, 22.7. LRMS (ESI): *m/z* 441.1 $(M + Na)^+$. HRMS (ESI): $(M + H)^+$ calcd for $C_{15}H_{19}N_2O_8S_2^+$, 419.0583; found 419.0580.



**Dimethyl 2,6-bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)heptanedioate (14)**

Compound **13** (0.500 g, 1.19 mmol) was coupled with 4-methoxybenzaldehyde (0.333 g, 2.51 mmol) according to General Procedure A. After 24 hours under reflux, the reaction was concentrated *in vacuo* and crude oil stored under vacuum where it partially crystallised. Hot methanol (8 mL) was added to the crude solid then allowed to cool to room temperature then on ice where a fine yellow solid precipitated out. This solid was collected via vacuum filtration and washed with cool methanol. The crude product was subjected to column chromatography (silica; 30:70 ethyl acetate/hexanes elution) to afford to afford an approximate 1:1 mixture of stereoisomers which was used in the next step (0.549 g, 70%), mp 68-72 ˚C. $\delta_H$ (400 MHz, $CDCl_3$) 7.85 (s, 2H, CH x2), 7.81 (s, 2H, CH x2), 7.44 (d, *J* 8.0, 4H, ArH), 7.38 (d, *J* 8.0, 4H, ArH), 6.97 (d, *J* 8.0, 4H, ArH), 6.95 (d, *J* 8.0, 4H, ArH), 4.96-4.91 (m, 4H, CH x4) 3.89 (s, 12H, $OCH_3$ x4), 3.76 (s, 6H, $CH_3$ x2), 3.75 (s, 6H, $CH_3$ x2), 2.35-2.12 (m, 8H, $CH_2$ x4), 1.34-1.28 (m, 4H, $CH_2$ x2). $\delta_C$ (100 MHz, $CDCl_3$) 168.7, 168.6, 167.5, 167.4, 165.81, 165.77, 161.6, 161.5, 134.6, 134.4, 132.32, 132.30, 125.77, 125.76, 117.65, 117.60, 114.79, 114.75, 55.48, 55.47, 54.7, 54.5, 52.9, 27.6, 27.3, 22.74, 22.72. LRMS (ESI): *m/z* 677.2 $(M + Na)^+$. HRMS (ESI): $(M + H)^+$ calcd for $C_{31}H_{31}N_2O_{10}S_2^+$, 655.1420; found 655.1420.



**2,6-Bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)heptanedioic acid (15)**

Ester **14** (0.200 g, 0.305 mmol) was hydrolysed according to General Procedure B to afford the desired compound as an approximate 1:1 mixture of *rac* and *meso* stereoisomers (0.163 g, 85%), mp 184-186 ˚C. $\delta_H$ (400 MHz, DMSO) 7.87 (s, 2H, CH x2), 7.84 (s, 2H, CH x2), 7.52 (d, *J* 8.0, 4H, ArH), 7.47 (s, *J* 8.0, 4H, ArH), 7.05 (d, J 8.0, 4H, ArH), 7.03 (d, J 8.0, 4H, ArH), 4.88-4.83 (m, 4H, CH), 3.84 (s, 12H, $OCH_3$ x4), 2.17-1.96 (m, 8H, $CH_2$ x4), 1.31-1.08 (m, 4H,

$CH_2$ x2). $\delta_C$ (100 MHz, $CDCl_3$) 169.95, 169.90, 167.4, 167.3, 165.7, 165.6, 161.8, 161.7, 134.6, 134.5, 132.82, 132.80, 125.64, 125.60, 117.2, 117.1, 115.41, 115.39, 55.9, 55.1, 54.9, 30.1, 27.4, 26.8, 22.9, 22.8. LRMS (ESI): *m/z* 665.1 $(M + K)^+$. HRMS (ESI): $(M + H)^+$ calcd for $C_{29}H_{27}N_2O_{10}S_2^+$, 627.1107; found, 627.1106.

## 1.3 Biological methods

### 1.3.1 DHDPS-DHDPR coupled enzyme assay

To determine $IC_{50}$ values for the inhibitors, DHDPS enzyme activity was determined using the coupled DHDPS-DHDPR assay in a Cary 4000 UV/Vis spectrophotometer at 340 nm in 1 cm acrylic cuvettes as previously described.[8,9] A master mix was prepared for each reaction as per Table 1. Reaction mixtures containing enzymes, pyruvate, buffer and NADPH were incubated at 30 ˚C for 12 mins before the addition of ASA to initiate the reaction. The oxidation of NADPH to $NADP^+$ was then monitored at 340 nm at 30 ˚C as a function of time. The initial rate ($\Delta A340 \cdot min^{-1}$) was calculated from the slope of the linear portion of the A340 versus time profile. All experiments were carried out in triplicate.

**Table 1** Coupled assay master mix.

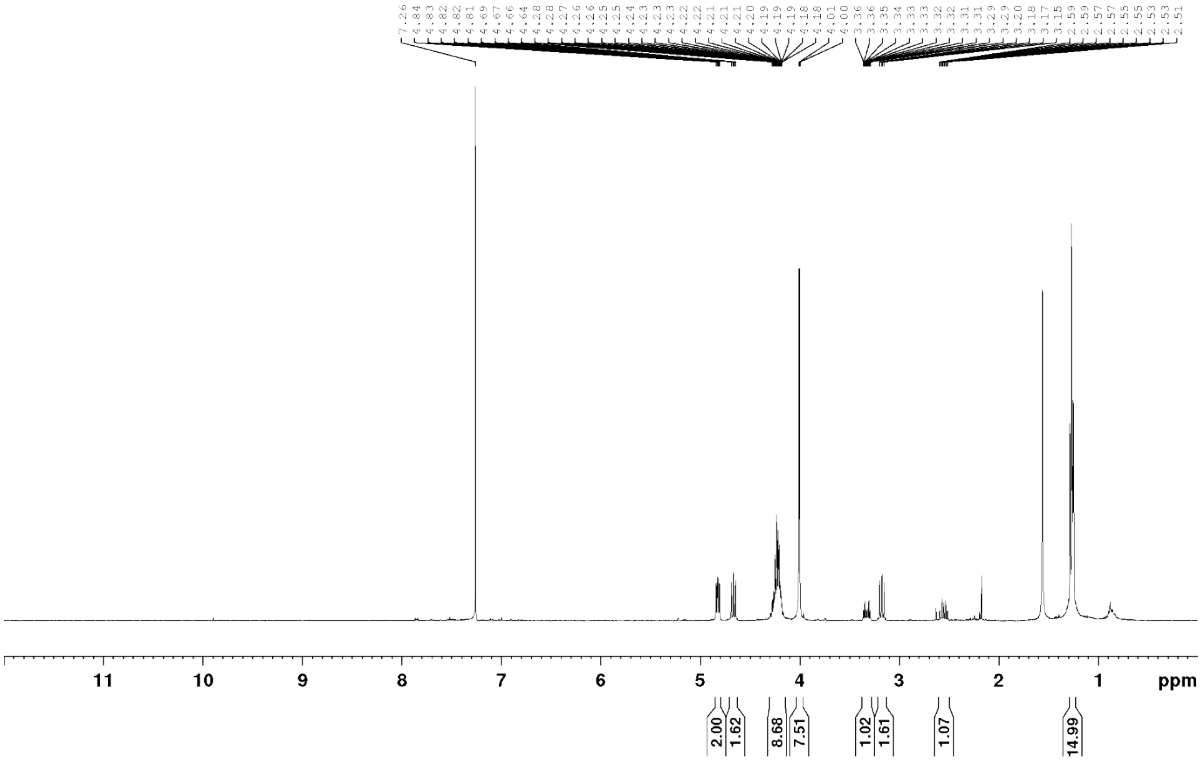
| Reagent | Volume (μL) | Final concentration (mM) |
|---|---|---|
| HEPES (pH 8.0) | 400 | 250 |
| NADPH | 20 | 0.2 |
| Pyruvate | 8 | 1 |
| ASA | 10 | 0.125 |
| EcDHDPR | 20 | 0.0009 |
| DHDPS | 10 | 0.00008 |
| Inhibitor | 8 | varied |
| Tween 20 | 80 | 0.05% |
| $H_2O$* | Up to 800 | - |
| Total | 800 | - |

*$H_2O$ volume was varied according to experiment.

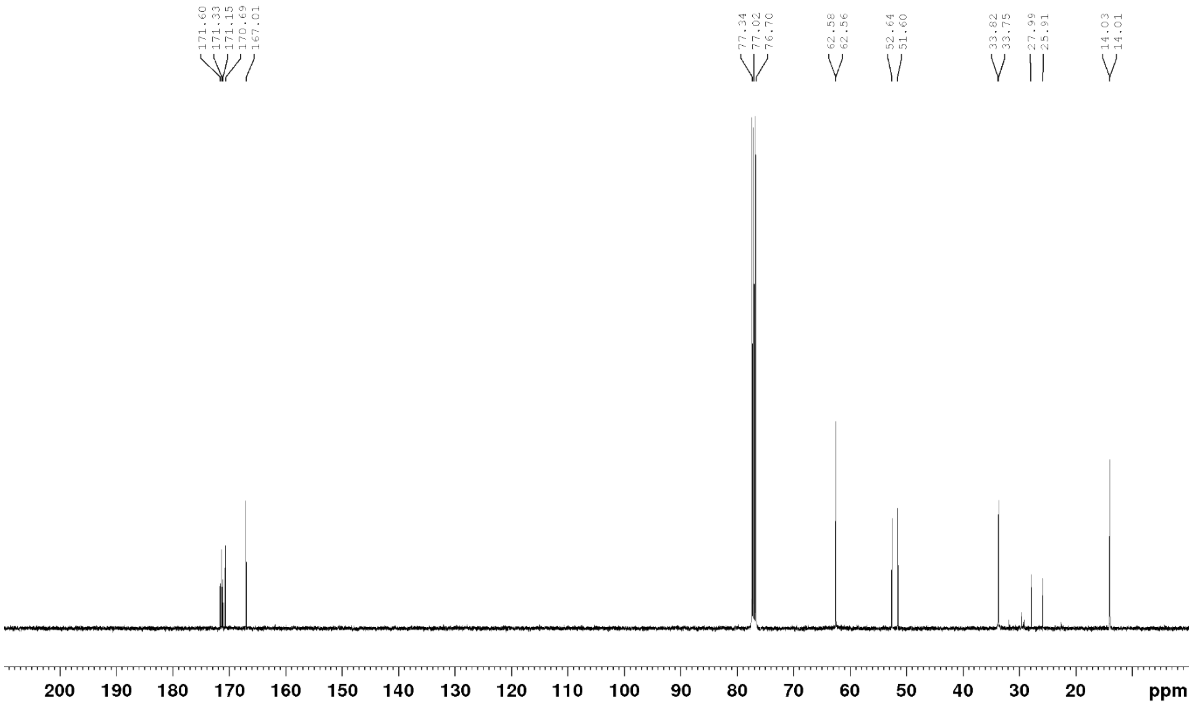
## 1.4 $^{1}$H-NMR and $^{13}$C-NMR spectra for novel compounds

**Diethyl 2,4-bis(2,4-dioxothiazolidin-3-yl)pentanedioate (2)** as the major product

$^{1}$H-NMR ($CDCl_3$, 400 MHz)

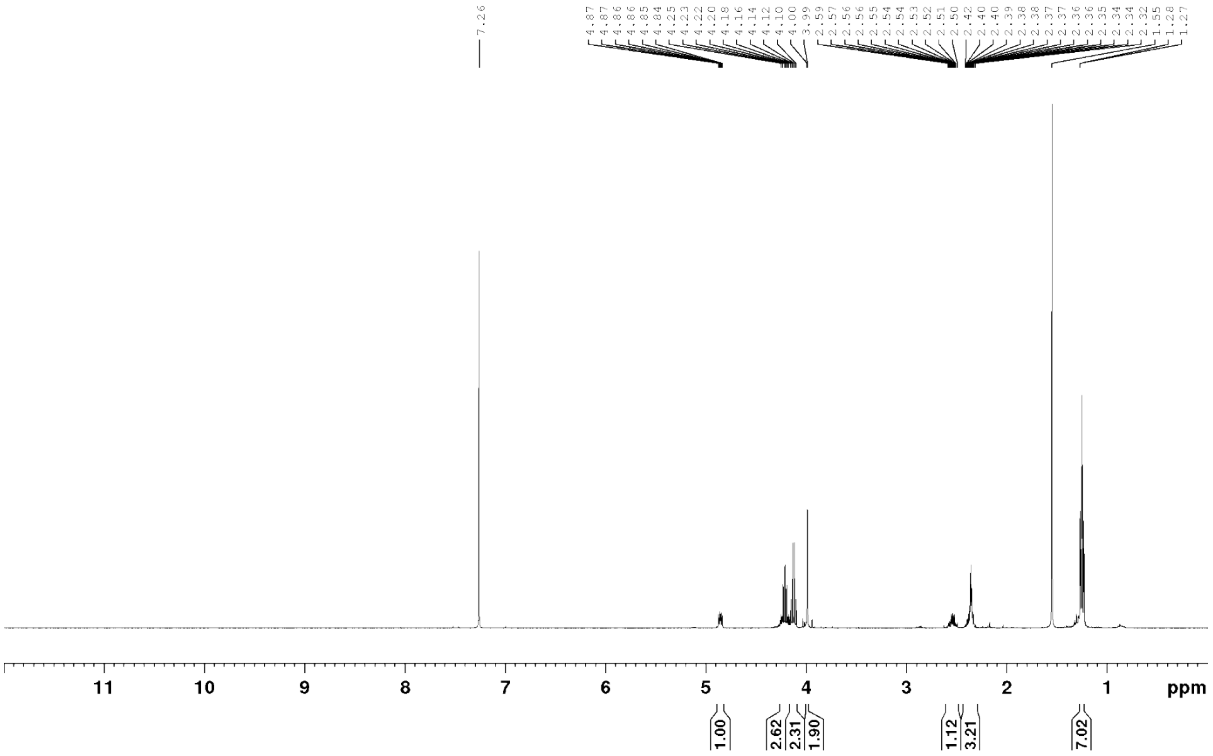



$^{13}$C-NMR ($CDCl_3$, 100 MHz)

