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Supporting Information

Induced Production, Synthesis, and Immunomodulatory Action of Clostrisulfone, a Diarylsulfone from *Clostridium acetobutylicum*

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Supplementary references

General analytical procedures

All 1D (^{1}H, ^{13}C, DEPT) and 2D NMR (^{1}H-^{1}H COSY, HSQC, NOESY, HMBC) have been recorded in deuterated solvents on a Bruker AVANCE II 300, AVANCE III 500 or 600 MHz instrument equipped with a Bruker Cryo Platform. The chemical shifts are reported in ppm relative to the solvent residual peak (^{1}H: δ ($CHCl_3$) 7.26 ppm, δ (DMSO) 2.50 ppm, ^{13}C: δ ($CHCl_3$) 77.16 ppm, δ (DMSO) 39.52 ppm). Following abbreviations are used for multiplicities of resonance signals: s, singlet, d, doublet, t, triplet, q, quartet, qt, quintet, br, broad. Spectra were normalized to the residual solvent signals. Preparative HPLC purification for synthetic compounds was achieved by using a Gilson Abimed device with Binary Pump 321 and DAD 156 (column: Phenomenex Luna C18, 10 µm, 250 × 21.2 mm, eluent: water, acetonitrile (10−100%)). The analytical reversed-phase HPLC analysis of the ethyl acetate extracts from *C. acetobutylicum* was carried out on a Shimadzu HPLC system using a Nucleosil 100-5C18 column (125 × 4.6 mm) at a flow rate of 1 mL min^{-1} with an gradient elution (MeCN/H_2O containing 0.1% (*v*/*v*) trifluoroacetic acid) 10/90 for 5 min, going up to 99.5/0.5 in 25 min, and kept for 1 min (Figure 1; the elution time of **1** is 39.4 min). LC-MS measurements were performed using an Exactive or Q Exactive Orbitrap High Performance Benchtop LC-MS with an electrospray ion source and an Accela HPLC system (Thermo Fisher Scientific, Bremen). HPLC conditions: C18 column (Betasil C18 3 µm 150 × 2.1 mm) and gradient elution (MeCN/0.1 % (*v/v*) HCOOH (H_2O) 5/95 for 1 min, going up to 98/2 in 15 min, then 98/2 for another 3 min; flow rate 0.2 mL min^{-1}; injection volume: 3 µL) (Figure 2).

General synthesis procedures

All reagents were obtained from commercial suppliers (Sigma Aldrich, TCI, Alfa Aesar, etc.) and used without further purification unless otherwise explained. Reactions were carried out under inert gas (Argon) by using the Schlenk technique in dried solvents. Acetonitrile was dried with phosphorous pentoxide, distilled and stored over molecular sieve (4 Å) under argon. Methanol, chloroform, dichloromethane, ethyl acetate and were distilled prior to use. Open column chromatographic separations were executed on silica gel (Kieselgel 60, 0.015–0.04 mm, Merck KGaA). Reaction progress was monitored by thin layer chromatography (TLC) (silica gel on aluminum sheets 20 × 20 cm with fluorescent dye 254 nm, Merck KGaA), GC-MS or HPLC-MS.

Cultivation of *C. acetobutylicum* for purification of clostrisulfone (1)

C. acetobutylicum DSM792 was obtained from DSMZ GmbH (Braunschweig). *C. acetobutylicum* was cultured in 500 mL by Biostat Q fermenters (B. Braun Biotech International) using P2 medium[1] (glucose 20 g L^{-1}; CH_3COONH_4 3.5 g L^{-1}; KH_2PO_4 0.5 g L^{-1}; K_2HPO_4 0.5 g L^{-1}; $FeSO_4 \cdot 7\,H_2O$ 0.01 g L^{-1}; $MgSO_4 \cdot 7\,H_2O$ 0.02 g L^{-1}; *p*-aminobenzoic acid 1 mg L^{-1}; biotin 0.01 mg L^{-1}, resazurin 1 mg L^{-1}; pH = 6.0.) with or without 1 mM L-cysteine. Anaerobic conditions were maintained by sparging

fermenters with a nitrogen gas (0.1 mL min^{-1}) during fermentation. Fermentation cultures were incubated at 37 °C with stirring at 150 rpm and pH 6.0 was maintained by automatic addition of 10% aq. NaOH solution (*w*/*v*).

Extraction and isolation of clostrisulfone (1)

C. acetobutylicum (0.5 L) was cultured in P2 medium with 1 mM L-cysteine at 37 °C for 72 h. The culture broth was extracted three times with ethyl acetate (0.5 L) and then the extracts were directly subjected to reversed-phase HPLC (Kromasil 100-C18, 20 × 250 mm, particle size 5 µm, Jasco) using a gradient program; solvent A (water), solvent B (acetonitrile), 10% B for 5 min, to 100% B in 30 min, and kept 100% B for 2.5 min, at flow rate 13 mL min^{-1} to yield 0.2 mg of clostrisulfone (**1**). HRMS (ESI^+) calcd. for $C_{28}H_{39}O_4S$ $[M+H]^+$: *m/z* 471.2564; found: 471.2559.

Preparation of 2,2,5,7,8-pentamethylchromane (3)

2,2,5,7,8-Pentamethylchromane (**3**) was synthesized in analogy to a previously described method.[4] To a stirred solution of 2,3,5-trimethylphenol (**4**) (13.62 g, 0.1 mol, 1 eq.) and zinc chloride (1.64 g, 12 mmol, 0.12 eq.) in glacial acetic acid (60 mL) were isoprene (6.81 g, 0.1 mol, 1 eq.) added and stirred over night at room temperature. The solution was warmed up to 80 °C for 7 h. After allowing to cool to room temperature water (300 mL) were added and the aqueous solution was extracted with cyclohexane (3 × 200 mL). The combined organic phases were washed with Claisen's alkali (3 × 200 mL, preparation: 350 g KOH were dissolved in 250 mL water and after cooling to room temperature diluted to 1 L with MeOH), water (3 × 200 mL) and brine (2 × 100 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum. The residue was distilled under vacuum affording a yellow oil (7.7 g), which contains the very nonpolar title compound as main part. For further purification the fraction was dissolved in cyclohexane and after gel filtration (SiO_2, 0.015 – 0.04 mm) evaporated under vacuum. This resulted in a colorless oil (3.08 g), which partially was purified by preparative column chromatography. A typical injection contained 185 mg (200 µL) of the oil and afforded 111 mg (9% extrapolated yield) of compound **3**.

2,2,5,7,8-Pentamethylchromane (3). ^{1}H NMR (500 MHz; $CDCl_3$): δ = 1.32 (s, 6H, 2,2-C**H**$_3$), 1.80 (t, 2H, $^3J_{HH}$ = 6.8 Hz, 3-C**H**$_2$), 2.08 (s, 3H, 8-C**H**$_3$), 2.17 (s, 3H, 5-C**H**$_3$), 2.21 (s, 3H, 7-C**H**$_3$), 2.61 (t, 2H, $^3J_{HH}$ = 6.8 Hz, 4-C**H**$_2$), 6.56 (s, 1H, 6-C**H**) ppm. ^{13}C NMR (125.8 MHz; $CDCl_3$): δ = 11.5 (1C, 8-**C**H$_3$), 19.0 (1C, 5-**C**H$_3$), 19.9 (1C, 7-**C**H$_3$), 20.6 (1C, 4-**C**H$_2$), 27.0 (2C, 2,2-**C**H$_3$), 32.9 (1C, 3-**C**H$_2$), 73.2 (1C, 2-**C**), 116.7 (1C, 4a-**C**), 122.1 (1C, 7-**C**), 122.4 (1C, 6-**C**H), 133.5 (1C, 5-**C**), 134.8 (1C, 8-**C**), 151.8 (1C, 8a-**C**) ppm. HRMS (ESI^+) calcd. for $C_{14}H_{21}O$: 205.1587; found: 205.1582.

