Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Supporting information

N-acylsulfonamide: a valuable moiety to design new sulfa drug analogues

Romain Amador,[a] Ali Tahrioui,[b] Magalie Barreau,[b] Olivier Lesouhaitier,[b] Michael Smietana*[a] and Guillaume Clavé*[a]

[a] *Institut des Biomolécules Max Mousseron, Université de Montpellier, CNRS, ENSCM, 1919 route de Mende, 34095 Montpellier, France.*
[b] *Unité de Recherche Communication Bactérienne et Stratégies Anti-infectieuses (CBSA), UR4312, Université de Rouen Normandie, Normandie Université, Évreux 27000, France*
E-mail: guillaume.clave@cnrs.fr ; michael.smietana@umontpellier.fr

Table of contents

General

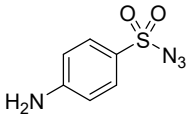
All dry solvents and reagents were purchased from commercial suppliers and were used without further purification. Thin-layer chromatography (TLC) analyses were carried out on 60 F-254 aluminium sheets. Purifications by column chromatography were performed using Biotage Isolera 1 system with Column Flash Pure from Büchi. NMR experiments were recorded on Bruker 400, 500 or 600 spectrometers at 20°C. Chemical shifts are expressed in parts per million (ppm) relative to the residual solvent signal: CD_3CN (δ_H = 1.96, δ_C = 1.79 (CH_3), 118.26 (CN)), $CDCl_3$ (δ_H = 7.26, δ_C = 77.36), CD_3OD (δ_H = 3.31, δ_C = 49.00 (CH_3), DMSO (δ_H = 2.5, δ_C = 39.52) or D_2O (δ_H = 4.79).[1] *J* values are in Hz. HRMS analyses were obtained with electrospray ionization (ESI) in positive or negative mode on a Q-TOF Micromass spectrometer. Analytical RP-HPLC was performed on a UHPLC Thermoscientific Ultimate3000 system equipped with a LPG-3400RS pump, a DAD 3000 detector and an WPS-3000TBRS Autosampler, Column Oven TCC-3000SD. Buffers and aqueous mobile-phases for RP-HPLC were prepared using water purified with a Milli-Q system (purified to 18.2 MΩ.cm).

RP-HPLC analysis: System A: RP-HPLC (Accucore™ C18 aQ column, 2.6 μm, 4.6×50 mm) with CH_3CN and 0.1% aqueous trifluoroacetic acid (aq. TFA, 0.1%, v/v, pH 2.0) as the eluents [0% CH_3CN (2 min), followed by linear gradient from 0 to 100% (25 min) of CH_3CN] at a flow rate of 1 $mL.min^{-1}$. Triple UV detection was achieved at 210, 260 and 650 nm. System B: System A with CH_3CN and aqueous triethylammonium acetate (aq. TEAA, 50 mM, pH 7.0) as the eluents.

The purity of the final compounds was determined by RP-HPLC analysis with detection at 260 nm.

Chemical synthesis

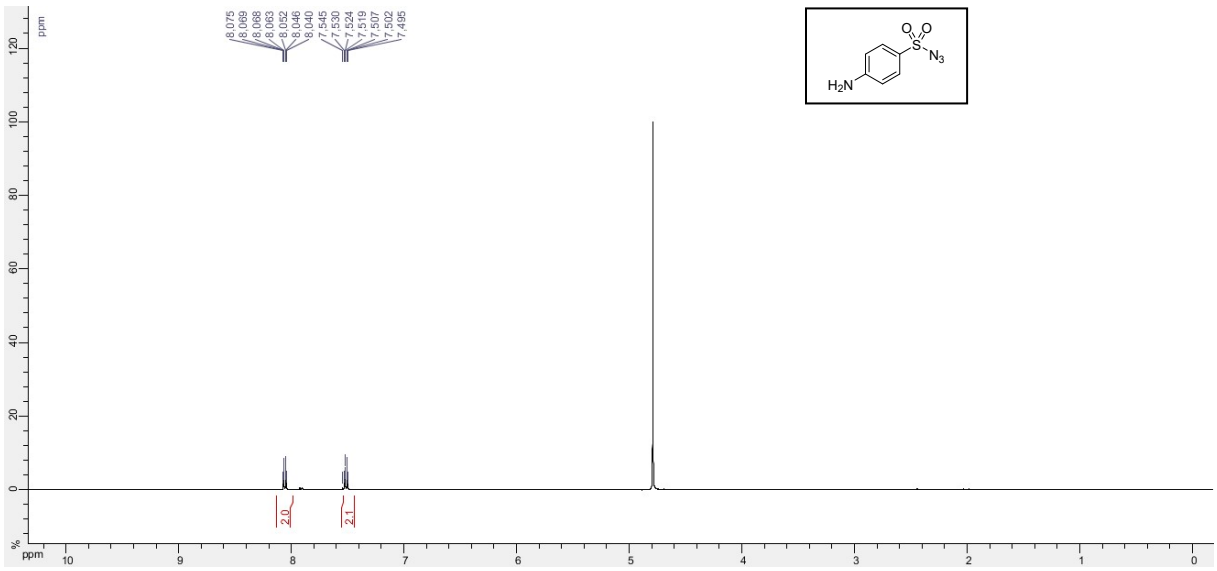
Synthesis of 4-aminobenzenesulfonyl azide 2