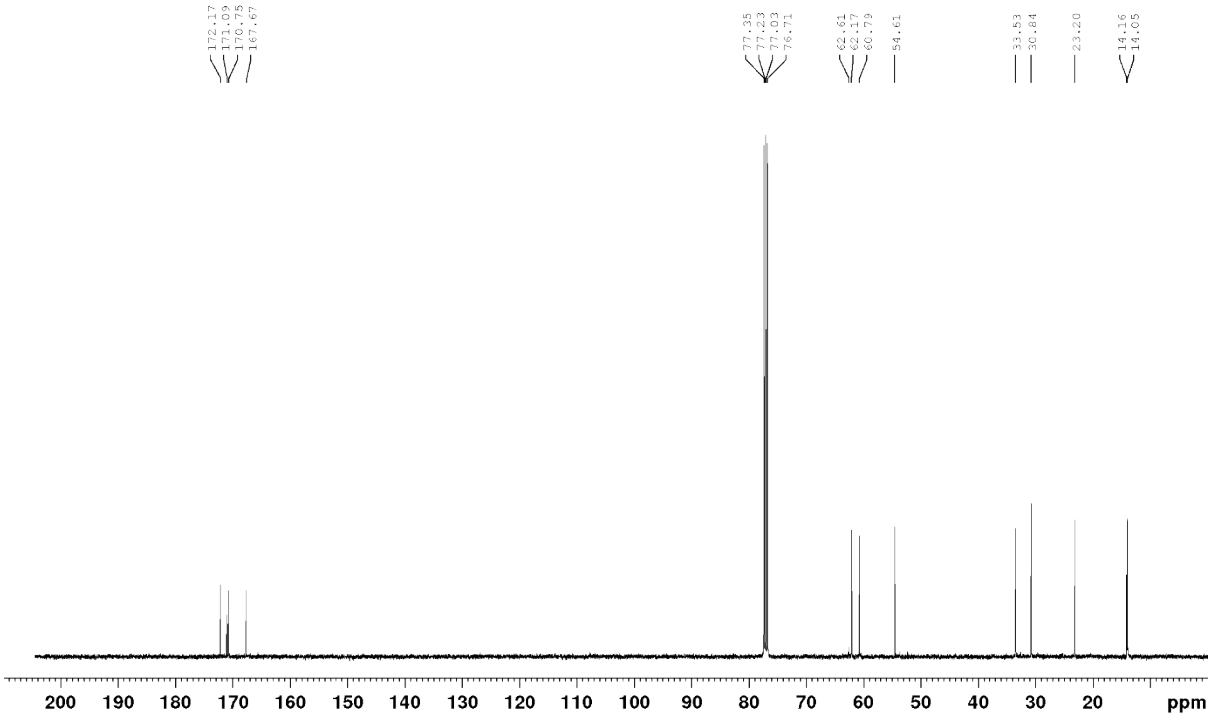


**Diethyl 2-(2,4-dioxothiazolidin-3-yl)pentanedioate (3)** as the minor product

$^{1}$H-NMR ($CDCl_3$, 400 MHz)

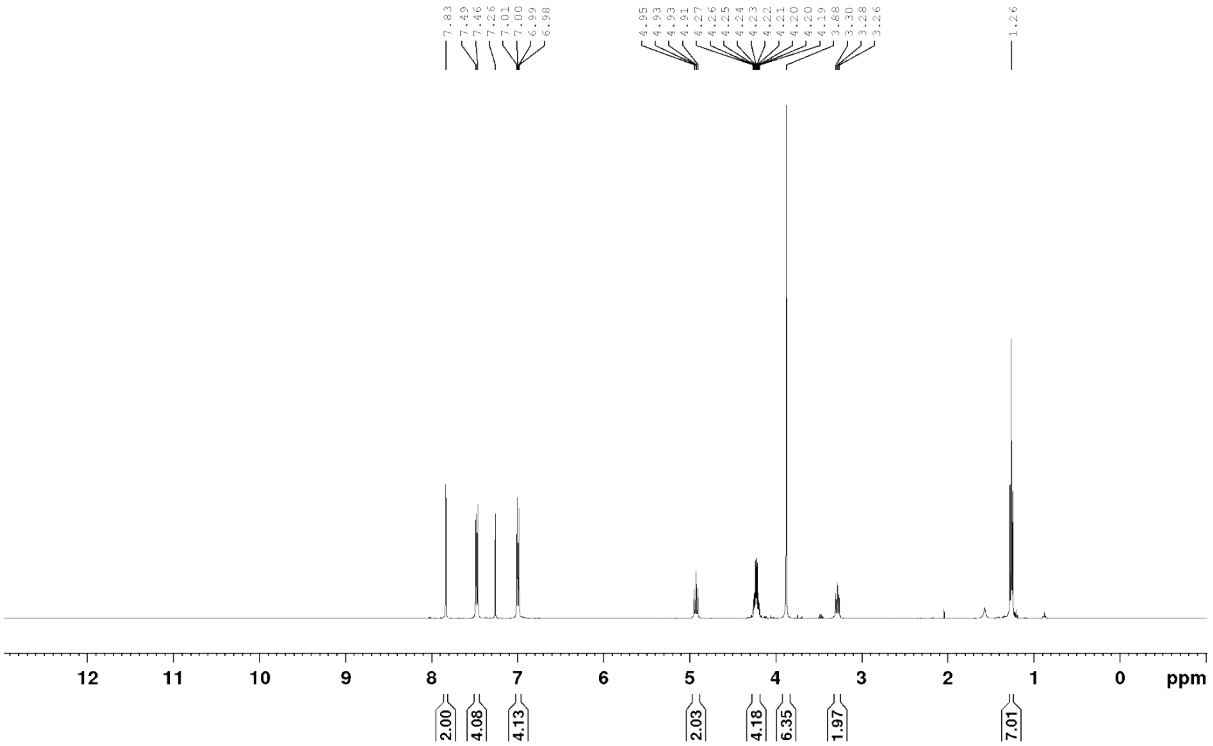



$^{13}$C-NMR ($CDCl_3$, 100 MHz)

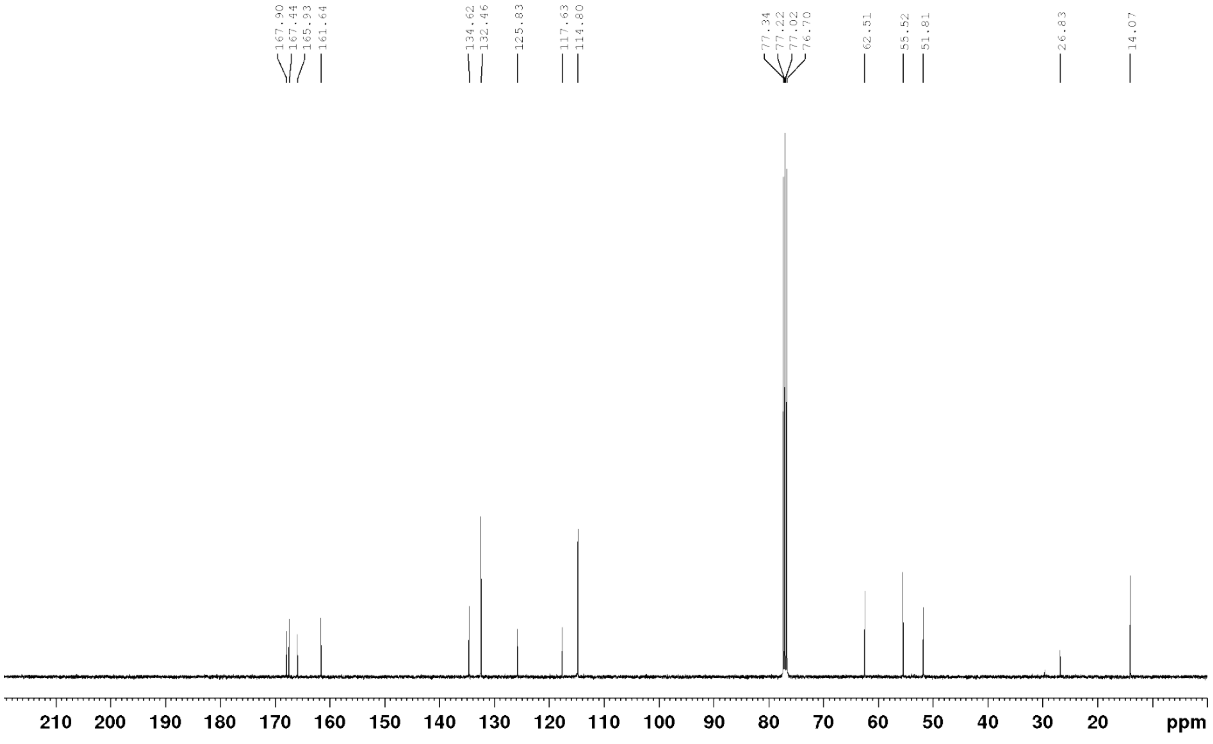


**Diethyl *rac*-2,4-bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)pentanedioate (4a)**

$^{1}$H-NMR (400 MHz, $CDCl_3$)

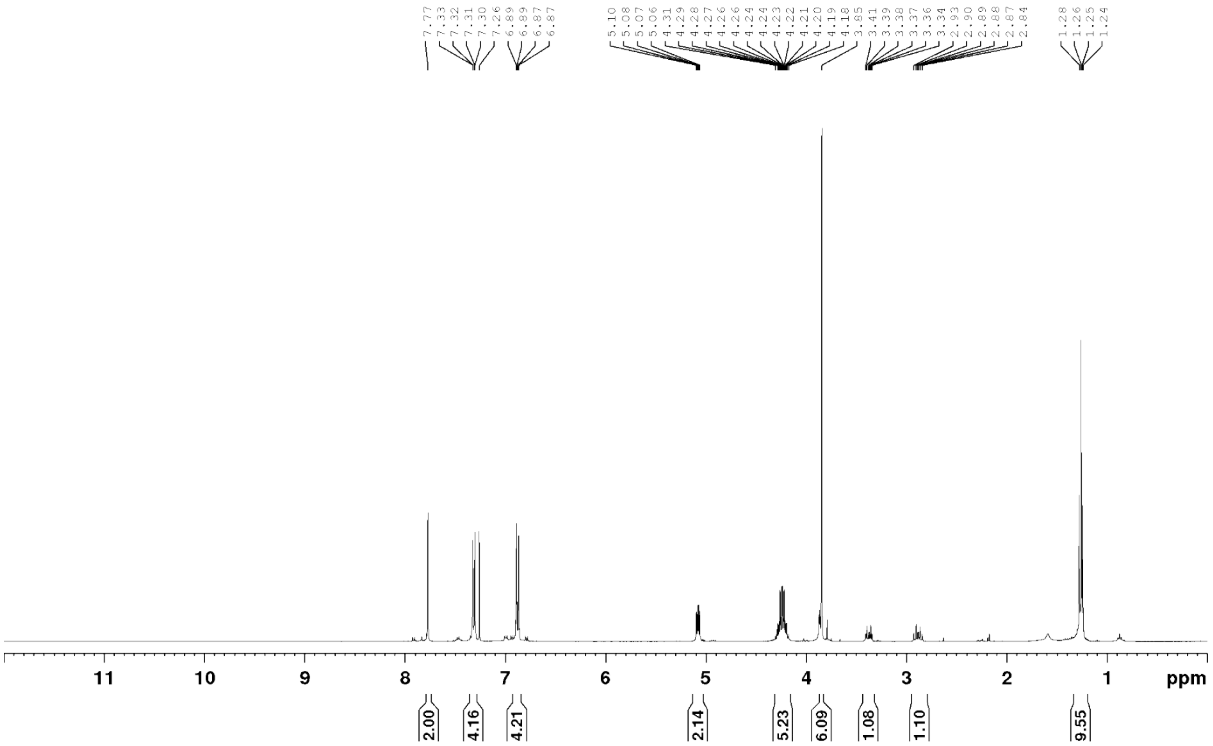



$^{13}$C-NMR (100 MHz, $CDCl_3$)