Synthesis of 6,6'-sulfonylbis(2,2,5,7,8-pentamethylchromane) (1)

Commercially available 2,2,5,7,8-pentamethylchromane-6-sulfonyl chloride (30.3 mg, 0.1 mmol, 1 eq.) and 2,2,5,7,8-pentamethylchromane (**3**) (20.4 mg, 0.1 mmol, 1 eq.) were solved in dry acetonitrile (0.4 mL) and triethylamine (20.2 mg, 0.2 mmol, 27.8 µL, 2 eq.) was added. Then, tin(IV) chloride (52.1 mg, 0.2 mmol, 23.4 µL, 2 eq.) was added slowly, and the solution was stirred over night at room temperature. The reaction mixture was poured to hydrochloric acid (10 mL, 0.3 M) and extracted with ethyl acetate (3 × 10 mL). The combined organic phases were washed with water (3 × 10 mL) and brine (2 × 10 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated under the reduced pressure. The residue was purified by recrystallization with MeOH, which afforded the title compound as white crystals (33 mg, 0.07 mmol, 70% yield). The filtrate was separated by preparative column chromatography for an entire recover of the remaining educt (**1**) (6.1 mg, 0.03 mmol).

6,6'-Sulfonylbis(2,2,5,7,8-pentamethylchromane) (1). ^{1}H NMR (500 MHz; DMSO-d_6): δ = 1.27 (s, 6H, 2,2-C**H**$_3$), 1.79 (t, 2H, $^3J_{HH}$ = 6.8 Hz, 3-C**H**$_2$), 2.00 (s, 3H, 8-C**H**$_3$), 2.18 (s, 3H, 7-C**H**$_3$), 2.21 (s, 3H, 5-C**H**$_3$), 2.56 (t, 2H, $^3J_{HH}$ = 6.8 Hz, 4-C**H**$_2$) ppm. ^{13}C NMR (125.8 MHz; DMSO-d6): δ = 11.5 (1C, 8-**C**H$_3$), 15.4 (1C, 5-**C**H$_3$), 16.4 (1C, 7-**C**H$_3$), 20.36 (1C, 4-**C**H$_2$), 26.2 (2C, 2,2-**C**H$_3$), 31.8 (1C, 3-**C**H$_2$), 73.8 (1C, 2-**C**), 118.4 (1C, 4a-**C**), 123.4 (1C, 7-**C**), 134.0 (1C, 8-**C**), 134.1 (1C, 5-**C**), 134.6 (1C, 6-**C**), 153.4 (1C, 8a-**C**) ppm. HRMS (ESI$^+$) calcd. for $C_{28}H_{39}O_4S$: 471.2564; found: 471.2554.

Biomimetic synthesis

For the biomimetic approach, oven-dried glass vials, caps and magnetic stir bars were used. 2,2,5,7,8-pentamethylchroman-6-ol (**5**; 11 mg; 50 µmol; 1 eq.), sodium sulfite (3 mg; 25 µmol; 0.5 eq.) and radical starter (AIBN or DBP, 5 mol-%) were added, mixed and dried under vacuum. After adding the pre-dried solvent (acetonitrile, benzene or carbon tetrachloride; 1 mL) the glass vial was heated to 50 °C. Samples for HPLC-HRMS were taken with a syringe under inert conditions. After 24 hours a product peak of clostrisulfone (**1**) was observed. Using 2,2,5,7,8-pentamethylchromane (**3**) as educt showed no conversion at all, even after changing the sulfur source to gaseous sulfur dioxide or 1,4-diazabicyclo[2.2.2]octane bis(sulfur dioxide) adduct (DABSO).

Production of clostrisulfone with different sulfur sources

The production of clostrisulfone by *C. acetobutylicum* was determined with different concentrations of L-homocysteine, L-methionine, sodium sulfide, sodium sulfite, sodium sulfate, dithiothreitol, and 2-mercaptoethanol. Concentrations of the sulfur sources during cultivation were selected around the MIC as follows: L-homocysteine 0.1 mM and 1 mM, L-methionine 5 mM and 50 mM, sodium sulfide 3 mM, sodium sulfite 1 mM and 5 mM, sodium sulfate 10 mM and 100 mM, dithiothreitol 0.05 mM

and 0.2 mM, 2-mercaptoethanol 0.2 mM and 1 mM. External sulfur sources were added to 100 ml P2 medium with *C. acetobutylicum* in the middle of the exponential growth phase. After 24 h cell suspensions were extracted with ethyl acetate and analyzed for clostrisulfone production as described. With none of the sulfur containing additives clostrisulfone production could be detected.

Fermentation for sulfur dioxide exposure

For each fermentation experiment 100 mL cell suspension with exponentially growing *C. acetobutylicum* DSM 792 (OD_{600nm} = 1) were used to inoculate 1 L of fermentation medium. All preculture steps were performed in anaerobic serum flasks in the anaerobic chamber. For 1 L cultures the *sixfors* bioreactor system by *Infors HT* was used. pH was controlled at 6.0 with 10% aq. NaOH, temperature at 37 °C and agitation was set at 200 rpm. Anaerobic conditions were ensured by overlaying the fermenter headspace with N_2 with a constant gassing rate of 0.2 L min^{-1} except fermentations with gaseous SO_2 where no gassing was used at all. Fermentation periods ranged from 1 to 3 days.

Serum flask cultivation for sulfur dioxide exposure

For cultivation experiments in serum flasks, precultures of all three strains were prepared analogously to fermentation cultures in the anaerobic chamber. The second preculture with OD_{600} of 1 was used to inoculate 10 mL P2 medium with 20 g L^{-1} glucose and corresponding additives either DABSO or DMSO. Cultivation was performed in a 37 °C incubation room with shaken at 200 rpm for 9 to 24 h. For OD measurements of samples were taken out of the serum flasks with syringe and needle in regular intervals.

Evaluation of clostrisulfone as product of a bacterial sulfur dioxide detoxification pathway

To test the hypothesis that clostrisulfone is produced as a result of a sulfur dioxide detoxification pathway, we evaluated cell stress after sulfur dioxide addition. Therefore, gaseous SO_2 was injected into the culture medium in different concentrations and the influence on cell growth, and clostrisulfone formation was monitored. However, precise addition of SO_2 proved to be difficult in this experimental setup because of the unknown amounts of SO_2 that remained in the gas phase in the headspace of the bioreactor and where therefore no longer available. Thus, a commercially available SO_2 donor, DABCO-bis(sulfur dioxide) (DABSO) was used. In a first attempt the addition of 100 µM DABSO resulted in a slight growth inhibition. To prove the potential for SO_2 detoxification the putative clostrisulfone precursor, pentamethylchromane (PMC) **3** was added to the bacterial culture in concentrations of 2 mM and 0.2 mM (solved in DMSO) with 500 µM DABSO. However, no bacterial growth could be detected neither after sole addition of DABSO nor after complementation with PMC.

Measuring of sulfur dioxide tolerance

SO_2 tolerance experiments were performed in shaken serum flasks. DABSO was added in concentrations between 0 and 110 µM. It could be shown that the concentration of DABSO correlates directly with the length of the adaption phase at the beginning of cultivation. This initial lag phase was always followed by exponential growth with growth rates similar to the non-treated culture. In parallel the influence of the corresponding nitrogen base DABCO **6** on cell growth was analyzed. Concentrations between 0 and 250 µM DABCO had no measurable influence on growth. Reduction of cell growth after addition of DABSO must therefore be the effect of the released SO_2.