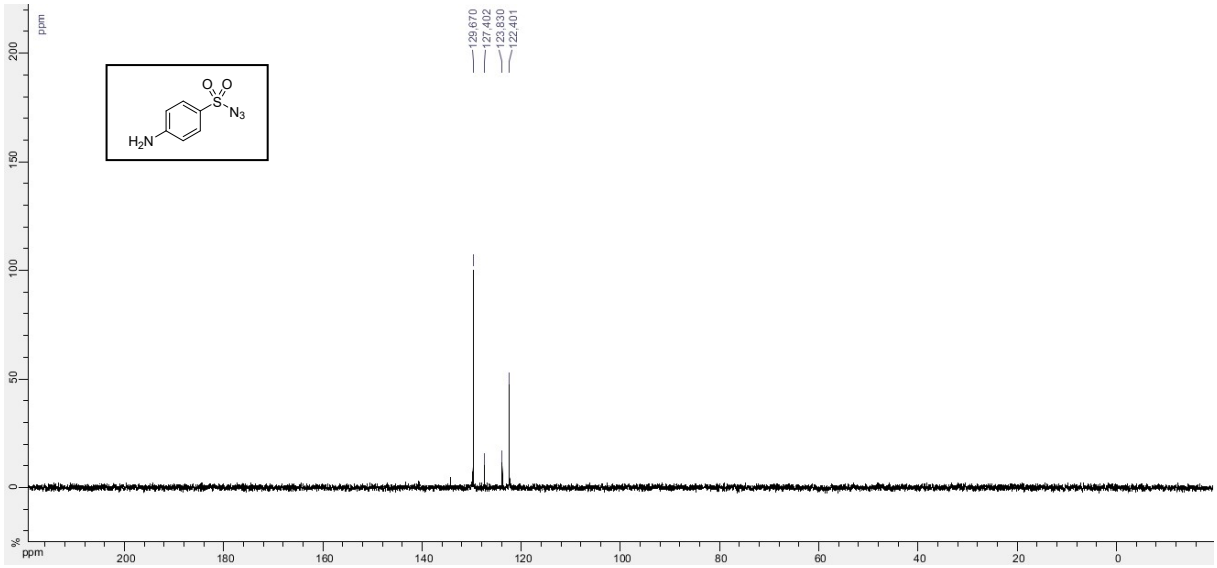


A modified version of a protocol previously describes was used to isolate the product as an hydrochloride salt.[2]

4-acetamidobenzenesulfonyl azide **1** (1.2 g, 5 mmol) was suspended in conc. aq. HCl (5 mL) and the resulting mixture was stirred at 90°C for 30 min. Thereafter, the mixture was diluted with THF (150 mL) and evaporated to dryness. The resulting residue was taken up in toluene and evaporated to dryness (2 x 50 mL). The resulting white hydrochloride salt was dissolved in H_2O and lyophilized giving the 4-aminobenzenesulfonyl azide **2** (1.15 g, 4.91 mmol, quant.) as a white amorphous powder. ^{1}H NMR (400 MHz, D_2O): δ = 7.50-7.55 (m, 2H), 8.04-8.08 (m, 2H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 122.4, 123.8, 127.4, 129.7 ppm.