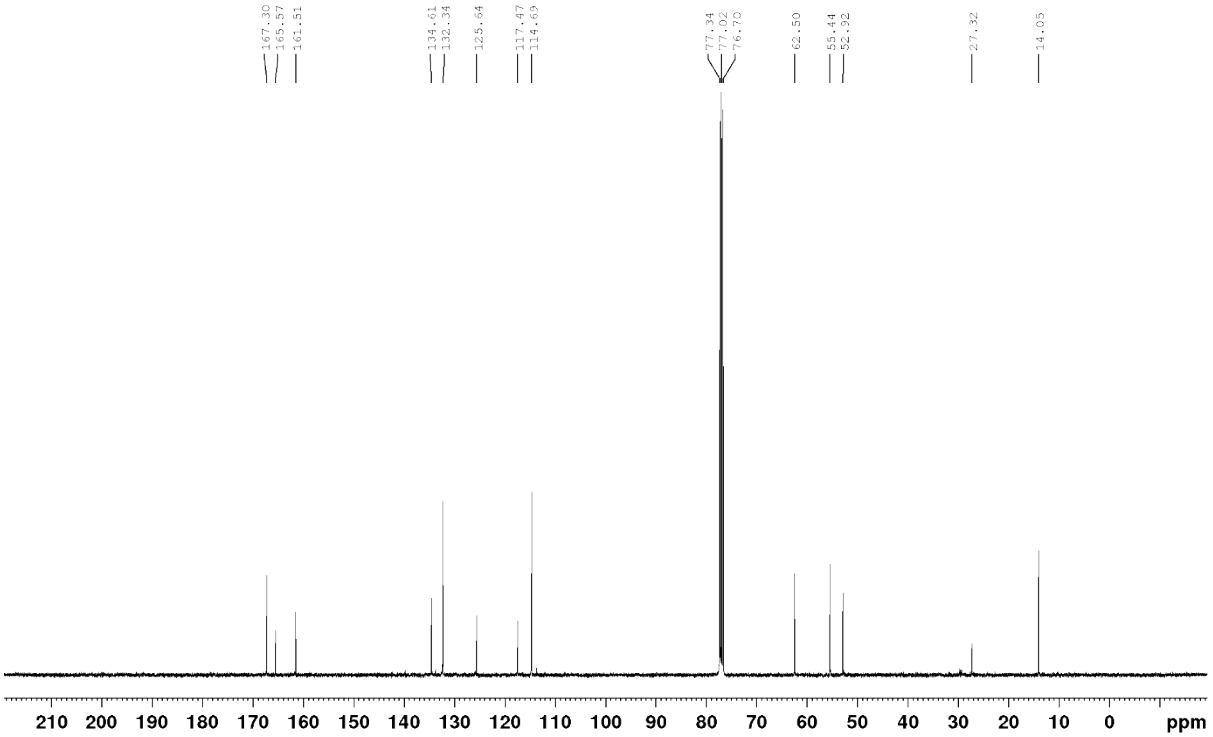


***meso*-Diethyl 2,4-bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)pentanedioate (4b)**

$^{1}$H-NMR (400 MHz, $CDCl_3$)

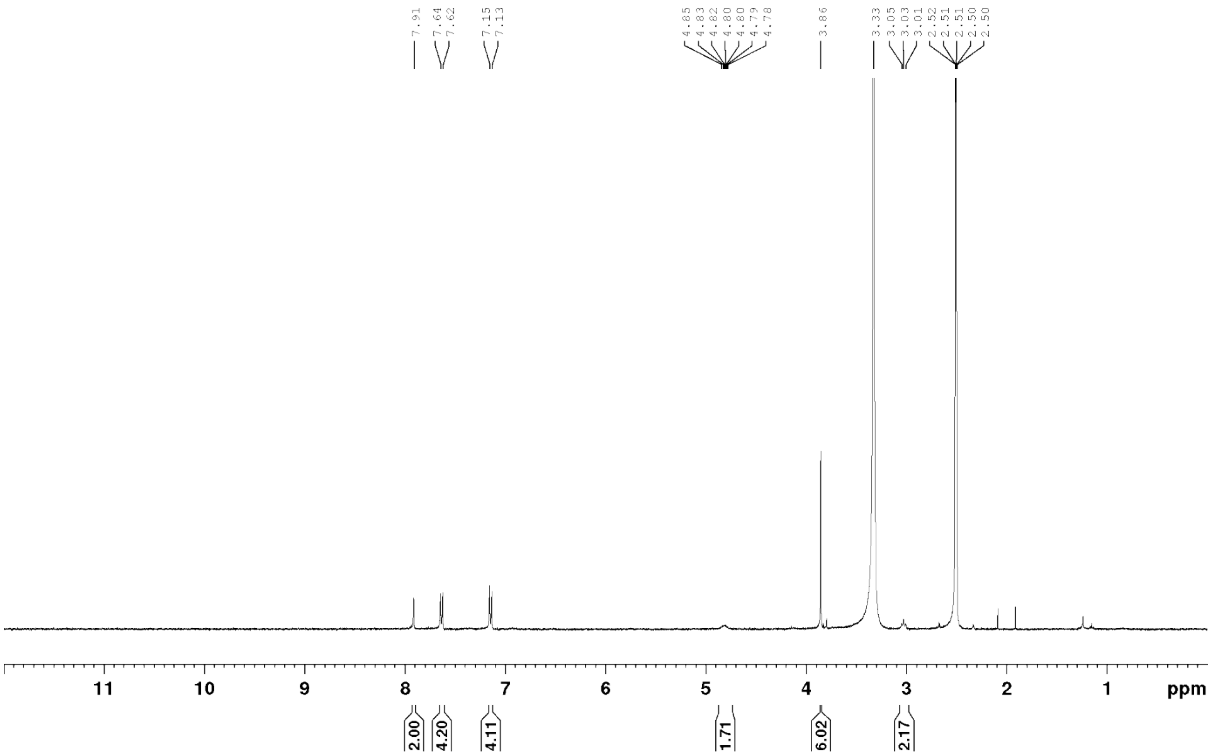



$^{13}$C-NMR (100 MHz, $CDCl_3$)

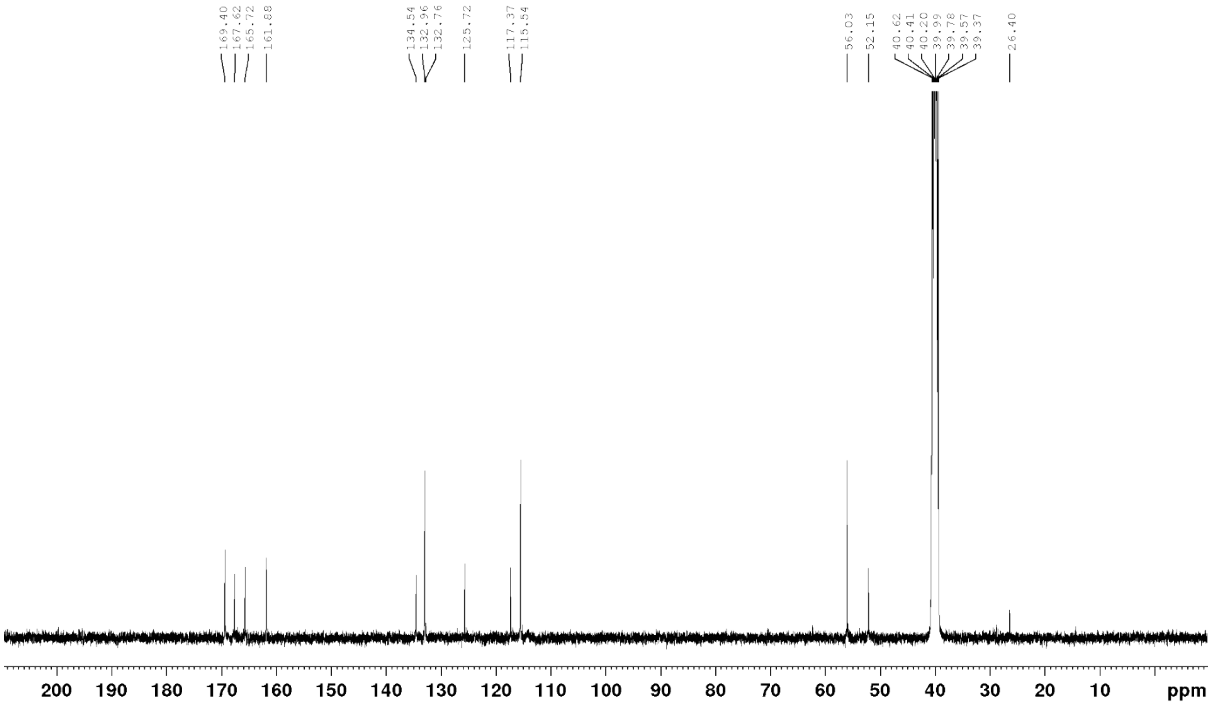


***rac*-2,4-Bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)pentanedioic acid (5a)**

$^{1}$H-NMR (400 MHz, DMSO)

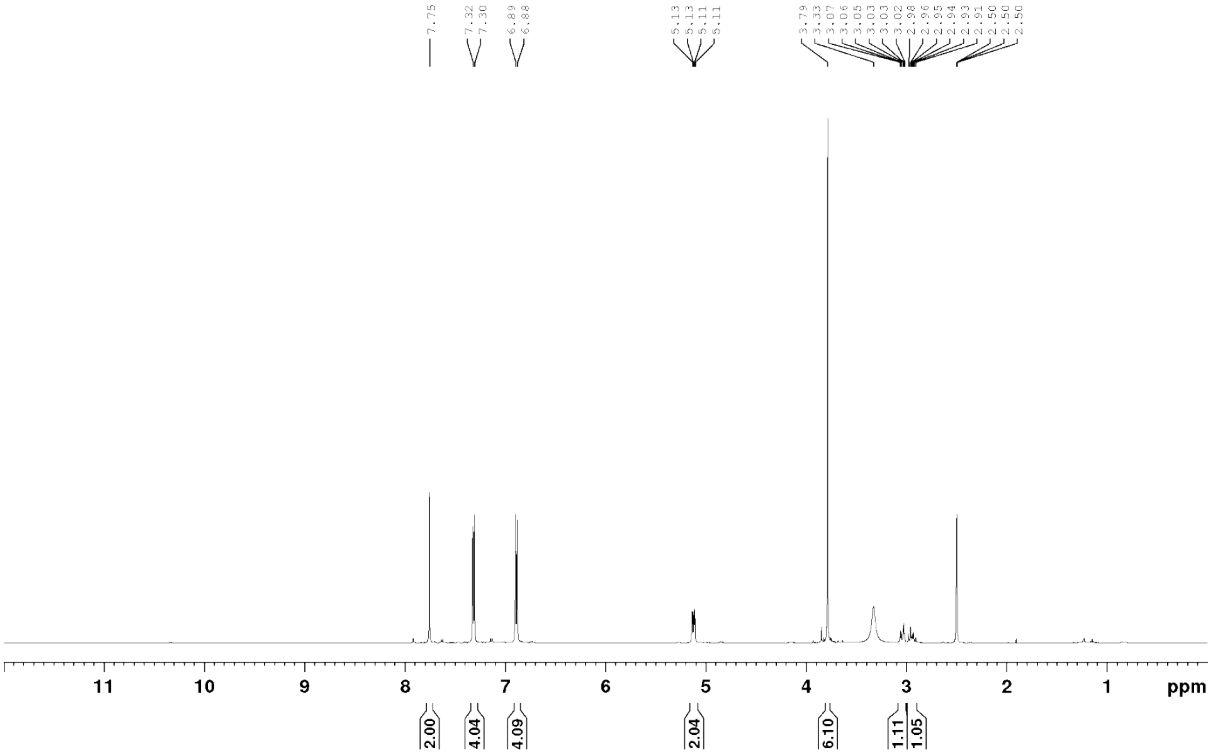



$^{13}$C-NMR (100 MHz, DMSO)

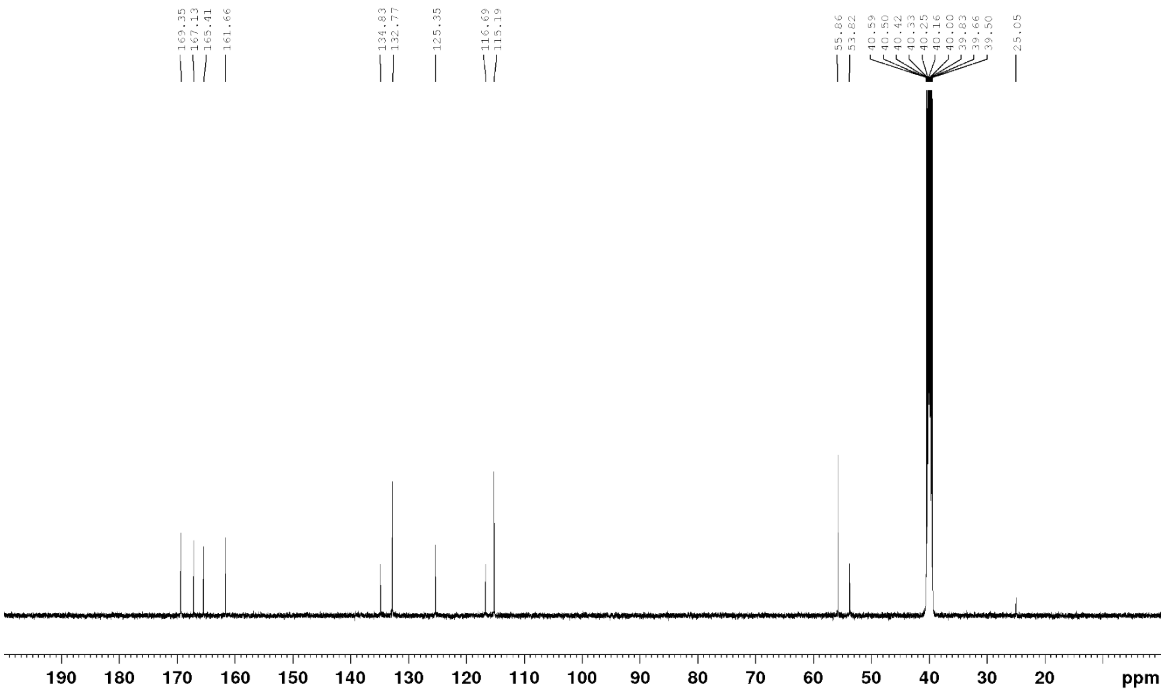


***meso*-2,4-Bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)pentanedioic acid (5b)**

$^{1}$H-NMR (500 MHz, DMSO)