For PMC feeding experiments a DABSO concentration of 90 µM was chosen where cell division is still detectable but obvious growth inhibition occurs. PMC as potential clostrisulfone precursor could subsequently support the bacterial sulfur dioxide detoxification and recover growth towards the untreated culture. However, addition of PMC in concentrations between 20 µM and 2 mM resulted more in the reduction of cell density. In concentrations of 200 µM and higher the addition of PMC resulted in a formation of a turbid suspension. Thereupon cell growth was monitored microscopically but the same reduction of cell concentration could be observed.

Effect on growth

As clostrisulfone could be involved in a bacterial SO_2 detoxification pathway it was hypothesized that clostrisulfone producing strains possess a growth advantage over non-producing strains in sulfur dioxide rich environment. Therefore 2 closely related strains, *Clostridium beijerinckii* DSM 51 and 53, where chosen and screened for clostrisulfone production. For the two *C. beijerinckii* strains no clostrisulfone production could be observed. Growth comparison coupled with sulfur dioxide tolerance testing between the strains should therefore being repeated in fermentation scale in bioreactors. The minimal inhibitory concentration (MIC) of DABSO was estimated and compared for all three strains. However, both *beijerinckii* strains showed higher MIC values than *C. acetobutylicum*. For *C. beijerinckii* DSM 51 slime production could be observed. This could be part of a bacterial protection method in sulfur dioxide rich environments which also explains the high tolerance of the strain. Inhibitory effects of sulfur dioxide depend a lot on the surrounding pH and the SO_2 donor that is used (e.g. metabisulfite or DABSO). Therefore results for sulfur dioxide tolerance of different microorganisms are hard to compare. For *C. acetobutylicum* growth could still be observed with SO_2 concentrations lower than 360 µM (180 µM DABSO).

Estimation of MIC values

For the estimation of the minimal inhibitory DABSO concentration 1 mL of exponentially growing cells in P2 medium with a starting OD_{600nm} of 0.05 were used. Defined volumes of sterile DABSO stock solution were added with final concentrations between 0 and 1 mM. Cell suspensions with DABSO were incubated standing under anaerobic conditions for 72 h at 37 °C. Cell growth was

detected photometrically. MIC values were defined as the lowest concentrations of DABSO where no cell growth could be detected after 72 h incubation. MIC measurements were performed in duplicates.

Analytical measurements

Cell growth was detected by measuring the optical density at 600 nm or by counting the cells in a Neubauer-improved counting chamber. For clostrisulfone (**1**) detection 10 mL samples were taken out of the fermenter, extracted with ethyl acetate, dried, resolved in a mixture of methanol and acetonitrile and analyzed via LC-MS measurement as described above.

Cell culture: murine RAW264.7 macrophages

RAW264.7 macrophages (ATCC, Manassas, VA) were cultivated in DMEM (Dulbecco's Modified Eagle Medium) high glucose (4.5 g L^{-1}) medium supplemented with 10% (*v*/*v*) FBS (fetal bovine serum) and 0.1 mg mL^{-1} penicillin/streptomycin/L-glutamine mixture. The cells were detached by scraping and excess of cells was aspirated. The remaining cells were grown in a mixture of 70% (*v*/*v*) fresh high glucose DMEM and 30% (*v*/*v*) conditioned DMEM, which was obtained by centrifugation from the previous passage of culture.

Nitric oxide detection by Griess assay

RAW264.7 macrophages were seeded in supplemented high glucose DMEM medium containing serum for 24 h. Attached cells were washed twice and incubated with test compounds in serum free DMEM medium for 4 h. Next, a combined incubation of the test compound and 100 ng mL^{-1} LPS (lipopolysaccharide) was performed for 20 h. After 24 h in total, cell culture supernatants were removed and centrifuged; aliquots of cell-free supernatants were transferred to a 96-well plate. After mixing with water and Griess reagent and incubation in the dark for 30 min, the concentration of nitrite in the supernatant was measured at 544 nm with a FLUOstar omega microplate reader (BMG Labtech, Ortenberg, Germany). Analyses were performed using MARS data analysis software version 2.41 (BMG Labtech, Ortenberg, Germany). The calculation was referred to a standard dilution row of $NaNO_2$.

Table S1. ^{1}H und ^{13}C NMR data for clostrisulfone (**1**) and Pmc chloride.

	Clostrisulfone (**1**)*		Pmc chloride (**2**)**	
Position	^{1}H (J Hz)	^{13}C	^{1}H (J Hz)	^{13}C
2/2′		74.0		74.9
2,2-Me/2′,2′-Me	1.27 (s)	26.3	1.34 (s)	26.7
3/3′	1.78 (t 6.9)	31.8	1.85 (t 6.8)	32.4
4/4′	2.55 (t 6.9)	20.4	2.68 (t 6.8)	21.2
4a/4a′		118.5		118.7
5/5′		134.23		135.6
5-Me/5′-Me	2.21 (s)	15.6	2.63 (s)	17.5
6/6′		134.6		137.3
7/7′		134.17		135.6
7-Me/7′-Me	2.16 (s)	16.5	2.61 (s)	18.5
8/8′		123.5		125.3
8-Me/8′-Me	2.00 (s)	11.7	2.14 (s)	12.0
8a/8a′		153.5		156.7

*: in DMSO-d_6 (600 MHz), **: 200 MHz in $CDCl_3$

Table S2. Signal intensity for clostrisulfone in LC-MS measurements after cultivation in serum flasks. Clostrisulfone production could not be detected for *C. beijerinckii* strains. Measured intensity deviated a lot for *C. acetobutylicum*. EIC (extracted ion chromatogram) intensity lower than 1E+06 was defined as noise. DABCO (1,4-diazabicyclo[2.2.2]octane) was used as a negative control of DABSO.

Strain	**Cultivation time**	**Additive**	**EIC intensity**
C. acetobutylicum DSM 792	1 day	none	noise
	2 days	none	noise
	3 days	none	5.00E+06
	3 days	1 mM cysteine	noise
	1 day	120 µM DABSO	0
	3 days	180 µM DABSO	noise
	1 day	200 µM DABSO	0
	1 day	225 µM DABSO	0
	1 day	250 µM DABSO	0
	2 days	70 µM DABCO	6.00E+06
	2 days	90 µM DABCO	noise
	2 days	110 µM DABCO	0
	2 days	130 µM DABCO	0
	2 days	150 µM DABCO	0
	2 days	250 µM DABCO	0
C. beijerinckii DSM 51	1 day	none	noise
	3 days	none	noise
	1 day	40 µM DABSO	0
	1 day	50 µM DABSO	0
	1 day	60 µM DABSO	0
	1 day	120 µM DABSO	0
	3 days	570 µM DABSO	noise
C. beijerinckii DSM 53	1 day	none	noise
	3 days	none	noise
	1 day	150 µM DABSO	noise
	1 day	200 µM DABSO	noise
	1 day	250 µM DABSO	0
	1 day	360 µM DABSO	0
	3 days	680 µM DABSO	noise

Table S3. Signal intensity for clostrisulfone (**1**) in LC-MS measurements for cultivation in bioreactors. Clostrisulfone concentration varied through the process. Only maximum intensities are shown with corresponding cultivation time points.