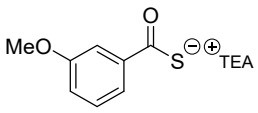
^{1}H and ^{13}C NMR Spectra (D_2O)





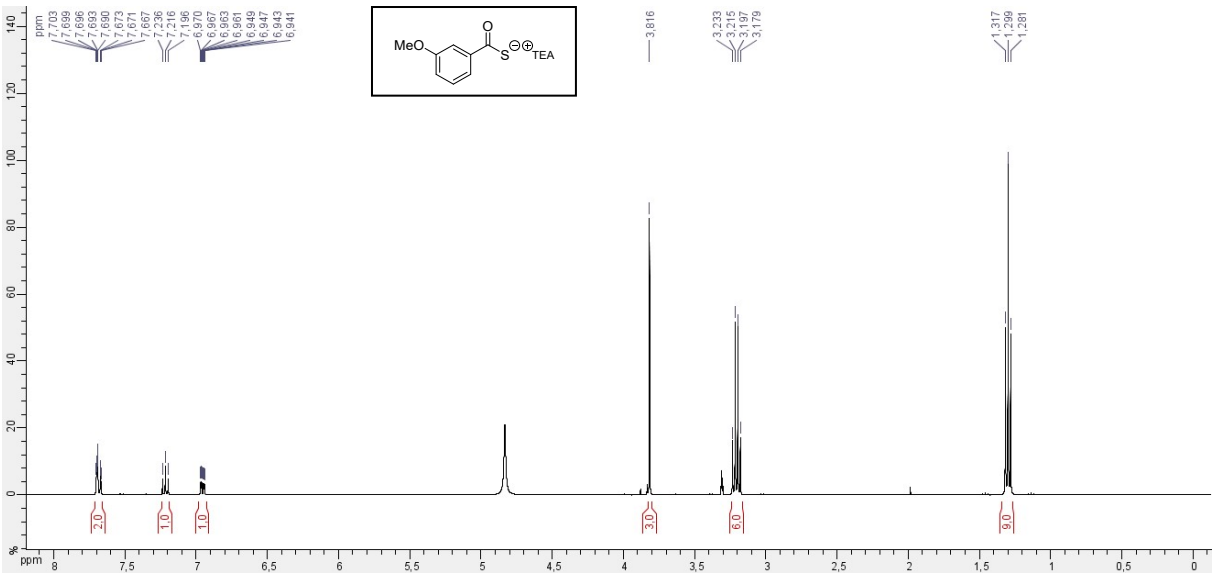
Synthesis of triethylammonium thioacetate derivatives

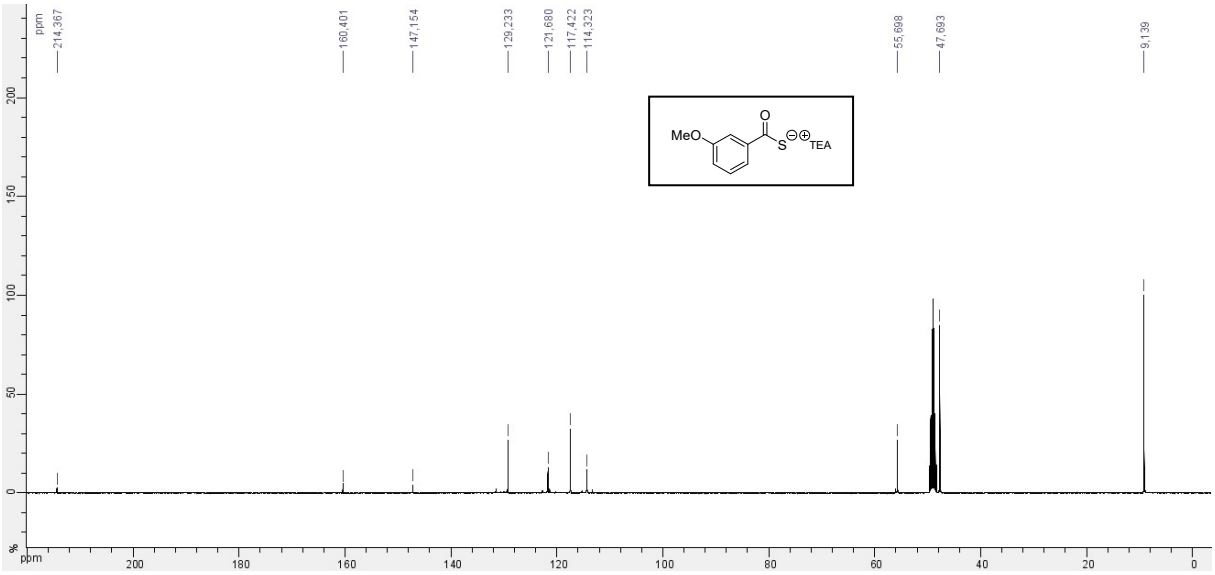
Triethylammonium 3-methoxybenzothioate (3a)



