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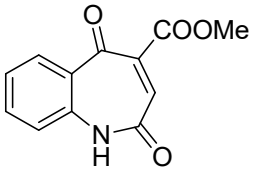
1. General experimental details

Unless otherwise noted, all chemicals were purchased from commercial sources and used without further purification. Column chromatography was carried out using silica 60 Å, Davisil, purchased from Fisher Chemicals. Reactions were monitored by thin-layer chromatography (TLC) using Macherey-Nagel's pre-

coated TLC sheets POLYGRAM SIL G/UV254, which were visualized under UV light (254 nm). HPLC analyses were performed on a Varian system using a Clarity DataApex software, UVIS-220 detector (at 220 nm), Varian ProStar 310 pump (1.0 mL/min flow), and Restec® Phenyl- Hexyl (250 × 4.0, 5 μm) column. ^{1}H and ^{13}C NMR spectra were recorded using Varian INOVA 300 MHz, Varian 400 MR, Varian VNMRS 600 MHz and/or Bruker Avance NEO 400 MHz spectrometers. Chemical shifts (δ) are given in parts per million (ppm). The ^{1}H NMR chemical shift scale is referenced to the TMS internal standard (δ = 0 ppm) or solvent residual peak (δ = 2.50 ppm for DMSO-d_6 and δ = 7.26 ppm for $CDCl_3$). The ^{13}C NMR chemical shift scale is referenced to the solvent residual peak (δ = 39.52 ppm for DMSO-d_6 and δ = 77.16 ppm for $CDCl_3$). Coupling constants (*J*) are given in hertz (Hz). The multiplicity of ^{1}H NMR signals is reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet. High-resolution mass spectra were measured using a Thermo Scientific mass spectrometer with an Orbitrap analyzer and HESI ionization. Retro–Michael reaction of adducts **10a-f** and **10h-m** was observed during the MS analysis and the mass peak of compound **9** (232 for $[M+H]^+$ and 254 for $[M+Na]^+$) was found in samples **10a-f** and **10h-m**. Compound **9** was formed by further reaction of **9** with methanol during analysis and mass peak of compound **10k** (286 for $[M+Na]^+$) was found in samples **9,10a,b,c,e,i,j,k,m**.

2. Synthesis

Synthesis of the starting material



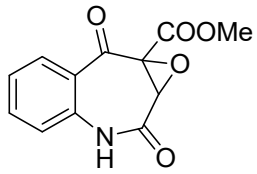
Methyl 2,5-dioxo-2,5-dihydro-1H-benzo[b]azepine-4-carboxylate 9

Bromination
1.161 g (5.210 mmol, 0.3 equiv.) of $Mg(ClO_4)_2$ was added to the suspension of **11** (4.050 g, 17.37 mmol) in ethyl acetate (400 mL) and the mixture was stirred for 10 min. *N*-Bromosuccinimide (3.400 g, 19.10 mmol, 1.1 equiv.) was added in one portion and resulting solution was stirred for 45 min and extracted twice with 200 mL of 5% $NaHCO_3$.Organic phase was dried with Na_2SO_4 and filtered.
Elimination
Triethylamine (2.049 g, 2.816 mL, 20.25 mmol, 1.166 equiv.) was added dropwise to the filtrate and the formation of a cloudy yellow solution was immediately observed. After 1 hour of stirring at rt, the mixture was dried with Na_2SO_4, filtered through a silica plug and washed with ethyl acetate (150 mL). Filtrate was concentrated *in vacuo*, until the crystallization of product was observed. Light yellow crystals of product were filtered off (2.88 g). Th filtrate was further concentrated (approx 100 mL) and another portion of light yellow crystals (0.6 g) was isolated. Combined yield of product **9** is 3.48 g, 87 %.

m.p. 192-194 °C
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.15 (s, 1H), 7.92 (d, *J* = 7.9 Hz, 1H), 7.62 (t, *J* = 7.7 Hz, 1H), 7.35 – 7.22 (m, 3H), 3.92 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 186.49, 164.98, 164.41, 142.17, 136.07, 134.80, 132.96, 130.56, 129.30, 125.27, 120.27, 53.49.
HRMS (ESI) m/z calcd. for $C_{12}H_{10}NO_4$ ([M+H$^+$) 232.06043, found 232.06053.

Epoxidation

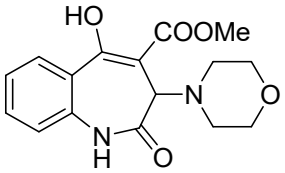


Methyl 2,8-dioxo-2,3,8,8a-tetrahydro-1aH-benzo[b]oxireno[2,3-e]azepine-8a-carboxylate 13

9 (76 mg, 0.330 mmol) was dissolved in DCM (14 mL), cooled to 0°C and *meta*-chloroperoxybenzoic acid (195 mg, 0.79 mmol, 2.4 equiv.) was added in one portion. After 5 min the cooling bath was removed, and the reaction mixture was stirred for 5 hours. The reaction mixture was quenched with 20 mL of 20% K_2CO_3 and extracted with DCM (2 x 10 mL). The combined organics were washed with saturated Na_2SO_3 (20 mL), brine (20 mL), dried (Na_2SO_4) and the volatiles removed *in vacuo* (without heating) to afford the title compound as a white solid in 82% yield (67 mg).

m.p. 236 °C decomposition
^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.83 (bs, 1H), 7.61 – 7.53 (m, 1H), 7.49 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.25 – 7.18 (m, 1H), 7.11 (d, *J* = 8.1 Hz, 1H), 4.51 (d, *J* = 2.1 Hz, 1H), 3.81 (s, 3H).
^{13}C NMR (101 MHz, DMSO-*d6*) δ 193.78, 165.20, 163.39, 135.89, 134.24, 129.75, 125.75, 124.07, 119.97, 67.93, 60.96, 53.21.
HRMS (ESI) m/z calcd. for $C_{12}H_9NO_5Na$ ([M+Na$^+$) 270.03729, found 270.03725.

aza-Michael addition

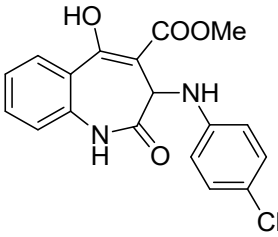


Methyl 5-hydroxy-3-morpholino-2-oxo-2,3-dihydro-1H-benzo[b]azepine-4-carboxylate 10a

To a stirred solution of morpholine (29 µL, 0.337 mmol, 1.02 equiv.) in dry acetonitrile (8 mL), was added **9** (76 mg, 0.330 mmol) at ambient temperature. The reaction mixture was then stirred at rt for 1h. The colorless solution was concentrated by rotary evaporation (without heating bath) to afford 101 mg (96%) of white solid.

m.p. 173-176 °C
^{1}H NMR (400 MHz, $CDCl_3$) δ 13.18 (bs, 1H), 9.47 (s, 1H), 7.86 – 7.76 (m, 1H), 7.47 – 7.40 (m, 1H), 7.20 (t, *J* = 7.6 Hz, 1H), 7.04 (d, *J* = 8.0 Hz, 1H), 4.33 (bs, 1H), 3.87 (s, 3H), 3.26 – 3.12 (m, 4H), 2.38 – 2.17 (m, 4H).
^{13}C NMR (100 MHz, $CDCl_3$) δ 172.35, 172.24, 169.66, 136.61, 131.83, 127.59, 126.49, 123.89, 119.77, 98.01, 66.72, 65.51, 52.71, 50.50.

HRMS (ESI) m/z calcd. for $C_{16}H_{18}N_2O_5Na$ ([M+Na$^+$) 341.11079, found 341.11079.

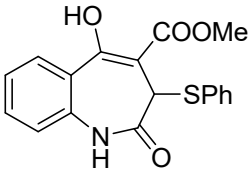


Methyl 3-((4-chlorophenyl)amino)-5-hydroxy-2-oxo-2,3-dihydro-1H-benzo[b]azepine-4-carboxylate 10b

To a stirred solution of 4-chloroaniline (43 mg, 0.337 mmol, 1.02 equiv.) in dry acetonitrile (8 mL), was added **9** (76 mg, 0.330 mmol) at ambient temperature. The reaction mixture was then stirred at rt for 20 h. The resulting precipitate was filtered, washed with ether (2 mL) and dried under reduced pressure to afford 105 mg (89%, 79% without acetonitrile) of white solid. The product crystallizes together with 1 equivalent of acetonitrile.

m.p. 159-161 °C decomposition
^{1}H NMR (400 MHz, DMSO-*d6*) δ 13.23 (s, 1H), 10.63 (s, 1H), 7.89 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.47 (t, *J* = 7.8 Hz, 1H), 7.22 (t, *J* = 7.7 Hz, 1H), 7.09 – 7.01 (m, 3H), 6.53 (d, *J* = 8.4 Hz, 2H), 5.47 (bs, 1H), 5.03 (s, 1H), 3.87 (s, 3H).
^{13}C NMR (101 MHz, DMSO-*d6*) δ 171.14, 170.66, 168.51, 146.75, 136.61, 132.15, 128.63, 128.55, 124.15, 122.92, 120.27, 120.20, 113.75, 98.96, 54.99, 52.95.
HRMS (ESI) m/z calcd. for $C_{18}H_{15}ClN_2O_4Na$ ([M+Na$^+$) 381.06126, found 381.06125

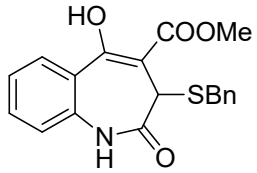
thia-Michael addition



Methyl 5-hydroxy-2-oxo-3-(phenylthio)-2,3-dihydro-1H-benzo[b]azepine-4-carboxylate 10c

To a stirred solution of thiophenol (34 µL, 0.337 mmol, 1.02 equiv.) in acetonitrile (8 mL), was added **9** (76 mg, 0.330 mmol) at ambient temperature. The reaction mixture was then stirred at rt for 1h. The resulting precipitate was filtered and dried under reduced pressure to afford 85 mg (76%) of white solid.

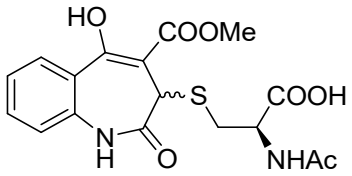
m.p. 190-192 °C decomposition
^{1}H NMR (400 MHz, DMSO-*d6*) δ 13.06 (s, 1H), 10.75 (bs, 1H), 7.85 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.63 – 7.57 (m, 1H), 7.37 – 7.22 (m, 7H), 5.19 (d, *J* = 1.7 Hz, 1H), 3.85 (s, 3H).
^{13}C NMR (100 MHz, DMSO-*d6*) δ 170.40, 169.20, 168.25, 137.36, 133.97, 132.89, 130.95, 129.28, 128.26, 127.70, 124.43, 123.74, 120.80, 97.84, 53.15, 46.71.
HRMS (ESI) m/z calcd. for $C_{18}H_{15}NO_4SNa$ ([M+Na$^+$) 364.06140, found 364.06145.



Methyl 3-(benzylthio)-5-hydroxy-2-oxo-2,3-dihydro-1H-benzo[b]azepine-4-carboxylate 10d

To a stirred solution of phenylmethanethiol (40 µL, 0.337 mmol, 1.02 equiv.) in acetonitrile (8 mL), was added **9** (76 mg, 0.330 mmol) at ambient temperature. The reaction mixture was then stirred at rt for 2h. The resulting colorless mixture was concentrated under reduced pressure to afford 115 mg (98%) of white solid.

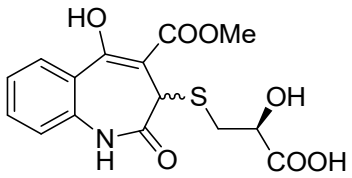
m.p. 136-139 °C
^{1}H NMR (400 MHz, DMSO- *d6*) δ 12.97 (s, 1H), 10.62 (bs, 1H), 7.81 – 7.75 (m, 1H), 7.57 – 7.51 (m, 1H), 7.36 – 7.16 (m, 7H), 4.67 (d, *J* = 1.8 Hz, 1H), 3.70 – 3.64 (m, 5H).
^{13}C NMR (100 MHz, DMSO- *d6*) δ 170.52, 170.10, 167.83, 137.51, 137.37, 132.57, 128.91, 128.29, 128.11, 126.99, 124.39, 123.51, 120.74, 98.44, 52.83, 42.13, 36.03.
HRMS (ESI) m/z calcd. for $C_{19}H_{17}NO_4SNa$ ([M+Na$^+$) 378.07705, found 378.07703.



(*R*)-2-acetamido-3-(((*R*,*S*)-5-hydroxy-4-(methoxycarbonyl)-2-oxo-2,3-dihydro-1H-benzo[b]azepin-3-yl)thio)propanoic acid 10e

To a stirred solution of *N*-acetyl-L-cysteine (55 mg, 0.337 mmol, 1.02 equiv.) in acetonitrile (8 mL), was added **9** (76 mg, 0.330 mmol) at ambient temperature. The reaction mixture was then stirred at rt for 2h. The resulting precipitate was filtered, washed with ether (10 mL) and dried under reduced pressure to afford 86 mg (66%) of white solid. According to the NMR spectra the product was isolated as a mixture of diastereomers in ratio 1:1.

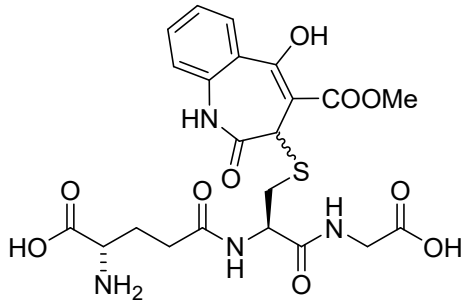
m.p. 164-165 °C
^{1}H NMR (400 MHz, DMSO- *d6*) δ 13.05 (s, 1H), 10.67 – 10.60 (m, 1H), 8.10 (t, *J* = 8.9 Hz, 1H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.53 (t, *J* = 7.7 Hz, 1H), 7.28 – 7.13 (m, 2H), 4.83 (d, *J* = 1.8 Hz, 0.5H), 4.77 (d, *J* = 1.9 Hz, 0.5H), 4.39 (td, *J* = 8.5, 4.6 Hz, 0.5H), 4.34 – 4.27 (m, 0.5H), 3.92 – 3.83 (m, 3H), 2.92 – 2.82 (m, 1H), 2.73 (dd, *J* = 13.6, 8.6 Hz, 0.5H), 2.65 (dd, *J* = 13.8, 9.0 Hz, 0.5H).
^{13}C NMR (100 MHz, DMSO- *d6*) δ 171.96, 171.79, 170.70, 170.66, 170.01, 169.97, 169.29, 169.23, 167.96, 167.81, 137.33, 137.28, 132.61, 128.14, 128.09, 124.36, 124.30, 123.57, 123.53, 120.86, 120.79, 98.24, 53.11, 53.07, 51.78, 51.26, 43.92, 43.38, 33.86, 33.74, 22.33.
HRMS (ESI) m/z calcd. for $C_{17}H_{18}N_2O_7SNa$ ([M+Na$^+$) 417.07269, found 417.07269.



(*S*)-2-hydroxy-3-(((*R,S*)-5-hydroxy-4-(methoxycarbonyl)-2-oxo-2,3-dihydro-1H-benzo[b]azepin-3-yl)thio)propanoic acid DBT10f

To a stirred solution of (*S*)-2-hydroxy-3-mercaptopropanoic acid (41 mg, 0.337 mmol, 1.02 equiv.) in acetonitrile (8 mL), was added **1** (76 mg, 0.330 mmol) at ambient temperature. The reaction mixture was then stirred at rt for 2h. The resulting precipitate was filtered, washed with ether (10 mL) and dried under reduced pressure to afford 77 mg (66%) of white solid. According to NMR spectra product was isolated as a mixture of diastereomers in ratio 1:1.

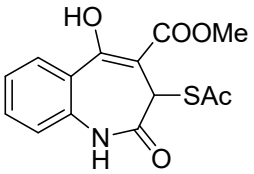
m.p. 200-204 °C decomposition
^{1}H NMR (400 MHz, DMSO- *d6*) δ 13.04 (bs, 1H), 10.63 – 10.59 (m, 1H), 7.78 (dt, *J* = 8.1, 1.8 Hz, 1H), 7.56 – 7.49 (m, 1H), 7.26 – 7.15 (m, 2H), 4.89 – 4.84 (m, 1H), 4.08 – 4.00 (m, 1H), 3.91 – 3.84 (m, 3H), 2.81 (dd, *J* = 13.4, 4.8 Hz, 0.5H), 2.75 (dd, *J* = 13.4, 4.9 Hz, 0.5H), 2.72 – 2.59 (m, 1H).
^{13}C NMR (100 MHz, DMSO- *d6*) δ 173.69, 173.66, 170.80, 170.77, 170.26, 170.24, 167.81, 167.79, 137.40, 137.37, 132.62, 132.58, 128.15, 128.10, 124.33, 124.32, 123.51, 123.48, 120.77, 120.71, 98.43, 98.40, 69.80, 69.35, 53.10, 53.07, 43.74, 43.71, 36.59, 36.37.
HRMS (ESI) m/z calcd. for $C_{15}H_{15}NO_7SNa$ ([M+Na$^+$) 376.04614, found 376.04616.



(*S*)-2-amino-5-(((*R*)-1-((carboxymethyl)amino)-3-(((*R,S*)-5-hydroxy-4-(methoxycarbonyl)-2-oxo-2,3-dihydro-1H-benzo[b]azepin-3-yl)thio)-1-oxopropan-2-yl)amino)-5-oxopentanoic acid 10g
L-Gluthathione (82 mg, 0.27 mmol, 1 equiv.) was dissolved in deionized water (4 mL) and suspension of **1** (62 mg, 0.27 mmol) in 4 mL of acetonitrile was added at ambient temperature. The resulting solution was stirred for 1 hour, concentrated under reduced pressure (no heating) to afford the title compound as a white solid in quantitative yield.

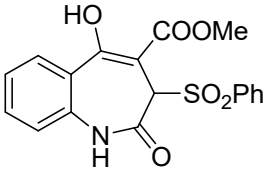
m.p. 179-180 °C decomposition
^{1}H NMR (400 MHz, D_2O) δ 7.72 (t, *J* = 8.7 Hz, 1H), 7.52 – 7.42 (m, 1H), 7.21 – 7.04 (m, 2H), 4.94 (s, 0.5H), 4.91 (s, 0.5H), 4.53 (dd, *J* = 8.9, 5.7 Hz, 0.5H), 4.46 (dd, *J* = 9.1, 5.3 Hz, 0.5H), 4.02 – 3.72 (m, 6H), 3.10 – 2.95 (m, 1H), 2.77 (dd, *J* = 14.5, 9.5 Hz, 0.5H), 2.67 (dd, *J* = 14.3, 9.3 Hz, 0.5H), 2.38 – 2.50 (m, 2H), 2.23 – 2.04 (m, 2H).
^{13}C NMR (100 MHz, D_2O) δ 174.30, 174.26, 173.39, 173.34, 173.33, 172.35, 172.11, 171.93, 171.84, 170.59, 170.56, 167.60, 135.63, 135.58, 132.74, 128.46, 124.79, 124.70, 124.64, 120.78, 120.73, 98.26,

98.06, 53.69, 53.66, 53.13, 52.98, 52.94, 52.39, 43.81, 43.15, 41.49, 34.08, 33.79, 31.34, 31.29, 26.12, 26.01.
HRMS (ESI) m/z calcd. for $C_{22}H_{27}N_4O_{10}S$ ([M+H$^+$) 539.14424, found 539.14398.



Methyl 3-(acetylthio)-5-hydroxy-2-oxo-2,3-dihydro-1H-benzo[b]azepine-4-carboxylate 10h
To a stirred solution of thioacetic acid (29 µL, 0.337 mmol, 1.02 equiv.) in acetonitrile (8 mL), was added **1** (76 mg, 0.330 mmol) at ambient temperature. The reaction mixture was then stirred at rt for 2h. The resulting mixture was concentrated to cca 3 mL, precipitate was filtered, washed with 1 mL of acetonitrile and dried under reduced pressure to afford 61 mg (60%) of white solid.

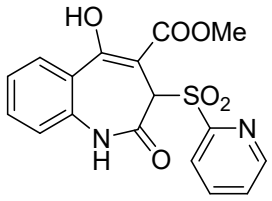
m.p. 204-207 °C decomposition
^{1}H NMR (400 MHz, DMSO- *d6*) δ 12.87 (bs, 1H), 10.72 (s, 1H), 7.85 – 7.78 (m, 1H), 7.64 – 7.56 (m, 1H), 7.30 (t, *J* = 7.6 Hz, 1H), 7.20 (d, *J* = 8.1 Hz, 1H), 5.64 (bs, 1H), 3.88 (s, 3H), 2.21 (s, 3H).
^{13}C NMR (100 MHz, DMSO- *d6*) δ 192.53, 170.17, 168.78, 168.39, 136.95, 133.13, 128.49, 124.30, 124.13, 121.44, 99.02, 53.13, 40.15, 29.97.
HRMS (ESI) m/z calcd. for $C_{14}H_{13}NO_5SNa$ ([M+Na$^+$) 330.04066, found 330.04068.



Methyl 5-hydroxy-2-oxo-3-(phenylsulfonyl)-2,3-dihydro-1H-benzo[b]azepine-4-carboxylate 10i

Sodium benzenesulfinate (65 mg, 0.40 mmol, 1.2 equiv.) was dissolved in deionized water (1 mL) and 0.4 mL of 1M HCl was added, forming a white suspension of benzenesulfinic acid. Suspension of **1** (76 mg, 0.330 mmol) in 2 mL of acetonitrile was added at ambient temperature. The resulting yellow suspension was stirred and the loss of yellow color was observed. After 10 minutes the resulting white suspension was filtered, washed with water and dried under reduced pressure to afford 81 mg (66%) of white solid.

m.p. 191-193 °C decomposition
^{1}H NMR (400 MHz, $CDCl_3$) δ 13.82 (s, 1H), 8.79 (bs, 1H), 8.04 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.77 (d, *J* = 7.3 Hz, 2H), 7.61 (t, *J* = 7.4 Hz, 1H), 7.50 (t, *J* = 7.6 Hz, 3H), 7.29 (t, *J* = 8.1 Hz, 1H), 7.01 (d, *J* = 8.1 Hz, 1H), 5.67 (bs, 1H), 3.64 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$) δ 171.51, 171.31, 165.96, 138.85, 135.34, 134.33, 133.23, 129.56, 129.25, 128.69, 125.07, 124.79, 121.20, 91.71, 69.57, 52.89.
HRMS (ESI) m/z calcd. for $C_{18}H_{15}NO_6SNa$ ([M+Na$^+$) 396.05123, found 396.05084.

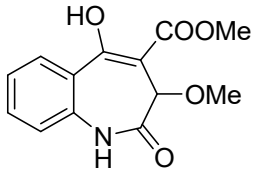


Methyl 5-hydroxy-2-oxo-3-(pyridin-2-ylsulfonyl)-2,3-dihydro-1H-benzo[b]azepine-4-carboxylate 10j

Sodium pyridine-2-sulfinate (65 mg, 0.40 mmol, 1.2 equiv.) was dissolved in deionized water (1 mL) and 0.4 mL of 1M HCl was added, forming a white suspension of 2-pyridinesulfinic acid. Suspension of **1** (76 mg, 0.330 mmol) in 2 mL of acetonitrile was added at ambient temperature. The resulting yellow suspension was stirred and the loss of yellow color was observed. After 10 minutes the resulting white suspension was filtered, washed with water and dried under reduced pressure to afford 105 mg (85%) of white solid.

m.p. 179-180 °C decomposition
^{1}H NMR (400 MHz, DMSO- *d6*) δ 13.53 (s, 1H), 11.11 (s, 1H), 8.84 (d, *J* = 3.9 Hz, 1H), 8.10 (td, *J* = 7.8, 1.7 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.82 (d, *J* = 7.8 Hz, 1H), 7.76 (dd, *J* = 7.6, 4.7 Hz, 1H), 7.59 – 7.52 (m, 1H), 7.27 (t, *J* = 7.6 Hz, 1H), 7.21 (d, *J* = 8.1 Hz, 1H), 5.94 (s, 1H), 3.63 (s, 3H).
^{13}C NMR (101 MHz, DMSO- *d6*) δ 170.62, 170.48, 163.79, 155.79, 150.62, 139.06, 136.25, 132.97, 128.76, 128.54, 123.98, 123.86, 122.97, 121.44, 91.23, 65.68, 53.02.
HRMS (ESI) m/z calcd. for $C_{17}H_{14}N_2O_6SNa$ ([M+Na$^+$) 397.04648, found 397.04651.

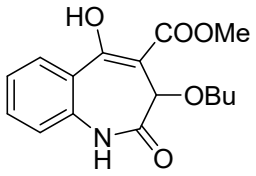
oxa-Michael addition



Methyl 5-hydroxy-3-methoxy-2-oxo-2,3-dihydro-1H-benzo[b]azepine-4-carboxylate 10k

9 (76 mg, 0.330 mmol) was dissolved in dry methanol (8 mL) and stirred at rt. After 20 h the white suspension was formed and concentrated *in vacuo* (without heating) to afford 82 mg (94%) of white solid.

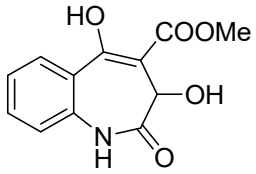
m.p. 167-168 °C
^{1}H NMR (400 MHz, $CDCl_3$) δ 13.36 (s, 1H), 9.47 (s, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.47 (t, *J* = 7.6 Hz, 1H), 7.28 – 7.20 (m, 1H), 7.13 (d, *J* = 8.0 Hz, 1H), 5.19 (bs, 1H), 3.90 (s, 3H), 3.17 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$) δ 171.81, 171.16, 170.28, 135.78, 132.27, 128.74, 125.49, 124.17, 120.45, 99.20, 76.10, 56.73, 52.74.
HRMS (ESI) m/z calcd. for $C_{13}H_{13}NO_5Na$ ([M+Na$^+$) 286.06859, found 286.06859.



Methyl 3-butoxy-5-hydroxy-2-oxo-2,3-dihydro-1H-benzo[b]azepine-4-carboxylate 10l

9 (76 mg, 0.330 mmol) was dissolved in butanol (8 mL) and stirred at rt. After 20 h the colorless solution was formed and concentrated *in vacuo* (bath temperature 30°C) to afford 88 mg (87%) of white solid.

m.p. 139-140 °C
^{1}H NMR (400 MHz, $CDCl_3$) δ 13.30 (s, 1H), 8.77 (s, 1H), 7.94 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.45 (ddd, *J* = 8.0, 7.3, 1.6 Hz, 1H), 7.22 (ddd, *J* = 8.3, 7.3, 1.2 Hz, 1H), 7.06 (dd, *J* = 8.0, 1.2 Hz, 1H), 5.25 (d, *J* = 1.7 Hz, 1H), 3.89 (s, 3H), 3.34 (dt, *J* = 9.0, 6.0 Hz, 1H), 3.26 (dt, *J* = 9.0, 6.5 Hz, 1H), 1.27 – 1.19 (m, 2H), 0.89 – 0.78 (m, 2H), 0.68 – 0.62 (m, 3H).
^{13}C NMR (100 MHz, $CDCl_3$) δ 171.76, 171.62, 170.26, 135.87, 131.97, 128.64, 125.85, 124.02, 120.43, 99.72, 74.46, 68.56, 52.69, 31.41, 18.87, 13.74.
HRMS (ESI) m/z calcd. for $C_{16}H_{19}NO_5Na$ ([M+Na$^+$) 328.11554, found 328.11540.

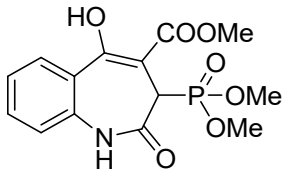


Methyl 3-hydroxy-2,5-dioxo-2,3,4,5-tetrahydro-1H-benzo[b]azepine-4-carboxylate 10m

To a stirred solution of **9** (76 mg, 0.330 mmol) in THF (4 mL), was added water (4 mL) at ambient temperature. The yellow solution was stirred at rt for 5h. THF was removed *in vacuo*, and resulting suspension was extracted with ethyl acetate (2x10 mL). The combined organic layers were dried (Na_2SO_4) and the volatiles removed *in vacuo* (without heating). Purification by silica gel chromatography (hexanes/EtOAc = 1/1) afforded the title compound as a white solid (65 mg, 79% yield). 4 mg of unreactected starting material were recovered.

m.p. 142-143 °C
^{1}H NMR (400 MHz, DMSO- *d6*) δ 13.09 (s, 1H), 10.52 (s, 1H), 7.81 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.52 – 7.46 (m, 1H), 7.21 – 7.14 (m, 2H), 5.51 (d, *J* = 3.5 Hz, 1H), 5.25 – 5.22 (m, 1H), 3.85 (s, 3H).
^{13}C NMR (100 MHz, DMSO- *d6*) δ 171.41, 171.36, 168.70, 137.41, 131.88, 127.92, 124.68, 122.70, 120.39, 101.59, 66.65, 52.72.
HRMS (ESI) m/z calcd. for $C_{12}H_{11}NO_5Na$ ([M+Na$^+$) 272.05294, found 272.05309.

phospha-Michael addition



Methyl 3-(dimethoxyphosphoryl)-5-hydroxy-2-oxo-2,3-dihydro-1H-benzo[b]azepine-4-carboxylate 10n
9 (76 mg, 0.330 mmol) was suspended in dimethyl phosphite (0.5 mL) and stirred at ambient temperature for 72 hours until white suspension was formed. The reaction mixture was concentrated *in vacuo*. The crude product was crystallized from acetonitrile (5 mL) and the product was isolated as a white solid (88 mg, 78 %).

m.p. 201-203 °C
^{1}H NMR (400 MHz, DMSO- *d6*) δ 13.13 (s, 1H), 10.68 (s, 1H), 7.82 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.52 (ddd, *J* = 8.1, 7.2, 1.6 Hz, 1H), 7.24 (ddd, *J* = 8.2, 7.2, 1.2 Hz, 1H), 7.16 (dd, *J* = 8.2, 1.2 Hz, 1H), 4.53 (dd, *J* = 28.8, 1.2 Hz, 1H), 3.88 (s, 3H), 3.33 (d, *J* = 11.0 Hz, 3H), 3.30 (d, *J* = 11.1 Hz, 3H).
^{13}C NMR (100 MHz, DMSO- *d6*) δ 170.85 (d, *J* = 5.0 Hz), 167.87 (d, *J* = 2.0 Hz), 167.66 (d, *J* = 5.3 Hz), 136.89, 132.35, 128.48, 124.42 (d, *J* = 2.0 Hz), 123.66, 121.3993.43 (d, *J* = 6.9 Hz), 53.22, 52.80 (d, *J* = 6.9 Hz), 52.76 (d, *J* = 6.8 Hz), 43.32 (d, *J* = 132.0 Hz).
^{31}P NMR (162 MHz, DMSO- *d6*) δ 21.98.
HRMS (ESI) m/z calcd. for $C_{14}H_{16}NO_7PNa$ ([M+Na$^+$) 364.05566, found 364.05563.