Strain	Cultivation time	Additive	Max EIC intensity
C. acetobutylicum DSM 792	4 h	none	0.00E+00
	14.5 h	none	2.00E+07
	4 h	1 mM cysteine	2.00E+06
	14.5 h	30 µM SO_2	3.00E+07
	24 h	30 µM SO_2	3.00E+08
	14.5 h	100 µM labeled SO_2	2.00E+07
	14.5 h	300 µM SO_2	5.00E+07
	17 h	30 µM Na_2SO_3	1.00E+06
	24 h	100 µM DABSO	7.00E+06
	24 h	100 µM DABSO (autoclaved)	2.00E+07

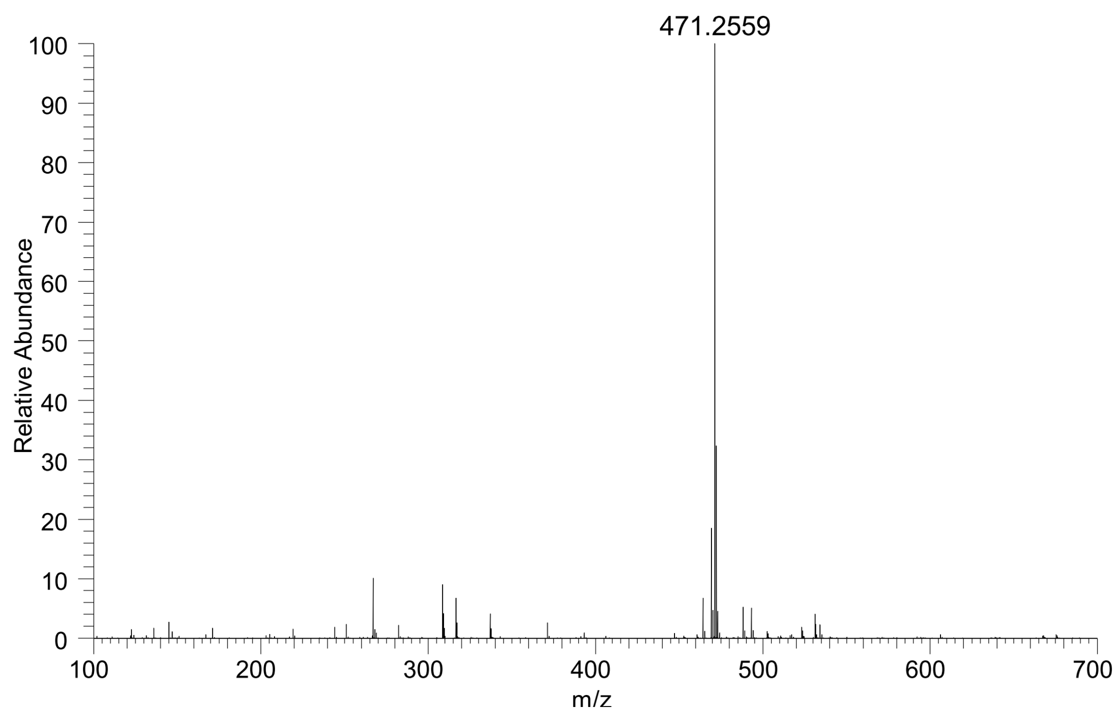

Figure S1. HR-ESIMS spectrum of **1** (Positive ion mode).

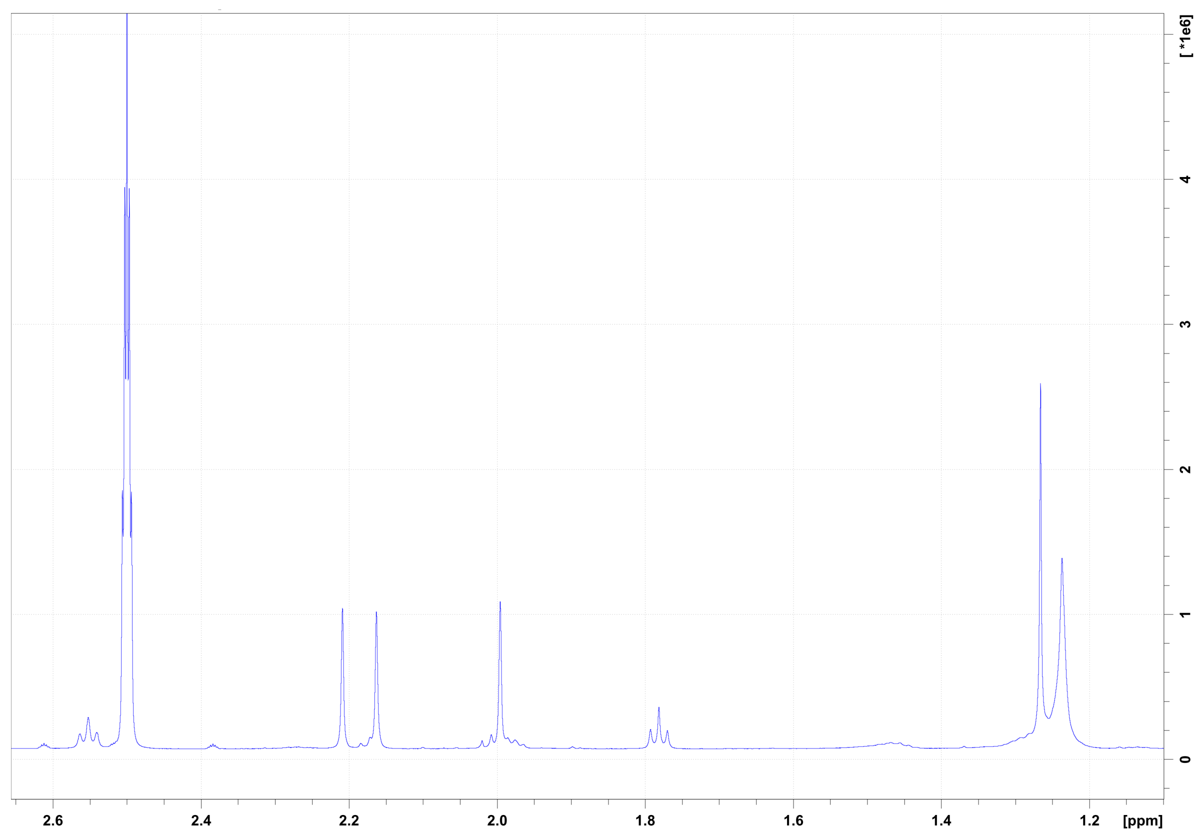

Figure S2. ^{1}H NMR spectrum of **1** in DMSO-d_6.