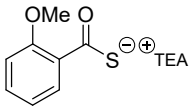
3-methoxybenzoic acid (228 mg, 1.5 mmol) was dissolved in dry DMF (6 mL). Thereafter, potassium thioacetate (513 mg, 4.5 mmol) and acetyl sulfide (30 μL, 300 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system B, conversion about 80%). Thereafter, aq. 50 mM TEAA (pH 7.0, 54 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-20%) in aq. 50 mM TEAA as the mobile phase, giving the triethylammonium 3-methoxybenzothioate **3a** as a yellow amorphous powder after lyophilization (210 mg, 780 µmol, 65% after recovery of starting material). ^{1}H NMR (400 MHz, MeOD): δ = 1.30 (t, *J* = 7.3 Hz, 9H), 3.21 (q, *J* = 7.3 Hz, 6H), 3.82 (s, 3H), 6.94-6.97 (m, 1H), 7.22 (t, *J* = 7.8 Hz, 1H), 7.67-7.70 (m, 2H), ppm. ^{13}C NMR (100 MHz, MeOD): δ = 9.1, 47.7, 55.7, 114.3, 117.4, 121.7, 129.2, 147.2, 160.4, 214.4 ppm. HRMS (ESI) *m/z*: $[M-H]^-$ Calcd for $C_8H_7O_2S$: 167,0172; found 167.0198.

^{1}H and ^{13}C NMR Spectra (MeOD)