3. Tested bacteria and determination of the antimicrobial efficiency

Biological experiments were performed on the reference susceptible strain *S. aureus* CCM 3953 and *E.coli* CCM 3988(Czech Collection of Microorganisms, Brno, Czech Republic) and the clinical MRSA L12 which originated from the hemoculture (Collection of microorganisms at the Department of Microbiology and Virology, Comenius University in Bratislava). The strains were maintained in skim-milk medium (Biolife, Italy) at −20 °C. First, the microorganisms were inoculated in the Mueller–Hinton Broth (MHB, Biolife, Italy) and cultivated in an incubator with shaking at 37 °C for 18 h. The antimicrobial susceptibility was tested in 96-well plates (Sarstedt, Germany) using the microdilution method according to the recommendation of the EUCAST, version 11.0 (The European Committee on Antimicrobial Susceptibility Testing, 2021). Briefly, overnight cultures of the tested bacteria were diluted in MHB to a density corresponding to the 0.5 McFarland standard (BioMérieux, France), representing approximately 10^8 cells per mL and subsequently diluted to obtain a final density of 5×10^5 cells per well.

The compounds were dissolved serially in MHB. The concentration ranged from 100 to 0.5 μg mL^{-1}. 100 μL of the prepared bacterial culture with 100 μL of the diluted compounds in MHB of an appropriate concentration was added into each well. The micro plate was incubated at 37 °C for 24 h.

The growth of bacteria was measured spectrophotometrically at OD630 using a microplate reader (Bio Tek ELx808). The susceptibility was evaluated in terms of MIC_{90} (representing the concentration at which 90 % of the growth is inhibited when compared to the control sample without an antimicrobial agent (1 % DMSO)). Each experiment was repeated at least 3 times with at least 3 parallel samples in each experiment.

4. Toxicity studies

Cytotoxicity test according to ISO 10993-5

Cytotoxicity testing was conducted according to the ISO 10993-5 protocol using the VERO6 cell line (ATCC, Sigma-Merck, USA). The IC_{50} concentrations (i.e., concentrations that cause a 50% decrease in viability compared to negative controls) were determined in four independent runs for each tested compound. The neutral red uptake test was used for viability determination. [1] The errors were calculated using the standard deviation (SD). The function =STDEV.S was used for the calculation.

Cytotoxicity assessment of 9 in VERO6 cell line:

Run 1

Test Article	Concentration µg/mL	% of viability	SD
Negative control	0.0	100.0	6.7
conc. 1	0.0	101.2	6.9
conc. 2	0.1	101.1	15.0
conc. 3	0.3	103.8	6.0
conc. 4	1.0	101.6	8.0
conc. 5	3.2	65.0	19.4
conc. 6	10.0	16.7	12.4
conc. 7	31.6	2.2	1.2
conc. 8	100.0	2.2	1.3
IC50	**5.3**	**y =-7,06x + 87,32**	

Run 2

Test Article	Concentration µg/mL	% of viability	SD
Negative control	0.0	100.0	8.5
conc. 1	0.0	102.0	4.5
conc. 2	0.1	94.4	3.9
conc. 3	0.3	104.8	9.1
conc. 4	1.0	98.5	10.4
conc. 5	3.2	96.7	8.6
conc. 6	10.0	65.6	20.9
conc. 7	31.6	5.1	1.1
conc. 8	100.0	3.7	1.4
IC50	**15.6**	**y =-2,8x + 93,56**	

[1] ISO, 2009: ISO 10993-5:2009(en) Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity

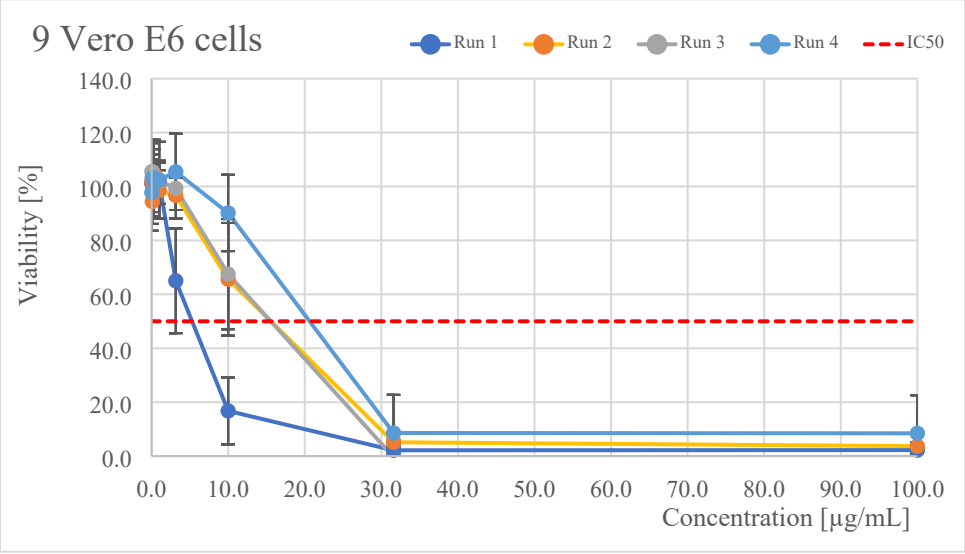
Run 3

Test Article	Concentration µg/mL	% of viability	SD
Negative control	0.0	100.0	4.1
conc.1	0.0	105.5	4.6
conc.2	0.1	103.6	2.9
conc.3	0.3	103.7	4.3
conc.4	1.0	102.1	4.0
conc.5	3.2	99.3	3.9
conc.6	10.0	67.5	20.4
conc.7	31.6	-0.4	0.6
conc.8	100.0	-0.3	0.6
IC50	**15.6**	**y =-3,14x + 98,92**	

Run 4

Test Article	Concentration µg/mL	% of viability	SD
Negative control	0.0	100.0	4.2
conc. 1	0.0	97.7	7.9
conc. 2	0.1	103.1	5.9
conc. 3	0.3	103.2	18.5
conc. 4	1.0	102.4	13.7
conc. 5	3.2	105.4	5.3
conc. 6	10.0	90.2	5.1
conc. 7	31.6	8.6	1.7
conc. 8	100.0	8.5	1.6
IC50	**20.6**	**y =-3,78x + 127,96**	

AVERAGE IC50	**14.3**	µg/mL
SD IC50	**6.4**	µg/mL



Cytotoxicity assessment of 10d in VERO6 cell line:

Run 1

Test Article	Concentration µg/mL	% of viability	SD
Negative control	0.0	100.0	6.6
conc. 1	0.0	102.8	6.7
conc. 2	0.1	93.9	10.7
conc. 3	0.3	98.3	7.6
conc. 4	1.0	103.4	2.3
conc. 5	3.2	98.6	6.0
conc. 6	10.0	84.8	9.2
conc. 7	31.6	1.4	1.1
conc. 8	100.0	1.7	1.3
IC50	**19.0**	**y =-3,86x + 123,43**	

Run 2

Test Article	Concentration µg/mL	% of viability	SD
Negative control	0.0	100.0	4.7
conc. 1	0.0	100.4	5.4
conc. 2	0.1	101.5	3.6
conc. 3	0.3	104.4	5.7
conc. 4	1.0	102.7	4.7
conc. 5	3.2	97.5	4.4
conc. 6	10.0	99.8	6.1
conc. 7	31.6	88.0	9.4
conc. 8	100.0	75.5	14.6
IC50		0.5485287	105.2917

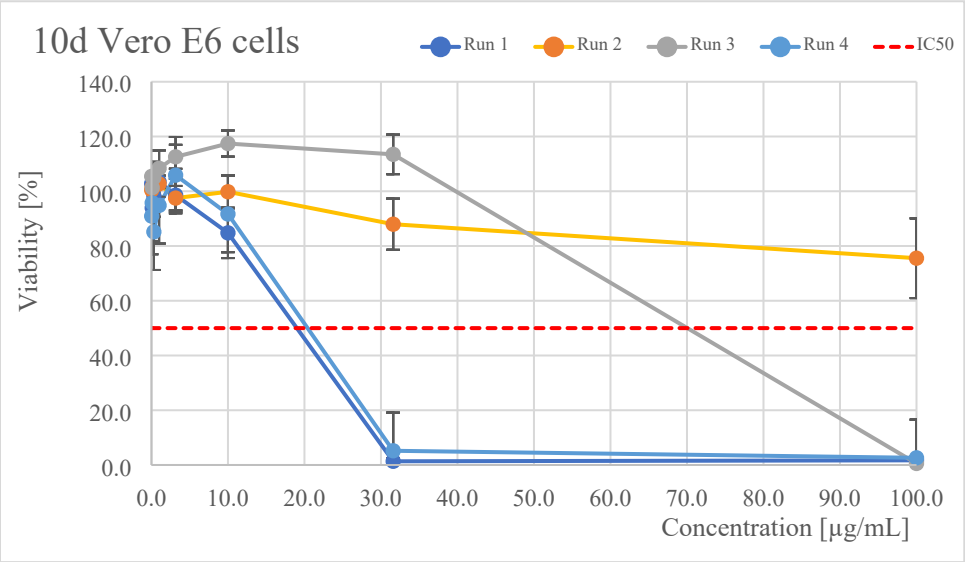
Run 3

Test Article	Concentration µg/mL	% of viability	SD
Negative control	0.0	100.0	11.8
conc. 1	0.0	105.4	5.3
conc. 2	0.1	101.3	4.5
conc. 3	0.3	105.0	4.2
conc. 4	1.0	108.4	6.5
conc. 5	3.2	112.6	4.4
conc. 6	10.0	117.4	4.8
conc. 7	31.6	113.5	7.2
conc. 8	100.0	0.6	0.8
IC50	**70.1**	**y =-1,65x + 165,64**	

Run 4

Test Article	Concentration µg/mL	% of viability	SD
Negative control	0.0	100.0	6.8
conc. 1	0.0	90.9	3.2
conc. 2	0.1	95.8	3.5
conc. 3	0.3	85.2	5.4
conc. 4	1.0	94.8	4.4
conc. 5	3.2	105.9	4.5
conc. 6	10.0	91.7	12.0
conc. 7	31.6	5.2	2.4
conc. 8	100.0	2.6	3.0
IC50	**20.4**	**y =-4x + 131,73**	

AVERAGE IC50	**36.5**	µg/mL
SD IC50	**29.1**	µg/mL



In vitro toxicity test in the 3D models of target tissues

Two routes of exposure were considered for the selected compounds: inhalation and oral exposure. For the follow-up work in pre-clinical screening of toxicity profiles (in compliance with the general strategy of using available in vitro systems instead of experimental animals in the early phases of testing), 3D human lung epithelia EpiAirway™ and a 3D reconstructed human small intestine model EpiIntestinal™ (MatTek Life Sciences, USA and Slovakia). Both tissue models are constructed from primary human cells and closely resemble barrier properties and histology comparable to native human tissues. Both models are metabolically and mitotically active and produce mucus as an additional protective layer against viral and bacterial infections or the toxic effects of xenobiotics.

The AlphaCloud exposure system (Vitrocell, Germany) was used for the inhalation route to resemble real-life exposure to aerosols. In both experiments, 100 µL of the substance was applied by pipetting (EpiIntestinal) or dispersed as an aerosol via AlphaCloud (EpiAirway) onto the apical side of the 3D models. Tissues were exposed for 21 ± 3 hours, after which the MTT viability test was conducted. The testing protocol is similar to the ISO 10993-23 protocol for medical device testing in reconstructed human tissue models. Additionally, changes in barrier properties were monitored by measuring the change in transepithelial electrical resistance (TEER) using the EVOM voltmeter (World Precision Instruments, USA) and STX4 electrode. Viability was assessed in the MTT viability assay. [2]

Biological response of EpiAirway to 9

Run 1

	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
NC	0.291	0.014	100.0	4.9	4.9
1% DMSO in DPBS	0.290	0.001	99.7	0.2	0.2
0,3% Triton	0.071	0.001	24.2	0.3	1.3
5,3	0.309	0.012	106.4	4.3	4.0
26,5	0.313	0.004	107.6	1.5	1.4
53,0	0.283	0.004	97.1	1.3	1.3

Run 2

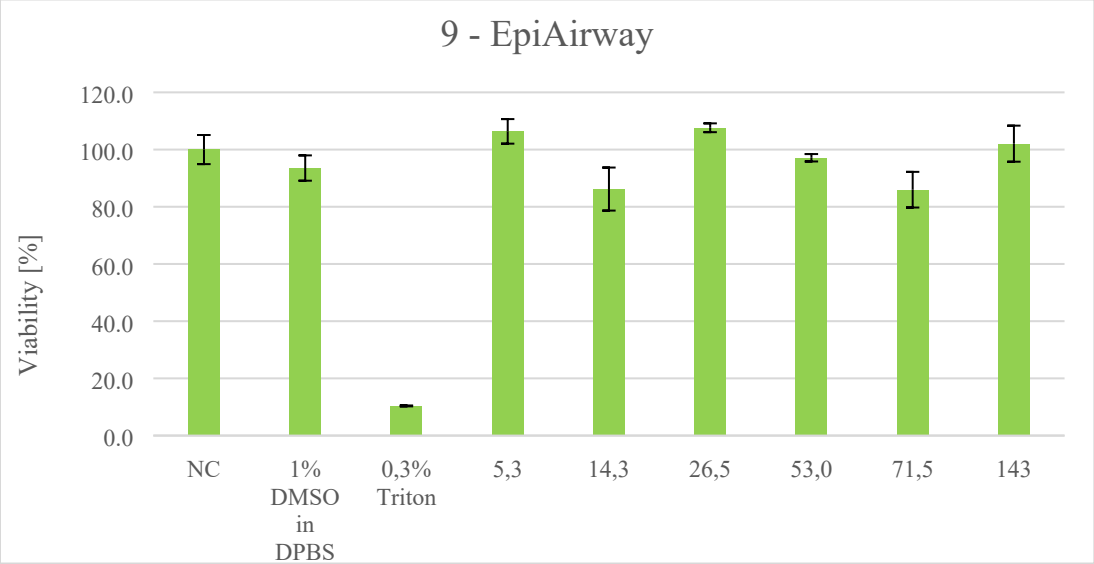
	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
NC	0.231	0.006	100.0	2.4	2.4
1% DMSO in DPBS	0.198	0.015	85.5	6.4	7.5
0,3% Triton	0.010	0.000	4.3	0.0	0.0
14,3	0.183	0.020	79.0	8.6	10.8
71,5	0.171	0.020	73.9	8.7	11.8
143	0.235	0.016	101.5	7.0	6.9

Run 3

	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
NC	1.746	0.139	100.0	8.0	8.0
1% DMSO in DPBS	1.666	0.115	95.4	6.6	6.9
0,3% Triton	0.045	0.003	2.5	0.2	6.4
14,3	1.631	0.113	93.4	6.5	6.9
71,5	1.712	0.065	98.0	3.7	3.8
143	1.792	0.097	102.6	5.6	5.4

[2] ISO, 2021: ISO 10993-23:2021(en) Biological evaluation of medical devices — Part 23: Tests for irritation.

	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
NC	0.756	0.053	100.0	5.1	5.1
1% DMSO in DPBS	0.718	0.044	93.5	4.4	4.9
0,3% Triton	0.042	0.001	10.4	0.2	2.6
5,3	0.309	0.012	106.4	4.3	4.0
14,3	0.907	0.066	86.2	7.5	8.9
26,5	0.313	0.004	107.6	1.5	1.4
53,0	0.283	0.004	97.1	1.3	1.3
71,5	0.941	0.043	86.0	6.2	7.8
143	1.013	0.057	102.1	6.3	6.2



Biological response of EpiIntestinal to 9

Run 1

	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
NC	0.293	0.012	100.0	4.2	4.2
1% DMSO in DPBS	0.292	0.002	99.7	0.7	0.7
0,3% Triton	0.070	0.002	24.0	0.8	3.5
5,3	0.310	0.009	106.0	3.0	2.9
26,5	0.314	0.000	107.3	0.1	0.1
53,0	0.283	0.001	96.7	0.2	0.3

Run 2

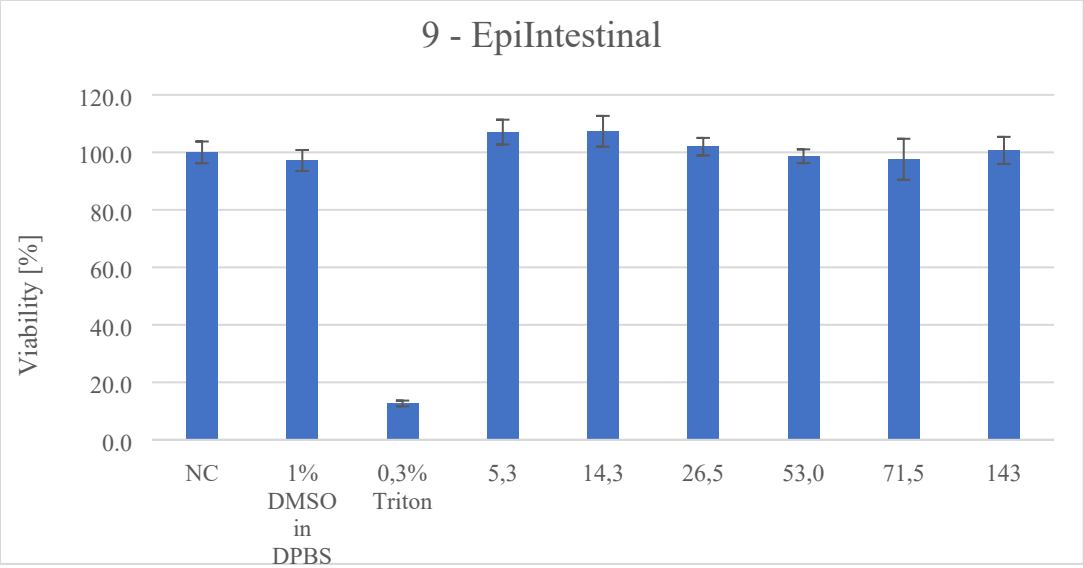
	mean	SD	mean of	SD	CV %

	of OD	of OD	viabilities [%]	of viabilities	[%]
NC	0.486	0.028	100.0	5.7	5.7
1% DMSO in DPBS	0.464	0.036	95.4	7.5	7.8
0,3% Triton	0.025	0.009	5.1	1.8	35.5
5,3	0.524	0.022	107.8	4.6	4.2
26,5	0.491	0.009	101.0	1.9	1.9
53,0	0.479	0.011	98.6	2.3	2.3

Run 3

	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
NC	0.218	0.003	100.0	1.5	1.5
1% DMSO in DPBS	0.210	0.006	96.3	2.8	2.9
0,3% Triton	0.019	0.001	8.9	0.3	3.7
14,3	0.234	0.012	107.3	5.3	5.0
71,5	0.213	0.016	97.6	7.1	7.3
143	0.220	0.010	100.7	4.7	4.7

	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
NC	0.332	0.014	100.0	3.8	3.8
1% DMSO in DPBS	0.322	0.015	97.2	3.7	3.8
0,3% Triton	0.038	0.004	12.6	1.0	14.2
5,3	0.417	0.016	107.1	4.3	4.0
14,3	0.234	0.012	107.3	5.3	5.0
26,5	0.403	0.005	102.0	3.0	3.1
53,0	0.381	0.006	98.6	2.4	2.4
71,5	0.213	0.016	97.6	7.1	7.3
143	0.220	0.010	100.7	4.7	4.7



Biological response of EpiAirway to **10d**

Run 1

	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
NC	0.418	0.016	100.0	3.8	3.8
1% DMSO	0.423	0.016	101.3	3.8	3.8
Triton 0,3%	0.010	0.002	2.3	0.6	24.4
100	0.440	0.027	105.4	6.5	6.2
500	0.407	0.002	97.6	0.5	0.5
1000	0.395	0.015	94.7	3.6	3.8

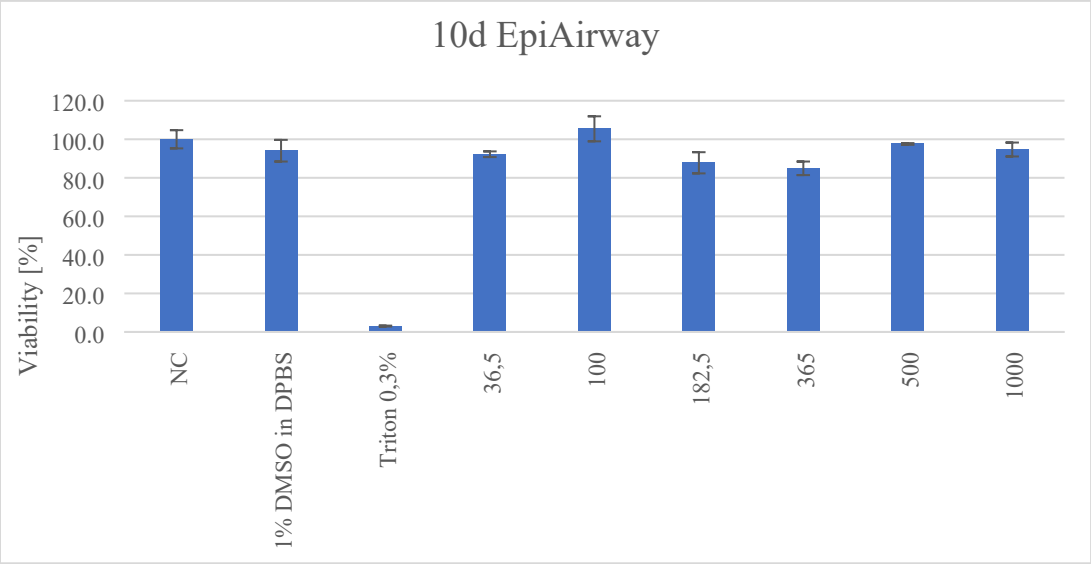
Run 2

	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
NC	0.231	0.006	100.0	2.4	2.4
1% DMSO in DPBS	0.198	0.015	85.5	6.4	7.5
0,3% Triton	0.010	0.000	4.3	0.0	0.0
36,5	0.224	0.002	97.2	0.8	0.8
182,5	0.214	0.019	92.7	8.3	8.9
365	0.194	0.013	83.8	5.5	6.6

Run 3

	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
NC	1.746	0.139	100.0	8.0	8.0
1% DMSO in DPBS	1.666	0.115	95.4	6.6	6.9
0,3% Triton	0.045	0.003	2.5	0.2	6.4
36,5	1.525	0.038	87.3	2.2	2.5
182,5	1.447	0.048	82.9	2.7	3.3
365	1.502	0.027	86.0	1.5	1.8

	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
NC	0.798	0.053	100.0	4.7	4.7
1% DMSO in DPBS	0.762	0.049	94.1	5.6	6.1
Triton 0,3%	0.021	0.002	3.1	0.2	10.2
36,5	0.875	0.020	92.2	1.5	1.6
100	0.440	0.027	105.4	6.5	6.2
182,5	0.831	0.033	87.8	5.5	6.1
365	0.848	0.020	84.9	3.5	4.2
500	0.407	0.002	97.6	0.5	0.5
1000	0.395	0.015	94.7	3.6	3.8



Biological response of EpiIntestinal to 10d

Run 1

	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
NC	0.293	0.012	100.0	4.2	4.2
1% DMSO in DPBS	0.292	0.002	99.7	0.7	0.7
0,3% Triton	0.070	0.002	24.0	0.8	3.5
19	0.287	0.011	98.1	3.7	3.8
95	0.338	0.021	115.4	7.3	6.3
190	0.290	0.008	99.2	2.7	2.7

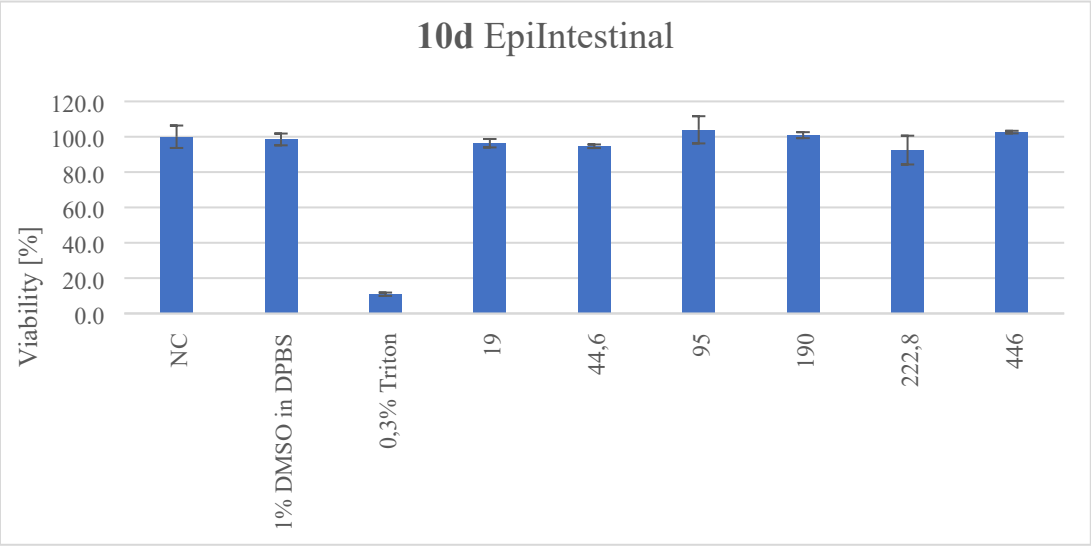
Run 2

	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
NC	0.486	0.028	100.0	5.7	5.7
1% DMSO in DPBS	0.464	0.036	95.4	7.5	7.8
0,3% Triton	0.025	0.009	5.1	1.8	35.5
19	0.460	0.005	94.6	1.0	1.1
95	0.450	0.040	92.5	8.1	8.8
190	0.499	0.004	102.6	0.7	0.7

Run 3

	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
NC	0.456	0.042	100.0	9.1	9.1
1% DMSO in DPBS	0.458	0.008	100.3	1.8	1.8
0,3% Triton	0.016	0.001	3.6	0.2	4.3
44,6	0.407	0.009	89.1	2.0	2.3
222,8	0.461	0.040	101.1	8.7	8.6
446	0.473	0.009	103.6	1.9	1.9

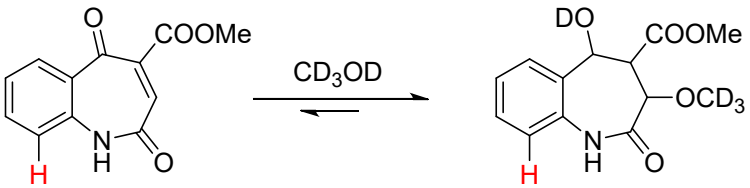
	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
NC	0.412	0.027	100.0	6.3	6.3
1% DMSO in DPBS	0.404	0.016	98.5	3.3	3.5
0,3% Triton	0.037	0.004	10.9	0.9	14.4
19	0.373	0.008	96.3	2.4	2.4
44,6	0.460	0.005	94.6	1.0	1.1
95	0.394	0.030	103.9	7.7	7.5
190	0.395	0.006	100.9	1.7	1.7
222,8	0.450	0.040	92.5	8.1	8.8
446	0.499	0.004	102.6	0.7	0.7



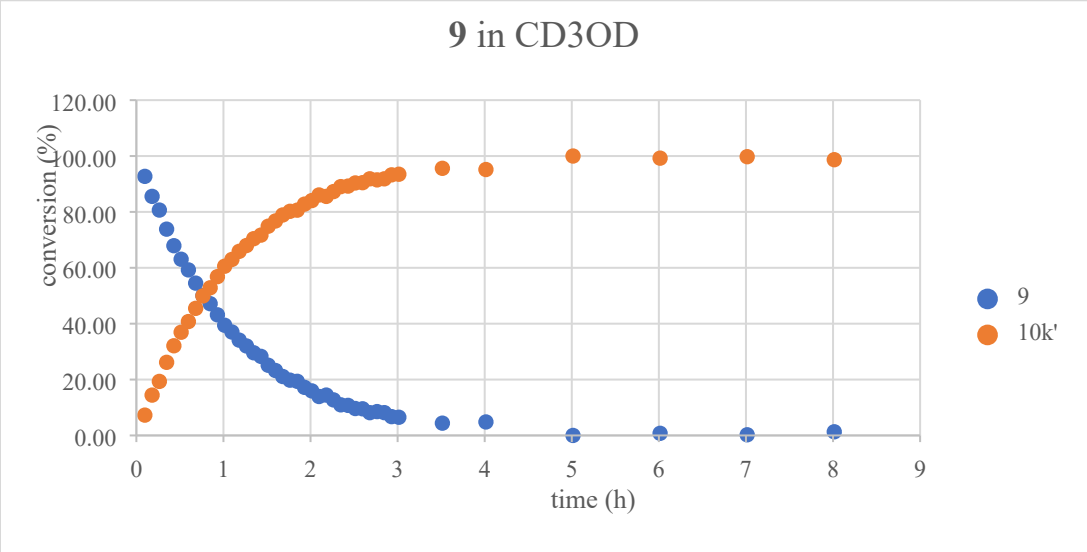
5. Characterization

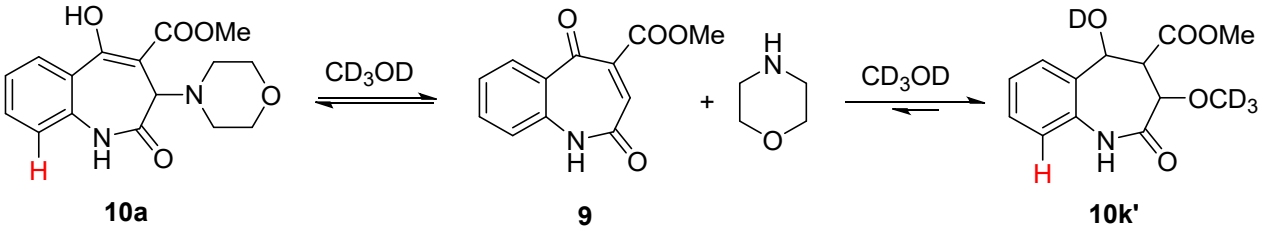
Stability in CD_3OD - NMR study

Samples were prepared by dissolving 2-3 mg of compounds **9, 10** in 0.65 mL CD_3OD. Sample was shaken and placed in spectrometer Varian VNMRS 600 MHz. ^1H NMR spectra were recorded in 5 minutes intervals for 3 hours, then in 30 min intervals for 60 minutes and finally in 60 min intervals for 10-18 hours. Then the sample was monitored regularly for further 7 days. Representative peaks of interest used for determining the conversion are marked in red below in Figures. The conversion (%) of **9** was calculated as the integral of the peak of **9** divided by the sum of the integrals of peaks **9** and **10k'**.