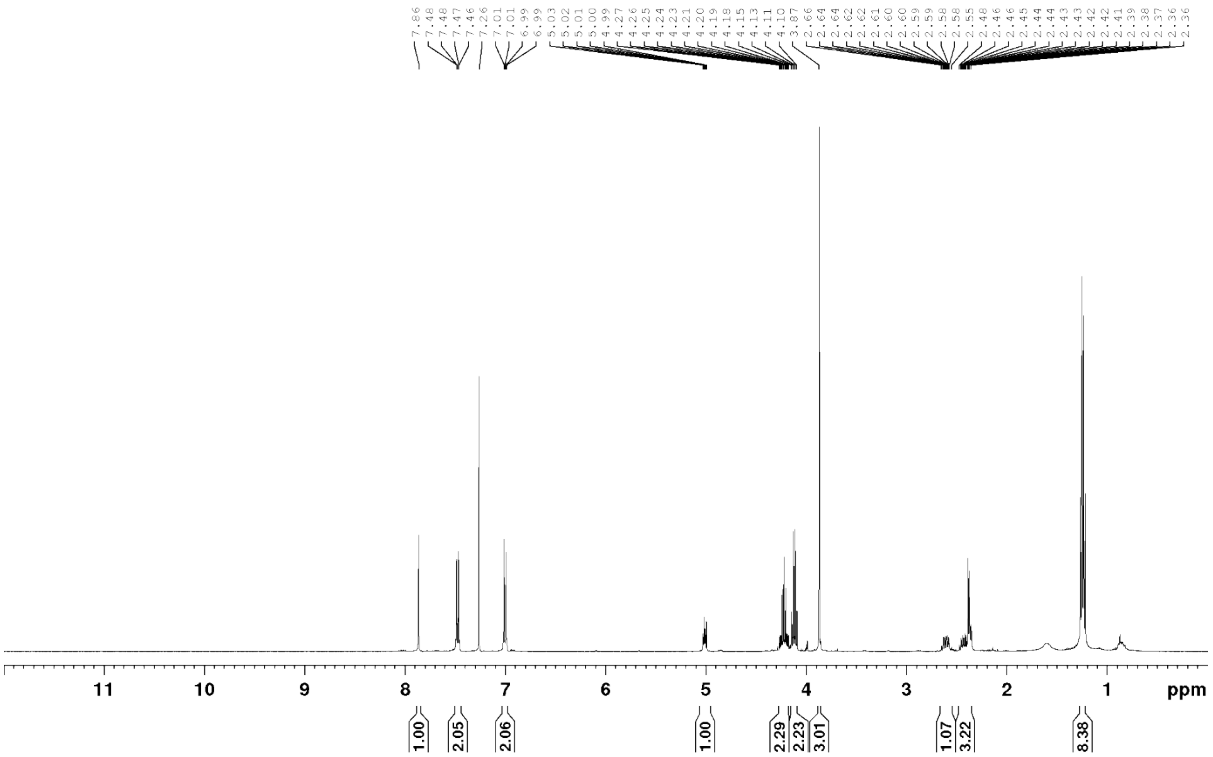



$^{13}$C-NMR (125 MHz, DMSO)

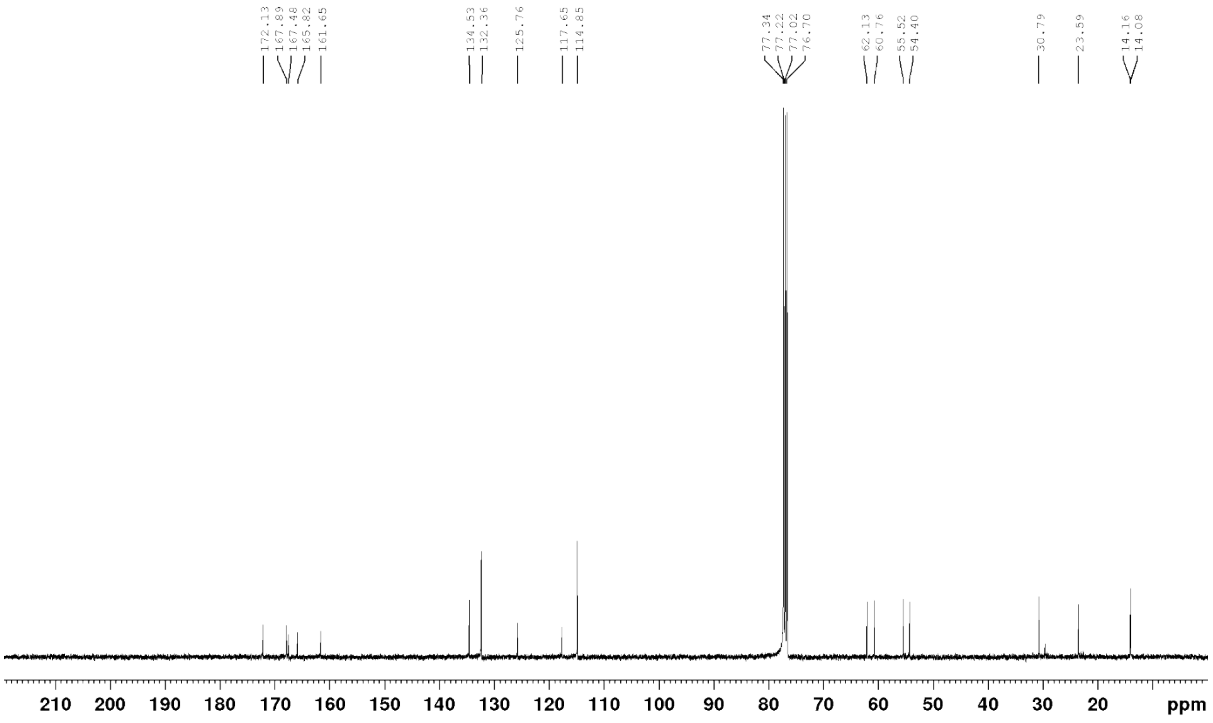


**Diethyl (*Z*)-2-(5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)pentanedioate (6)**

$^{1}$H-NMR ($CDCl_3$, 400 MHz)

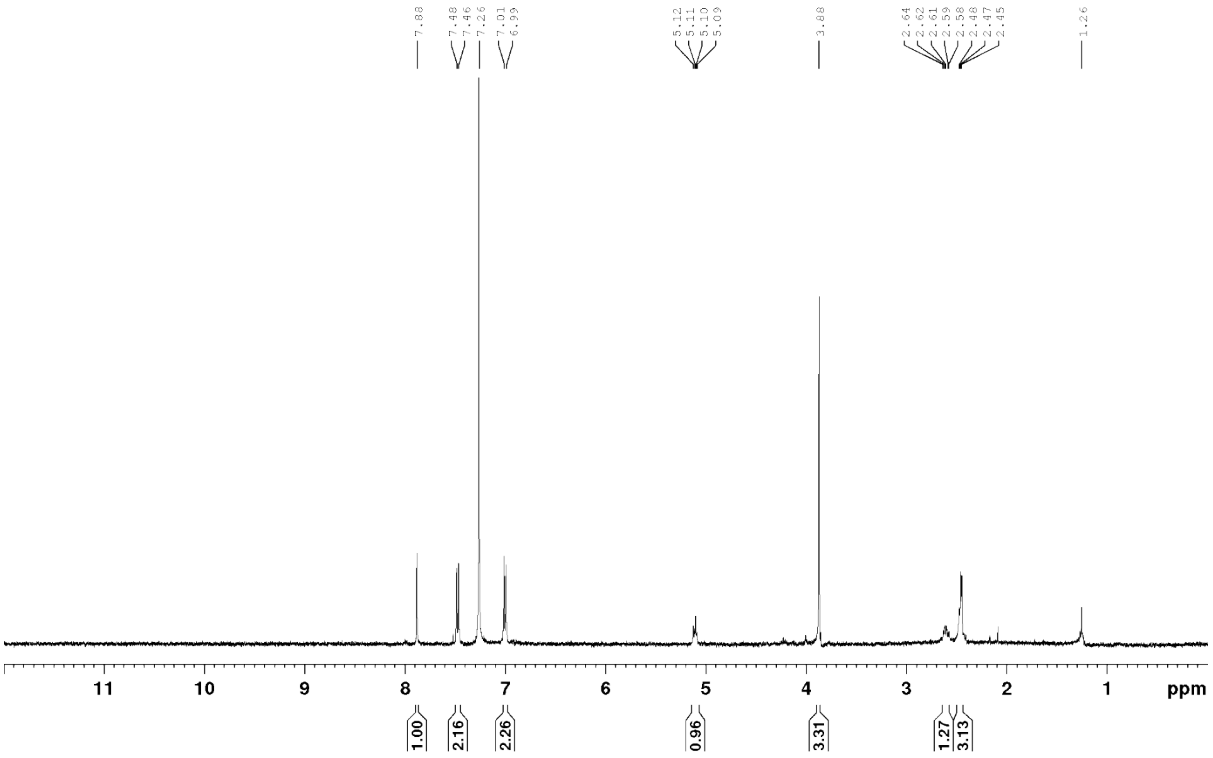



$^{13}$C-NMR ($CDCl_3$, 100 MHz)

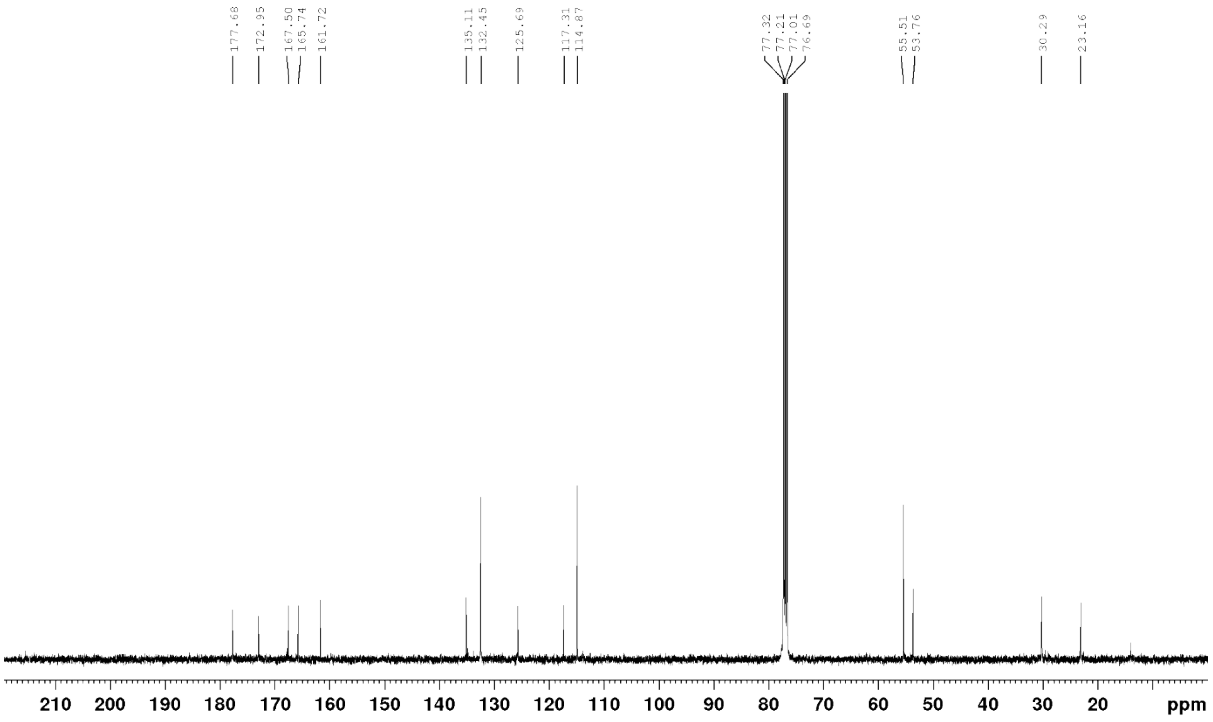


**(*Z*)-2-(5-(4-Methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)pentanedioic acid (7)**

$^{1}$H-NMR ($CDCl_3$, 400 MHz)

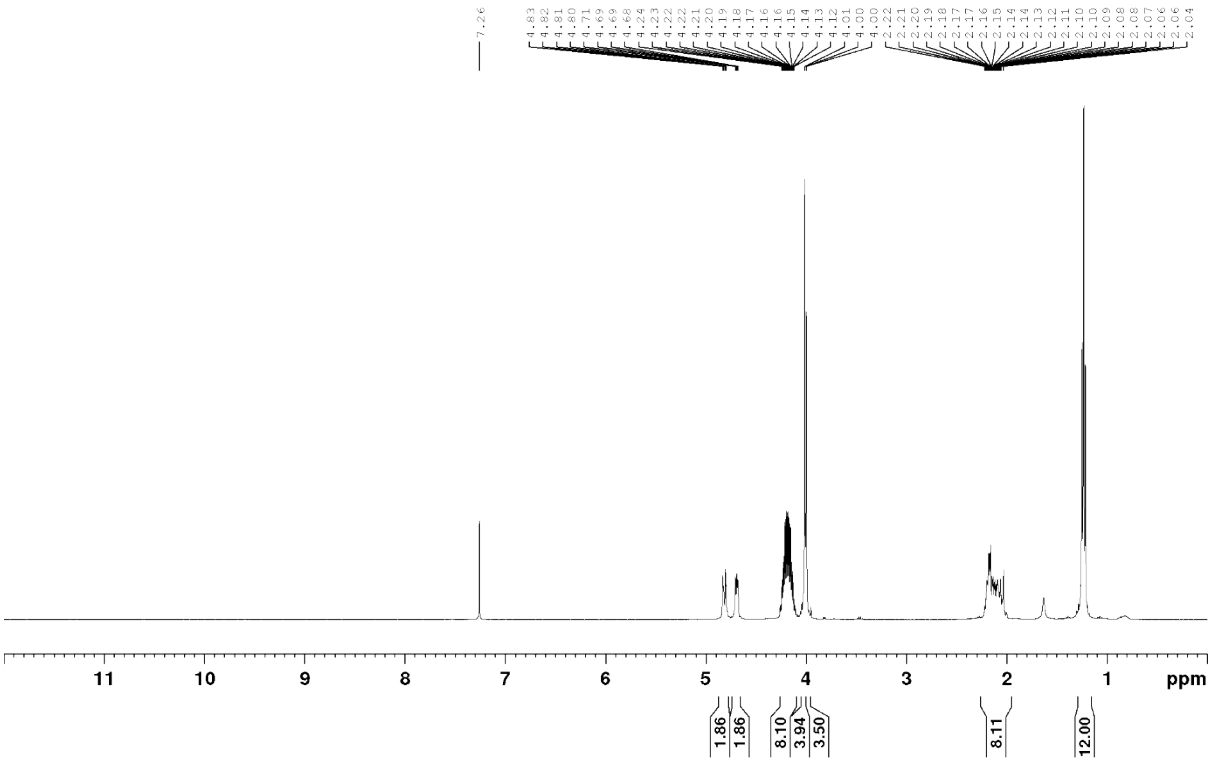



$^{13}$C-NMR ($CDCl_3$, 100 MHz)