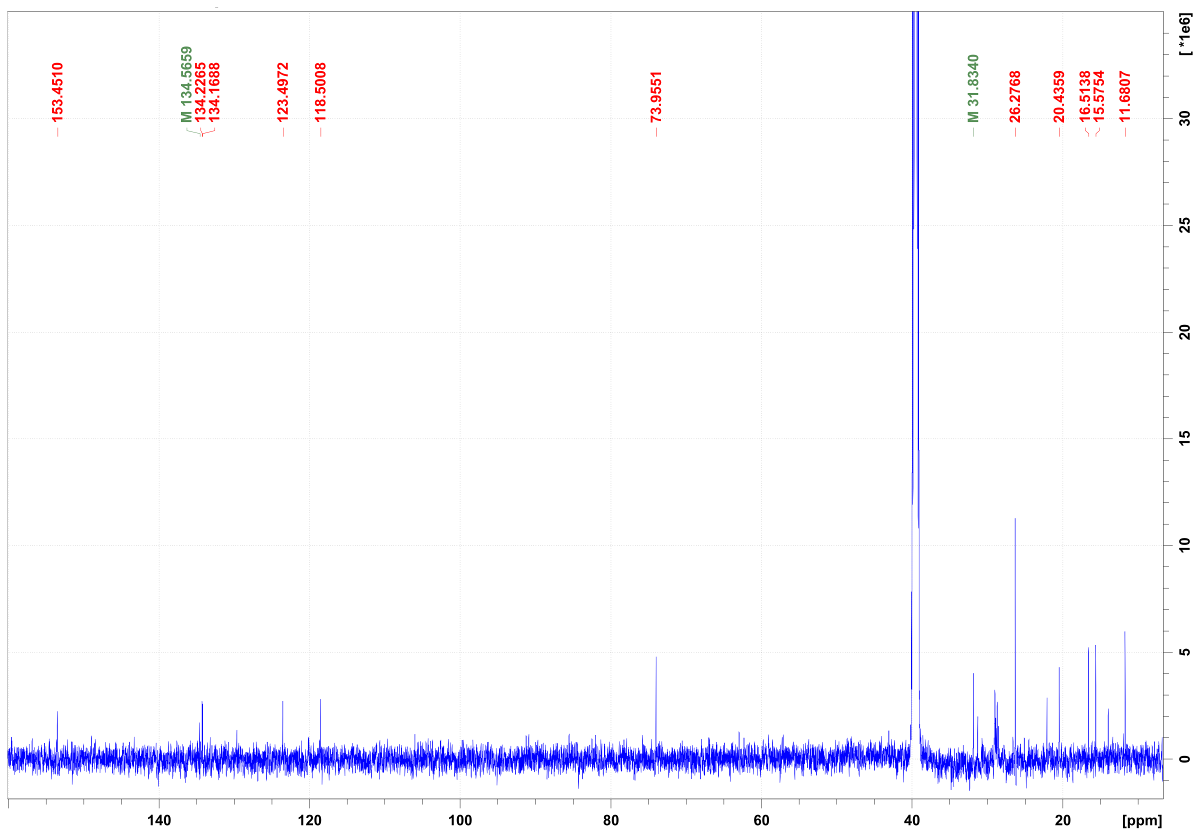

Figure S3. ^{13}C NMR spectrum of **1** in DMSO-d_6.

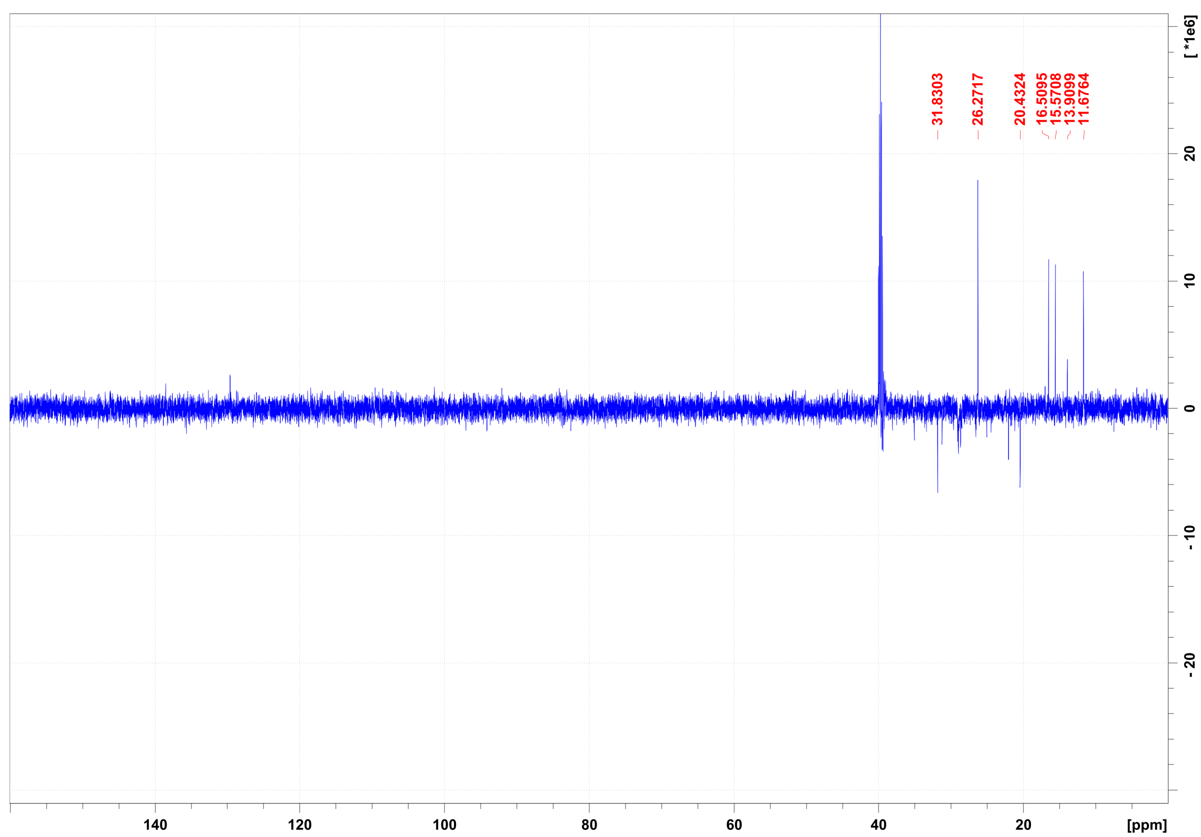

Figure S4. DEPT135 spectrum of **1** in DMSO-d_6.

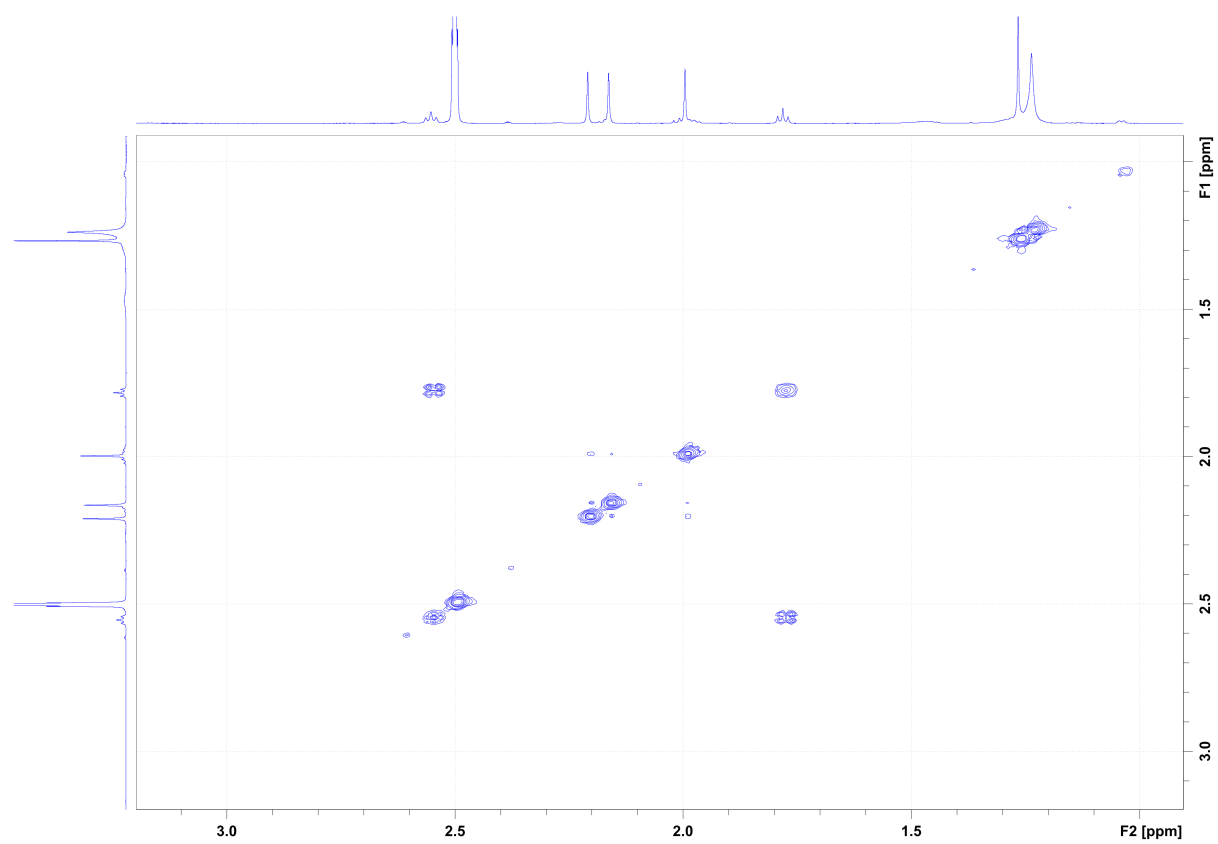

Figure S5. ^{1}H-^{1}H COSY spectrum of **1** in DMSO-d_6.