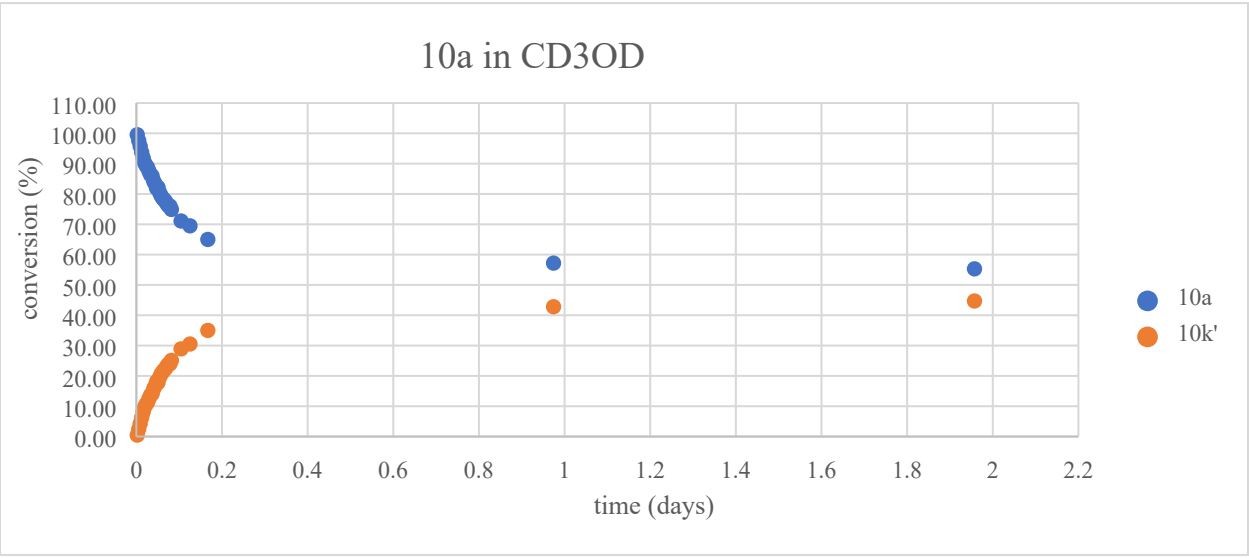


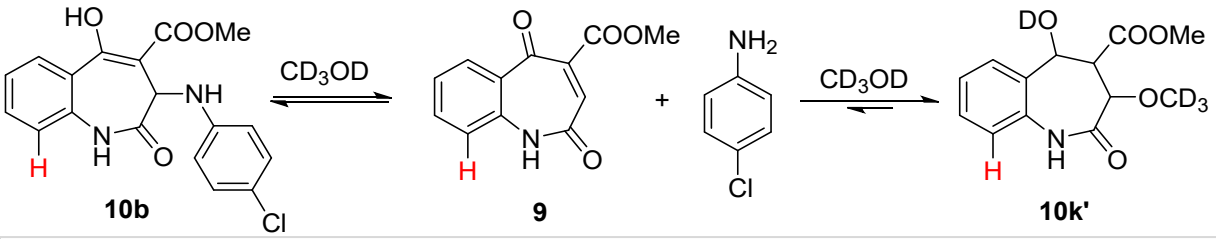
9 **10k'**

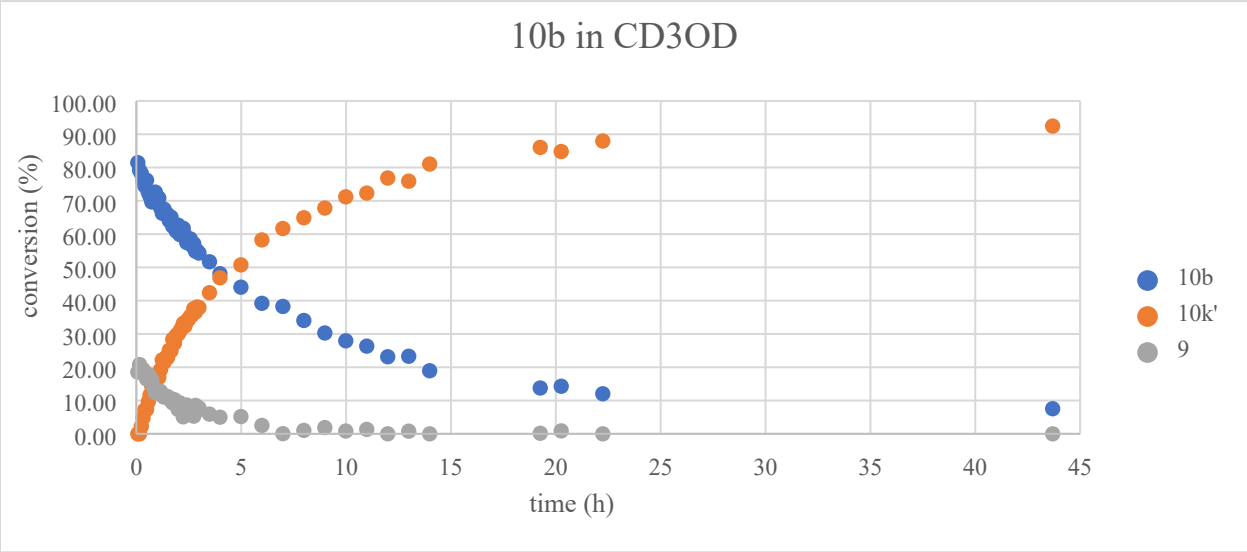


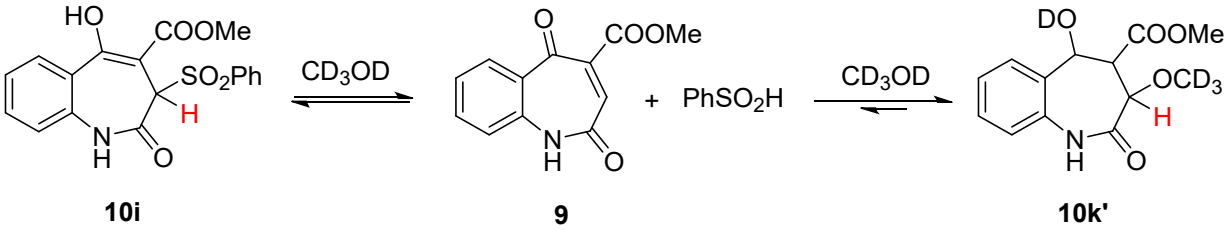


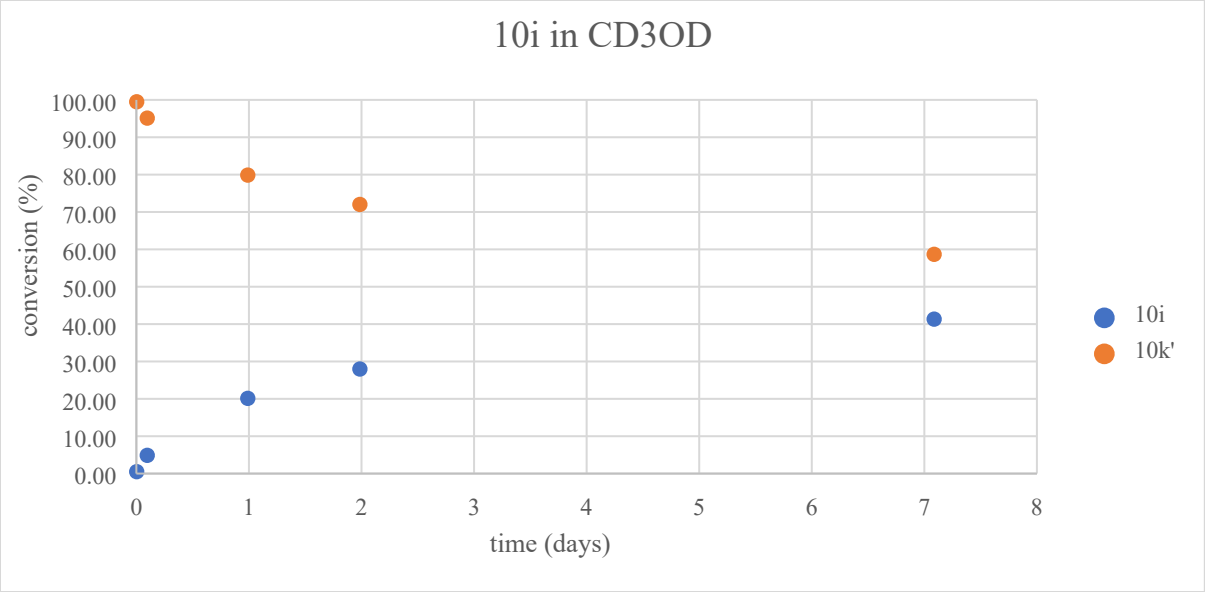
10a **9** **10k'**

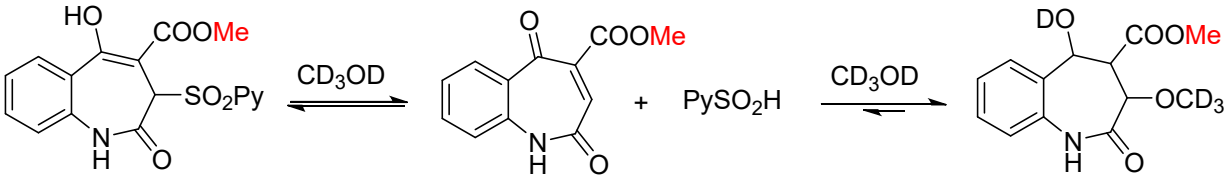



HO
COOMe
NH
CD3OD
O
COOMe
NH2
CD3OD
DO
COOMe
OCD3
10b
9
10k'

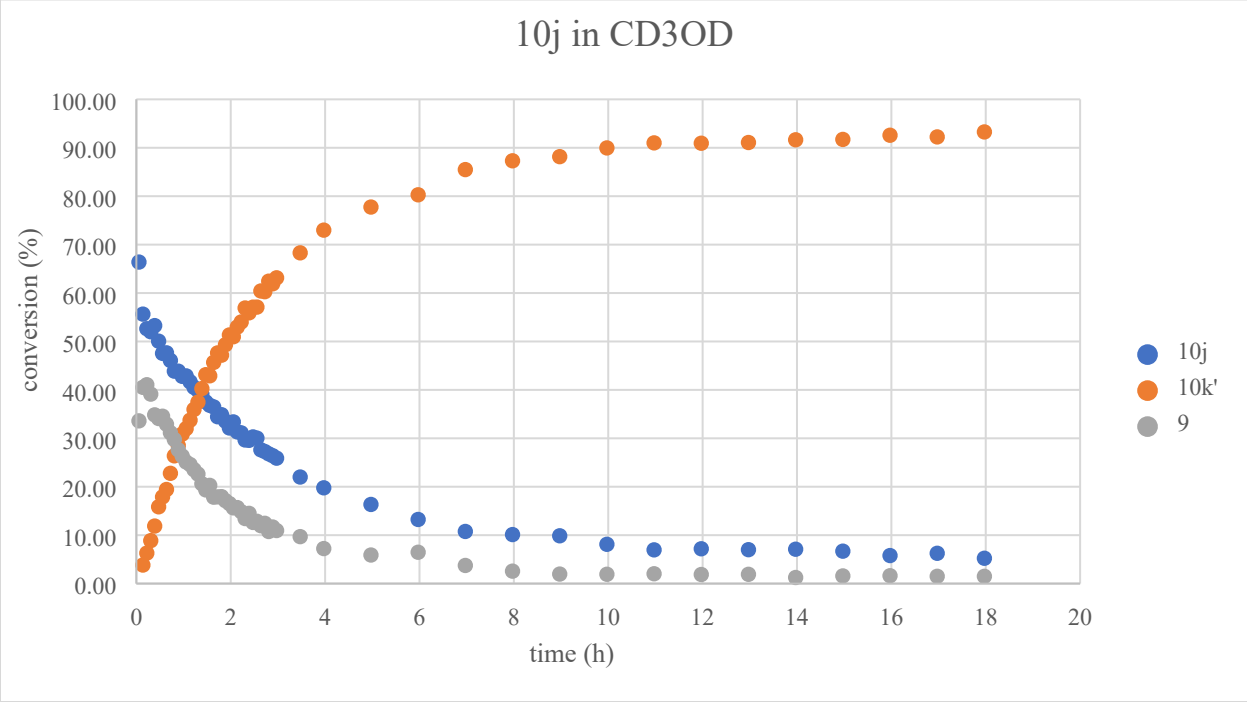

10b in CD3OD
conversion (%)
time (h)
10b
10k'
9

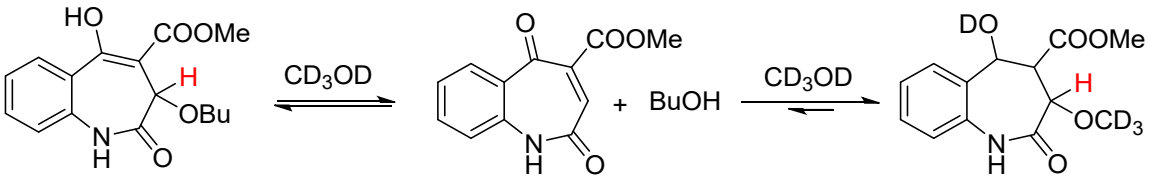

HO
COOMe
SO2Ph
CD3OD
O
COOMe
PhSO2H
CD3OD
DO
COOMe
OCD3
10i
9
10k'


10i in CD3OD
conversion (%)
time (days)
10i
10k'

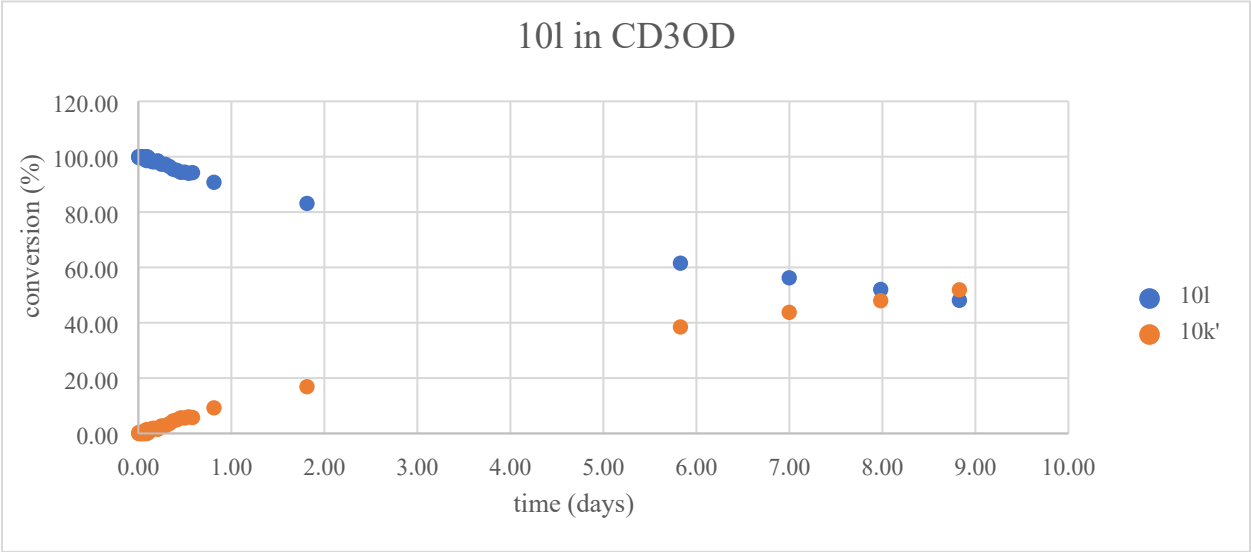

HO
COOMe
SO2Py
CD3OD
O
COOMe
PySO2H
CD3OD
DO
COOMe
OCD3

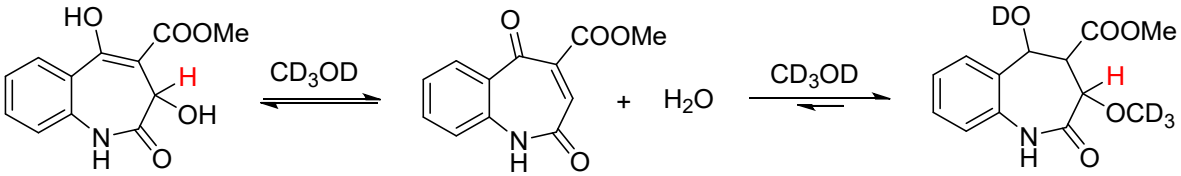
10j **9** **10k'**


10j in CD3OD
conversion (%)
time (h)
10j
10k'
9

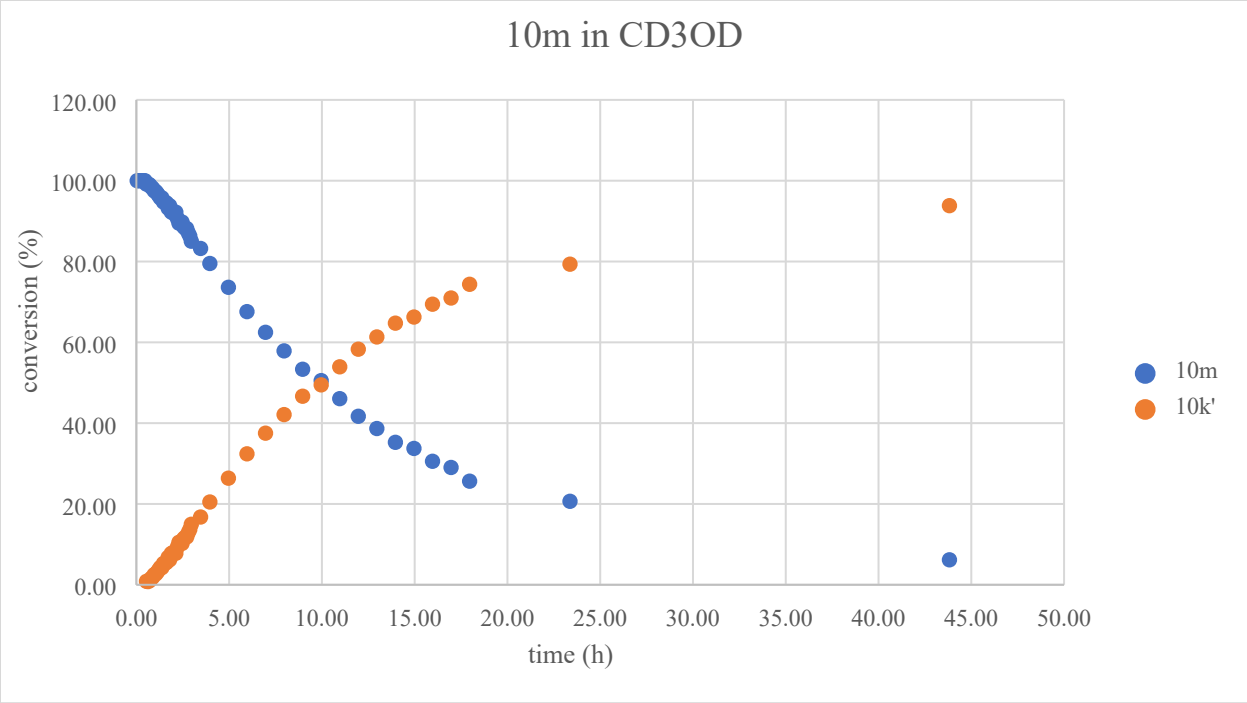


10l **9** **10k'**





10m **9** **10k'**



thia-Michael addition – reversibility study

NMR study

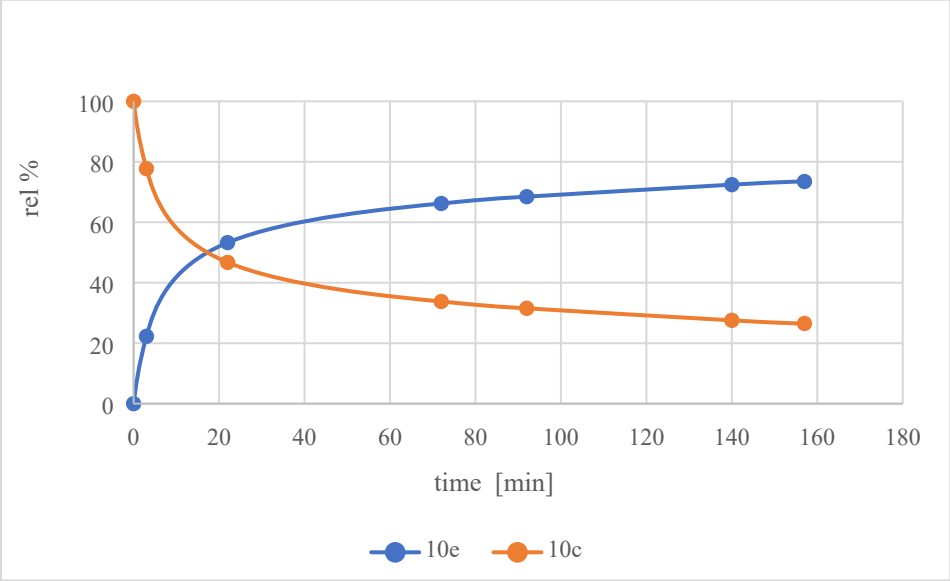
A stock solution of **9** (50mM, 0.35 mL) in DMSO-*d6* and a stock solution of 1-undecanethiol (50mM, 0.35 mL) in DMSO- *d6* were shaken in NMR tube. Within minutes of the thiol addition, a clear colorimetric shift from yellow to colorless was spotted. ^{1}H NMR spectrum of undecane thiol adduct **10o** was recorded. A stock solution of benzyl mercaptan (50mM, 0.35 mL) in DMSO-*d6* was then added and the sample was regularly monitored via NMR. No reaction was observed for 24 hours. After the addition of Et_3N (0.1 equiv. 50mM in DMSO-*d6*, 35 µL), thiol exchange was observed, and the sample was regularly monitored via NMR for 7 days.

HPLC study

[3] preparation of 1 L of solution: 8 g NaCl, 0.2 g KCl, 1.44g Na_2HPO_4, 0.245 g of KH_2PO_4 were dissolved in distilled water.

[4] Sample preparation: 20 µL of reaction mixture was dissolved in 1 mL acetonitrile, Water-acetonitrile eluent (v/v 2:3) was used, with 5 mL of Et_3N and 5 mL of 85% aqueous H_3PO_4 as additives for 1 L of the solution, pH 2.5)

10c (5.3 mg, 0.015 mmol) and *N*-acetyl-L-cysteine (11.1 mg, 0.068 mmol, 4.4 equiv.) were dissolved in DMSO (2 mL) at ambient temperature. Phosphate buffered saline[3] (1 mL, pH 7.4) was added and the mixture was stirred at 37°C. Reaction was monitored by reverse-phase HPLC.[4]

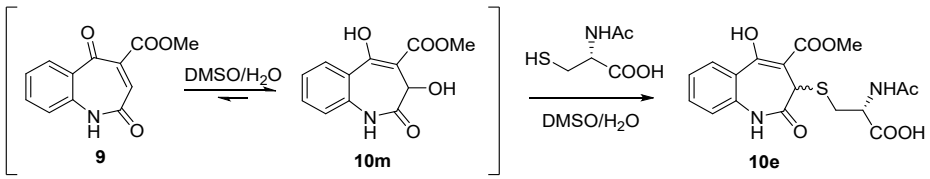


Reaction time [min]	Relative %*	
	10e	**10c**
0	0	100
3	22.27	77.73
22	53.27	46.73
72	66.21	33.79
92	68.46	31.54
140	72.44	27.56
157	73.48	26.52

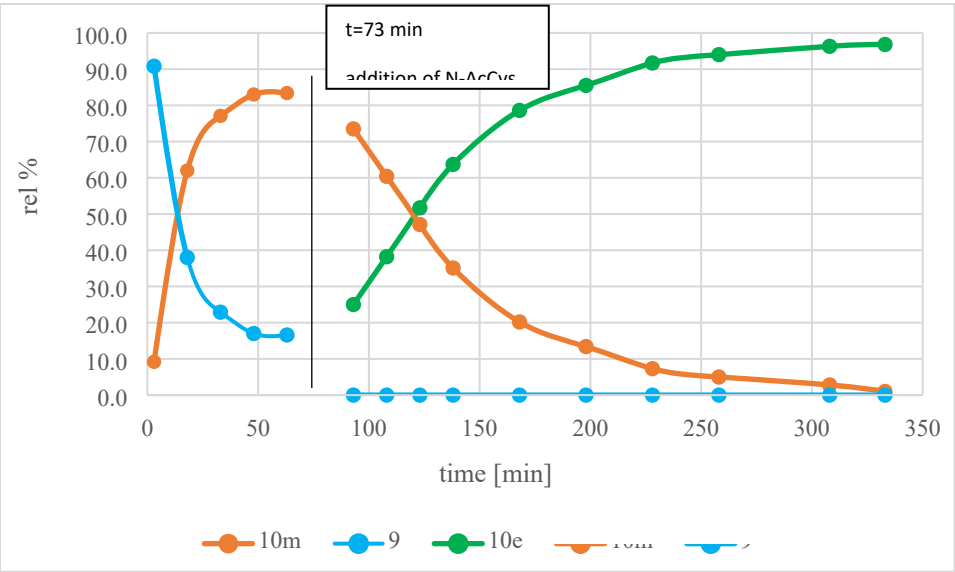
*The relative percentage of **10e** was calculated as the integral of the peak of **10e** divided by the sum of the integrals of peaks **10c** and **10e.**

oxa- versus thia-Michael addition – HPLC study

9 (4.6 mg, 0.02 mmol) was dissolved in mixture of DMSO (2 mL) and water (1 mL) at 30°C. Reaction was monitored by reverse-phase HPLC.[5] After 73 minutes *N*-acetyl-L-cysteine (3.3 mg, 0.022 mmol, 1.1 equiv.) was added and the mixture was stirred at 30°C and monitored by HPLC.



[5] Sample preparation: 100 µL of reaction mixture was dissolved in 1.5 mL acetonitrile, Water-acetonitrile eluent (v/v 1:1) was used, with 5 mL of Et_3N and 5 mL of 85% aqueous H_3PO_4 as additives for 1 L of the solution, pH 2.5)



Reaction time [min]	Relative % *		
	10m	**9**	**10e**
3	9.2	90.8	
18	62.0	38	
33	77.1	22.9	
48	83.0	17	
63	83.4	16.6	
93	74.5	0	25.5
108	61.4	0	38.6
123	48.1	0	51.9
138	36.1	0	63.9
168	20.7	0	79.3
198	13.9	0	86.1
228	7.7	0	92.3
258	5.3	0	94.7
308	3.2	0	96.8
333	1.2	0	98.8

*The relative percentage of **10m** was calculated as the integral of the peak of **10m** divided by the sum of the integrals of peaks **10m, 9** and **10e.**

X-ray single-crystal analysis of 9, 10b,c,h,i,k,l

Single-crystal diffraction data for **9** and **10b,c,h,i,k,l** were collected on a Bruker D8 VENTURE Kappa Duo diffractometer equipped with a PHOTON III detector and two IμS microfocus sealed tubes (Cu, Mo). Data were collected at 120K. The primary radiation was monochromated CuKα (λ= 1.54178 Å) for **9**, **10b,c,h,i** and MoKα (λ= 0.71073 Å) for **10k,l**. Data reduction was carried out using the diffractometer software. The phase problem was solved by intrinsic phasing (SHELXT) and the structural models were refined by full-

matrix least-squares against F^2 (SHELXL). Non-hydrogen atoms were refined anisotropically and with no constraints imposed. Hydrogen atoms were refined isotropically in idealized positions.

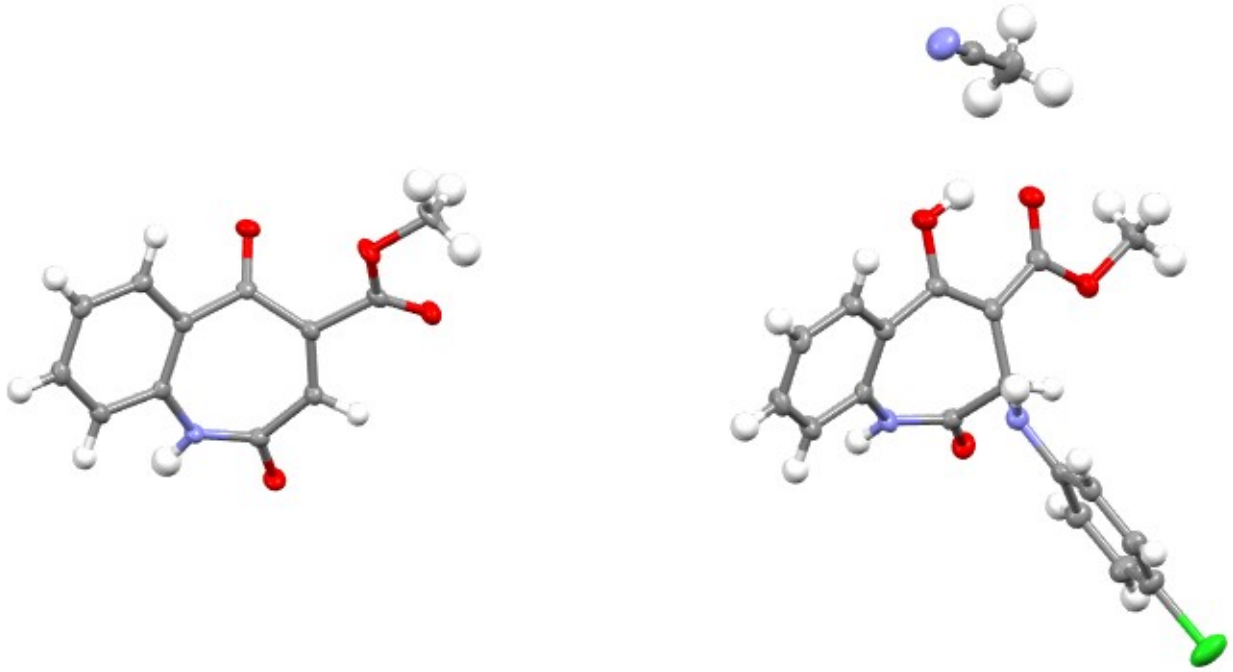


Figure 1. The molecular structure of **9** and **10b.CH_3CN** in solid state. Non-hydrogen atoms are displayed by thermal ellipsoids on 30% probability level.