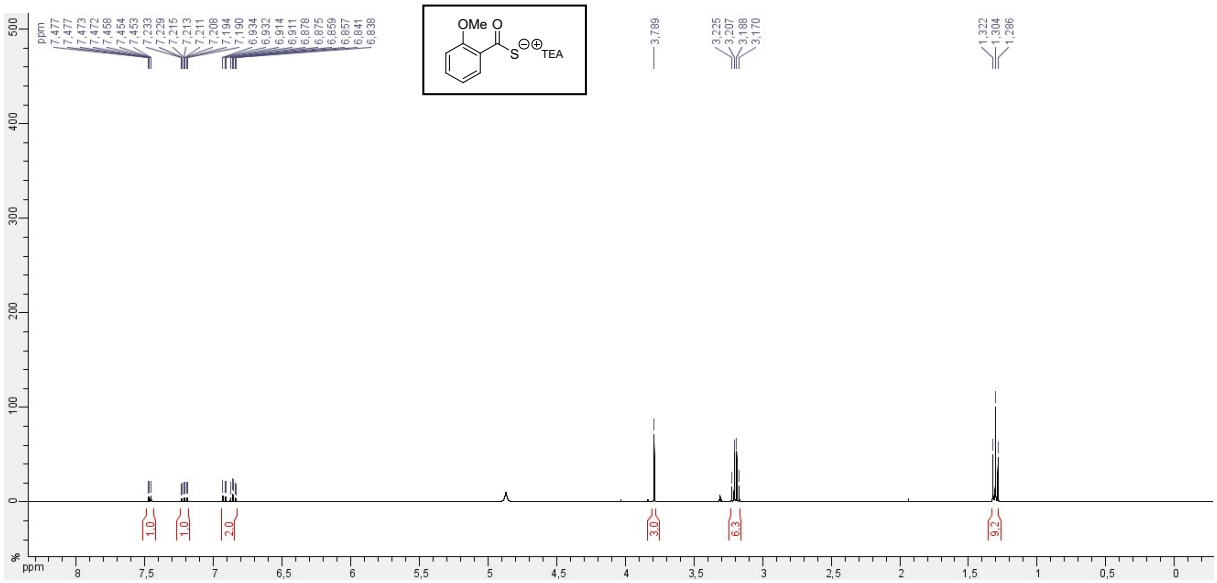


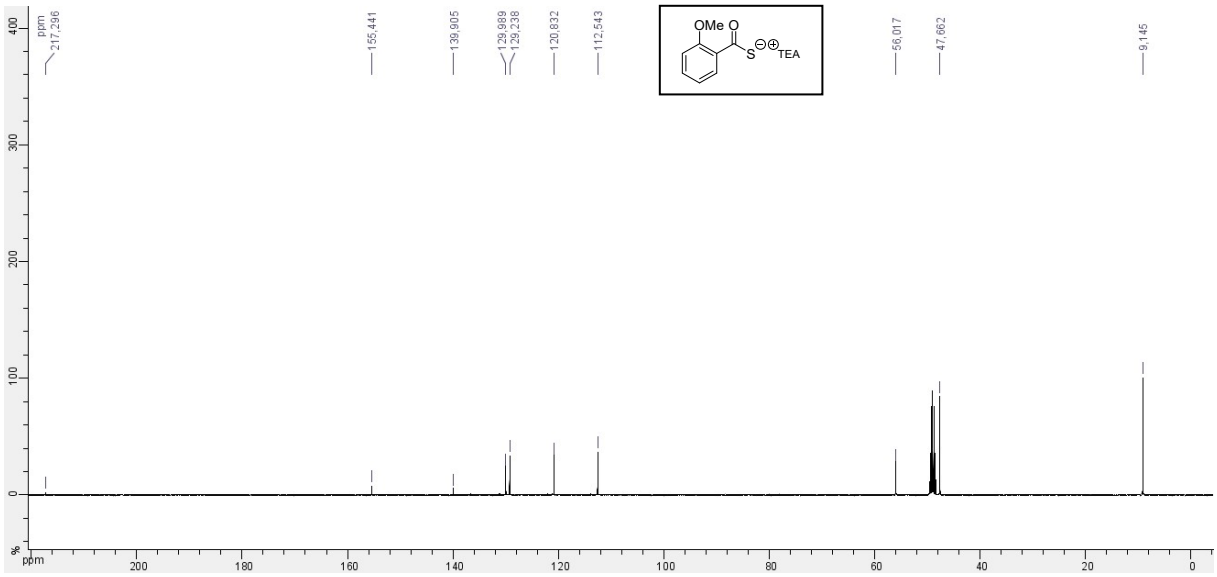
Triethylammonium 2-methoxybenzothioate (3b)



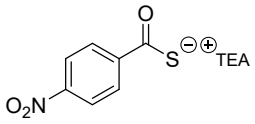
2-methoxybenzoic acid (228 mg, 1.5 mmol) was dissolved in dry DMF (6 mL). Thereafter, potassium thioacetate (513 mg, 4.5 mmol) and acetyl sulfide (30 μL, 300 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system B, conversion about 65%). Thereafter, aq. 50 mM TEAA (pH 7.0, 54 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-20%) in aq. 50 mM TEAA as the mobile phase, giving the triethylammonium 2-methoxybenzothioate **3b** as a yellow amorphous powder after lyophilization (182 mg, 677 µmol, 69% after recovery of starting material). ^{1}H NMR (400 MHz, MeOD): δ = 1.30 (t, *J* = 7.3 Hz, 9H), 3.20 (q, *J* = 7.3 Hz, 6H), 3.79 (s, 3H), 6.84-7.48 (m, 4H) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 9.2, 47.7, 56.0, 112.5, 120.8, 129.2, 130.0, 139.9, 155.4, 217.3 ppm. HRMS (ESI) *m/z*: $[M-H]^-$ Calcd for $C_8H_7O_2S$: 167,0172; found 167.0197.