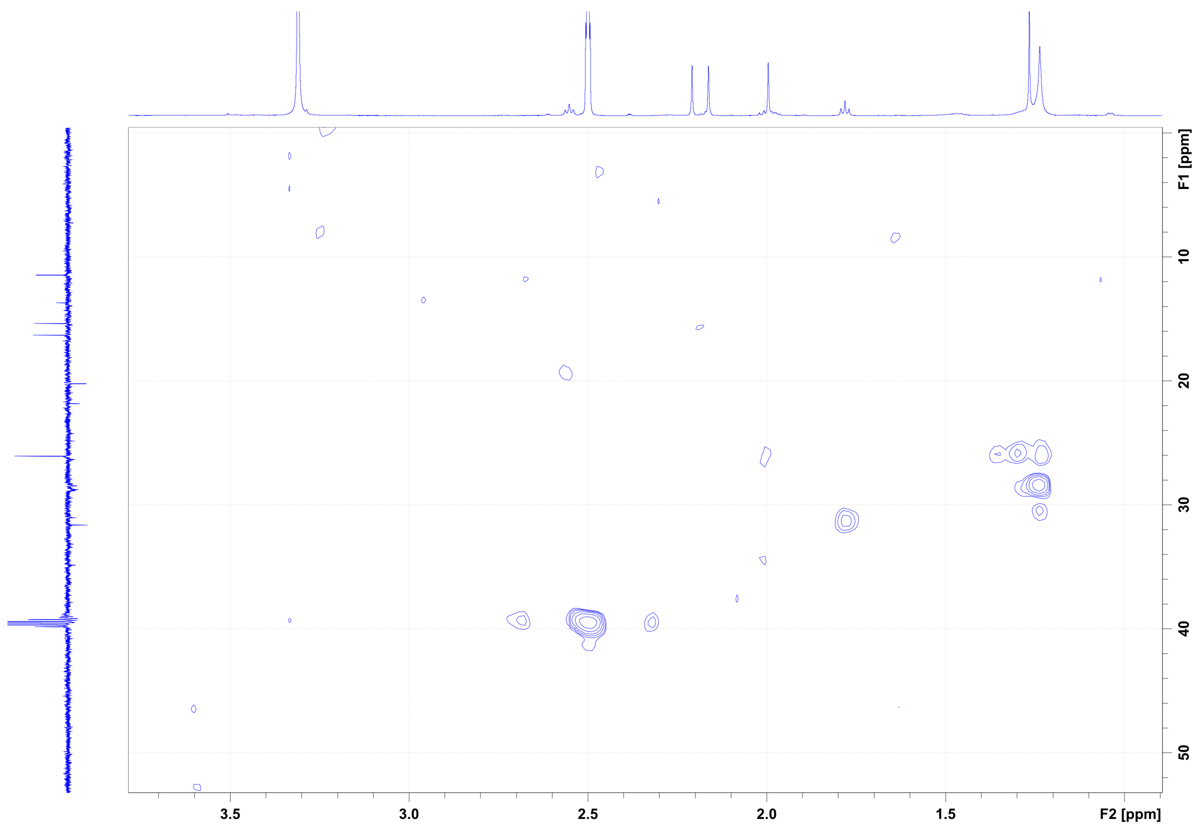

Figure S6. HSQC spectrum of **1** in DMSO-d_6.

Figure S7. HMBC spectrum of **1** in DMSO-d_6.

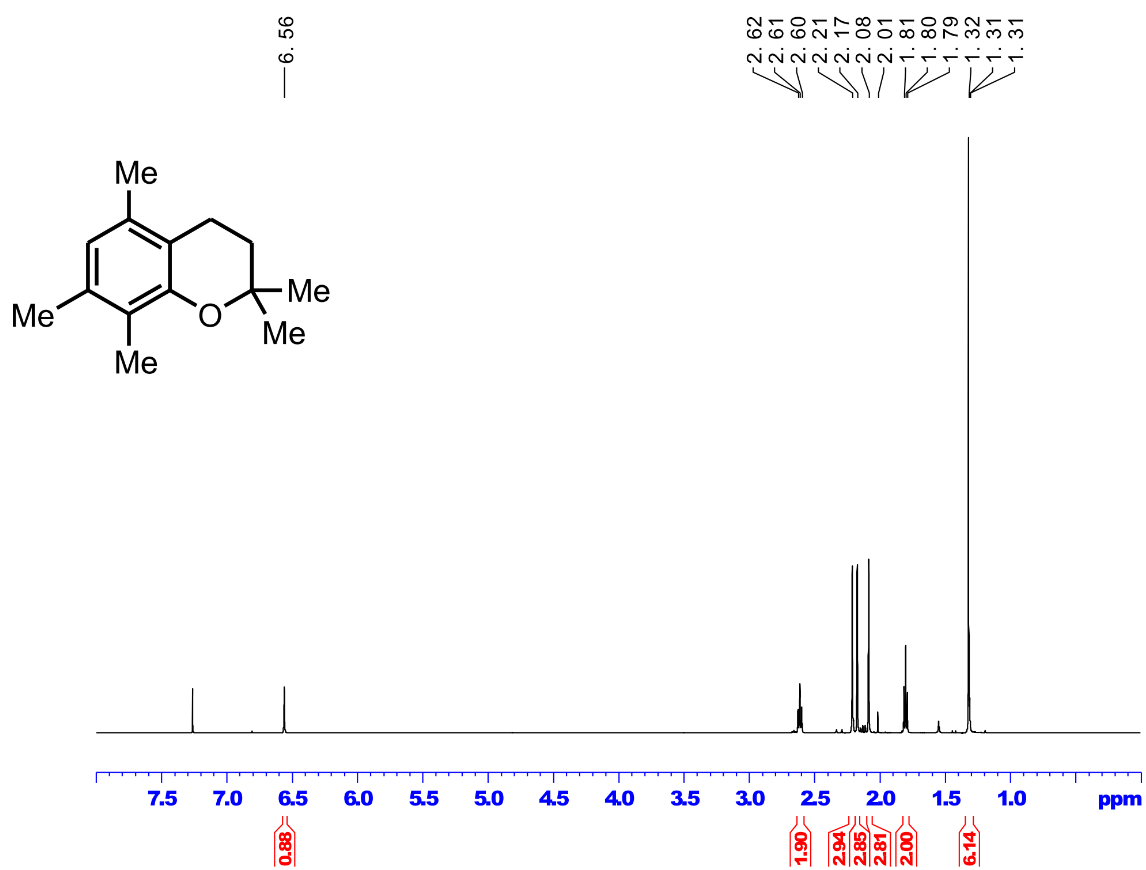

Figure S8. ^{1}H NMR spectrum of 2,2,5,7,8-pentamethylchromane (**3**) in $CDCl_3$.