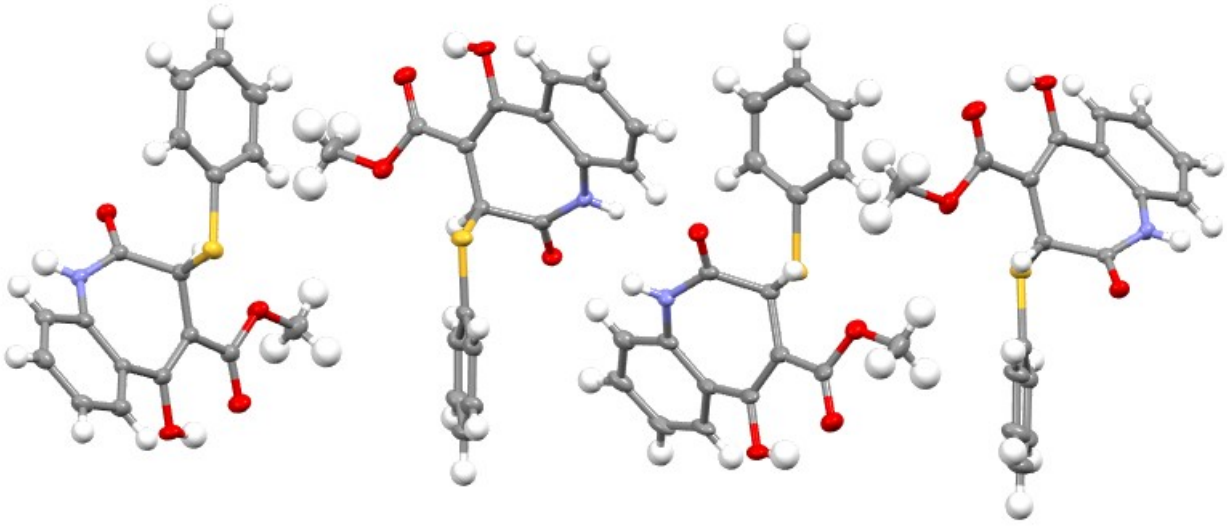


Figure 2. The molecular structure of **10c** in solid state. Non-hydrogen atoms are displayed by thermal ellipsoids on 30% probability level.

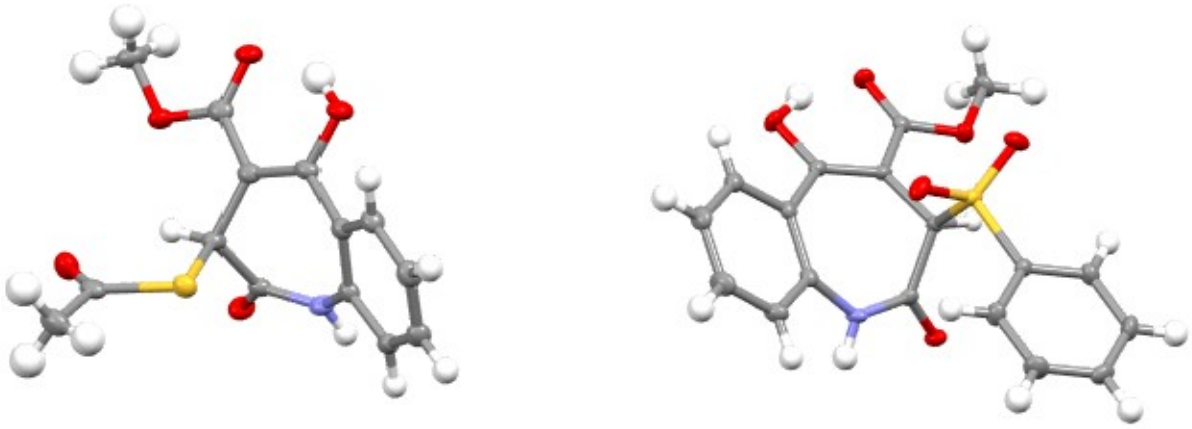


Figure 3. The molecular structure of **10h** and **10i** in solid state. Non-hydrogen atoms are displayed by thermal ellipsoids on 30% probability level.

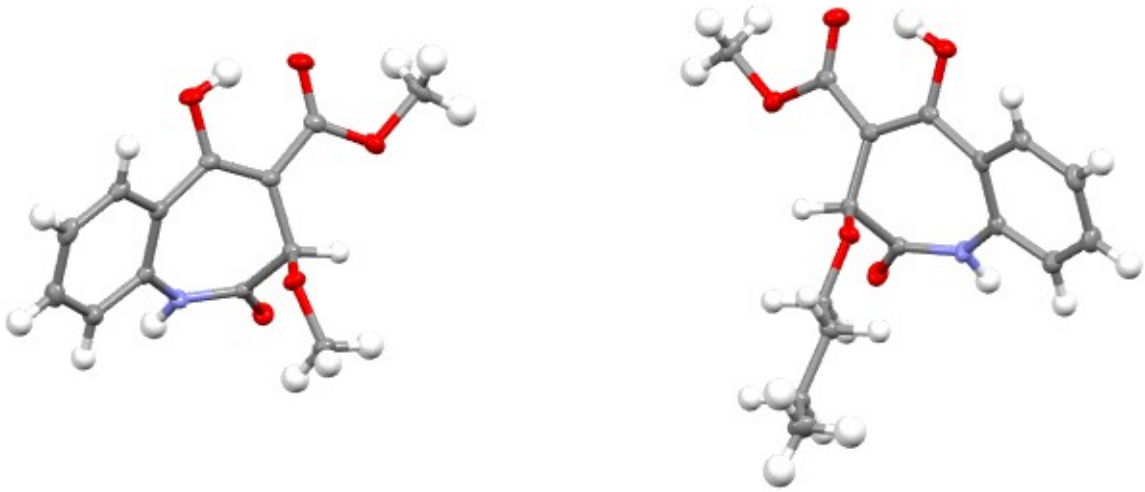
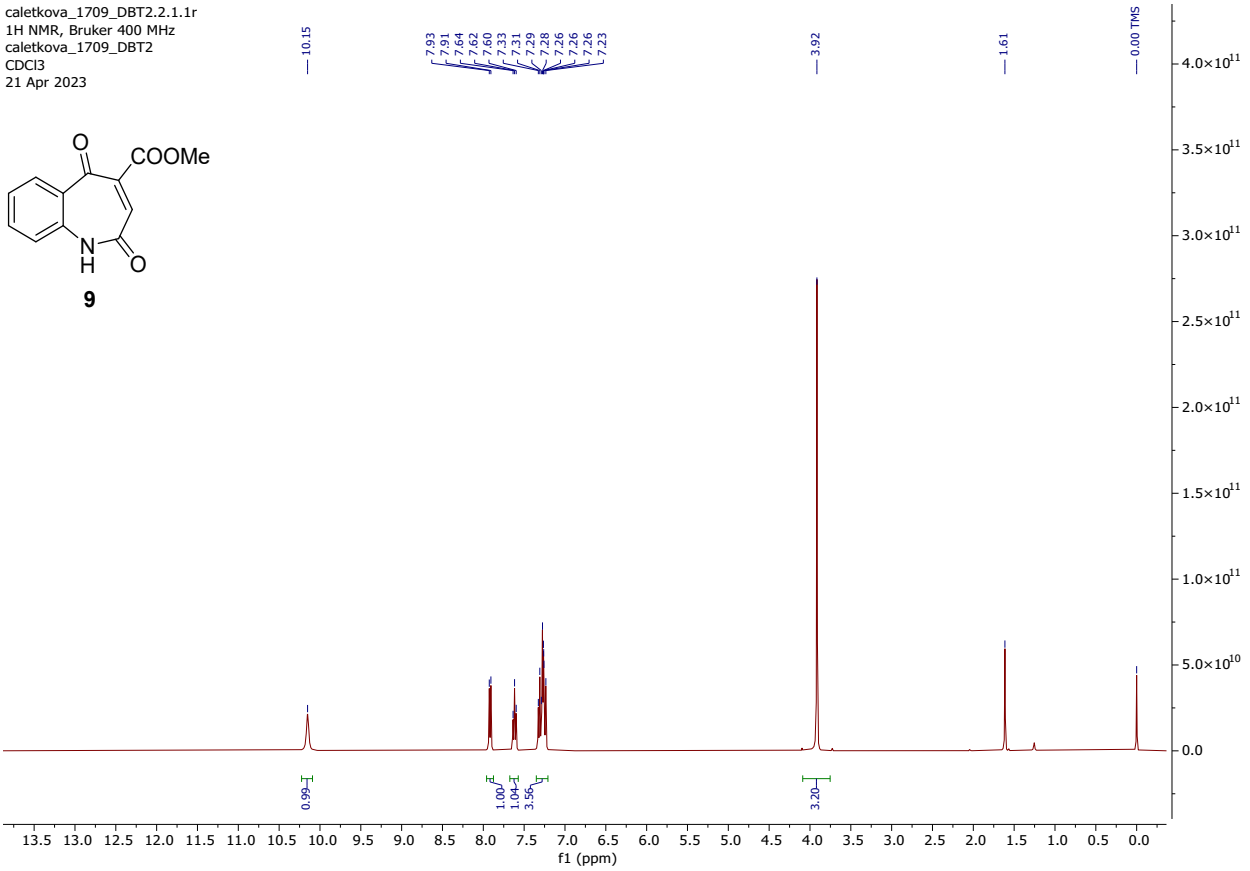
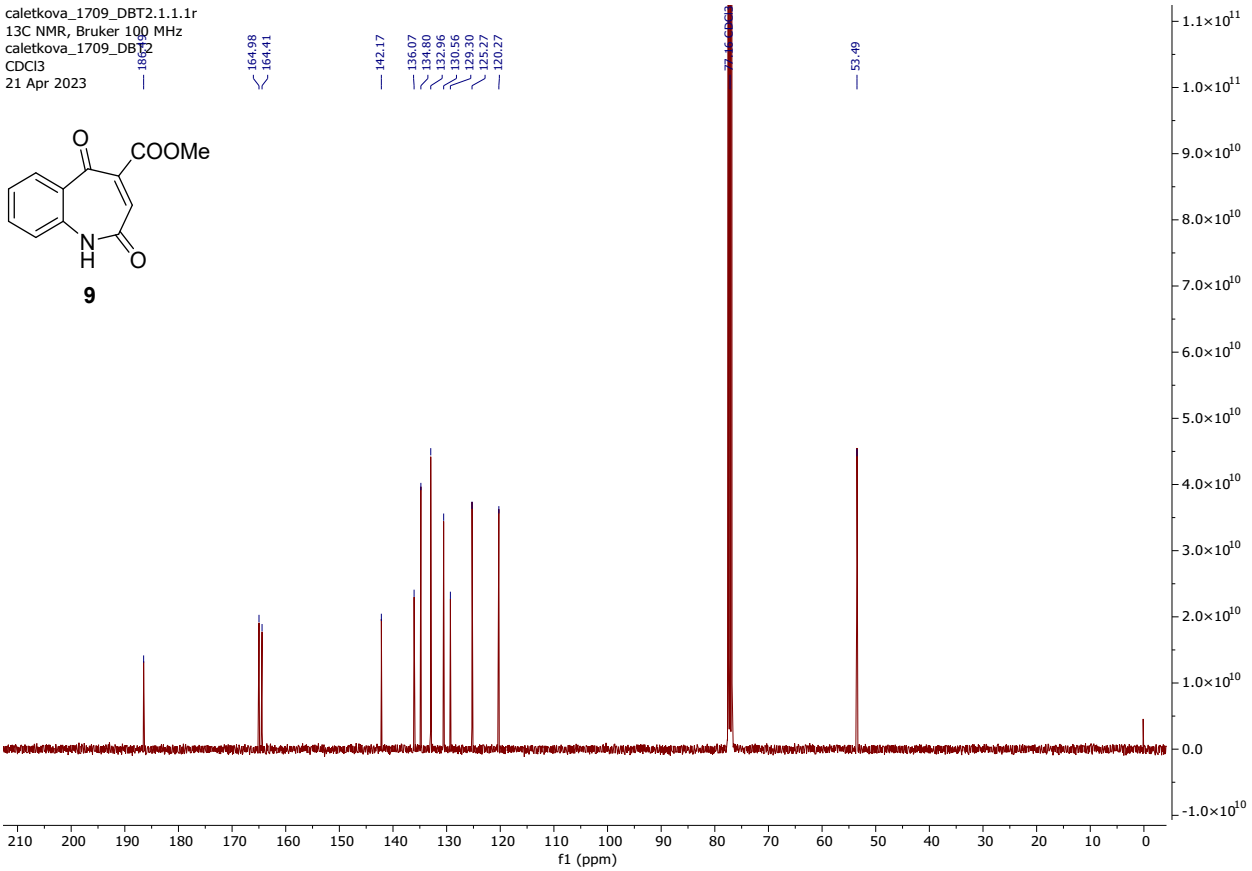
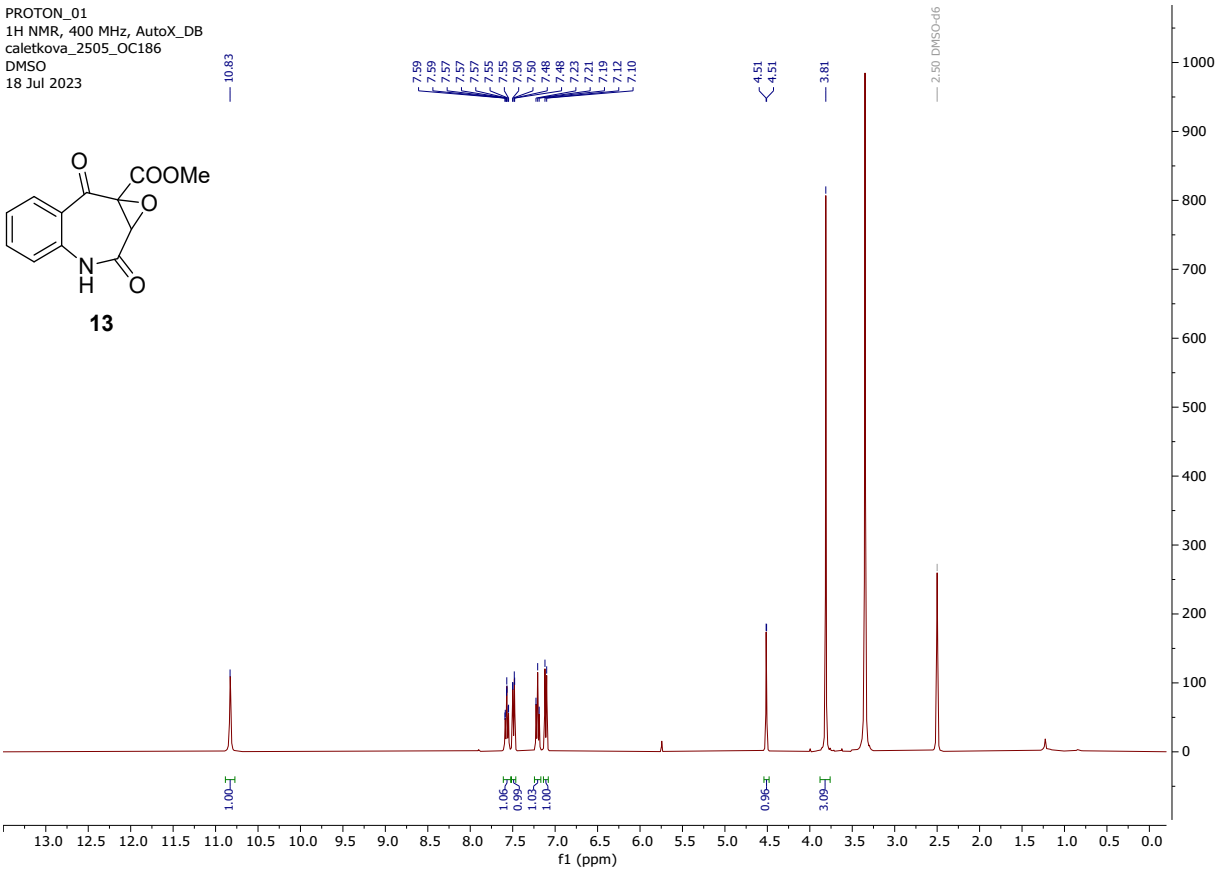


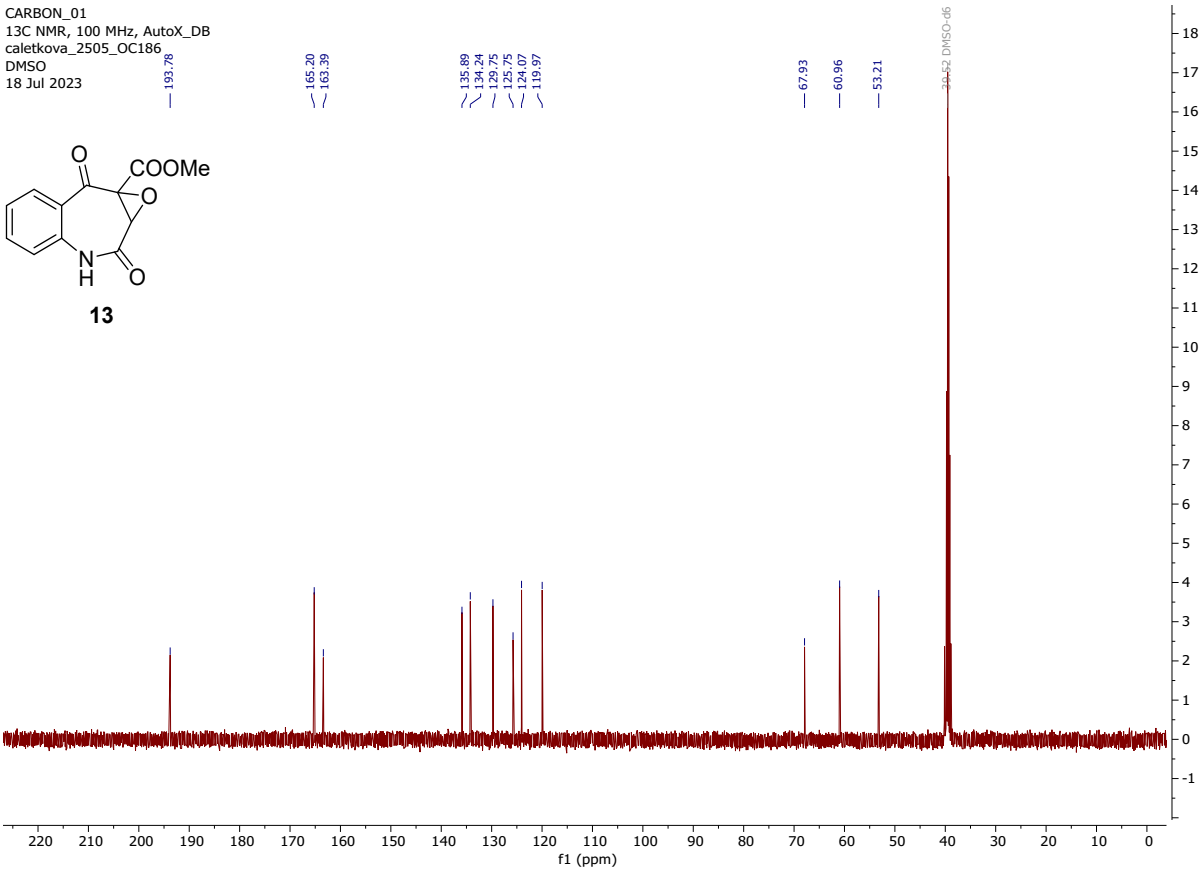
Figure 4. The molecular structure of **10k** and **10l** in solid state. Non-hydrogen atoms are displayed by thermal ellipsoids on 30% probability level.

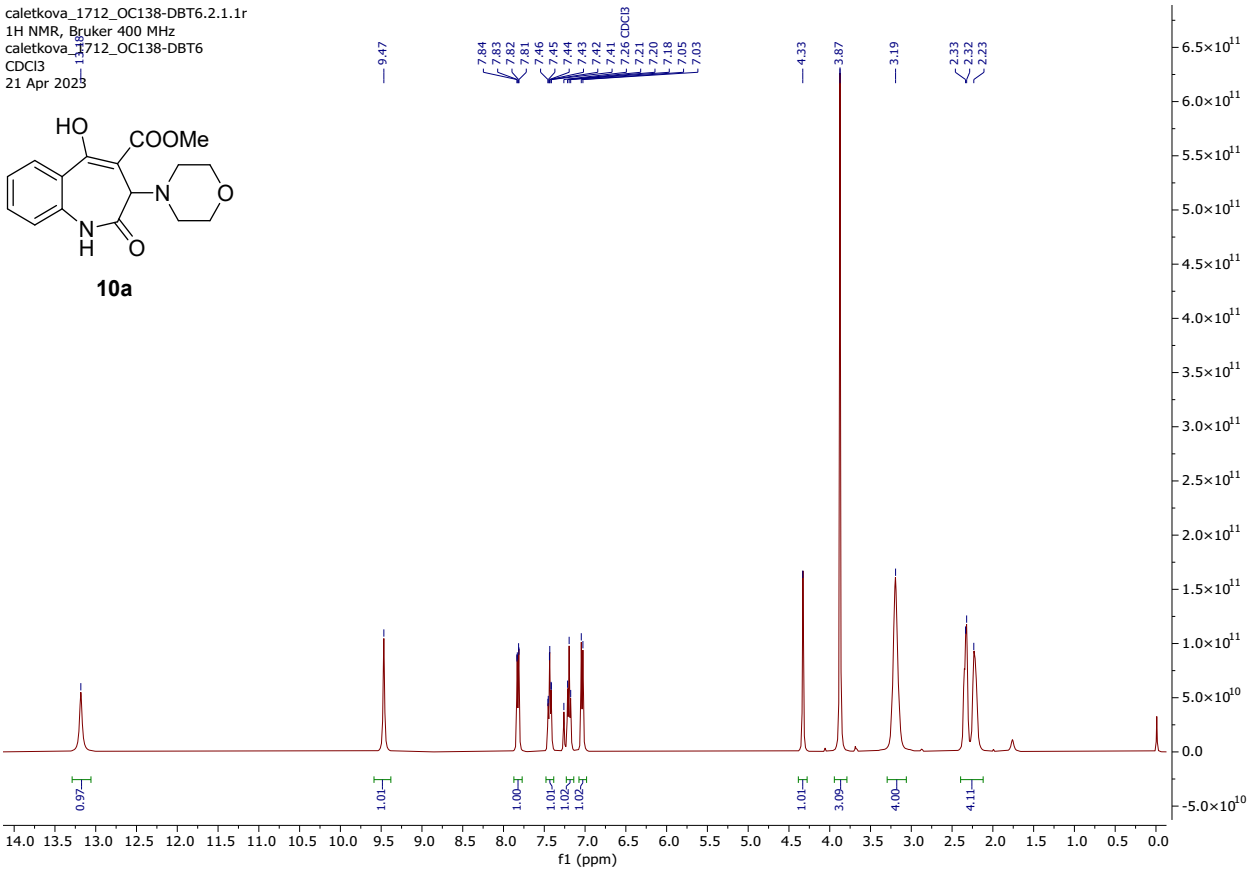
^{1}H and ^{13}C NMR spectra of prepared compounds

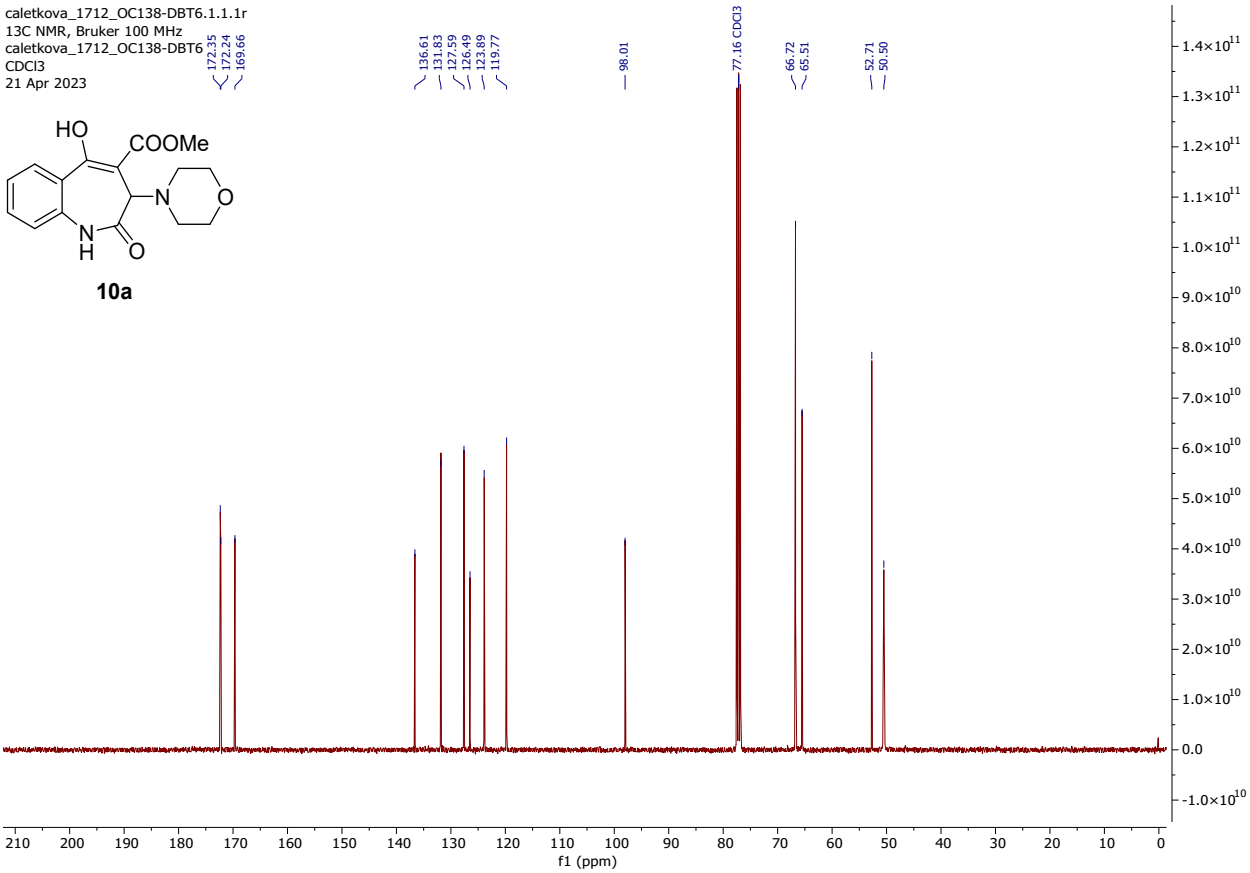


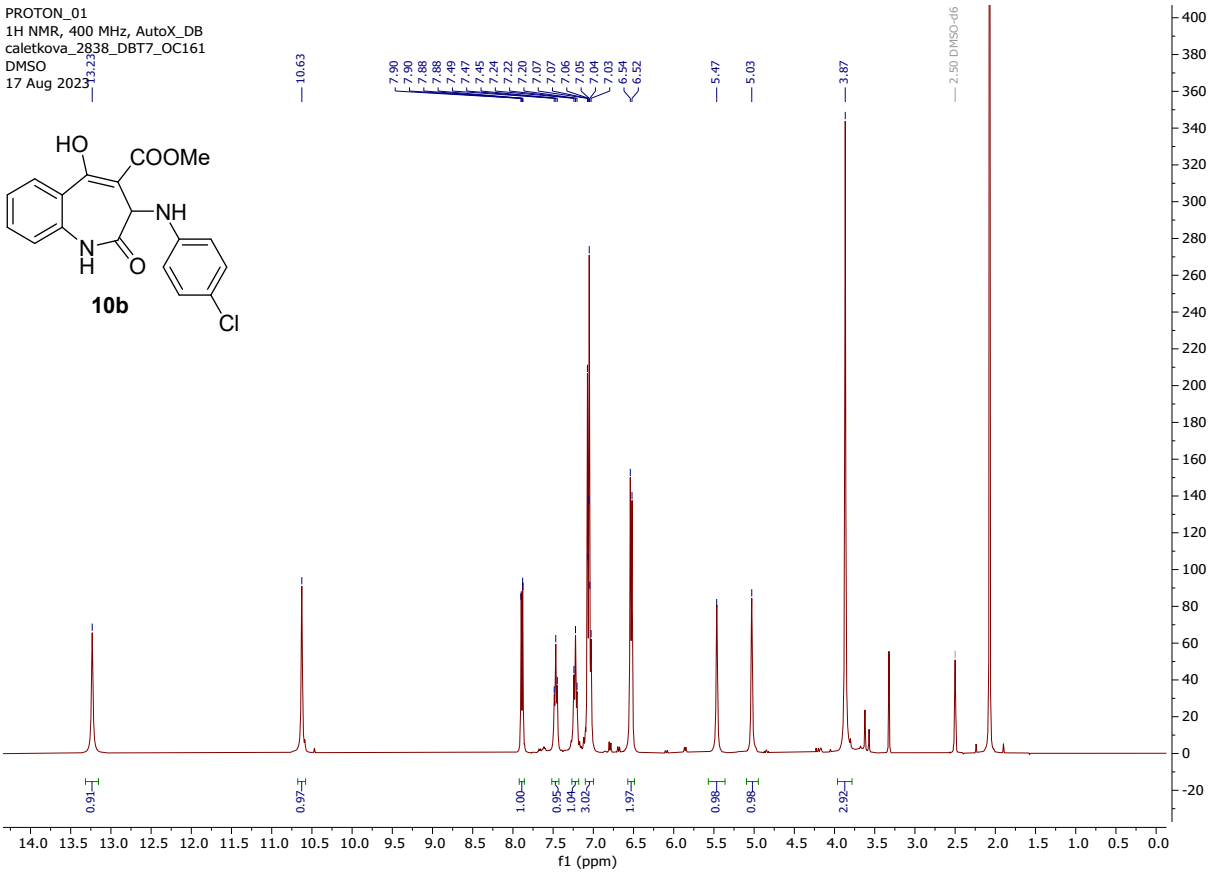

caletkova_1709_DBT2.1.1.1r
13C NMR, Bruker 100 MHz
caletkova_1709_DBT2
CDCl3
21 Apr 2023
193.45
164.98
164.41
142.17
136.07
134.80
132.96
130.56
129.30
125.27
120.27
77.16 CDCl3
53.49
O
COOMe
N
H
O
9
f1 (ppm)