^{1}H and ^{13}C NMR Spectra (MeOD)



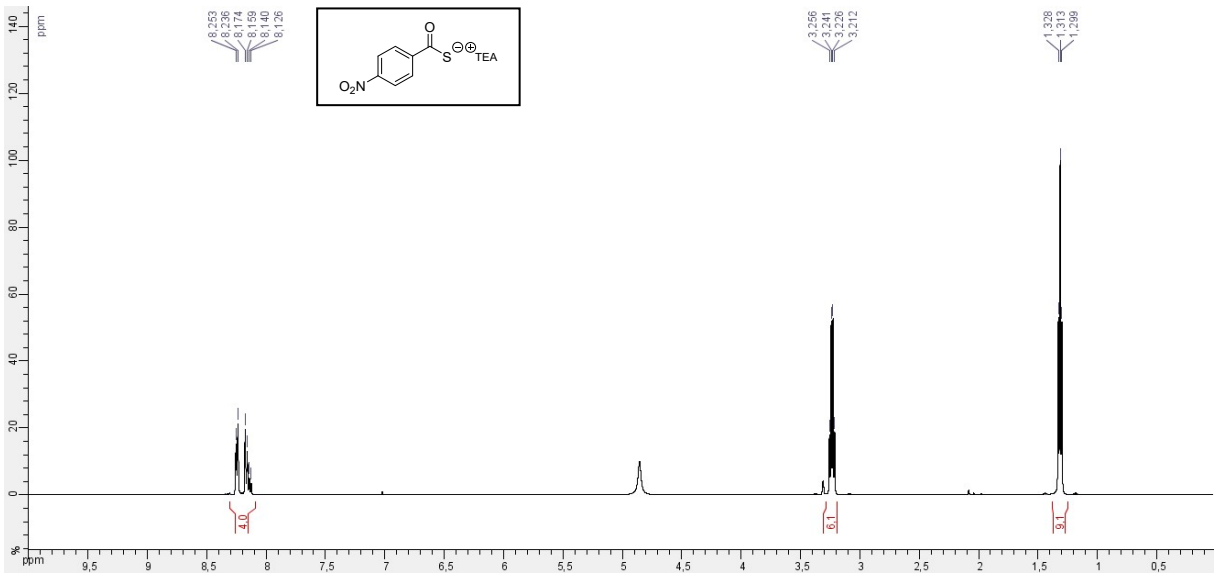


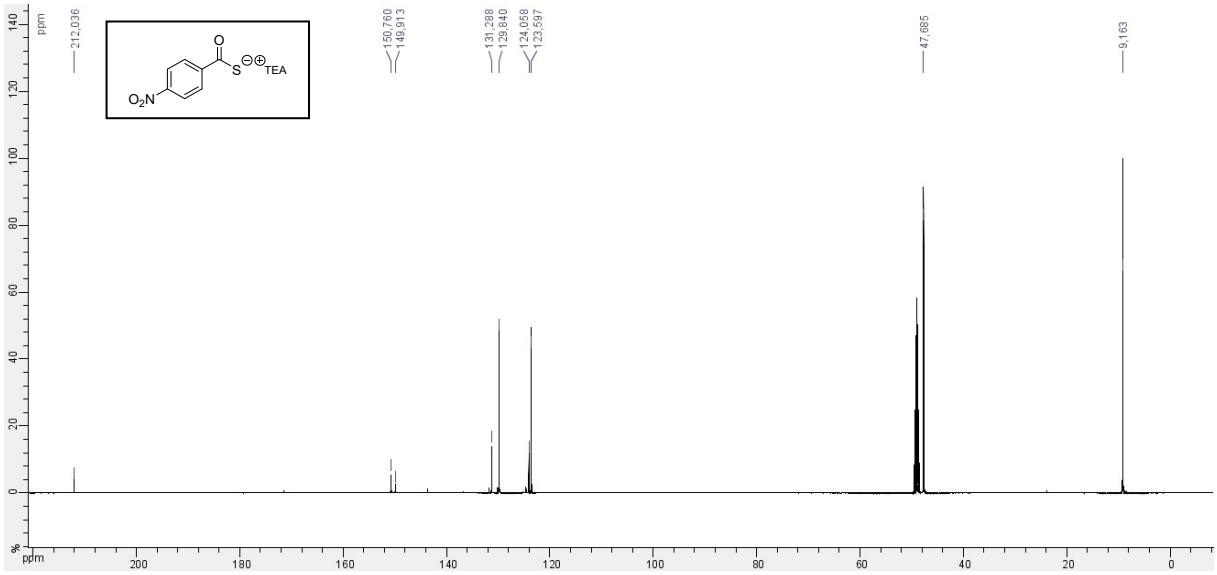
Triethylammonium 4-nitrobenzothioate (3c)