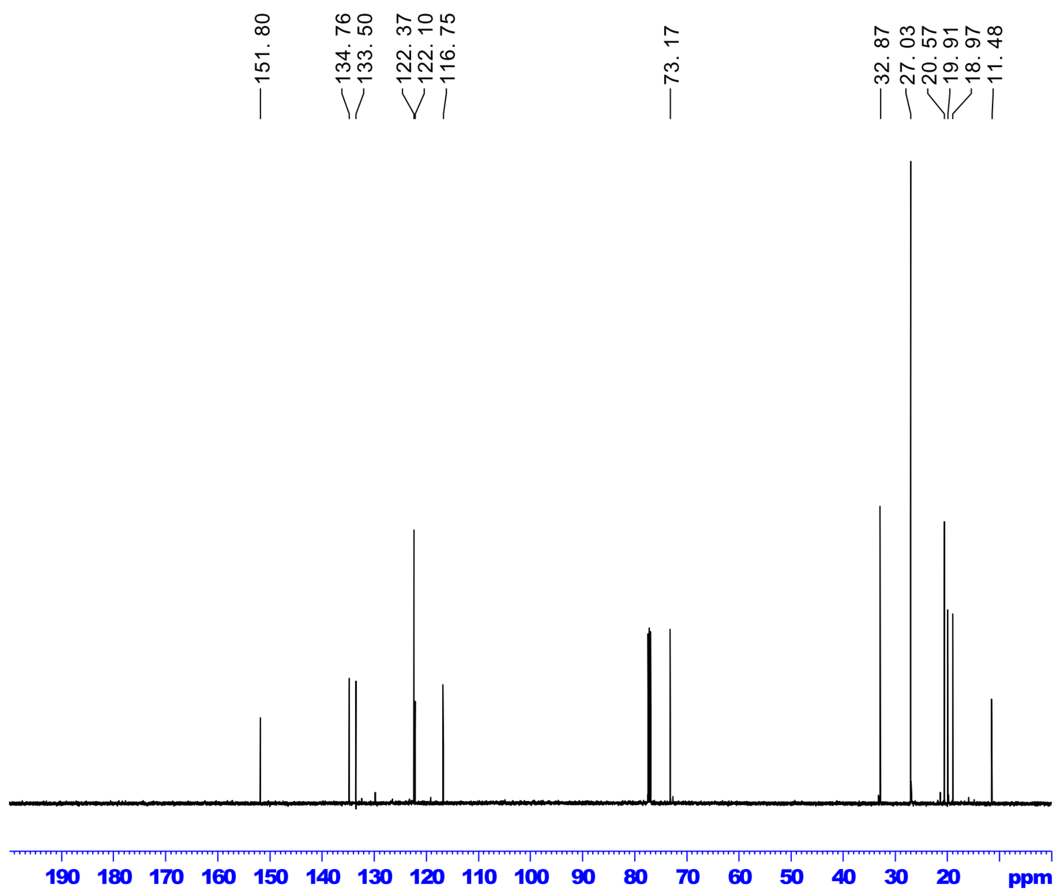

Figure S9. ^{13}C NMR spectrum of 2,2,5,7,8-pentamethylchromane (**3**) in $CDCl_3$.

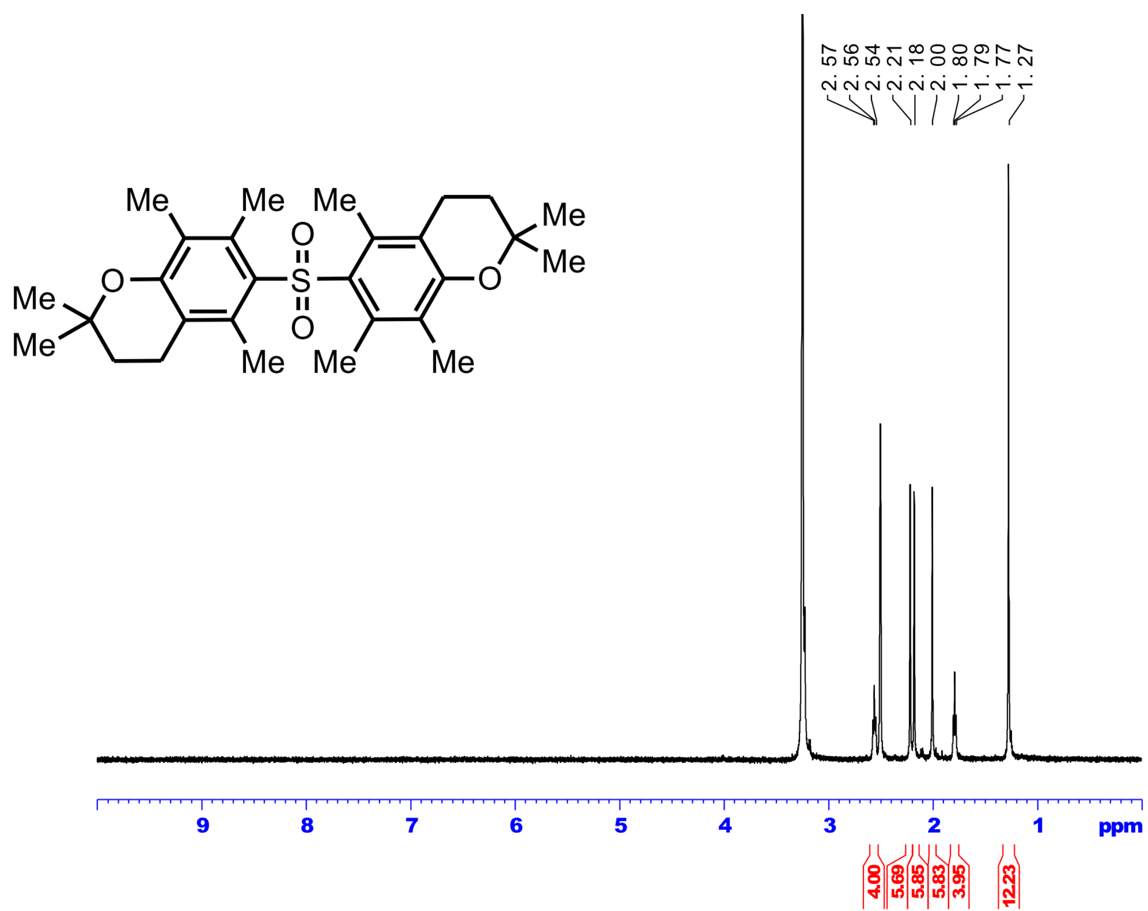

Figure S10. ^{1}H NMR spectrum of synthetic clostrisulfone (**1**) in DMSO-d_6.