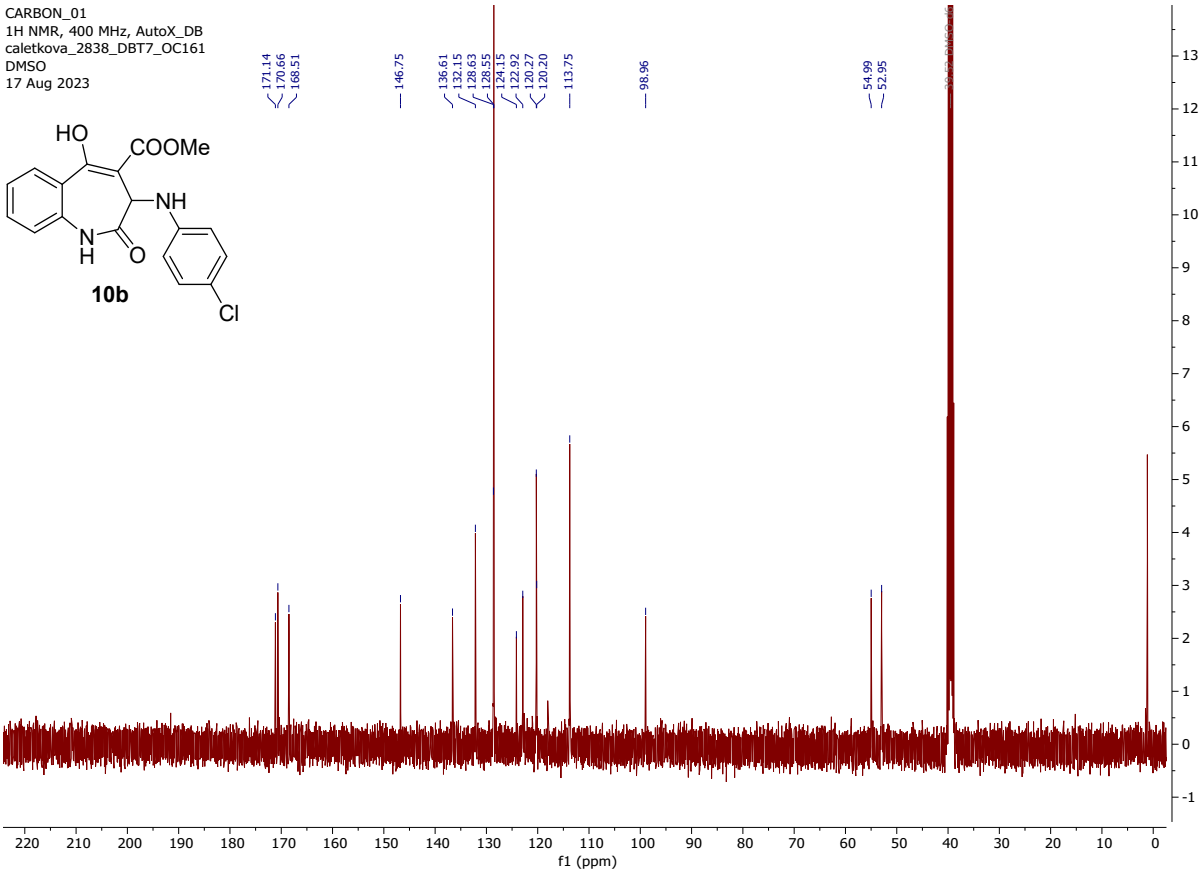

PROTON_01
1H NMR, 400 MHz, AutoX_DB
caletkova_2505_OC186
DMSO
18 Jul 2023
10.83
7.59
7.59
7.57
7.57
7.57
7.55
7.55
7.50
7.50
7.48
7.48
7.23
7.21
7.19
7.12
7.10
4.51
4.51
3.81
2.50 DMSO-d6
O
COOMe
O
N
H
O
13
1.00
1.06
0.99
1.03
1.00
0.96
3.09
f1 (ppm)

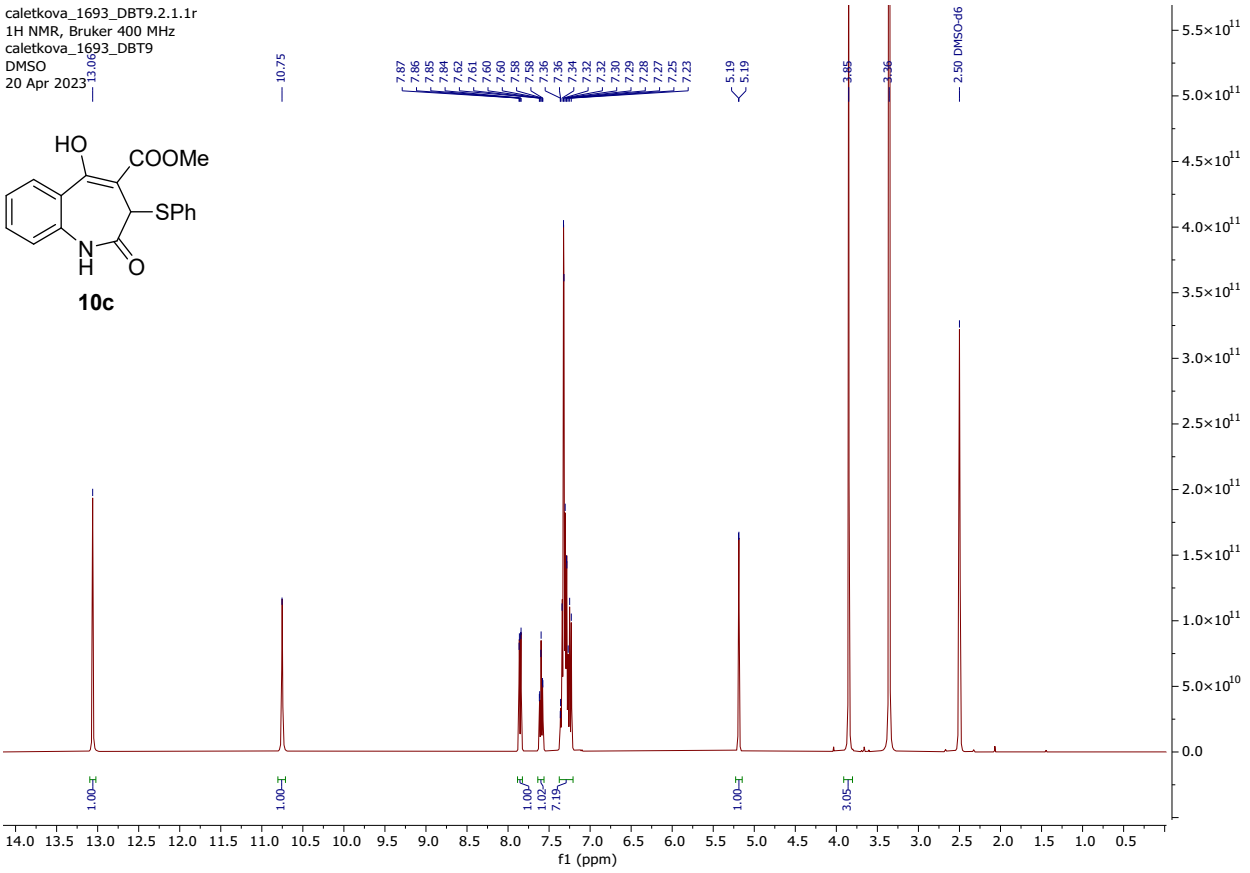

CARBON_01
13C NMR, 100 MHz, AutoX_DB
caletkova_2505_OC186
DMSO
18 Jul 2023
193.78
165.20
163.39
135.89
134.24
129.75
125.75
124.07
119.97
67.93
60.96
53.21
39.52 DMSO-d6
O
COOMe
O
N
H
O
13
f1 (ppm)

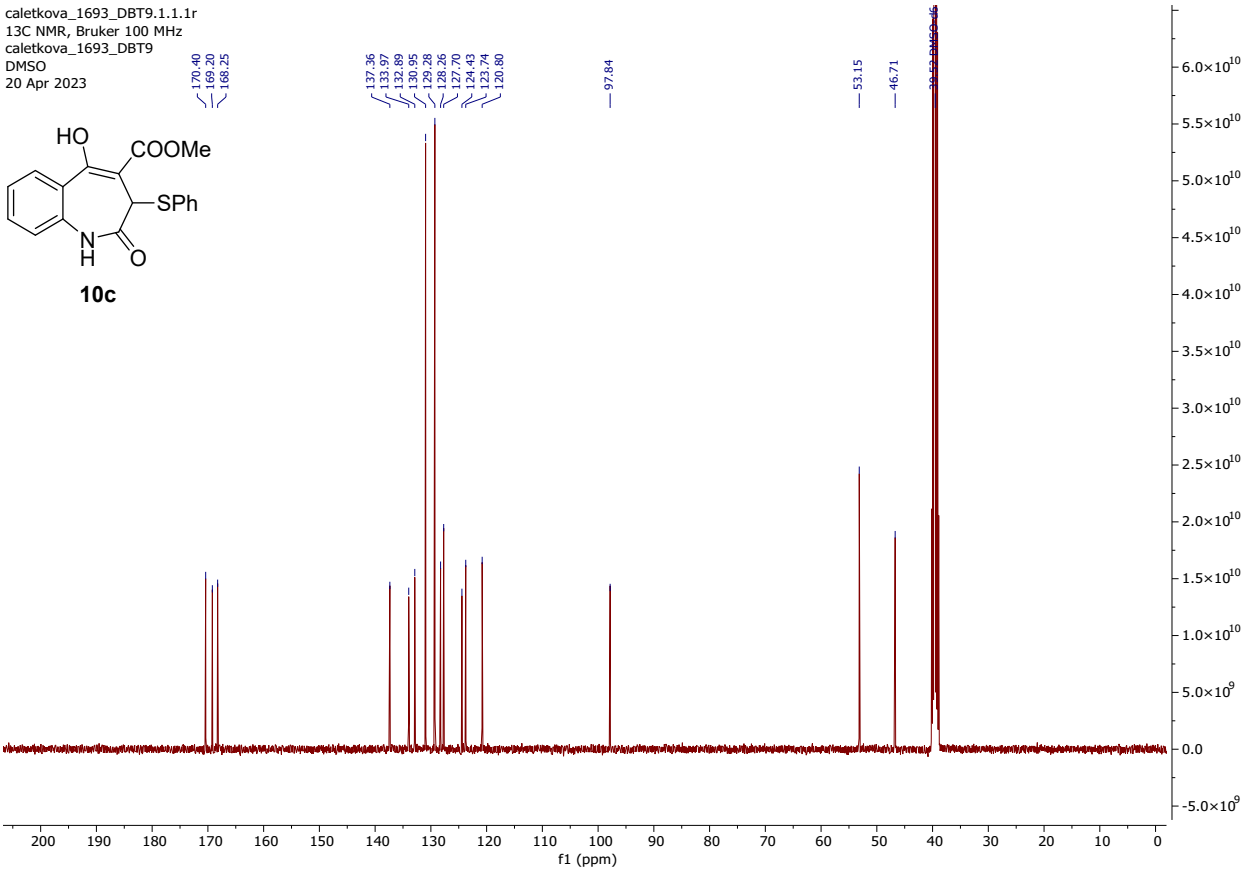

caletkova_1712_OC138-DBT6.2.1.1r
1H NMR, Bruker 400 MHz
caletkova_1712_OC138-DBT6
CDCl3
21 Apr 2023
HO
COOMe
N
O
N
H
O
10a
13.18
9.47
7.84
7.83
7.82
7.81
7.46
7.45
7.44
7.43
7.42
7.41
7.26 CDCl3
7.21
7.20
7.18
7.05
7.03
4.33
3.87
3.19
2.33
2.32
2.23
0.97
1.01
1.00
1.01
1.02
1.02
1.01
3.09
4.00
4.11
f1 (ppm)

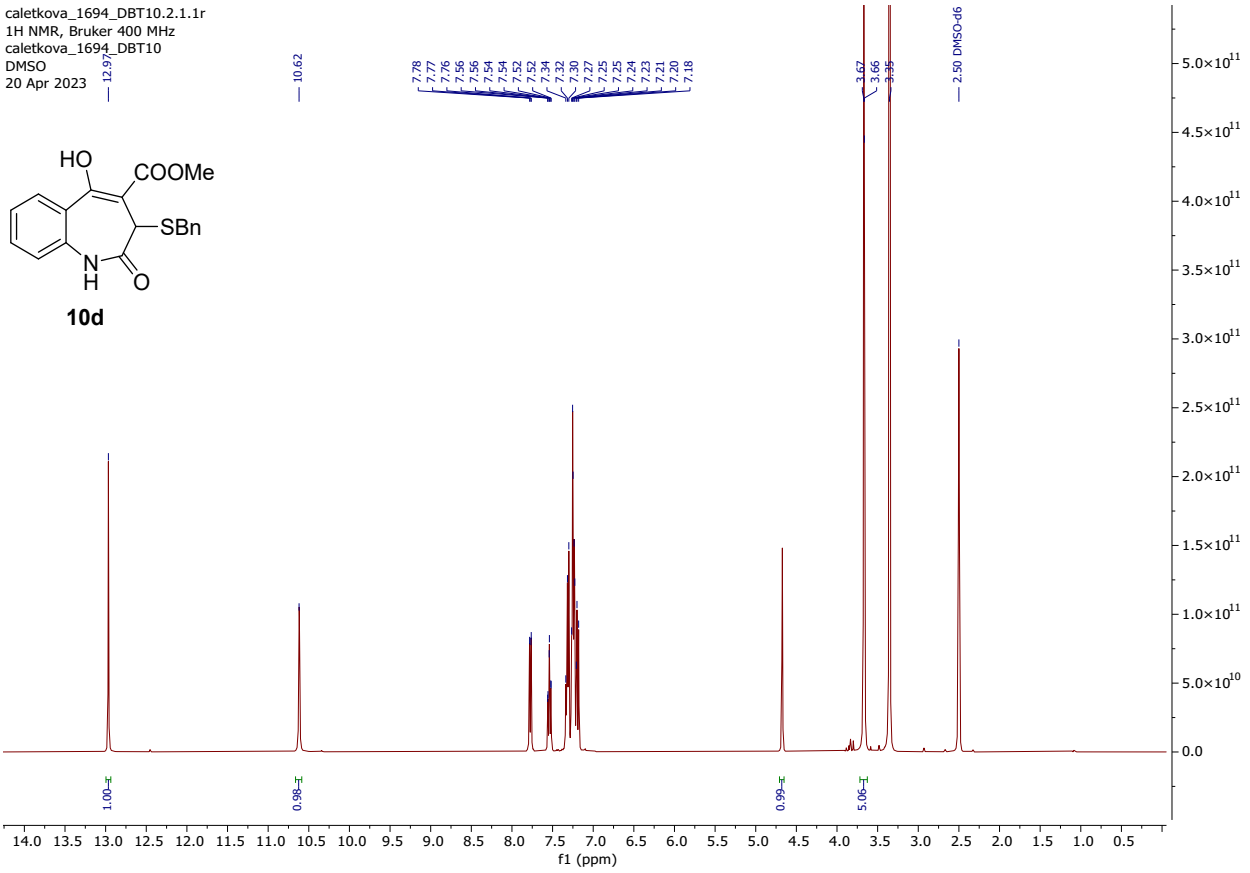

caletkova_1712_OC138-DBT6.1.1.1r
13C NMR, Bruker 100 MHz
caletkova_1712_OC138-DBT6
CDCl3
21 Apr 2023
172.35
172.24
169.66
136.61
131.83
127.59
126.49
123.89
119.77
98.01
77.16 CDCl3
66.72
65.51
52.71
50.50
HO
COOMe
N
O
N
H
O
10a
f1 (ppm)

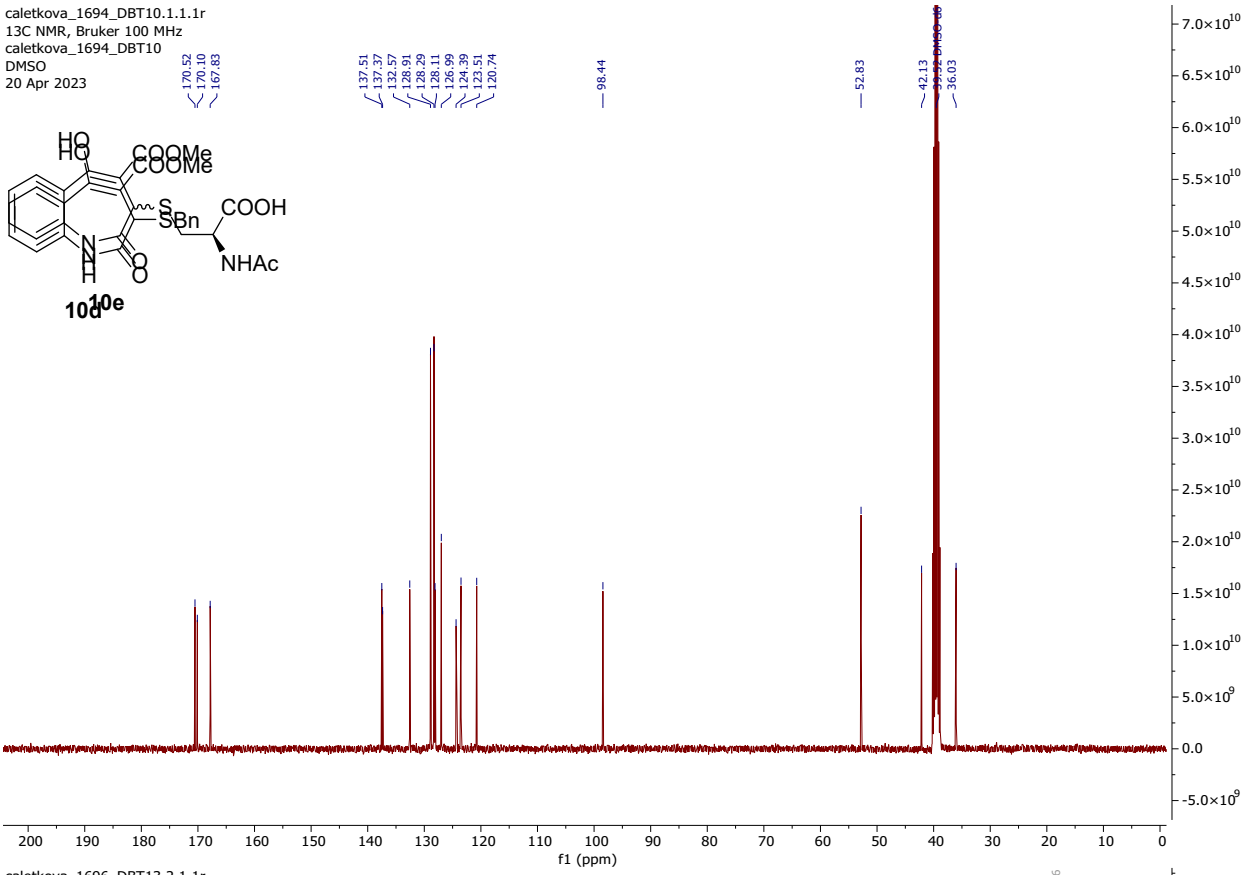

PROTON_01
1H NMR, 400 MHz, AutoX_DB
caletkova_2838_DBT7_OC161
DMSO
17 Aug 2023
HO
COOMe
NH
N
H
O
Cl
10b
13.23
10.63
7.90
7.90
7.88
7.88
7.49
7.47
7.45
7.24
7.22
7.20
7.07
7.07
7.06
7.05
7.04
7.03
6.54
6.52
5.47
5.03
3.87
2.50 DMSO-d6
0.91
0.97
1.00
0.95
1.04
3.02
1.97
0.98
0.98
2.92
f1 (ppm)

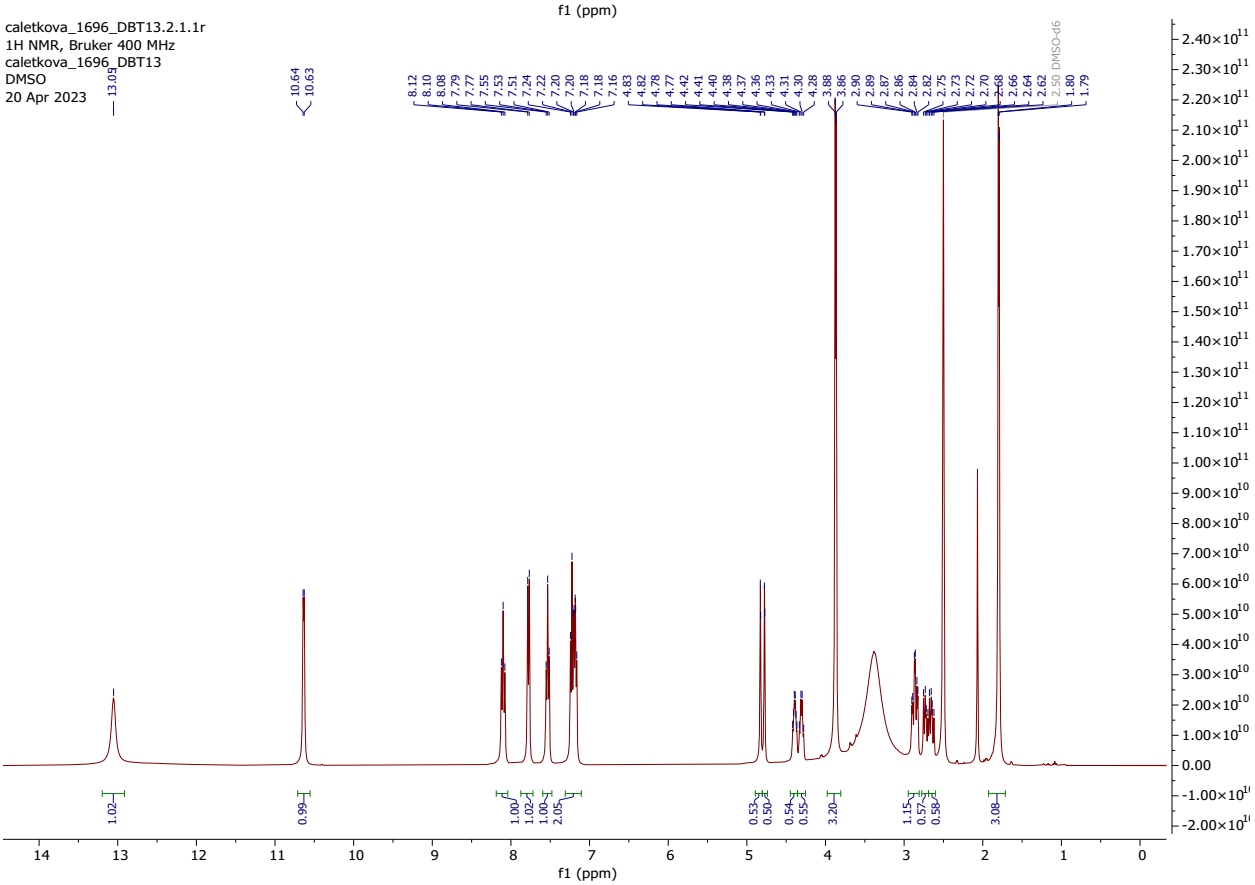

CARBON_01
1H NMR, 400 MHz, AutoX_DB
caletkova_2838_DBT7_OC161
DMSO
17 Aug 2023
HO
COOMe
NH
N
H
O
Cl
10b
171.14
170.66
168.51
146.75
136.61
132.15
128.63
128.55
124.15
122.92
120.27
120.20
113.75
98.96
54.99
52.95
f1 (ppm)

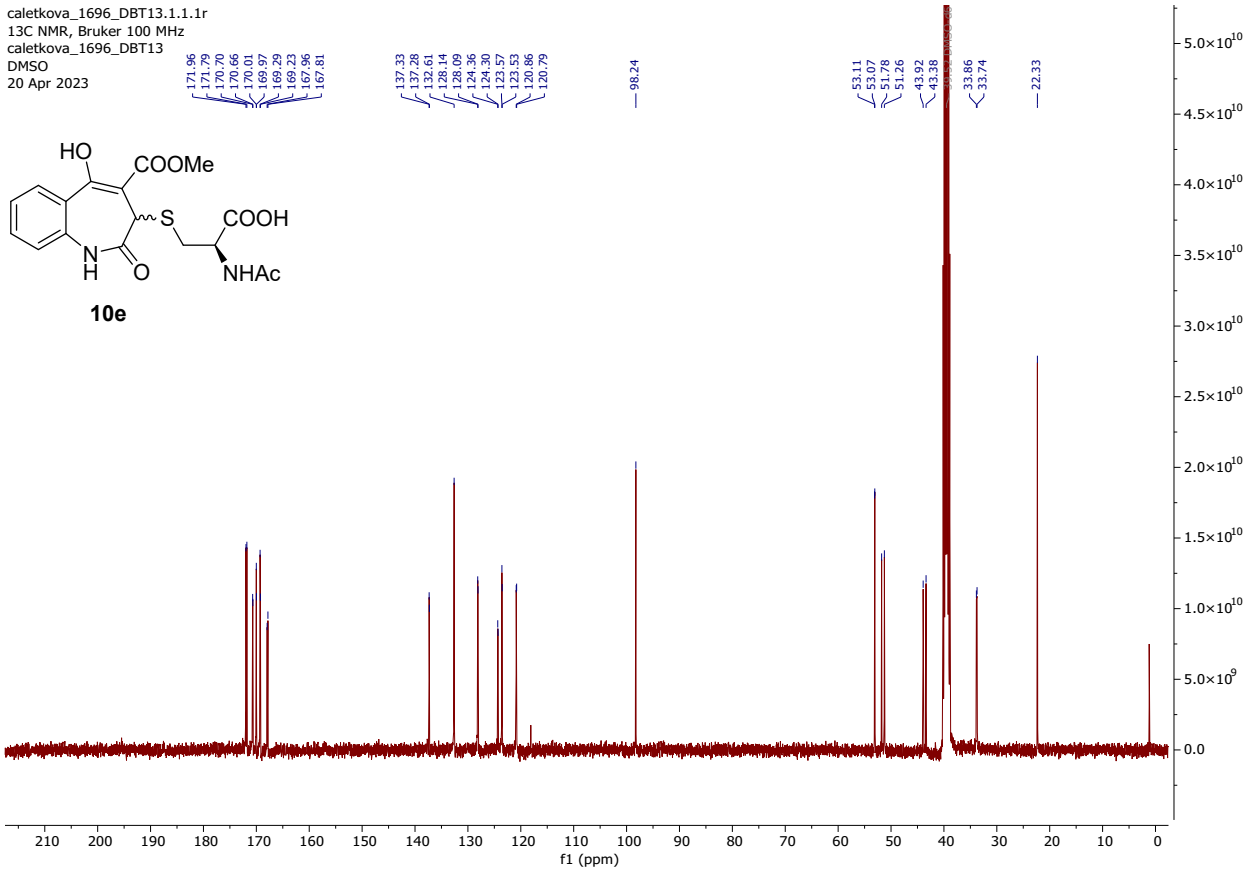

caletkova_1693_DBT9.2.1.1r
1H NMR, Bruker 400 MHz
caletkova_1693_DBT9
DMSO
20 Apr 2023
HO
COOMe
SPh
N
H
O
10c
13.06
10.75
7.87
7.86
7.85
7.84
7.62
7.61
7.60
7.60
7.58
7.58
7.36
7.34
7.32
7.32
7.30
7.29
7.28
7.27
7.25
7.23
5.19
5.19
3.85
3.36
2.50 DMSO-d6
1.00
1.00
1.00
1.02
7.19
1.00
3.05
f1 (ppm)

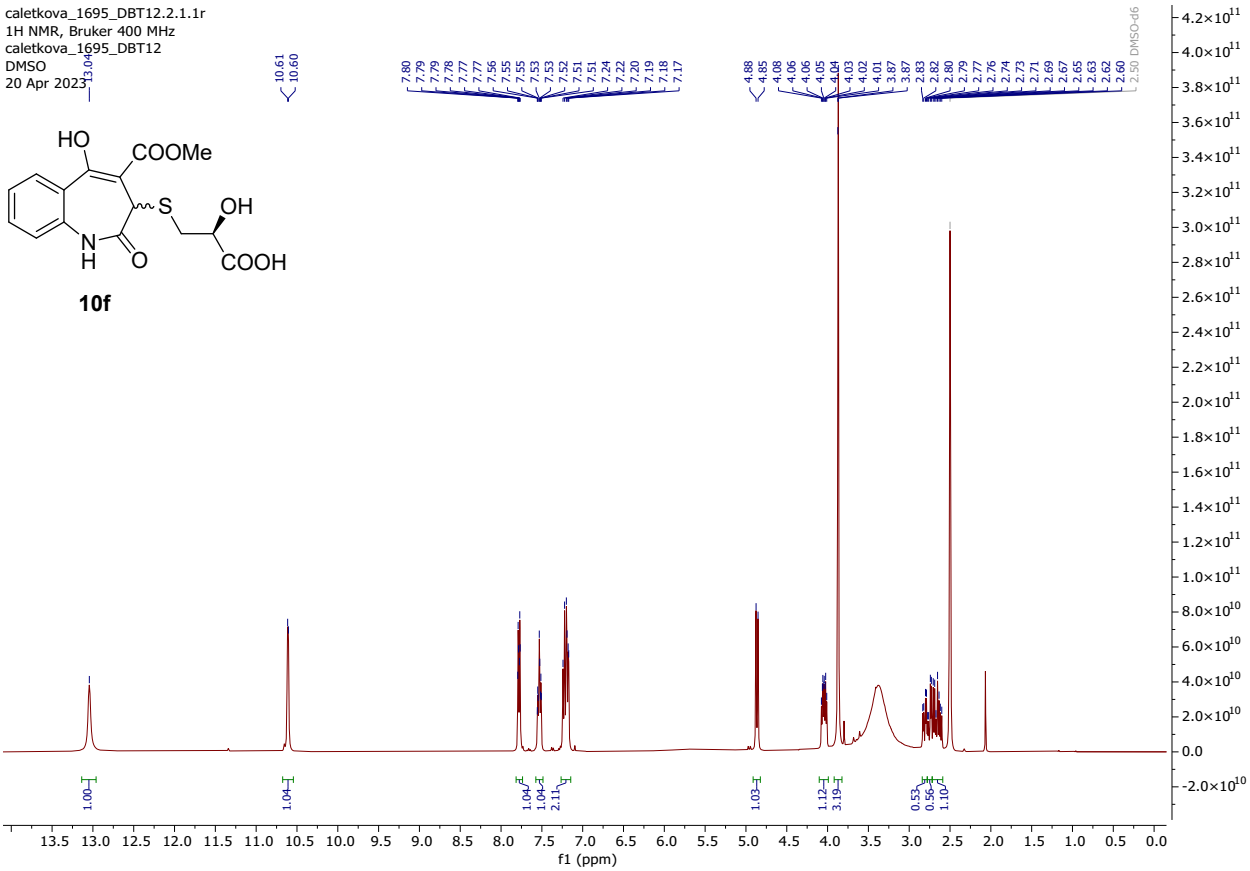

caletkova_1693_DBT9.1.1.1r
13C NMR, Bruker 100 MHz
caletkova_1693_DBT9
DMSO
20 Apr 2023
170.40
169.20
168.25
137.36
133.97
132.89
130.95
129.28
128.26
127.70
124.43
123.74
120.80
97.84
53.15
46.71
HO
COOMe
SPh
N
H
O
10c
f1 (ppm)