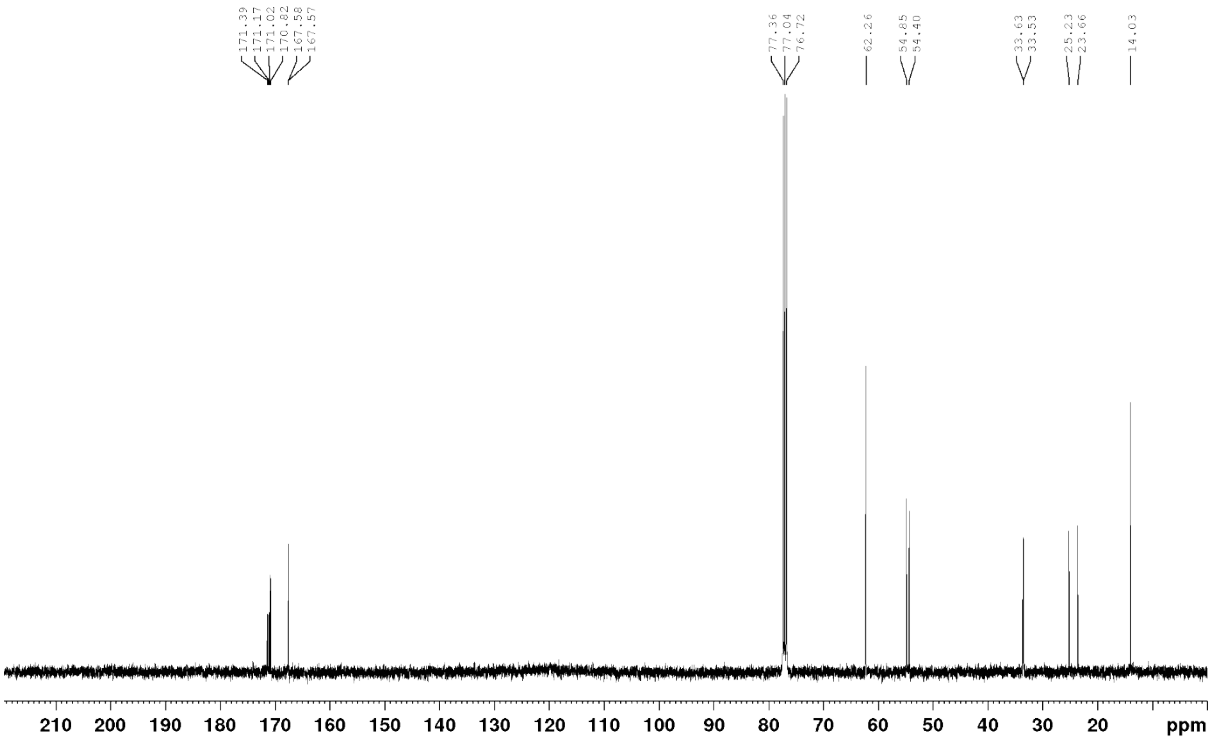


**Diethyl 2,5-bis(2,4-dioxothiazolidin-3-yl)hexanedioate (9)**

$^{1}$H-NMR (400 MHz, $CDCl_3$)

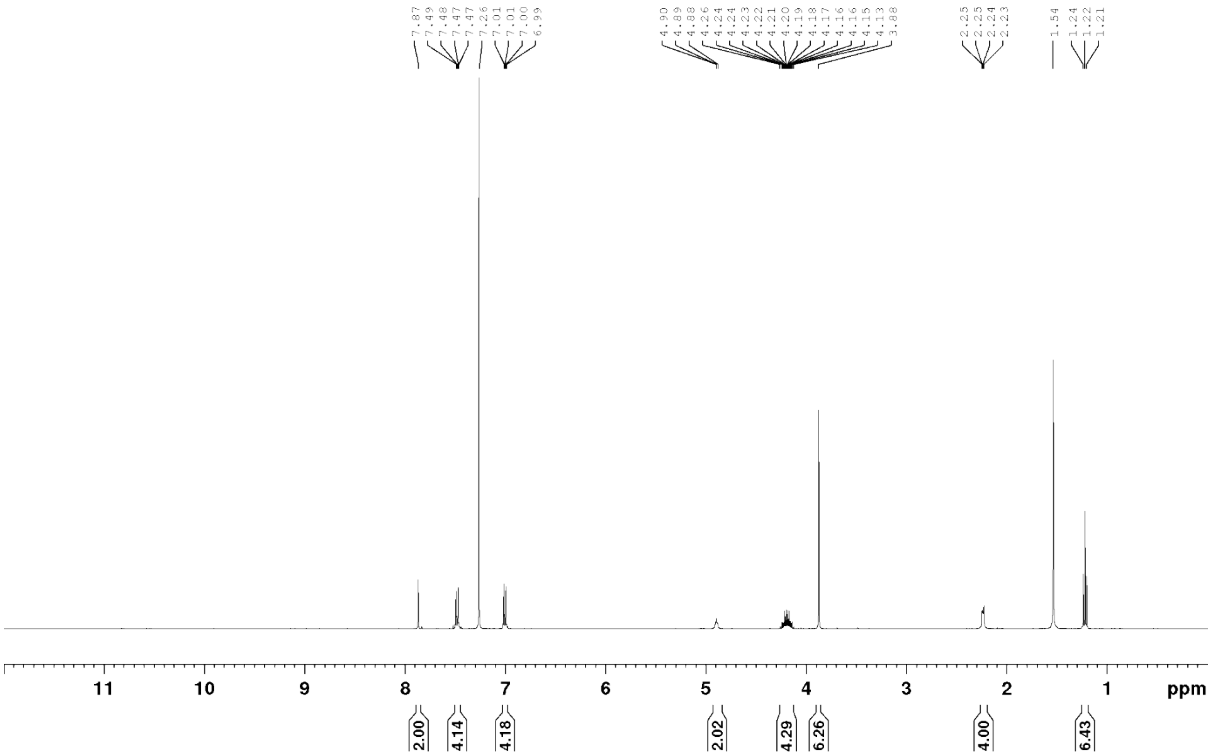



$^{13}$C-NMR (100 MHz, $CDCl_3$)

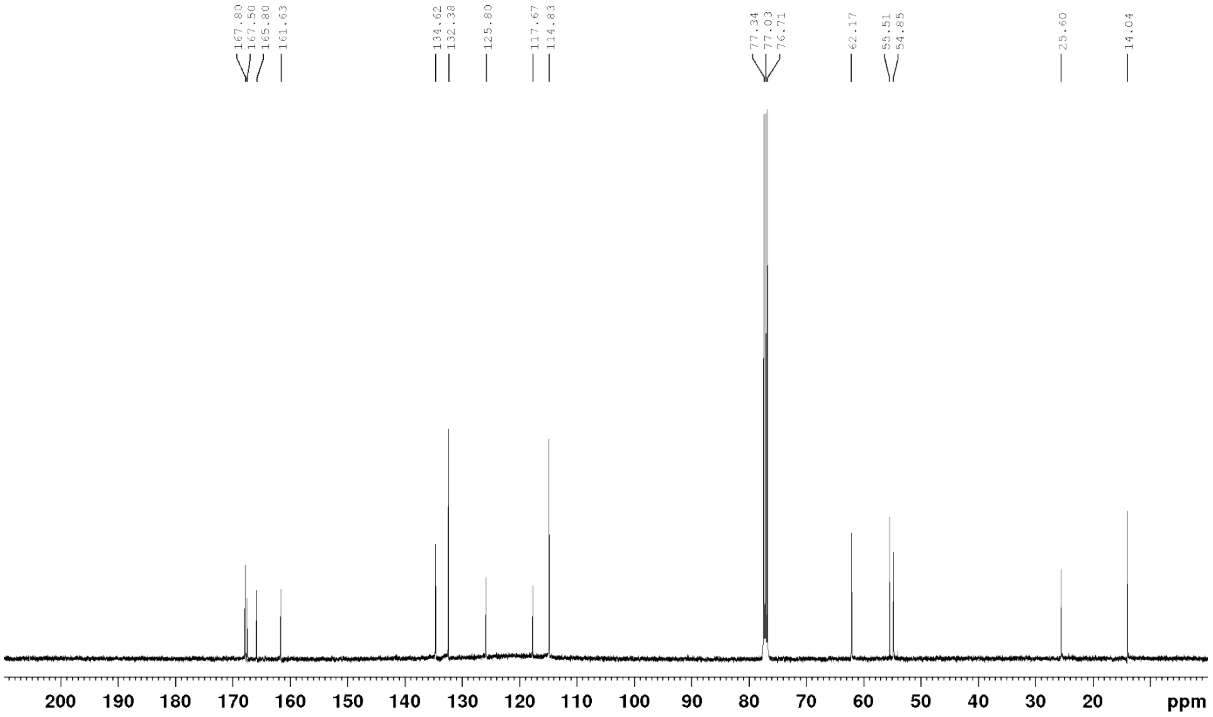


**Diethyl *meso*-2,5-Bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)hexanedioate (10a)**

$^{1}$H-NMR (400 MHz, $CDCl_3$)

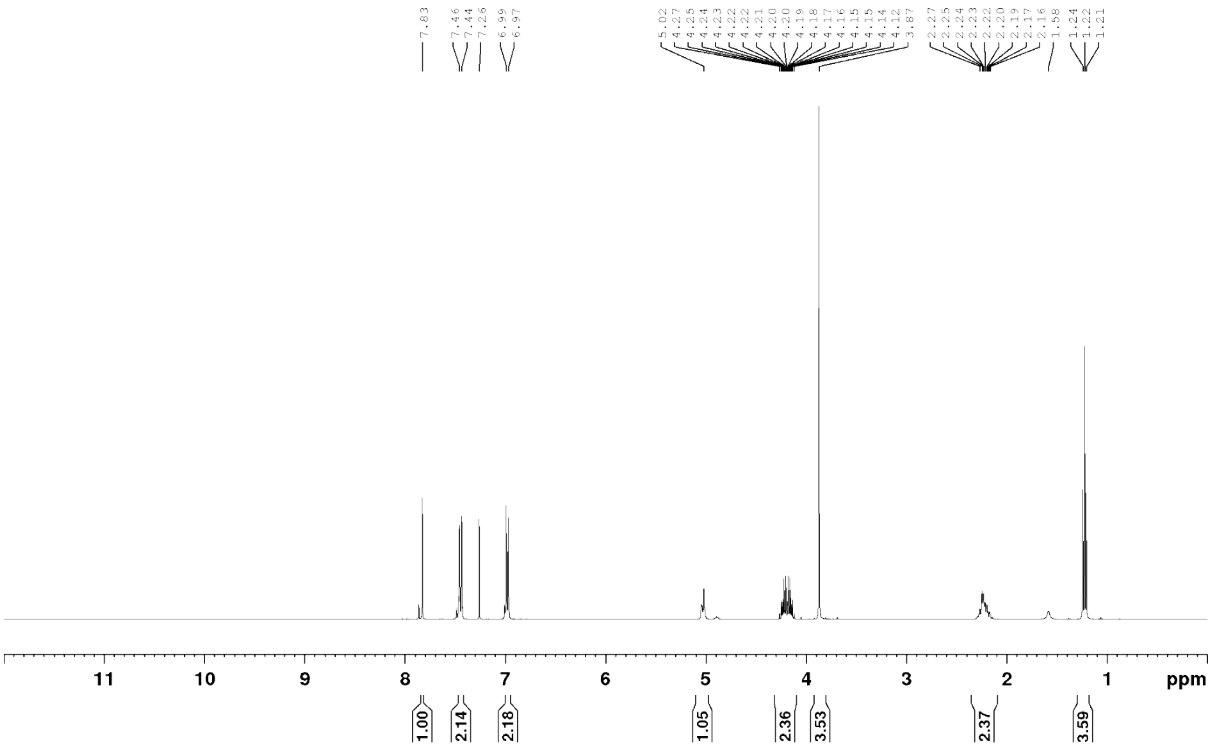



$^{13}$C-NMR (100 MHz, $CDCl_3$)