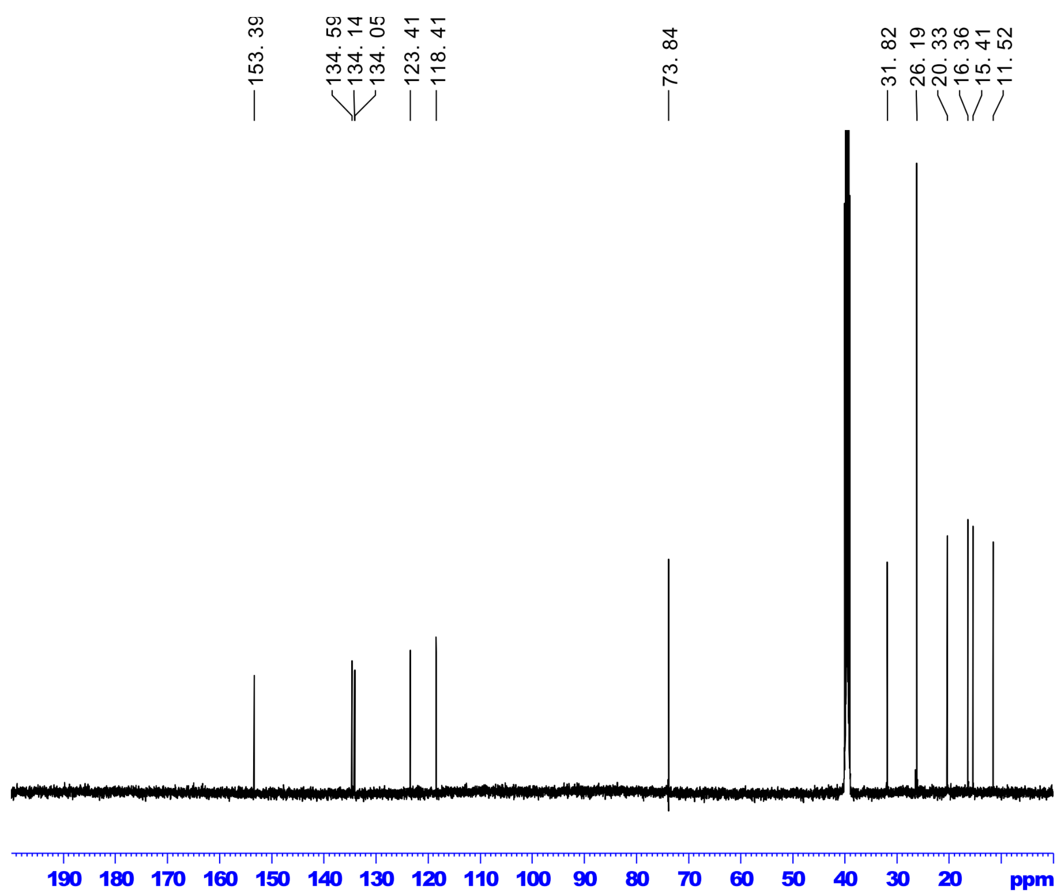

Figure S11. ^{13}C NMR spectrum of synthetic clostrisulfone (**1**) in DMSO-d_6.

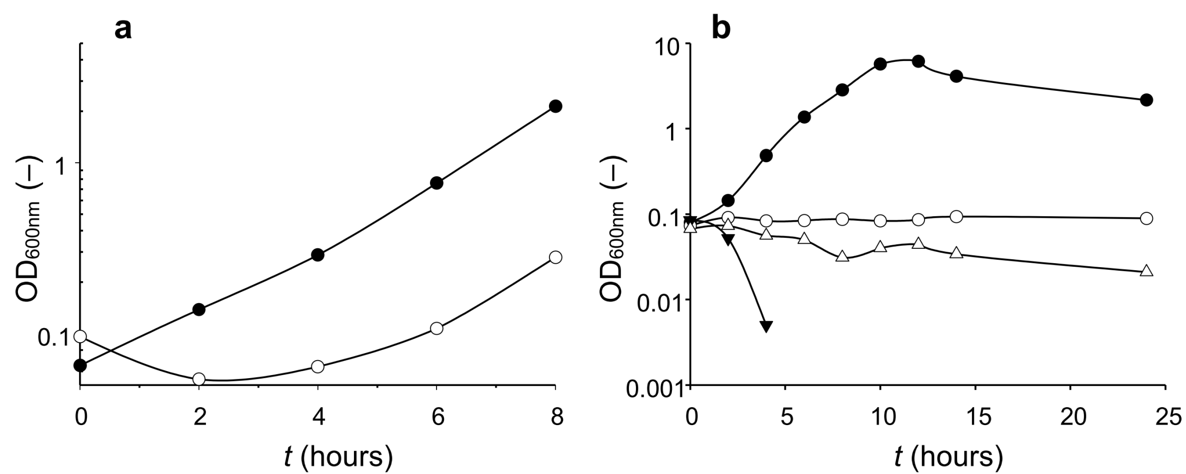

Figure S12. The effect of DABSO and PMC against growth of *C. acetobutylicum*. Growth curve of *C. acetobutylicum* without (filled circle), with addition of 0.1 mM (**a**) and 0.5 mM (**b**) DABSO (open circle). To *C. acetobutylicum* culture with 0.5 mM DABSO, 0.2 mM (filled reverse triangle) and 2 mM (open triangle) PMC were added. No growth improvement could be detected after addition of the preliminary precursor.

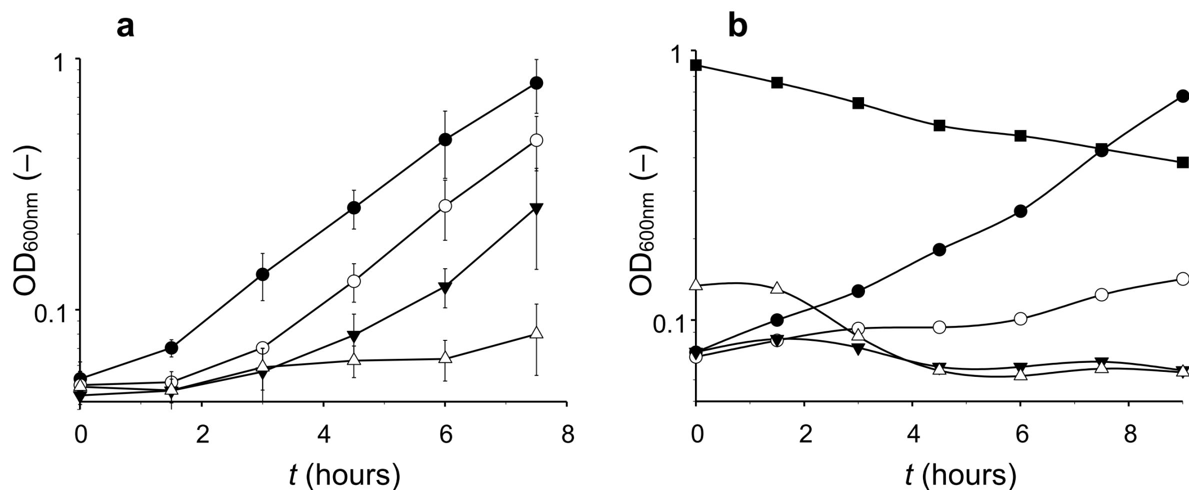

Figure S13. Dose-dependent growth of *C. acetobutylicum* against DABSO and PMC. **a**: Growth curve of *C. acetobutylicum* without (filled circle) and with addition of 70 µM (open circle), 90 µM (filled reverse triangle), and 110 µM (open circle) DABSO. Concentration of DABSO directly correlates with the length of the initial lag phase. **b**: Growth of *C. acetobutylicum* without addition (filled circle), with addition of 90 µM DABSO (open circle) and 90 µM DABSO with 20 µM (filled reverse circle), 200 µM (open circle) and 2 mM (filled square) PMC. Addition of PMC results in a decrease of cell density. Higher starting values of optical density are reduced to the insoluble PMC in suspension.

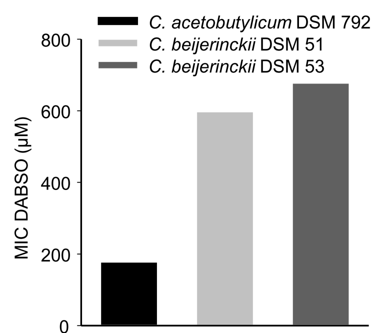

Figure S14. Minimal inhibitory concentrations (MICs) of DABSO against the strains *C. acetobutylicum*, *C. beijerinckii* DSM 51 and 53. *C. beijerinckii* strains possess a far higher tolerance for SO_2.

Supplementary references

[1] F. Monot, J. R. Martin, H. Petitdemange, R. Gay, *Appl. Environ. Microbiol.* **1982**, *44*, 1318-1324.

[2] R. Ramage, J. Green, A. J. Blake, *Tetrahedron* **1991**, *47*, 6353-6370.