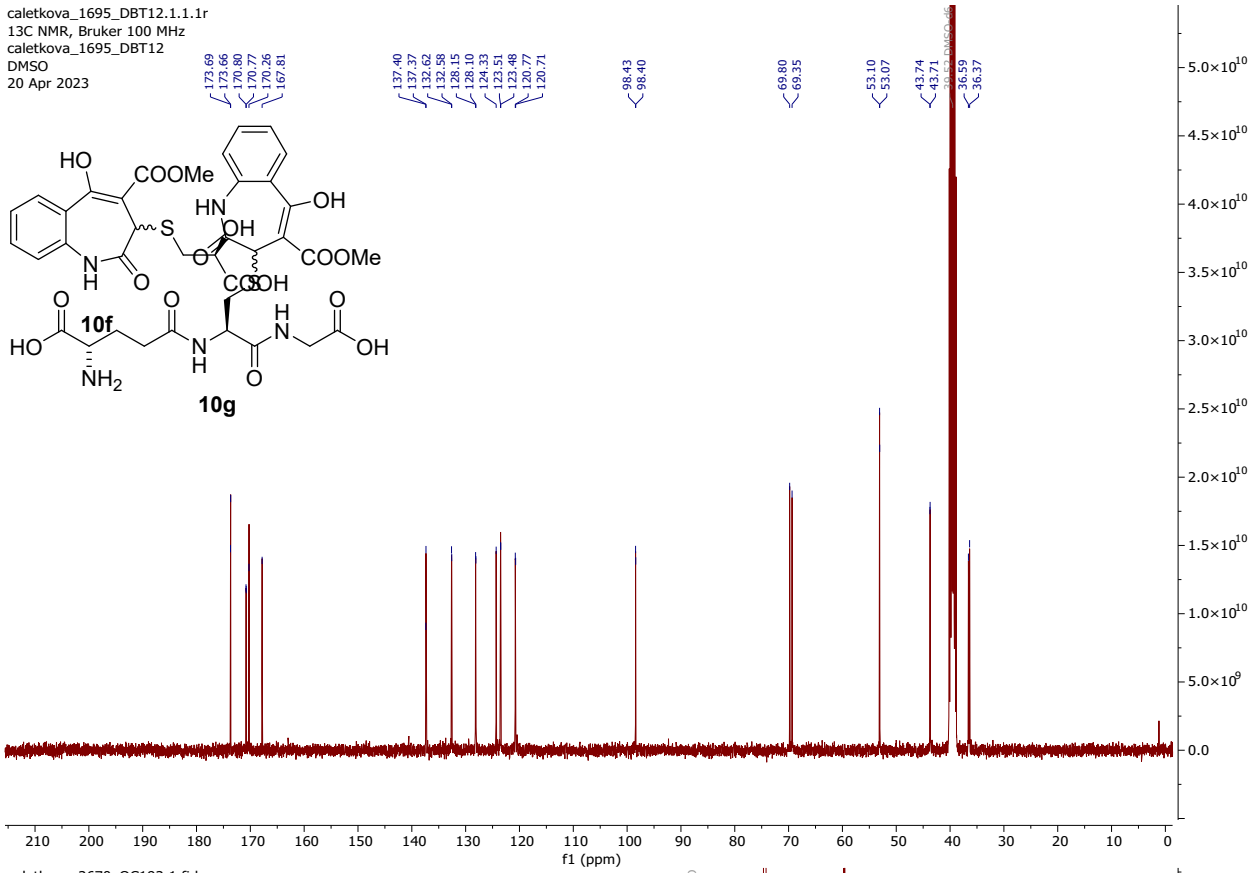

caletkova_1694_DBT10.2.1.1r
1H NMR, Bruker 400 MHz
caletkova_1694_DBT10
DMSO
20 Apr 2023
12.97
10.62
7.78
7.77
7.76
7.56
7.56
7.54
7.54
7.52
7.52
7.34
7.32
7.30
7.27
7.25
7.25
7.24
7.23
7.21
7.20
7.18
3.67
3.66
3.35
2.50 DMSO-d6
HO
COOMe
SBn
N
H
O
10d
1.00
0.98
0.99
5.06
f1 (ppm)

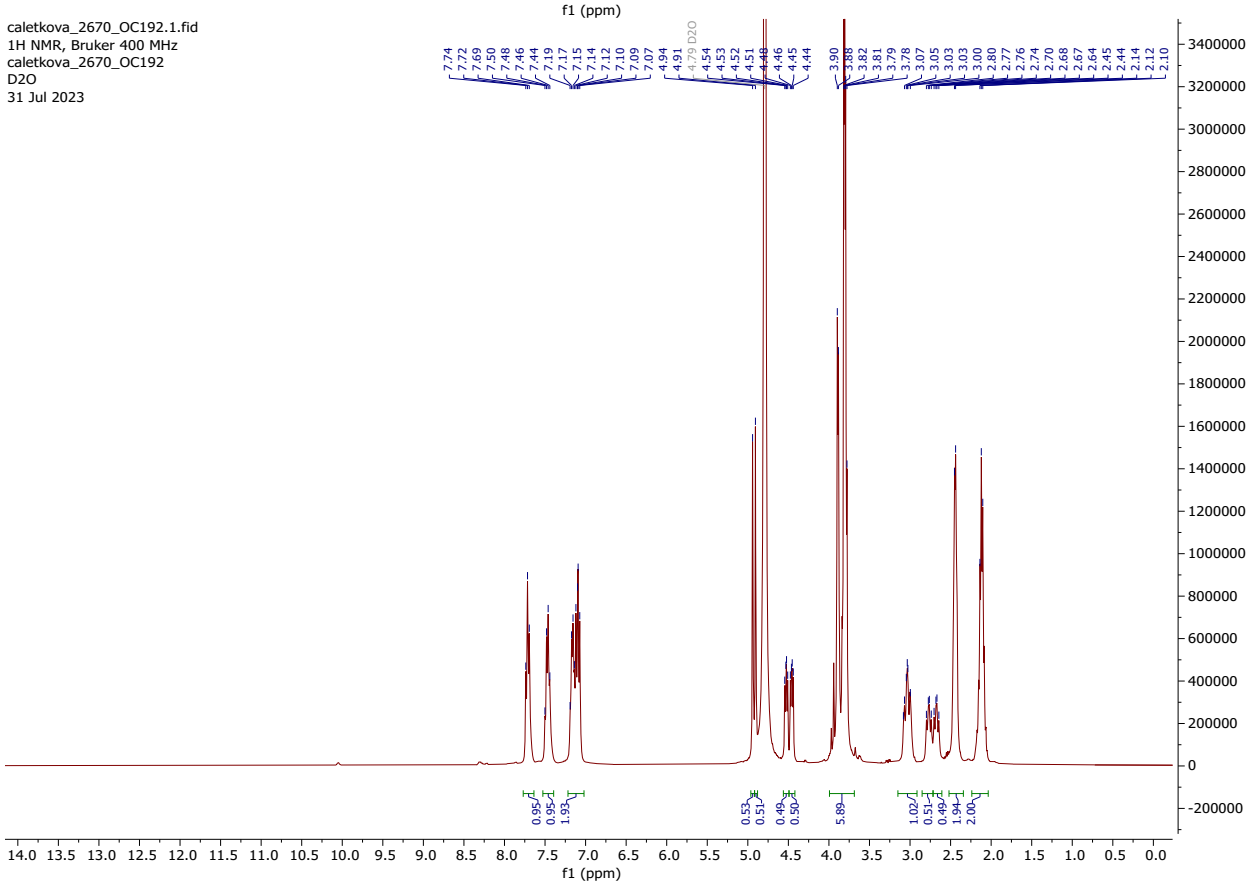

caletkova_1694_DBT10.1.1.1r
13C NMR, Bruker 100 MHz
caletkova_1694_DBT10
DMSO
20 Apr 2023
170.52
170.10
167.83
137.51
137.37
132.57
128.91
128.29
128.11
126.99
124.39
123.51
120.74
98.44
52.83
42.13
39.52 DMSO-d6
36.03
HO
HO
COOMe
COOMe
S
SBn
COOH
N
H
NHAc
O
O
10d
10e
f1 (ppm)


caletkova_1696_DBT13.2.1.1r
1H NMR, Bruker 400 MHz
caletkova_1696_DBT13
DMSO
20 Apr 2023
13.09
10.64
10.63
8.12
8.10
8.08
7.79
7.77
7.55
7.53
7.51
7.24
7.22
7.20
7.20
7.18
7.16
4.83
4.82
4.78
4.77
4.42
4.41
4.40
4.38
4.37
4.36
4.33
4.31
4.30
4.28
3.88
3.86
2.90
2.89
2.87
2.86
2.84
2.82
2.75
2.73
2.72
2.70
2.68
2.66
2.64
2.62
2.50 DMSO-d6
1.80
1.79
1.02
0.99
1.00
1.02
1.00
2.05
0.53
0.50
0.54
0.55
3.20
1.15
0.57
0.58
3.08
f1 (ppm)

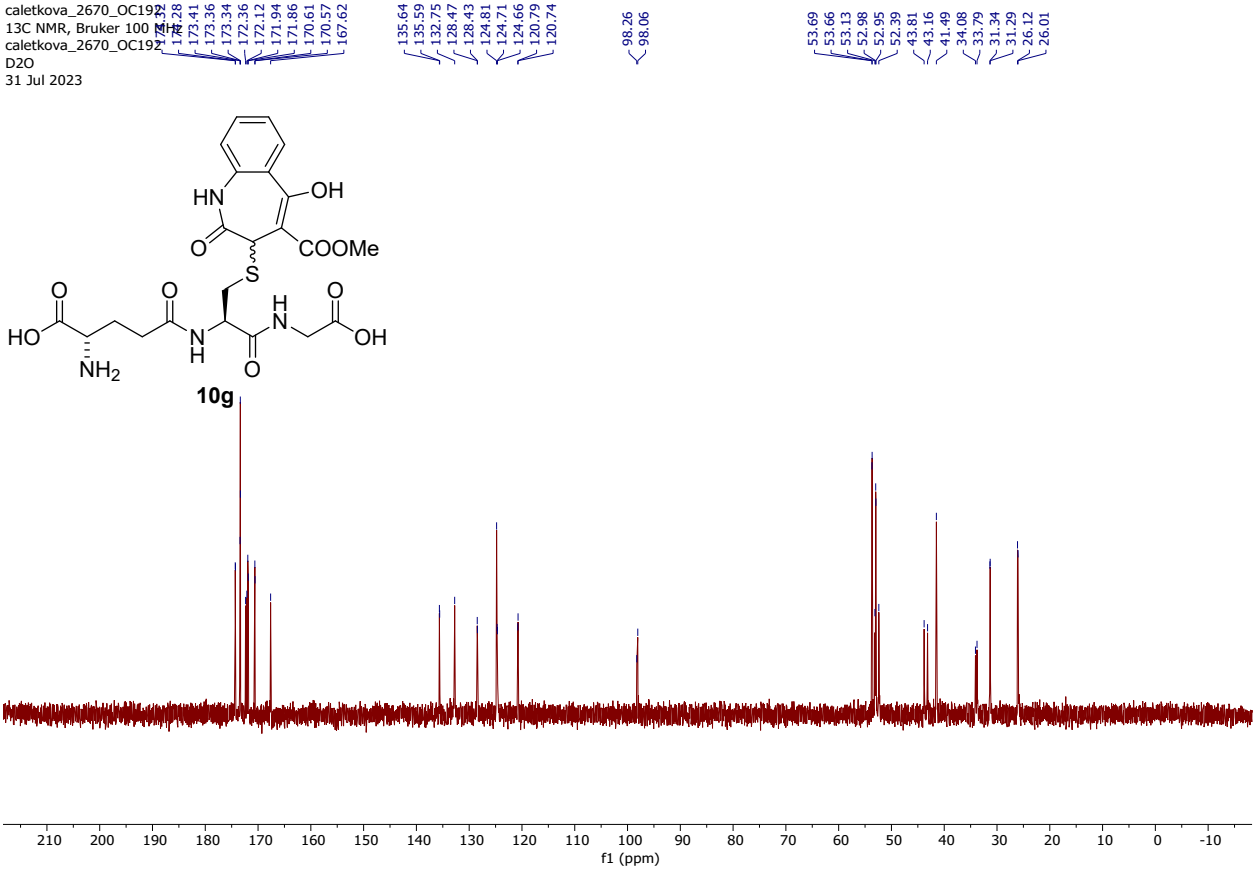

caletkova_1696_DBT13.1.1.1r
13C NMR, Bruker 100 MHz
caletkova_1696_DBT13
DMSO
20 Apr 2023
171.96
171.79
170.70
170.66
170.01
169.97
169.29
169.23
167.96
167.81
137.33
137.28
132.61
128.14
128.09
124.36
124.30
123.57
123.53
120.86
120.79
98.24
53.11
53.07
51.78
51.26
43.92
43.38
33.86
33.74
22.33
HO
COOMe
S
COOH
N
H
O
NHAc
10e
f1 (ppm)

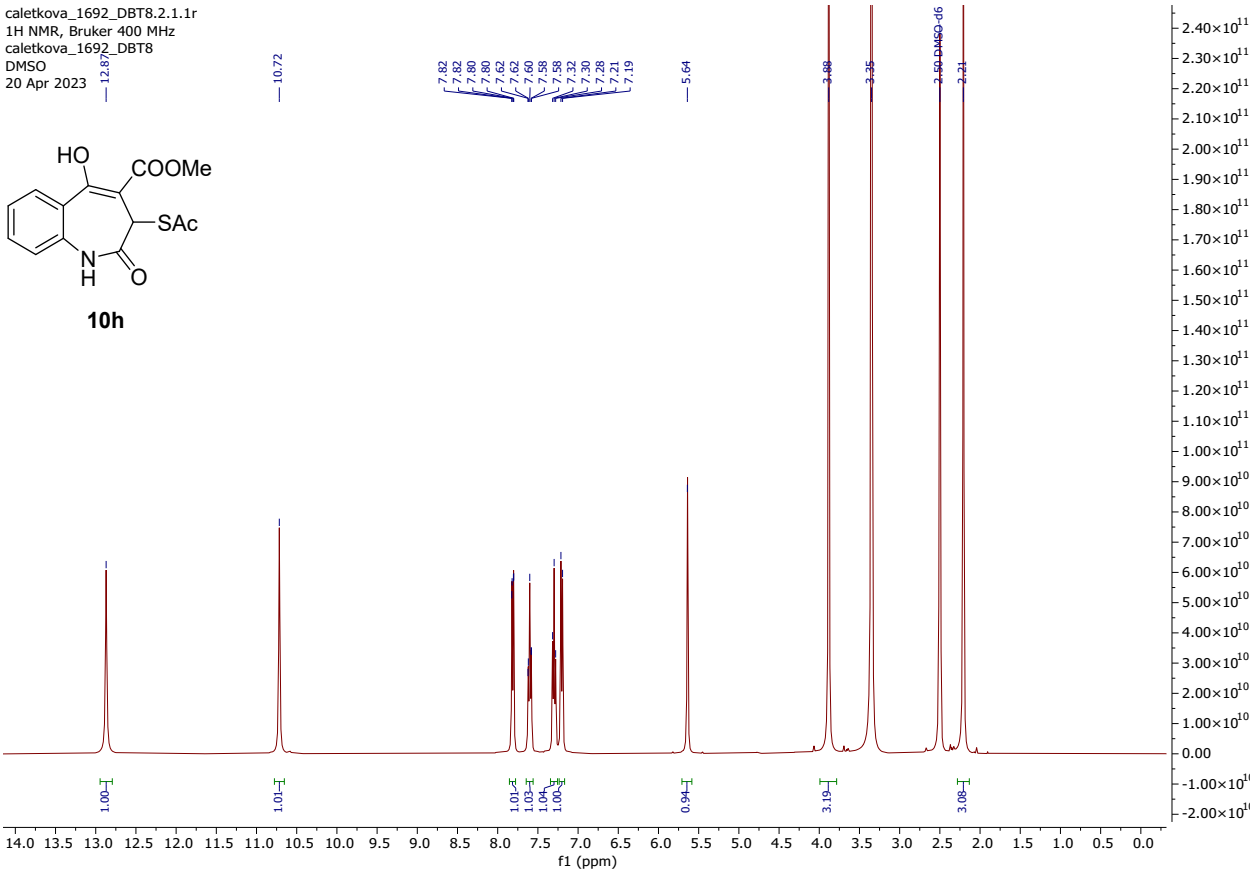

caletkova_1695_DBT12.2.1.1r
1H NMR, Bruker 400 MHz
caletkova_1695_DBT12
DMSO
20 Apr 2023
HO
COOMe
S
OH
N
H
O
COOH
10f
f1 (ppm)

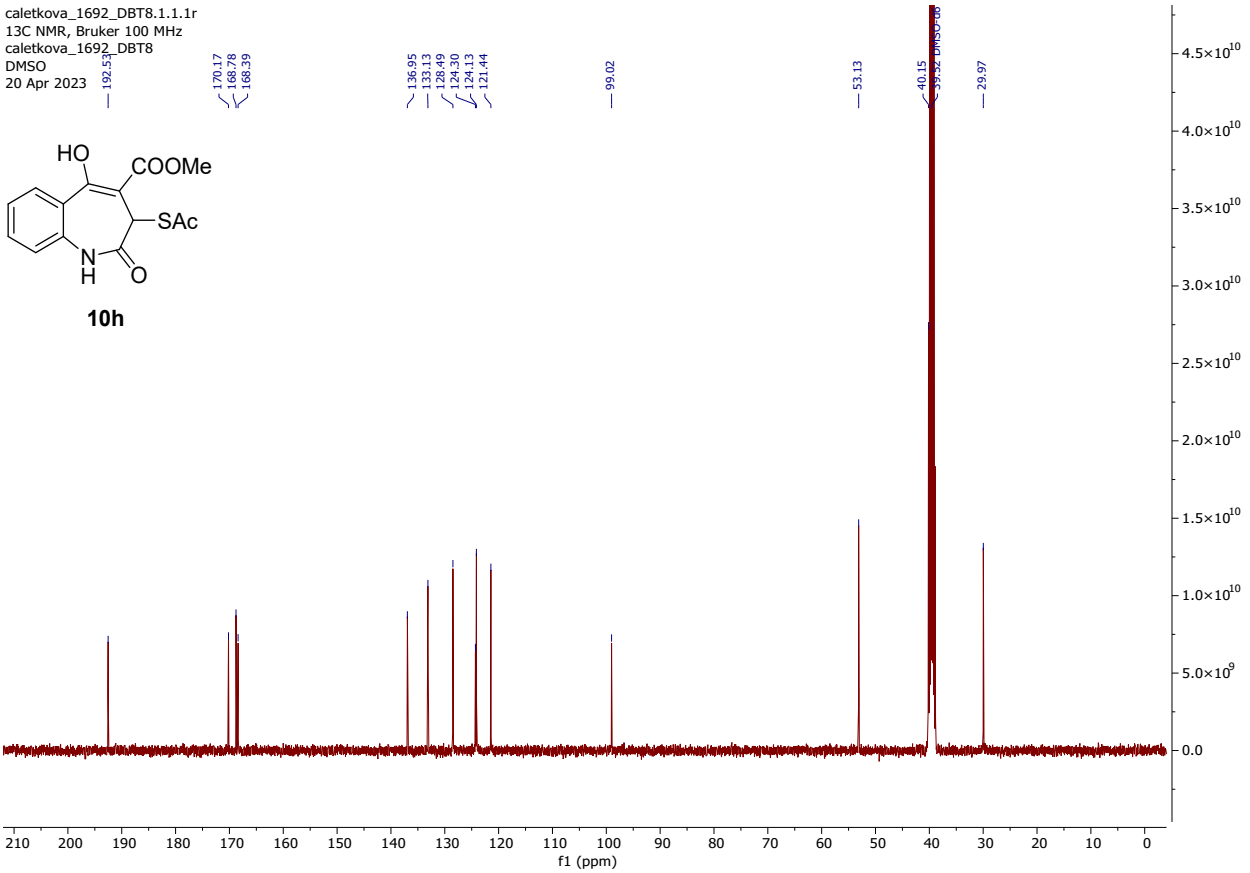

caletkova_1695_DBT12.1.1.1r
13C NMR, Bruker 100 MHz
caletkova_1695_DBT12
DMSO
20 Apr 2023
10f
10g
f1 (ppm)

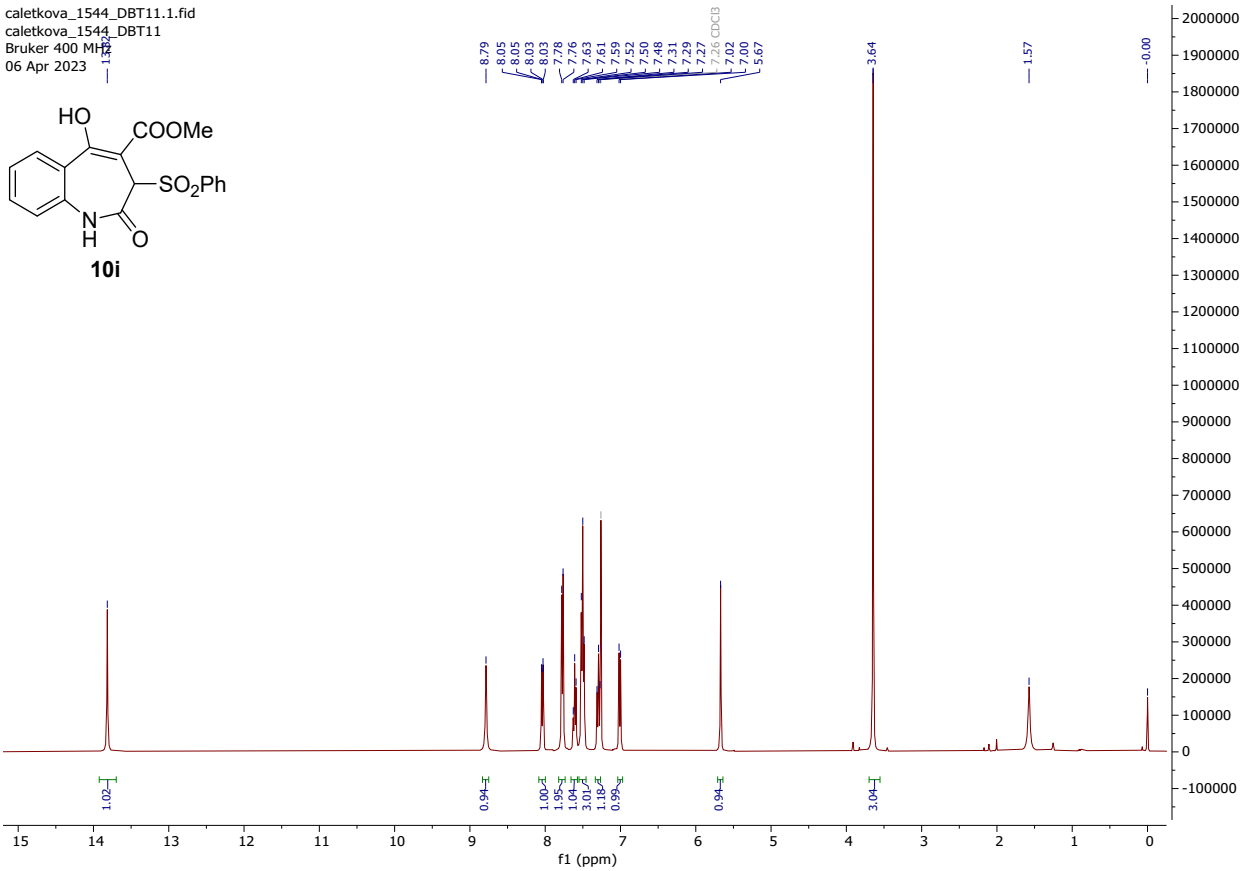

caletkova_2670_OC192.1.fid
1H NMR, Bruker 400 MHz
caletkova_2670_OC192
D2O
31 Jul 2023
f1 (ppm)

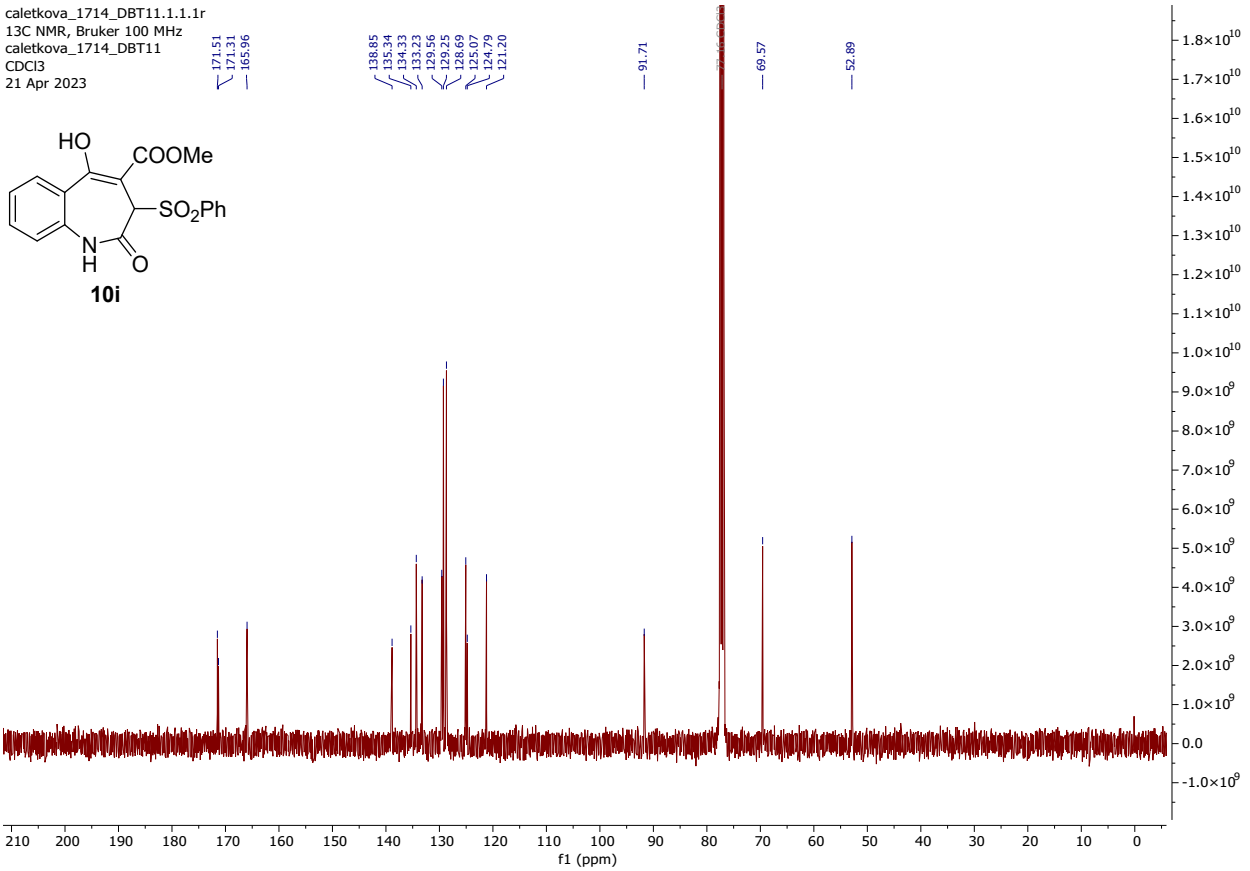
caletkova_2670_OC192
13C NMR, Bruker 100 MHz
caletkova_2670_OC192
D2O
31 Jul 2023