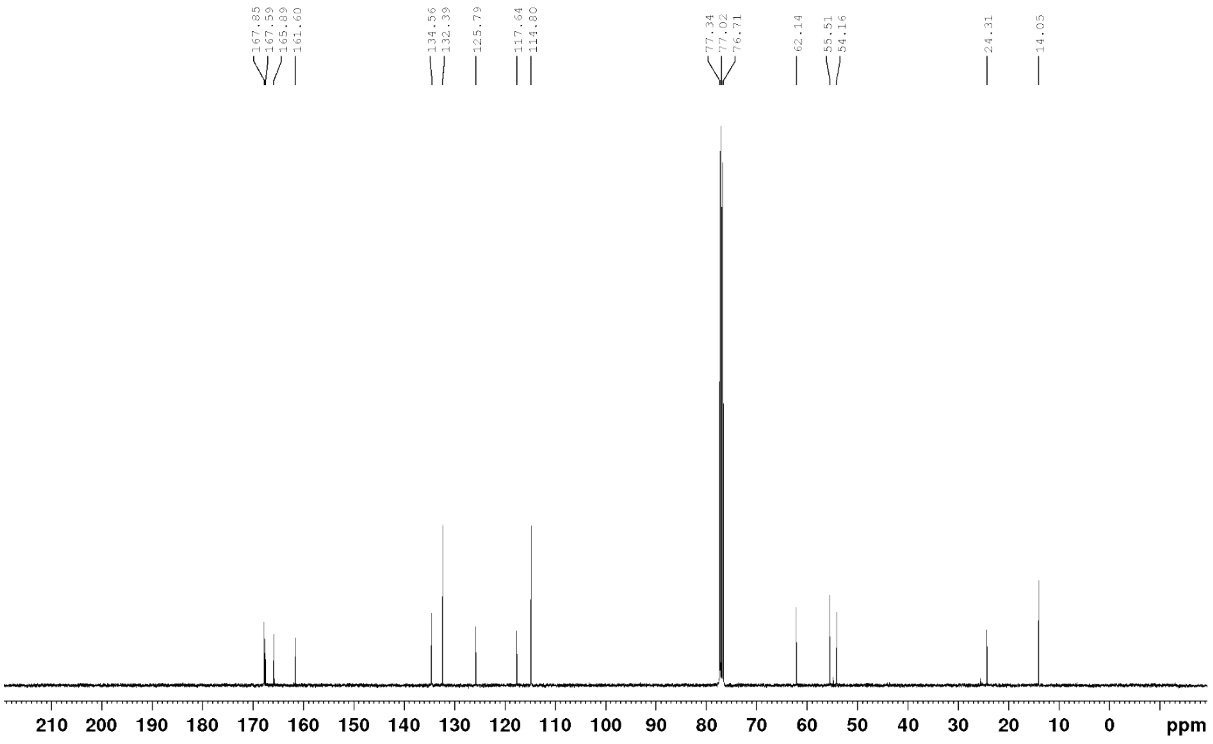


***rac*-Diethyl 2,5-bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)hexanedioate (10b)**

$^{1}$H-NMR (400 MHz, $CDCl_3$)

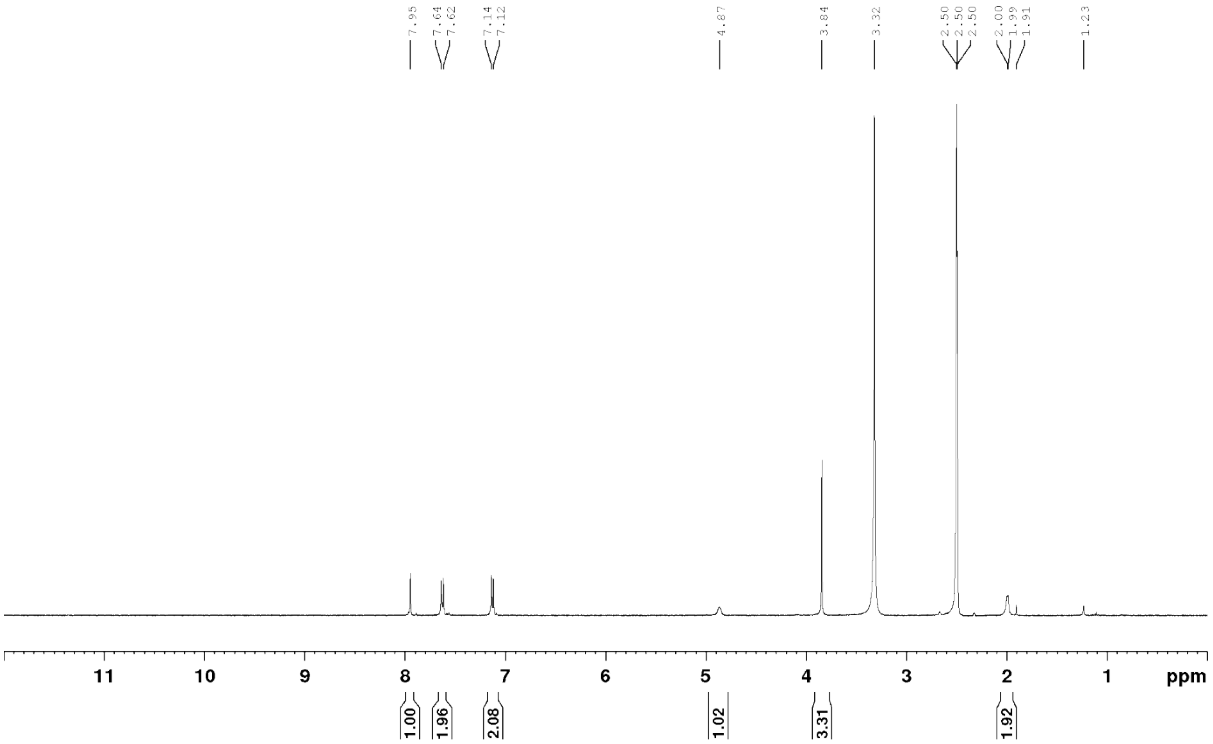



$^{13}$C-NMR (100 MHz, $CDCl_3$)

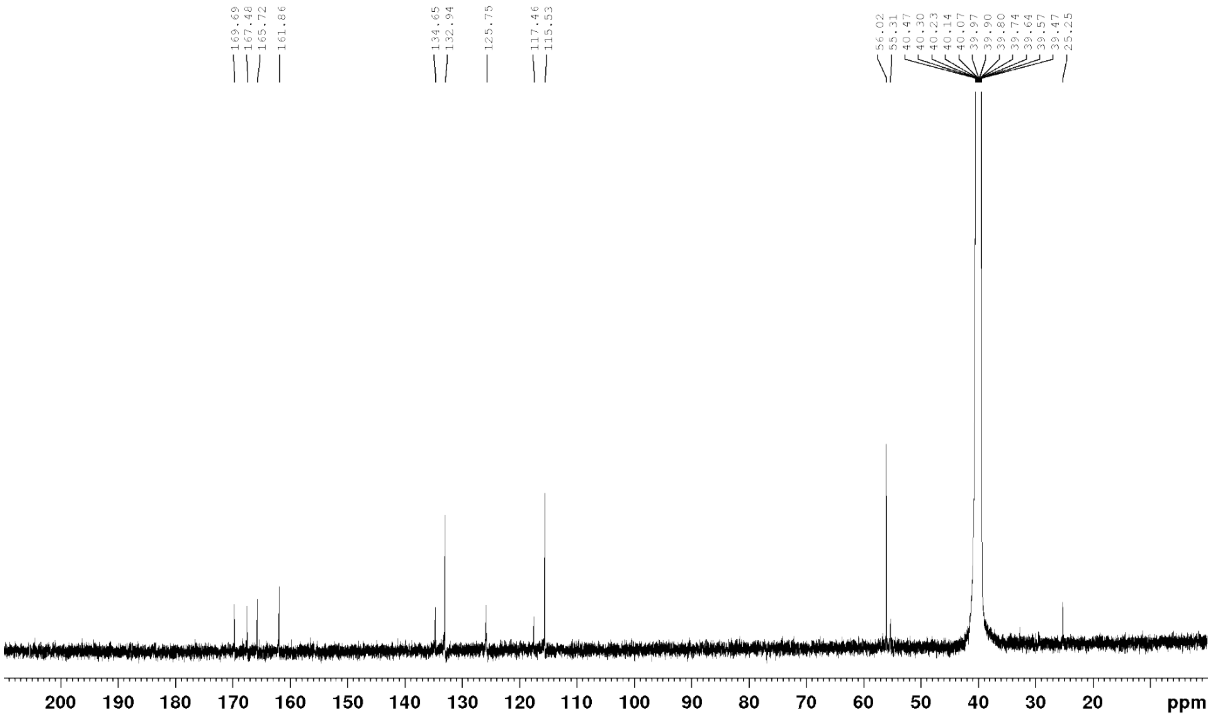


***meso*-2,5-Bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)hexanedioic acid (11a)**

$^{1}$H-NMR (100 MHz, DMSO)

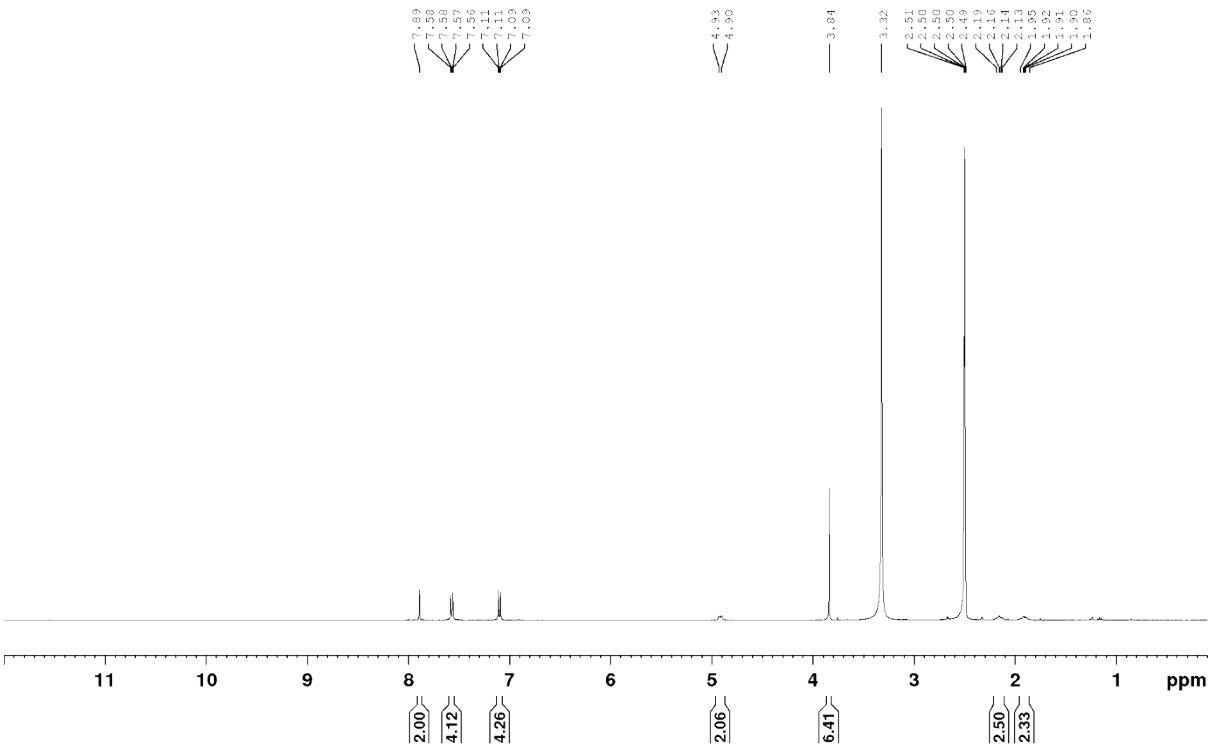



$^{13}$C-NMR (125 MHz, DMSO)

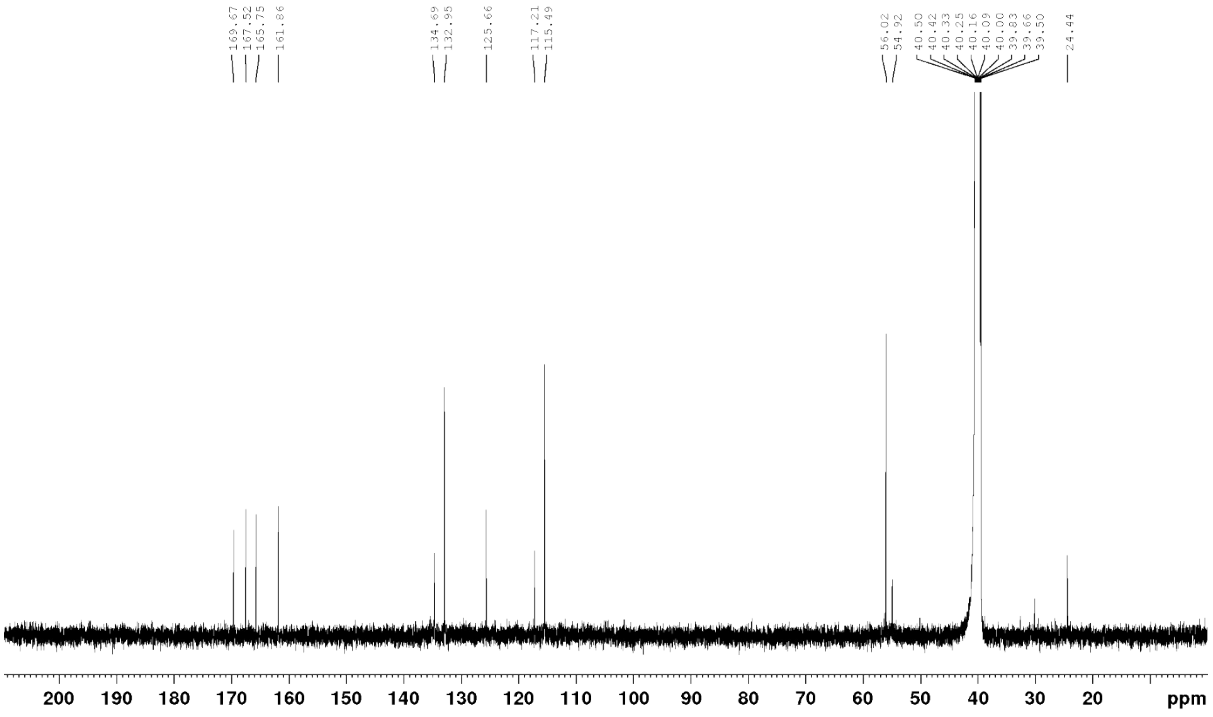


***rac*-2,5-Bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)hexanedioic acid (11b)**

$^{1}$H-NMR (400 MHz, DMSO)

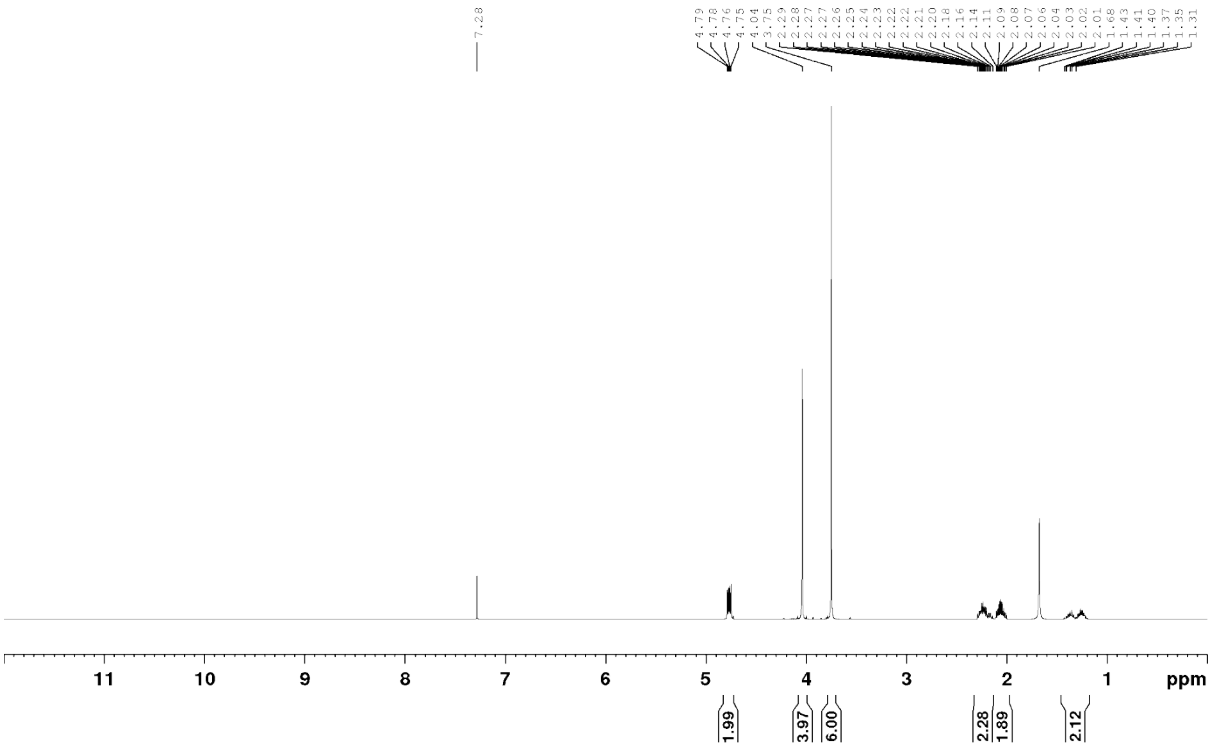



$^{13}$C-NMR (125 MHz, DMSO)

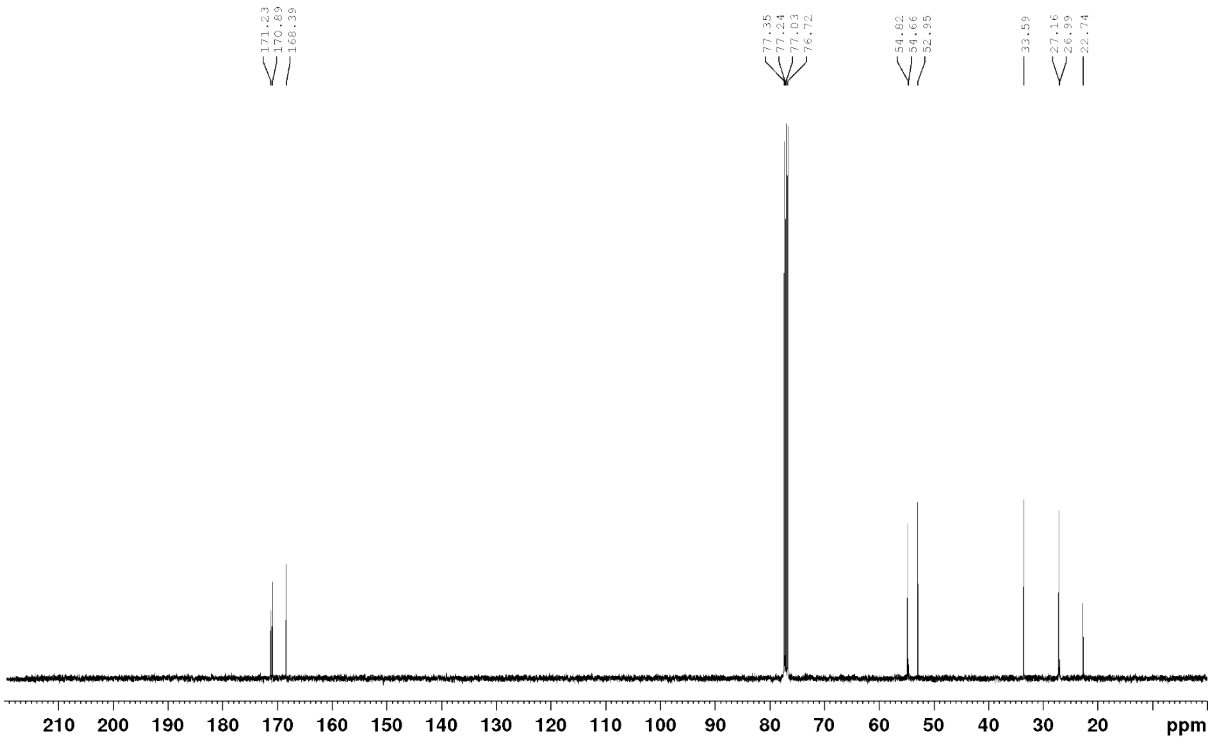


**Dimethyl 2,6-bis(2,4-dioxothiazolidin-3-yl)heptanedioate (13)**

$^{1}$H-NMR (400 MHz, $CDCl_3$)

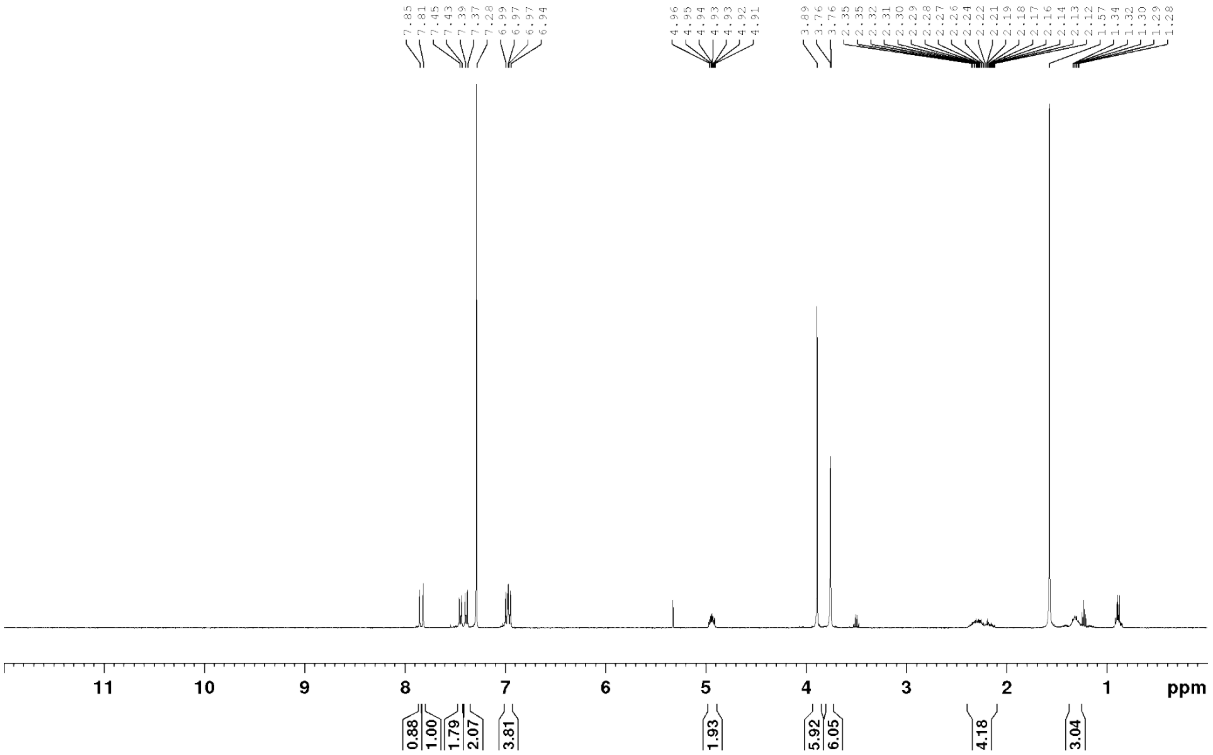



$^{13}$C-NMR (100 MHz, $CDCl_3$)

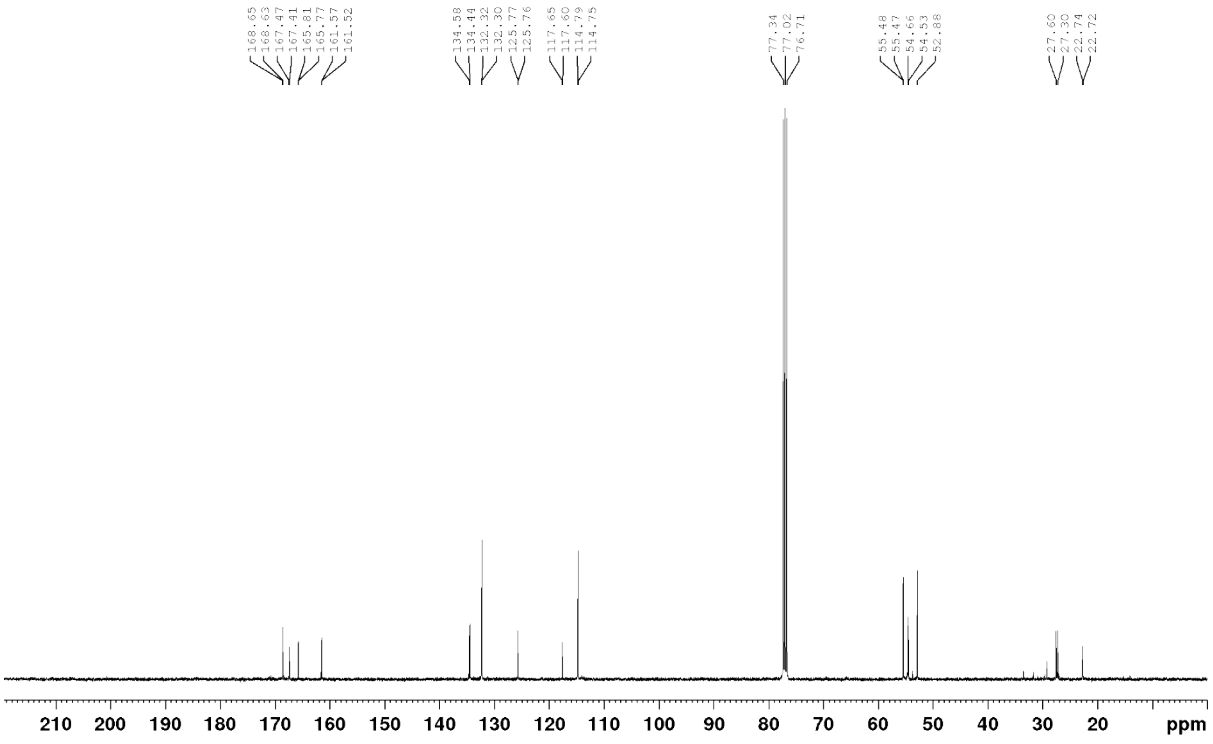


**Dimethyl 2,6-bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)heptanedioate (14)**

$^{1}$H-NMR (400 MHz, $CDCl_3$)