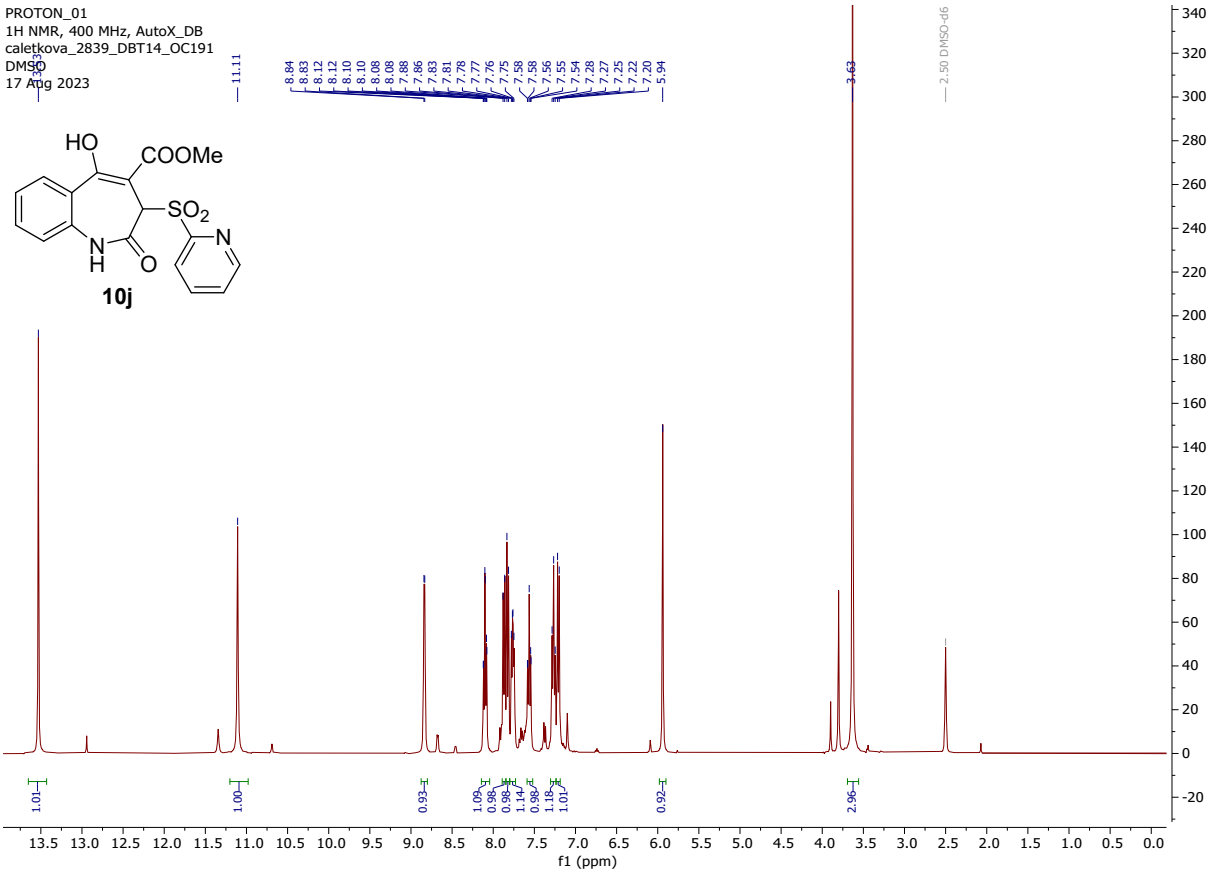


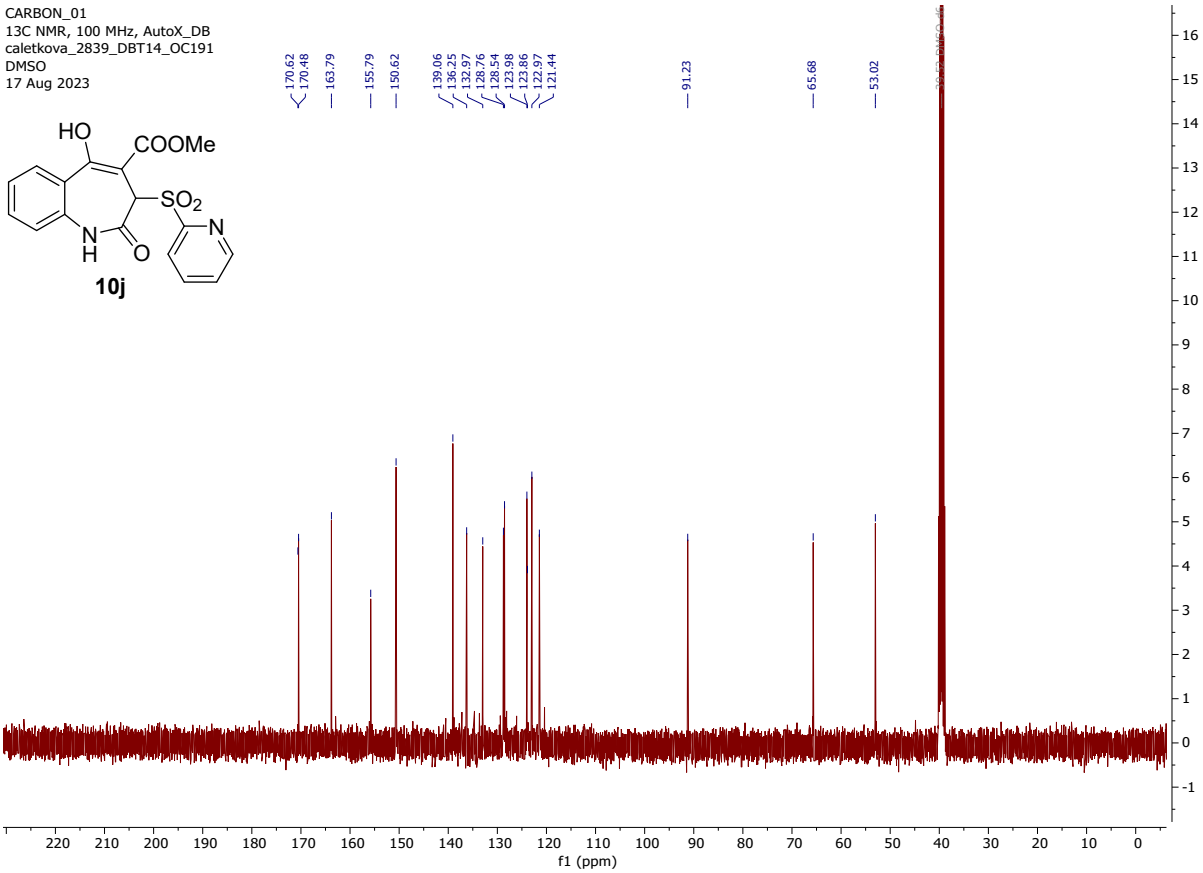

caletkova_1692_DBT8.2.1.1r
1H NMR, Bruker 400 MHz
caletkova_1692_DBT8
DMSO
20 Apr 2023
HO
COOMe
SAc
N
H
O
10h
12.87
10.72
7.82
7.82
7.80
7.80
7.62
7.62
7.60
7.58
7.58
7.32
7.30
7.28
7.21
7.19
5.64
3.88
3.35
2.50 DMSO-d6
2.21
1.00
1.01
1.01
1.03
1.04
1.00
0.94
3.19
3.08
f1 (ppm)

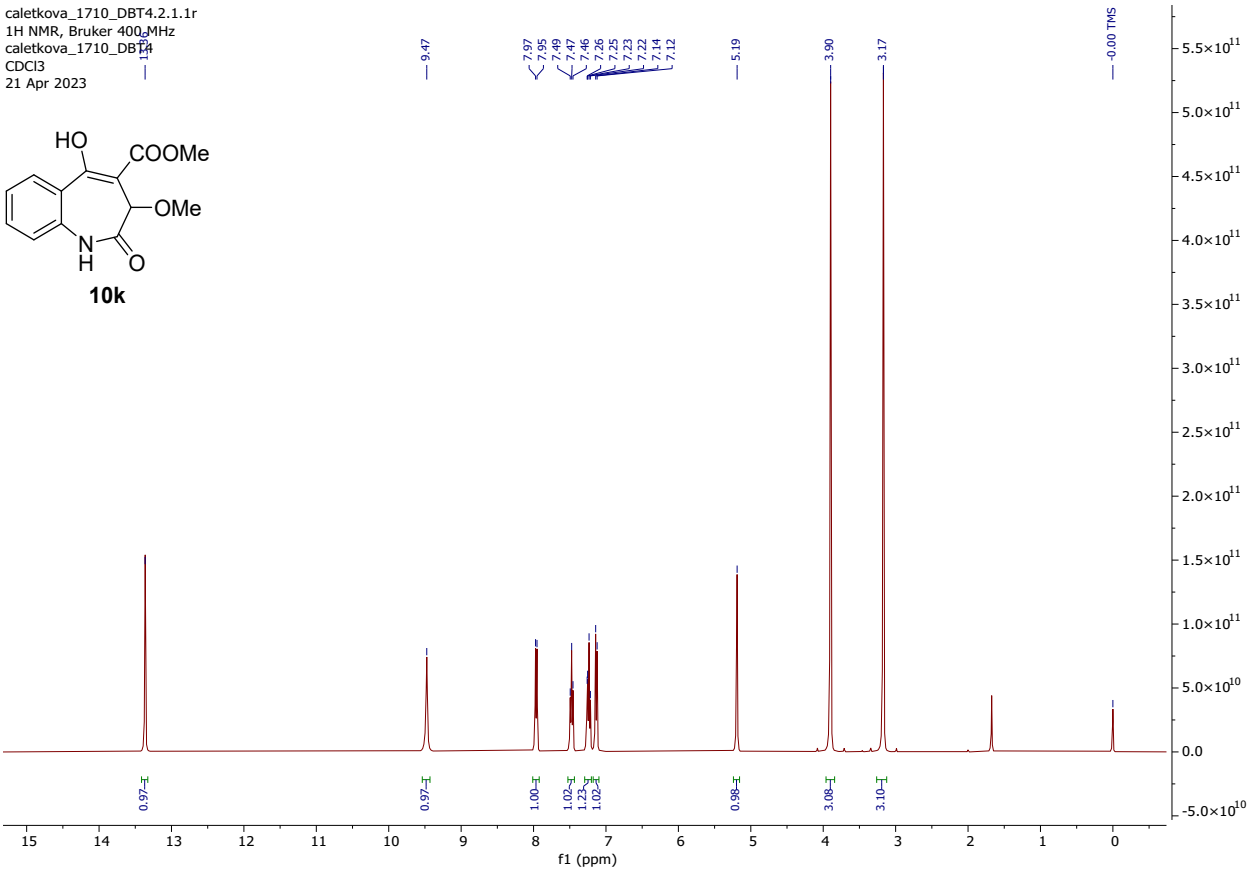

caletkova_1692_DBT8.1.1.1r
13C NMR, Bruker 100 MHz
caletkova_1692_DBT8
DMSO
20 Apr 2023
192.53
170.17
168.78
168.39
136.95
133.13
128.49
124.30
124.13
121.44
99.02
53.13
40.15
39.52 DMSO-d6
29.97
HO
COOMe
SAc
N
H
O
10h
4.5×10^10
4.0×10^10
3.5×10^10
3.0×10^10
2.5×10^10
2.0×10^10
1.5×10^10
1.0×10^10
5.0×10^9
0.0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

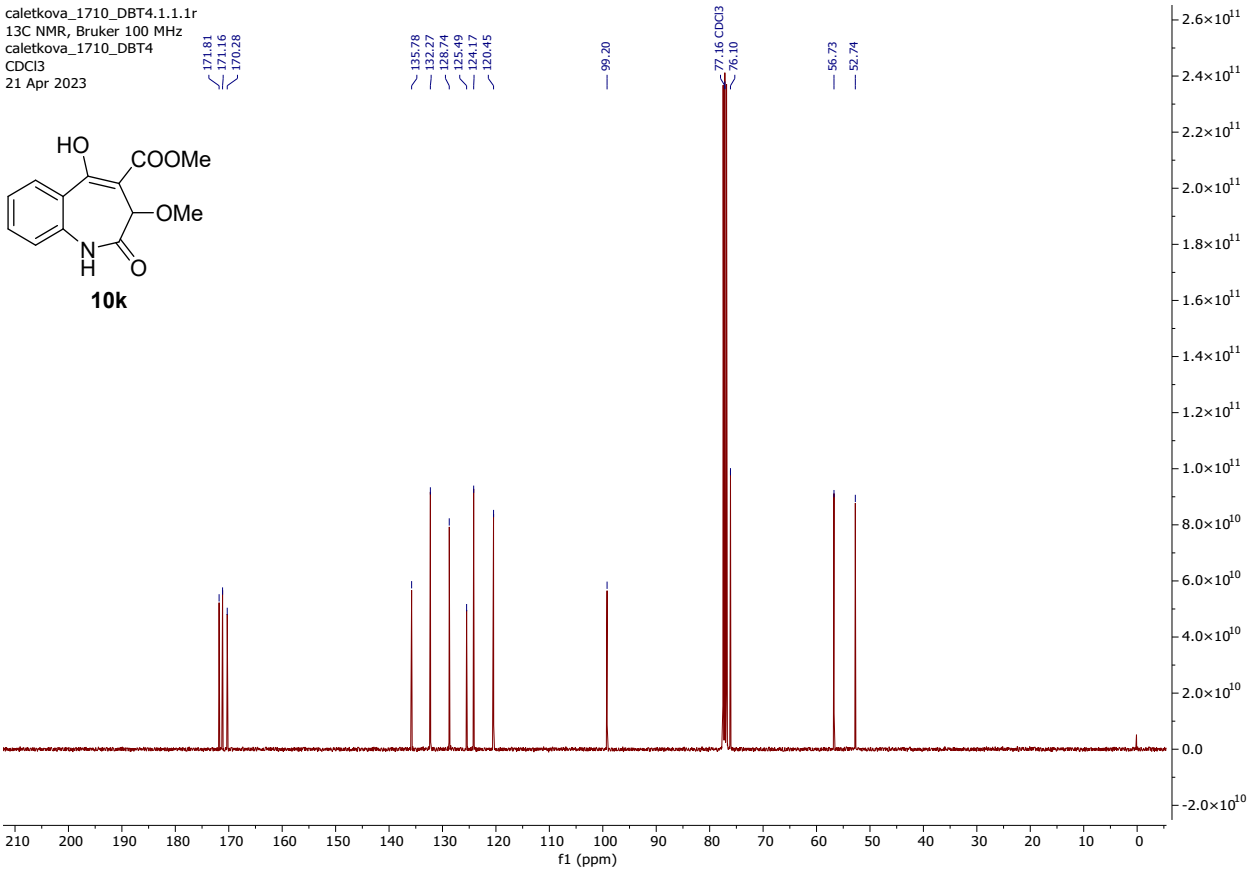

caletkova_1544_DBT11.1.fid
caletkova_1544_DBT11
Bruker 400 MHz
06 Apr 2023
HO
COOMe
SO2Ph
N
H
O
10i
f1 (ppm)

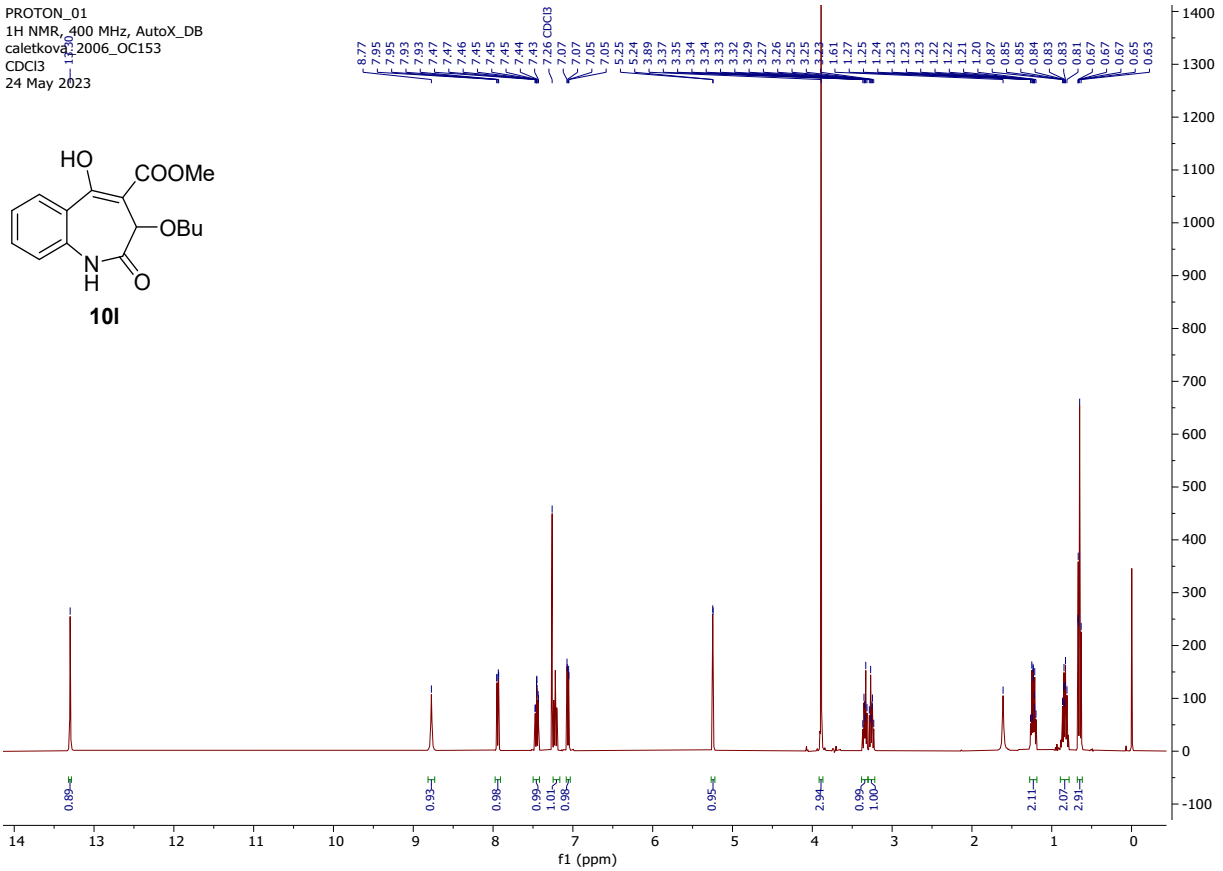

caletkova_1714_DBT11.1.1.1r
13C NMR, Bruker 100 MHz
caletkova_1714_DBT11
CDCl3
21 Apr 2023
171.51
171.31
165.96
138.85
135.34
134.33
133.23
129.56
129.25
128.69
125.07
124.79
121.20
91.71
69.57
52.89
HO
COOMe
SO2Ph
N
H
O
10i
f1 (ppm)

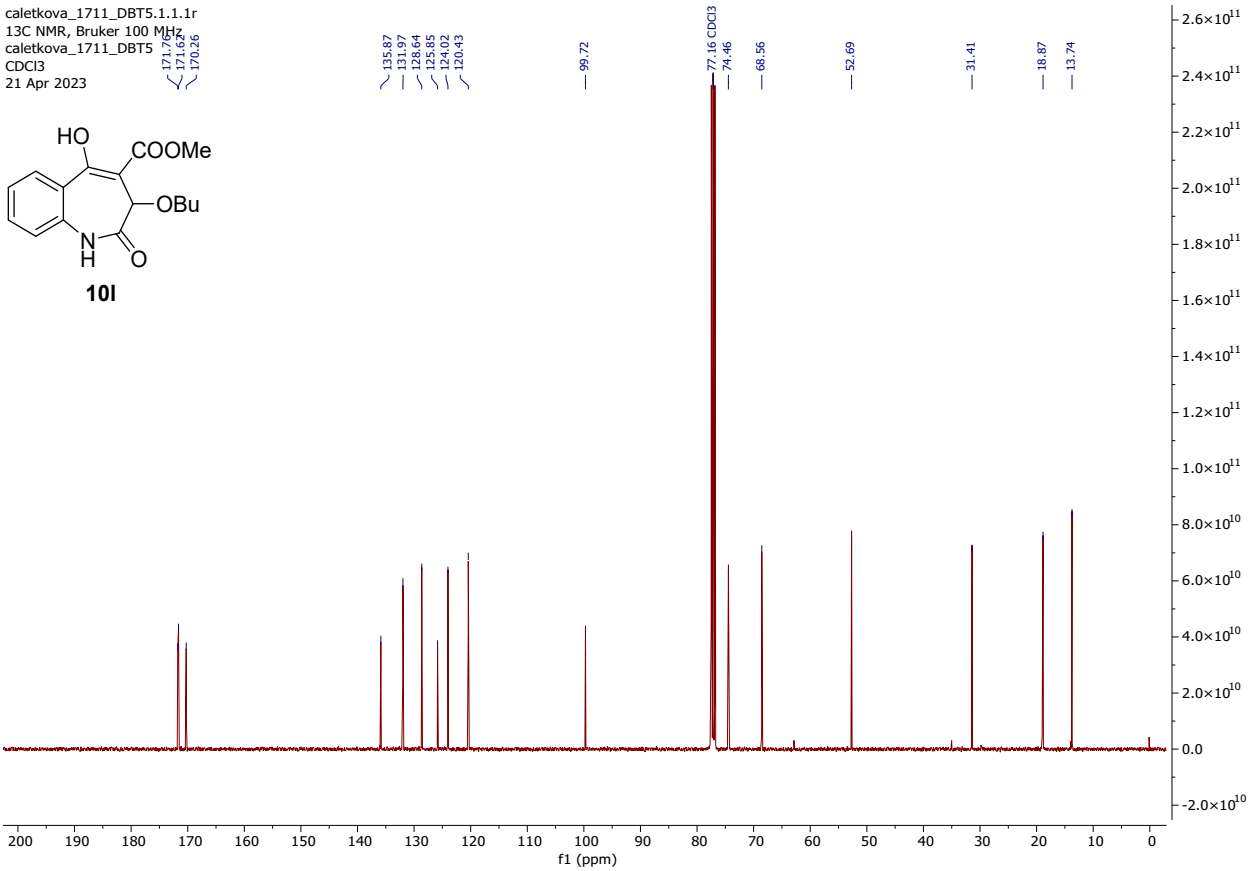

PROTON_01
1H NMR, 400 MHz, AutoX_DB
caletkova_2839_DBT14_OC191
DMSO
17 Aug 2023
HO
COOMe
SO2
N
H
N
O
10j
f1 (ppm)

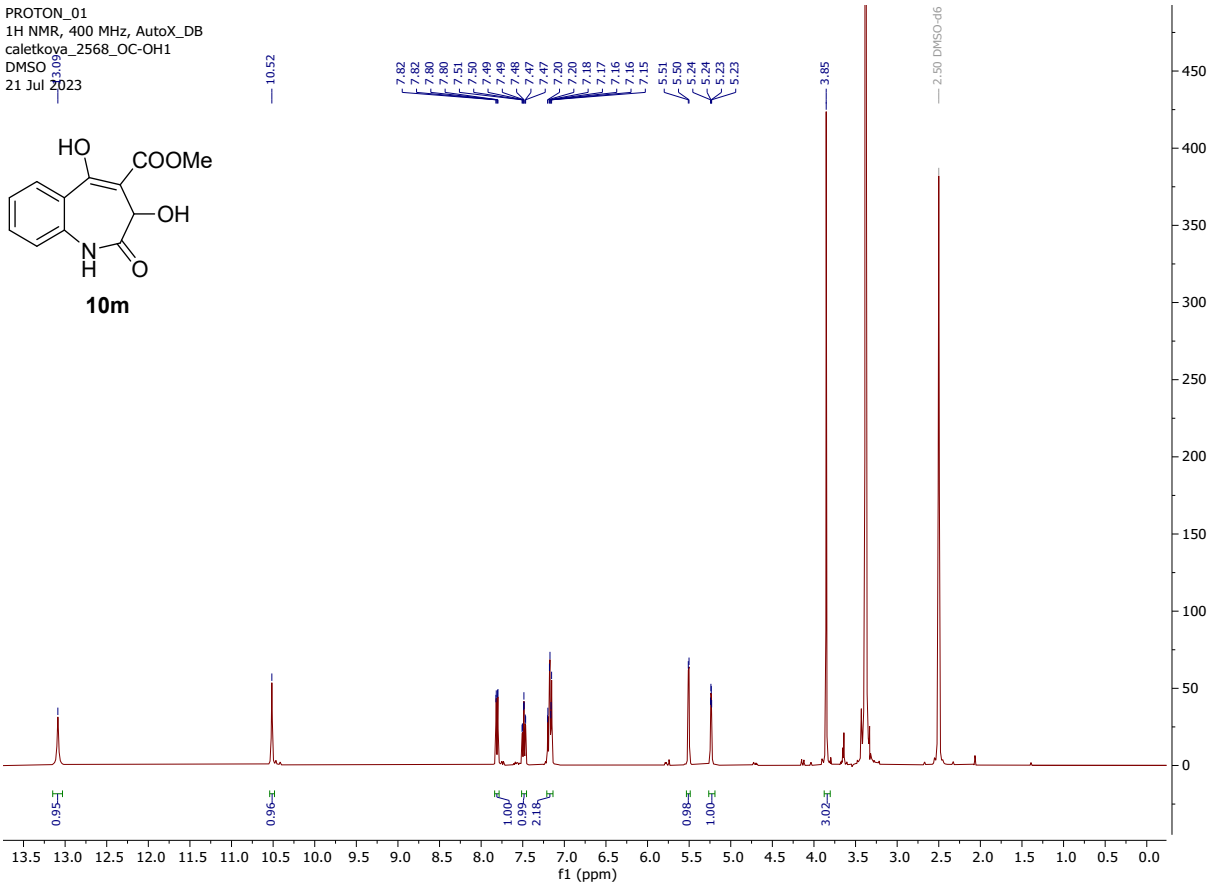

CARBON_01
13C NMR, 100 MHz, AutoX_DB
caletkova_2839_DBT14_OC191
DMSO
17 Aug 2023
HO
COOMe
SO2
N
H
N
O
10j
170.62
170.48
163.79
155.79
150.62
139.06
136.25
132.97
128.76
128.54
123.98
123.86
122.97
121.44
91.23
65.68
53.02
f1 (ppm)

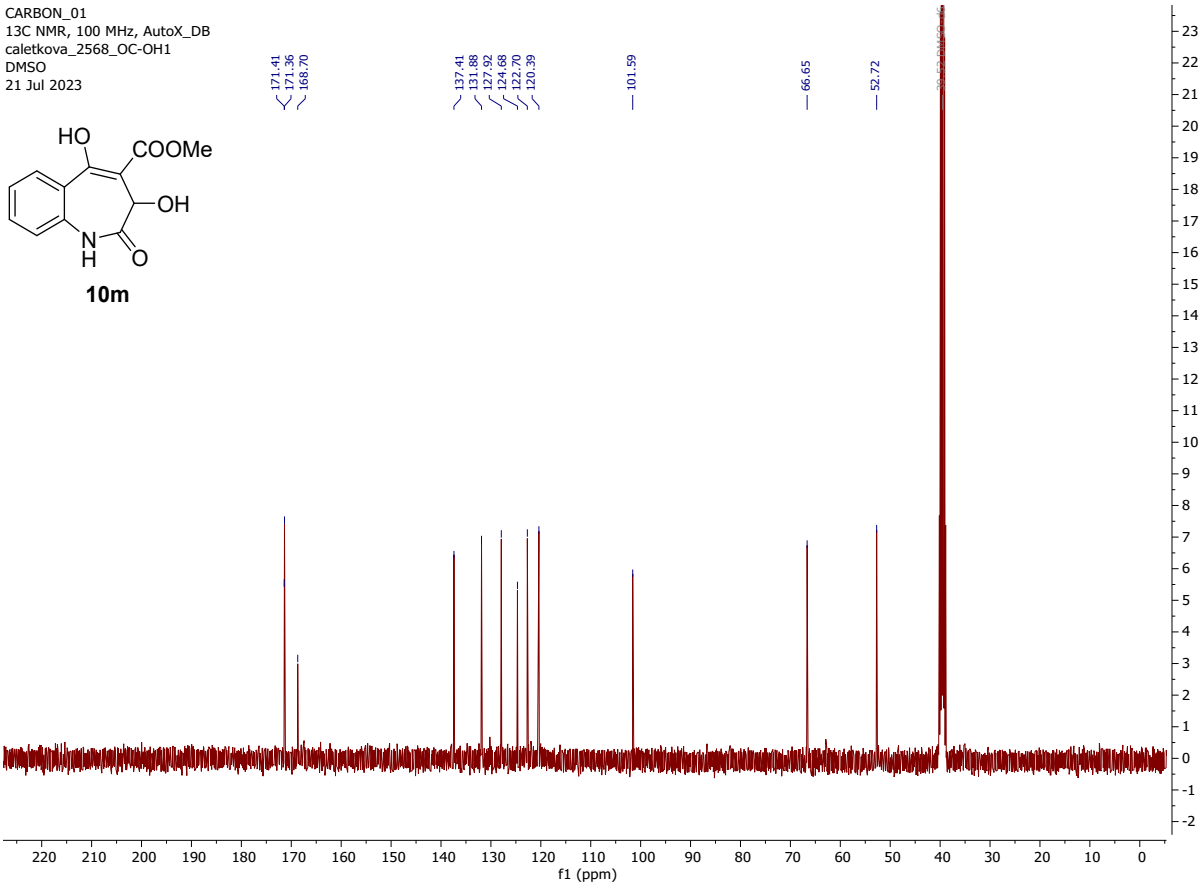

caletkova_1710_DBT4.2.1.1r
1H NMR, Bruker 400 MHz
caletkova_1710_DBT4
CDCl3
21 Apr 2023
HO
COOMe
OMe
N
H
O
10k
13.45
9.47
7.97
7.95
7.49
7.47
7.46
7.26
7.25
7.23
7.22
7.14
7.12
5.19
3.90
3.17
-0.00 TMS
0.97
0.97
1.00
1.02
1.23
1.02
0.98
3.08
3.10
f1 (ppm)

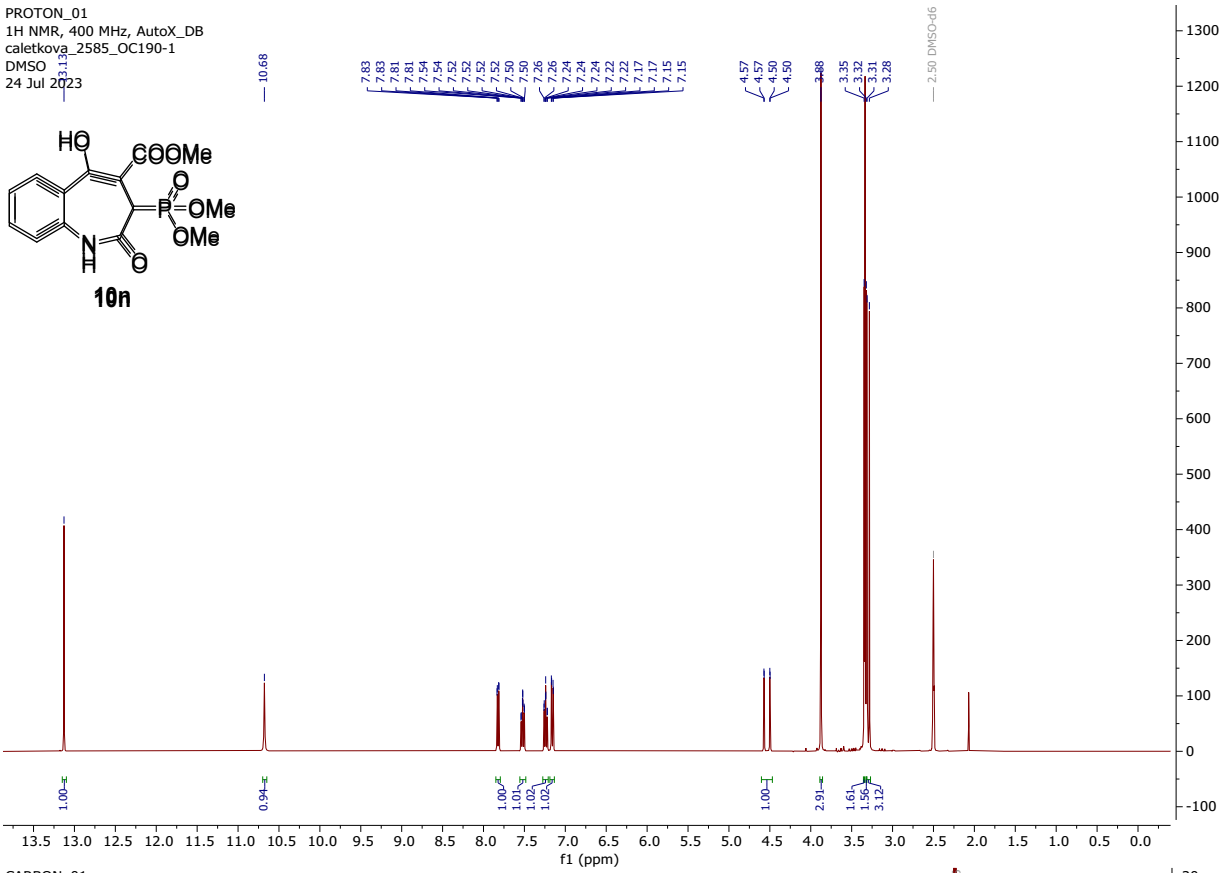

caletkova_1710_DBT4.1.1.1r
13C NMR, Bruker 100 MHz
caletkova_1710_DBT4
CDCl3
21 Apr 2023
171.81
171.16
170.28
135.78
132.27
128.74
125.49
124.17
120.45
99.20
77.16 CDCl3
76.10
56.73
52.74
HO
COOMe
OMe
N
H
O
10k
f1 (ppm)