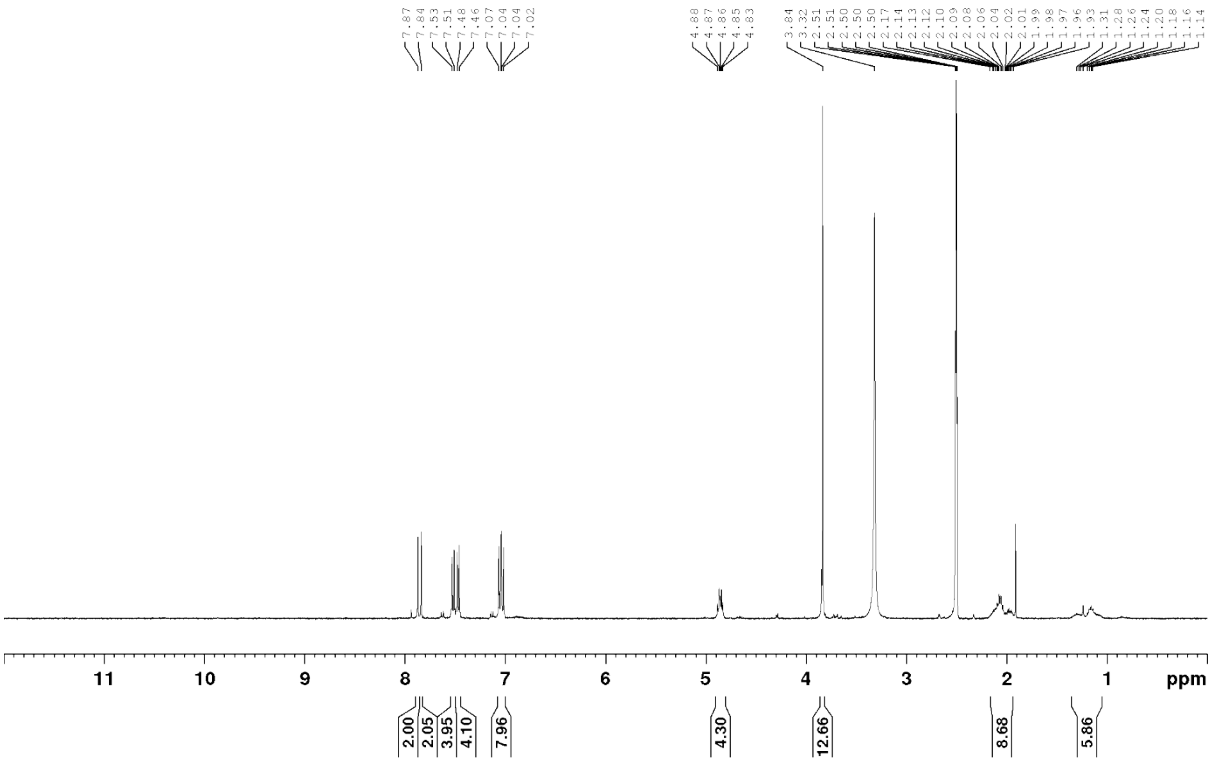



$^{13}$C-NMR (100 MHz, $CDCl_3$)

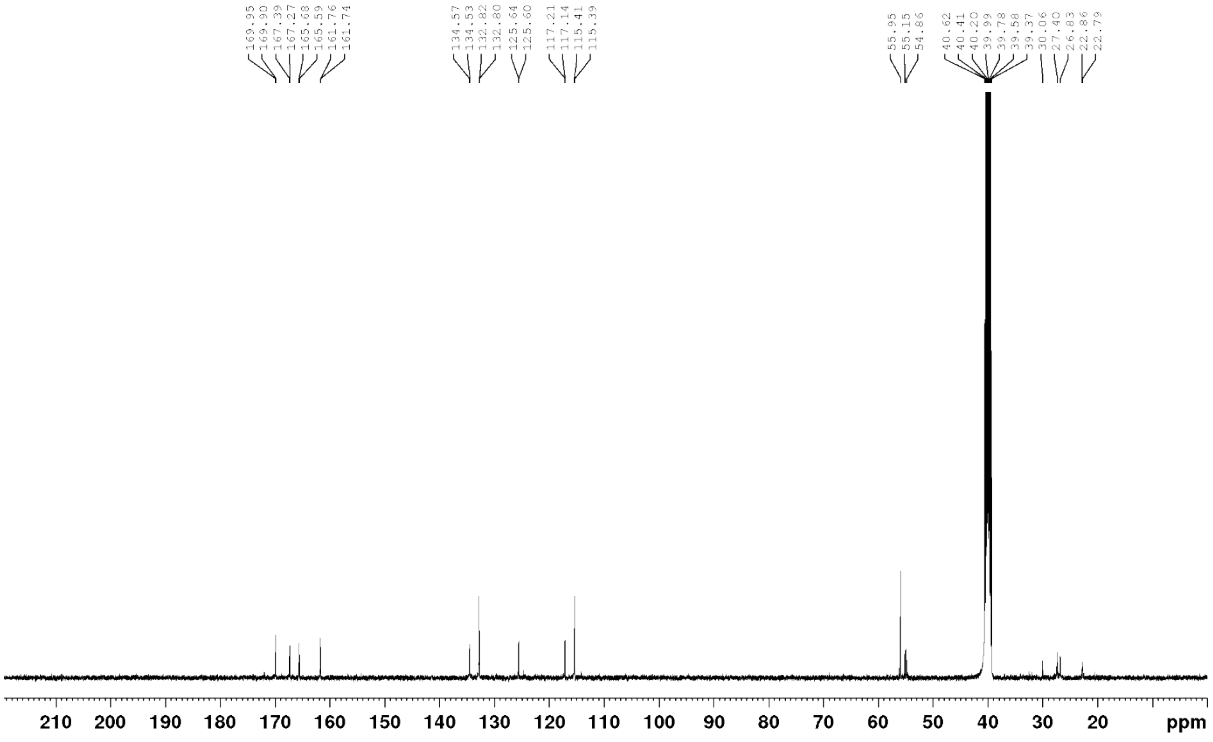


**2,6-Bis(5-((*Z*)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)heptanedioic acid (15)**

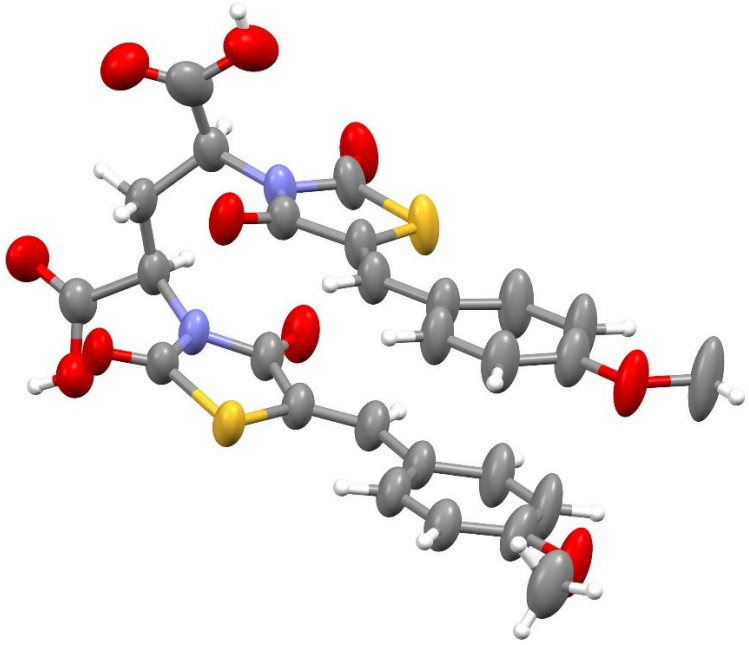
$^{1}$H-NMR (400 MHz, DMSO)



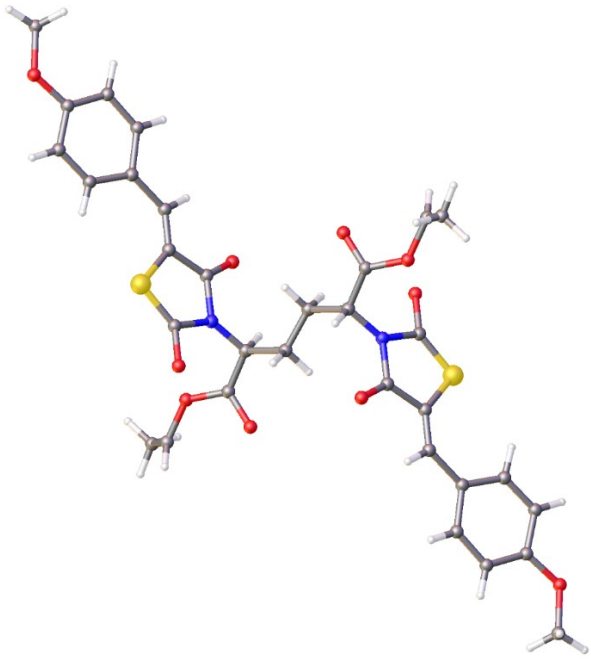

$^{13}$C-NMR (100 MHz, DMSO)



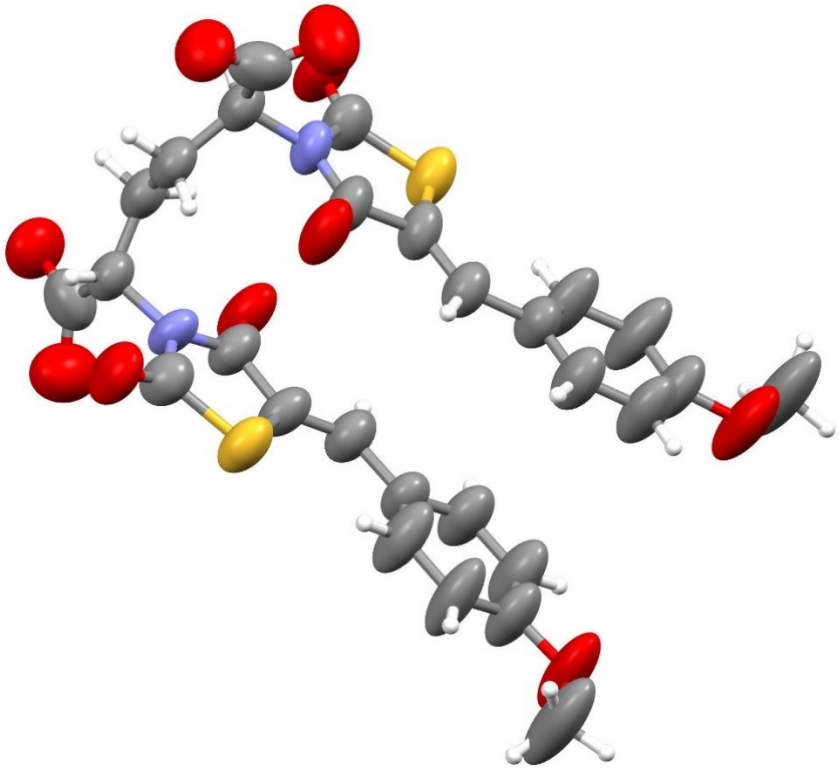

## 1.5 X-ray crystal structures



**Supplementary Figure 1** Crystal structure of compound **4b**, grown via slow evaporation from a concentrated solution of the material in a mixture of chloroform and methanol, confirming a "closed" configuration and *meso* stereochemistry with the *R,S* enantiomer shown.



**Supplementary Figure 2** Crystal structure of compound **10a,** grown via slow evaporation from a concentrated solution of the material in a mixture of chloroform and methanol, indicating a "open" configuration and *meso* stereochemistry with the *R,S* enantiomer shown.



**Supplementary Figure 3** Crystal structure of compound **11b**, grown via slow evaporation from a concentrated solution of the material in a mixture of chloroform and methanol, confirming "closed" configuration with *rac* stereochemistry with the *S,S* enantiomer shown.

## References


1. Armarego, W. L. F.; Chai, C. L. L., *Purification of Laboratory Chemicals*. Butterworth-Heinemann: Burlington, 2003.

2. Sheldrick, G. M., A short history of SHELX. *Acta Crystallographica Section A* **2008,** *64* (Pt 1), 112-22.

3. Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H., OLEX2: a complete structure solution, refinement and analysis program. *Journal of Applied Crystallography* **2009,** *42* (2), 339-341.

4. Bhat, B. A.; Ponnala, S.; Sahu, D. P.; Tiwari, P.; Tripathi, B. K.; Srivastava, A. K., Synthesis and antihyperglycemic activity profiles of novel thiazolidinedione derivatives. *Bioorganic & Medicinal Chemistry* **2004,** *12* (22), 5857-64.

5. Christoff, R. M.; Soares da Costa, T. P.; Bayat, S.; Holien, J. K.; Perugini, M. A.; Abbott, B. M., Synthesis and structure-activity relationship studies of 2,4-thiazolidinediones and analogous heterocycles as inhibitors of dihydrodipicolinate synthase. *Bioorganic & Medicinal Chemistry* **2021,** *52*, 1-9.

6. Das, S.; Das, U.; Varela-Ramírez, A.; Lema, C.; Aguilera, R. J.; Balzarini, J.; De Clercq, E.; Dimmock, S. G.; Gorecki, D. K. J.; Dimmock, J. R., Bis[3,5-bis(benzylidene)-4-oxo-1-piperidinyl]amides: A novel class of potent cytotoxins. *ChemMedChem* **2011,** *6* (10), 1892-1899.

7. Guanti, G.; Riva, R., Synthesis of chiral non-racemic azetidines by lipase-catalysed acetylations and their transformation into amino alcohols: precursors of chiral catalysts. *Tetrahedron: Asymmetry* **2001,** *12* (4), 605-618.

8. Dobson, R. C. J.; Gerrard, J. A.; Pearce, F. G., Dihydrodipicolinate synthase is not inhibited by its substrate, (*S*)-aspartate beta-semialdehyde. *Biochemistry Journal* **2004,** *377* (Pt 3), 757-762.

9. Soares da Costa, T. P.; Hall, C. J.; Panjikar, S.; Wylie, J. A.; Christoff, R. M.; Bayat, S.; Hulett, M. D.; Abbott, B. M.; Gendall, A. R.; Perugini, M. A., Towards novel herbicide modes of action by inhibiting lysine biosynthesis in plants. *eLife* **2021,** *10*, e69444