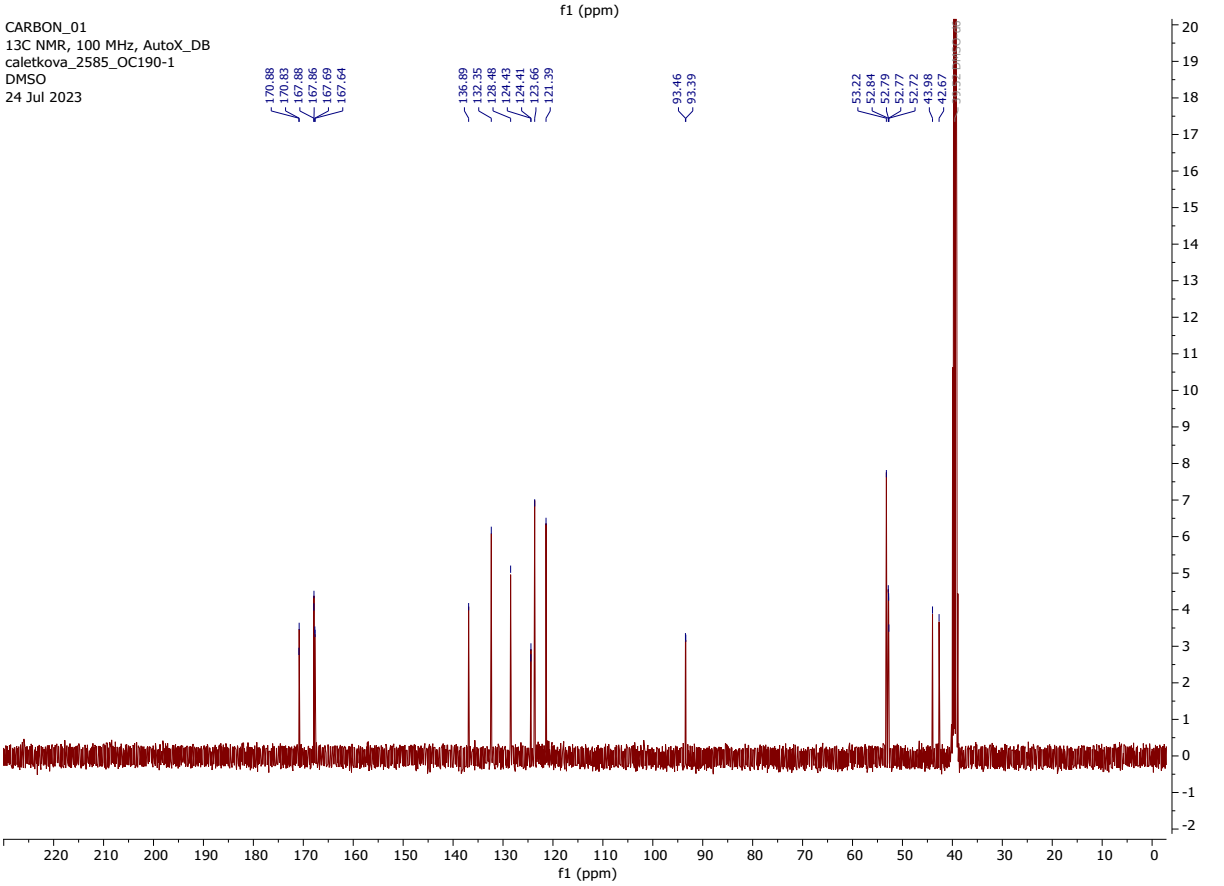

PROTON_01
1H NMR, 400 MHz, AutoX_DB
caletkova_2006_OC153
CDCl3
24 May 2023
HO
COOMe
OBu
N
H
O
10l
f1 (ppm)


caletkova_1711_DBT5.1.1.1r
13C NMR, Bruker 100 MHz
caletkova_1711_DBT5
CDCl3
21 Apr 2023
171.76
171.62
170.26
135.87
131.97
128.64
125.85
124.02
120.43
99.72
77.16 CDCl3
74.46
68.56
52.69
31.41
18.87
13.74
HO
COOMe
OBu
N
H
O
10l
f1 (ppm)

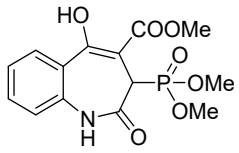

PROTON_01
1H NMR, 400 MHz, AutoX_DB
caletkova_2568_OC-OH1
DMSO
21 Jul 2023
HO
COOMe
OH
N
H
O
10m
13.09
10.52
7.82
7.82
7.80
7.80
7.51
7.50
7.49
7.49
7.48
7.47
7.47
7.20
7.18
7.17
7.16
7.16
7.15
5.51
5.50
5.24
5.24
5.23
5.23
3.85
2.50 DMSO-d6
0.95
0.96
1.00
0.99
2.16
0.98
1.00
3.02
f1 (ppm)


CARBON_01
13C NMR, 100 MHz, AutoX_DB
caletkova_2568_OC-OH1
DMSO
21 Jul 2023
HO
COOMe
OH
N
H
O
10m
171.41
171.36
168.70
137.41
131.88
127.92
124.68
122.70
120.39
101.59
66.65
52.72
f1 (ppm)


PROTON_01
1H NMR, 400 MHz, AutoX_DB
caletkova_2585_OC190-1
DMSO
24 Jul 2023
HO
COOMe
O
P
OMe
OMe
N
H
O
10n
f1 (ppm)


CARBON_01
13C NMR, 100 MHz, AutoX_DB
caletkova_2585_OC190-1
DMSO
24 Jul 2023
f1 (ppm)

PHOSPHORUS_01
31P NMR, 400 MHz, AutoX_DB
caletkova_2585_OC190-1
DMSO
24 Jul 2023



10n

Toxicity test in the 3D models of target tissues - data from the measurement and calculation of the standard deviation

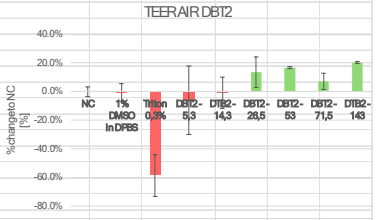
Biological response of EpiAirway to **9** – data from the measurement and calculation of the standard deviation

AIR

10/11/2023

Code N°	Tissue n	Raw data Before	Blank corr Before	TEER value Before (Ω·cm2)	Raw data After	Blak corr. After	TEER value After (Ω·cm2)	% change	Average	Change to NC	AVG change to NC	SD
NC	1	2.184	2.140	1.284	1.456	1.423	0.854	-33.5%	-29.7%	-3.8%	0.0%	0.053377
	2	3.456	3.412	2.047	2.56	2.527	1.516	-25.9%		3.8%		
1% DMSO in DPBS	1	2.243	2.199	1.319	1.905	1.872	1.123	-14.9%	-19.4%	14.9%	10.3%	0.064863
	2	3.103	3.059	1.835	2.357	2.324	1.394	-24.0%		5.7%		
Triton 0,3%	1	2.009	1.965	1.179	0.228	0.195	0.117	-90.1%	-69.9%	-60.4%	-40.2%	0.284641
	2	0.513	0.469	0.281	0.268	0.235	0.141	-49.8%		-20.1%		
DBT2 - 5,3	1	2.068	2.024	1.214	1.674	1.641	0.985	-18.9%	-35.7%	10.9%	-6.0%	0.238439
	2	2.785	2.741	1.645	1.333	1.3	0.780	-52.6%		-22.9%		
DBT2 - 26,5	1	2.686	2.642	1.585	2.049	2.016	1.210	-23.7%	-16.2%	6.1%	13.5%	0.105626
	2	3.121	3.077	1.846	2.841	2.808	1.685	-8.7%		21.0%		
DBT2 - 53	1	2.129	2.085	1.251	1.84	1.807	1.084	-13.3%	-12.9%	16.4%	16.8%	0.006098
	2	3.248	3.204	1.922	2.836	2.803	1.682	-12.5%		17.2%		

Code N°	Average	AVG change to NC	SD
NC	-22.2%	0.0%	3.41%
1% DMSO in DPBS	-22.7%	-0.6%	6.10%
Triton 0,3%	-80.6%	-58.5%	14.57%
DBT2 - 5,3	-35.7%	-6.0%	23.84%
DTB2 - 14,3	-15.3%	-0.7%	10.81%
DBT2 - 26,5	-16.2%	13.5%	10.56%
DBT2 - 53	-12.9%	16.8%	0.61%
DBT2 - 71,5	-7.3%	7.3%	5.73%
DTB2 - 143	5.9%	20.5%	0.72%



15/12/2023

Code N°	Tissue n	Raw data Before	Blank corr Before	TEER value Before (Ω·cm2)	Raw data After	Blak corr. After	TEER value After (Ω·cm2)	% change	Average	Change to NC		SD
NC	1	2.201	2.167	1.300	0.837	0.807	0.484	-62.8%	-54.8%	-11.0%	0.0%	0.15559
	2	1.776	1.742	1.045	1.062	1.032	0.619	-40.8%		11.0%		
1% DMSO	1	1.739	1.705	1.023	1.348	1.318	0.791	-22.7%	-22.6%	29.1%	29.2%	0.001572
	2	1.934	1.900	1.140	1.503	1.473	0.884	-22.5%		29.3%		
Triton 0,3%	1	2.212	2.178	1.307	0.161	0.131	0.079	-94.0%	-91.8%	-42.2%	-40.0%	0.030933
	2	1.506	1.472	0.883	0.183	0.153	0.092	-89.6%		-37.8%		
DTB2	1	1.732	1.698	1.019	0.575	0.545	0.327	-67.9%	-62.5%	-16.1%	-10.8%	0.076133
	2	2.578	2.544	1.526	1.12	1.09	0.654	-57.1%		-5.4%		
DTB2	1	1.744	1.710	1.026	1.391	1.361	0.817	-20.4%	-16.6%	31.4%	35.2%	0.053889
	2	2.374	2.340	1.404	2.072	2.042	1.225	-12.7%		39.0%		
DTB2	1	2.268	2.234	1.340	0.44	0.41	0.246	-81.6%	-86.4%	-29.9%	-34.6%	0.066722
	2	1.921	1.887	1.132	0.199	0.169	0.101	-91.1%		-39.3%		

21/12/2023

Code N°	Tissue n	Raw data Before	Blank corr Before	TEER value Before (Ω·cm2)	Raw data After	Blak corr. After	TEER value After (Ω·cm2)	% change	Average	Change to NC		SD
NC	1	2.062	2.028	1.217	1.783	1.753	1.052	-13.6%	-14.6%	1.1%	0.0%	0.014922
	2	2.565	2.531	1.519	2.165	2.135	1.281	-15.7%		-1.1%		
1% DMSO	1	2.185	2.151	1.291	1.535	1.505	0.903	-30.1%	-26.0%	-15.4%	-11.4%	0.05717
	2	2.742	2.708	1.625	2.143	2.113	1.268	-22.0%		-7.4%		
Triton 0,3%	1	2.282	2.248	1.349	0.215	0.185	0.111	-91.8%	-91.3%	-77.2%	-76.7%	0.006817
	2	1.974	1.940	1.164	0.209	0.179	0.107	-90.8%		-76.2%		
DTB2 - 14,3	1	2.260	2.226	1.336	2.086	2.056	1.234	-7.6%	-15.3%	7.0%	-0.7%	0.108089
	2	1.677	1.643	0.986	1.297	1.267	0.760	-22.9%		-8.3%		
DBT2 - 71,5	1	2.276	2.242	1.345	2.016	1.986	1.192	-11.4%	-7.3%	3.2%	7.3%	0.057343
	2	2.177	2.143	1.286	2.104	2.074	1.244	-3.3%		11.3%		
DTB2 - 143	1	2.727	2.693	1.616	2.868	2.838	1.703	5.4%	5.9%	20.0%	20.5%	0.007212
	2	2.273	2.239	1.343	2.412	2.382	1.429	6.4%		21.0%		

Biological response of EpiIntestinal to **9** - data from the measurement and calculation of the standard deviation

SMI

10/11/2023

Code N°	Tissue n	Raw data Before	Blank corr Before	TEER value Before (Ω·cm2)	Raw data After	Blak corr. After	TEER value After (Ω·cm2)	% change	Average	Change to N	AVG change to NC	SD
NC	1	0.397	0.345	0.207	0.348	0.311	0.187	-9.7%	-2.6%	-7.1%	0.0%	0.100461
	2	0.382	0.330	0.198	0.382	0.345	0.207	4.5%		7.1%		
1% DMSO in DPBS	1	0.365	0.313	0.188	0.328	0.291	0.175	-6.9%	-11.8%	-4.4%	-9.2%	0.068955
	2	0.392	0.340	0.204	0.32	0.283	0.170	-16.7%		-14.1%		
Triton 0,3%	1	0.395	0.343	0.206	0.22	0.183	0.110	-46.6%	-44.5%	-44.0%	-41.9%	0.029774
	2	0.358	0.306	0.184	0.214	0.177	0.106	-42.4%		-39.8%		
DBT2 - 5,3	1	0.419	0.367	0.220	0.415	0.378	0.227	3.2%	2.3%	5.7%	4.9%	0.011892
	2	0.386	0.334	0.200	0.376	0.339	0.203	1.5%		4.1%		
DBT2 - 26,5	1	0.385	0.333	0.200	0.393	0.356	0.214	7.0%	4.9%	9.6%	7.5%	0.029764
	2	0.410	0.358	0.215	0.406	0.369	0.221	2.8%		5.3%		
DBT2 - 53	1	0.388	0.336	0.202	0.393	0.356	0.214	5.9%	4.7%	8.5%	7.3%	0.017623
	2	0.391	0.339	0.203	0.387	0.35	0.210	3.4%		6.0%		

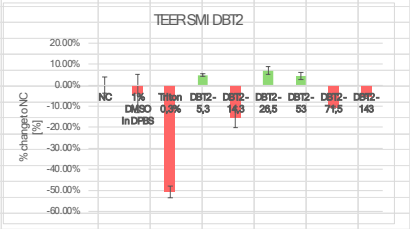
17/11/2023

Code N°	Tissue n	Raw data Before	Blank corr Before	TEER value Before (Ω·cm2)	Raw data After	Blak corr. After	TEER value After (Ω·cm2)	% change	Average	Change to N	AVG change to NC	SD
NC	1	0.405	0.370	0.222	0.374	0.331	0.199	-10.4%	-10.6%	0.2%	0.0%	0.002842
	2	0.407	0.372	0.223	0.374	0.331	0.199	-10.8%		-0.2%		
1% DMSO	1	0.363	0.328	0.197	0.353	0.31	0.186	-5.6%	-15.4%	5.0%	-4.8%	0.138731
	2	0.445	0.410	0.246	0.349	0.306	0.184	-25.2%		-14.6%		
Triton 0,3%	1	0.397	0.362	0.217	0.2	0.157	0.094	-56.7%	-55.4%	-46.1%	-44.8%	0.018057
	2	0.399	0.364	0.218	0.21	0.167	0.100	-54.1%		-43.6%		
DBT2 - 5,3	1	0.347	0.312	0.187	0.336	0.293	0.176	-5.9%	-5.9%	4.7%	4.7%	0.000205
	2	0.373	0.338	0.203	0.362	0.319	0.191	-5.9%		4.7%		
DBT2 - 26,5	1	0.359	0.324	0.194	0.352	0.309	0.185	-4.6%	-4.1%	5.9%	6.4%	0.007158
	2	0.357	0.322	0.193	0.353	0.31	0.186	-3.6%		6.9%		
DBT2 - 53	1	0.373	0.338	0.203	0.346	0.303	0.182	-10.3%	-9.2%	0.2%	1.4%	0.016178
	2	0.387	0.352	0.211	0.366	0.323	0.194	-8.1%		2.5%		

15/12/2023

Code N°	Tissue n	Raw data Before	Blank corr Before	TEER value Before (Ω·cm2)	Raw data After	Blak corr. After	TEER value After (Ω·cm2)	% change	Average	Change to N	AVG change to NC	SD
NC	1	0.337	0.297	0.178	0.397	0.359	0.215	20.8%	19.9%	0.9%	0.0%	0.012672
	2	0.338	0.298	0.179	0.393	0.355	0.213	19.0%		-0.9%		
1% DMSO	1	0.350	0.310	0.186	0.402	0.364	0.218	17.2%	21.8%	-2.7%	1.9%	0.065283
	2	0.330	0.290	0.174	0.404	0.366	0.220	26.4%		6.5%		
Triton 0,3%	1	0.320	0.280	0.168	0.184	0.146	0.088	-47.6%	-45.2%	-67.5%	-65.1%	0.03428
	2	0.316	0.276	0.166	0.197	0.159	0.095	-42.8%		-62.7%		
DBT2 - 14,3	1	0.349	0.309	0.185	0.35	0.312	0.187	1.1%	4.6%	-18.8%	-15.3%	0.049689
	2	0.349	0.309	0.185	0.371	0.333	0.200	8.1%		-11.8%		
DBT2 - 71,5	1	0.344	0.304	0.182	0.371	0.333	0.200	9.9%	9.6%	-10.0%	-10.3%	0.004536
	2	0.328	0.288	0.173	0.353	0.315	0.189	9.2%		-10.6%		
DBT2 - 143	1	0.315	0.275	0.165	0.352	0.314	0.188	13.9%	14.5%	-6.0%	-5.4%	0.007926
	2	0.316	0.276	0.166	0.357	0.319	0.191	15.1%		-4.8%		

Code N°	Average	AVG change to NC	SD
NC	2.3%	0.0%	3.87%
1% DMSO in DPBS	-1.8%	-4.0%	9.10%
Triton 0,3%	-48.4%	-50.6%	2.74%
DBT2 - 5,3	-1.8%	4.8%	0.60%
DBT2 - 14,3	4.6%	-15.3%	4.97%
DBT2 - 26,5	0.4%	6.9%	1.85%
DBT2 - 53	-2.3%	4.3%	1.69%
DBT2 - 71,5	9.6%	-10.3%	0.45%
DBT2 - 143	14.5%	-5.4%	0.79%



Biological response of EpiAirway to **10d** – data from the measurement and calculation of the standard deviation

AIR

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Code N°	Tissue n	Raw data Before	Blank corr Before	TEER valu Before (Ω	Raw data After	Blak corr. After	TEER valu After (Ω·cm2)	% change	Average	Change to	AVG change to NC	SD
NC	1	1.976	1.932	1.159	2.115	2.083	1.250	7.9%	9.0%	-1.1%	0.0%	0.016209
	2	2.361	2.317	1.390	2.584	2.552	1.531	10.1%		1.1%		
Triton 0,3%	1	1.600	1.556	0.934	0.19	0.158	0.095	-89.8%	-92.1%	-98.8%	-101.1%	0.032419
	2	2.729	2.685	1.611	0.182	0.15	0.090	-94.4%		-103.4%		
1% DMSO	1	2.507	2.463	1.478	2.424	2.392	1.435	-2.9%	8.9%	-11.9%	-0.1%	0.167173
	2	2.729	2.685	1.611	3.274	3.242	1.945	20.7%		11.7%		
DBT10-10	1	1.743	1.699	1.019	2.238	2.206	1.324	29.9%	13.1%	20.9%	4.1%	0.238057
	2	3.390	3.346	2.008	3.254	3.222	1.933	-3.7%		-12.7%		
DBT10-50	1	2.106	2.062	1.237	2.625	2.593	1.556	25.8%	11.2%	16.8%	2.2%	0.206272
	2	2.950	2.906	1.744	2.841	2.809	1.685	-3.4%		-12.4%		
DBT10-1(	1	2.620	2.576	1.546	2.349	2.317	1.390	-10.1%	-3.5%	-19.1%	-12.5%	0.093651
	2	2.950	2.906	1.744	3.031	2.999	1.799	3.2%		-5.8%		

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Code N°	Tissue n	Raw data Before	Blank corr Before	TEER valu Before (Ω	Raw data After	Blak corr. After	TEER valu After (Ω·cm2)	% change	Average	Change to NC		SD
NC	1	2.201	2.167	1.300	0.837	0.807	0.484	-62.8%	-51.8%	-11.0%	0.0%	0.15559
	2	1.776	1.742	1.045	1.062	1.032	0.619	-40.8%		11.0%		
1%DMSO	1	1.739	1.705	1.023	1.348	1.318	0.791	-22.7%	-22.6%	29.1%	29.2%	0.001572
	2	1.934	1.900	1.140	1.503	1.473	0.884	-22.5%		29.3%		
Triton 0,3%	1	2.212	2.178	1.307	0.161	0.131	0.079	-94.0%	-91.8%	-42.2%	-40.0%	0.030933
	2	1.506	1.472	0.883	0.183	0.153	0.092	-89.6%		-37.8%		
DBT10	1	2.039	2.005	1.203	1.188	1.158	0.695	-42.2%	-36.3%	9.5%	15.5%	0.083723
	2	2.184	2.150	1.290	1.526	1.496	0.898	-30.4%		21.4%		
DBT10	1	2.087	2.053	1.232	1.551	1.521	0.913	-25.9%	-24.5%	25.9%	27.3%	0.019983
	2	2.577	2.543	1.526	1.987	1.957	1.174	-23.1%		28.7%		
DBT10	1	1.038	1.004	0.602	1.632	1.602	0.961	59.6%	13.6%	111.4%	65.6%	0.647979
	2	3.753	3.719	2.231	2.558	2.528	1.517	-32.0%		19.8%		

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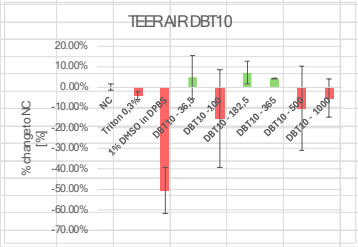
Code N°	Tissue n	Raw data Before	Blank corr Before	TEER valu Before (Ω	Raw data After	Blak corr. After	TEER valu After (Ω·cm2)	% change	Average	Change to NC		SD
NC	1	2.062	2.028	1.217	1.783	1.753	1.052	-13.6%	-14.6%	1.1%	0.0%	0.014922
	2	2.565	2.531	1.519	2.165	2.135	1.281	-15.7%		-1.1%		
Triton 0,3%	1	2.282	2.248	1.349	0.215	0.185	0.111	-91.8%	-91.3%	-77.2%	-76.7%	0.006817
	2	1.974	1.940	1.164	0.209	0.179	0.107	-90.8%		-76.2%		
1% DMSO	1	2.185	2.151	1.291	1.535	1.505	0.903	-30.1%	-26.0%	-15.4%	-11.4%	0.05717
	2	2.742	2.708	1.625	2.143	2.113	1.268	-22.0%		-7.4%		
DBT10-36	1	1.614	1.580	0.948	1.776	1.746	1.048	10.5%	3.0%	25.2%	17.7%	0.106219
	2	1.710	1.676	1.006	1.632	1.602	0.961	-4.5%		10.1%		
DBT10-18	1	1.853	1.819	1.091	2.073	2.043	1.226	12.4%	16.3%	27.0%	30.9%	0.055399
	2	1.312	1.278	0.767	1.567	1.537	0.922	20.2%		34.8%		
DBT10-36	1	2.073	2.039	1.223	2.084	2.054	1.232	0.7%	0.7%	15.3%	15.3%	0.001052
	2	1.737	1.703	1.022	1.743	1.713	1.028	0.6%		15.2%		

Code N°	Average	AVG change to NC	SD
NC	-2.8%	0.0%	1.56%
Triton 0,3%	-91.7%	-88.9%	1.96%
1% DMSO	-8.6%	-5.7%	11.22%
DBT10-36	3.0%	17.7%	10.62%
DBT10-10	13.1%	4.1%	23.81%
DBT10-18	16.3%	30.9%	5.54%
DBT10-36	0.7%	15.3%	0.11%
DBT10-50	11.2%	2.2%	20.63%
DBT10-1(	-3.5%	-12.5%	9.37%



Biological response of EpiIntestinal to **10d** – data from the measurement and calculation of the standard deviation

SMI

10/11/2023

Code N°	Tissue n	Raw data Before	Blank corr. Before	TEER valu Before (Ω	Raw data After	Blak corr. After	TEER value After (Ω·cm2)	% change	Average	Change to N	AVG change to NC	SD
NC	1	0.397	0.345	0.207	0.348	0.311	0.187	-9.7%	-2.6%	-7.1%	0.0%	0.100461
	2	0.382	0.330	0.198	0.382	0.345	0.207	4.5%		7.1%		
1% DMSO in	1	0.365	0.313	0.188	0.328	0.291	0.175	-6.9%	-11.8%	-4.4%	-9.2%	0.068955
	2	0.392	0.340	0.204	0.32	0.283	0.170	-16.7%		-14.1%		
Triton 0,3%	1	0.395	0.343	0.206	0.22	0.183	0.110	-46.6%	-44.5%	-44.0%	-41.9%	0.029774
	2	0.358	0.306	0.184	0.214	0.177	0.106	-42.4%		-39.8%		
DBT10 - 19	1	0.384	0.332	0.199	0.306	0.269	0.161	-19.1%	-19.4%	-16.5%	-16.9%	0.004493
	2	0.423	0.371	0.223	0.336	0.299	0.179	-19.7%		-17.2%		
DBT10 - 95	1	0.476	0.424	0.254	0.356	0.319	0.191	-24.8%	-6.3%	-22.2%	-3.7%	0.262166
	2	0.418	0.366	0.220	0.449	0.412	0.247	12.3%		14.8%		
DBT10 - 190	1	0.425	0.373	0.224	0.366	0.329	0.197	-12.1%	-15.5%	-9.5%	-13.0%	0.049306
	2	0.429	0.377	0.226	0.342	0.305	0.183	-19.0%		-16.5%		

17/11/2023

Code N°	Tissue n	Raw data Before	Blank corr. Before	TEER valu Before (Ω	Raw data After	Blak corr. After	TEER value After (Ω·cm2)	% change	Average	Change to N	AVG change to NC	SD
NC	1	0.405	0.370	0.222	0.374	0.331	0.199	-10.4%	-10.6%	0.2%	0.0%	0.002842
	2	0.407	0.372	0.223	0.374	0.331	0.199	-10.8%		-0.2%		
1% DMSO	1	0.363	0.328	0.197	0.353	0.31	0.186	-5.6%	-15.4%	5.0%	-4.8%	0.138731
	2	0.445	0.410	0.246	0.349	0.306	0.184	-25.2%		-14.6%		
Triton 0,3%	1	0.397	0.362	0.217	0.2	0.157	0.094	-56.7%	-55.4%	-46.1%	-44.8%	0.018057
	2	0.399	0.364	0.218	0.21	0.167	0.100	-54.1%		-43.6%		
DBT10 - 19	1	0.375	0.340	0.204	0.382	0.339	0.203	-0.5%	-4.5%	10.1%	6.1%	0.056512
	2	0.408	0.373	0.224	0.384	0.341	0.205	-8.5%		2.1%		
DBT10 - 95	1	0.371	0.336	0.202	0.352	0.309	0.185	-8.4%	-6.6%	2.1%	3.9%	0.025513
	2	0.382	0.347	0.208	0.373	0.33	0.198	-4.8%		5.8%		
DBT10 - 190	1	0.385	0.350	0.210	0.366	0.323	0.194	-7.6%	3.6%	2.9%	14.1%	0.158476
	2	0.316	0.281	0.169	0.367	0.324	0.194	14.8%		25.4%		

24/11/2023

Code N°	Tissue n	Raw data Before	Blank corr. Before	TEER valu Before (Ω	Raw data After	Blak corr. After	TEER value After (Ω·cm2)	% change	Average	Change to N	AVG change to NC	SD
NC	1	0.436	0.392	0.235	0.353	0.321	0.193	-17.9%	-19.0%	1.1%	0.0%	0.015662
	2	0.425	0.381	0.229	0.337	0.305	0.183	-20.1%		-1.1%		
1% DMSO	1	0.464	0.420	0.252	0.362	0.33	0.198	-21.4%	-18.3%	-2.4%	0.7%	0.044194
	2	0.417	0.373	0.224	0.348	0.316	0.190	-15.2%		3.8%		
Triton 0,3%	1	0.380	0.336	0.202	0.174	0.142	0.085	-57.9%	-61.9%	-38.9%	-42.9%	0.056163
	2	0.459	0.415	0.249	0.173	0.141	0.085	-65.9%		-46.9%		
DBT10 - 44,6	1	0.390	0.346	0.208	0.33	0.298	0.179	-13.9%	-17.4%	5.0%	1.5%	0.049482
	2	0.434	0.390	0.234	0.341	0.309	0.185	-20.9%		-2.0%		
DBT10 - 222,8	1	0.395	0.351	0.211	0.326	0.294	0.176	-16.6%	-18.1%	2.4%	0.9%	0.021659
	2	0.425	0.381	0.229	0.339	0.307	0.184	-19.7%		-0.7%		
DBT10 - 446	1	0.426	0.382	0.229	0.356	0.324	0.194	-15.3%	-15.4%	3.7%	3.5%	0.00221
	2	0.408	0.364	0.218	0.338	0.306	0.184	-15.6%		3.4%		

Code N°	Average	AVG change to NC	SD
NC	-10.7%	0.0%	3.97%
1% DMSO in DPBS	-15.2%	-4.5%	8.40%
Triton 0,3%	-53.9%	-43.2%	3.47%
DBT10 - 19	-11.9%	-5.4%	3.05%
DBT10 - 44,6	-17.4%	1.5%	4.95%
DBT10 - 95	-6.4%	0.1%	14.38%
DBT10 - 190	-6.0%	0.6%	10.39%
DBT10 - 222,8	-18.1%	0.9%	2.17%
DBT10 - 446	-15.4%	3.5%	0.22%