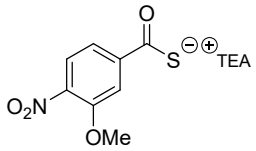
4-nitrobenzoic acid (84 mg, 500 μmol) was dissolved in dry DMF (3 mL). Thereafter, potassium thioacetate (171 mg, 1.5 mmol) and acetyl sulfide (10 μL, 100 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system B, conversion about 61%). Thereafter, aq. 50 mM TEAA (pH 7.0, 28 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-30%) in aq. 50 mM TEAA as the mobile phase, giving the triethylammonium 4-nitrobenzothioate **3c** as a red amorphous powder after lyophilization (80 mg, 281 μmol, 94% after recovery of starting material). Noteworthy, the product is likely to degrade at RT in solution preventing the obtaining of clean spectra. ^{1}H NMR (500 MHz, MeOD): δ = 1.31 (t, *J* = 7.3 Hz, 9H), 3.23 (q, *J* = 7.3 Hz, 6H), 3.99 (s, 3H), 8.13-8.25 (m, 4H) ppm. ^{13}C NMR (125 MHz, MeOD): δ = 9.2, 47.7, 123.6, 124.1, 129.8, 131.3, 149.9, 150.8, 212.0 ppm. HRMS (ESI) *m/z*: $[M-H]^-$ Calcd for $C_7H_4NO_3S$: 181.9912; found 181.9916.

^{1}H and ^{13}C NMR Spectra (MeOD)



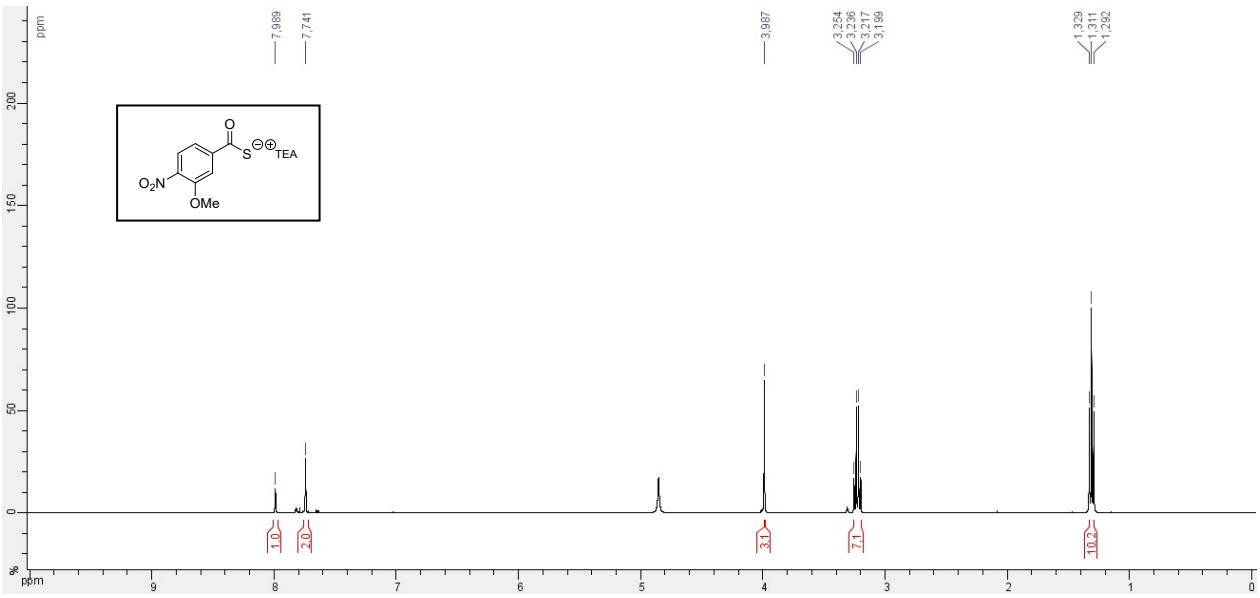


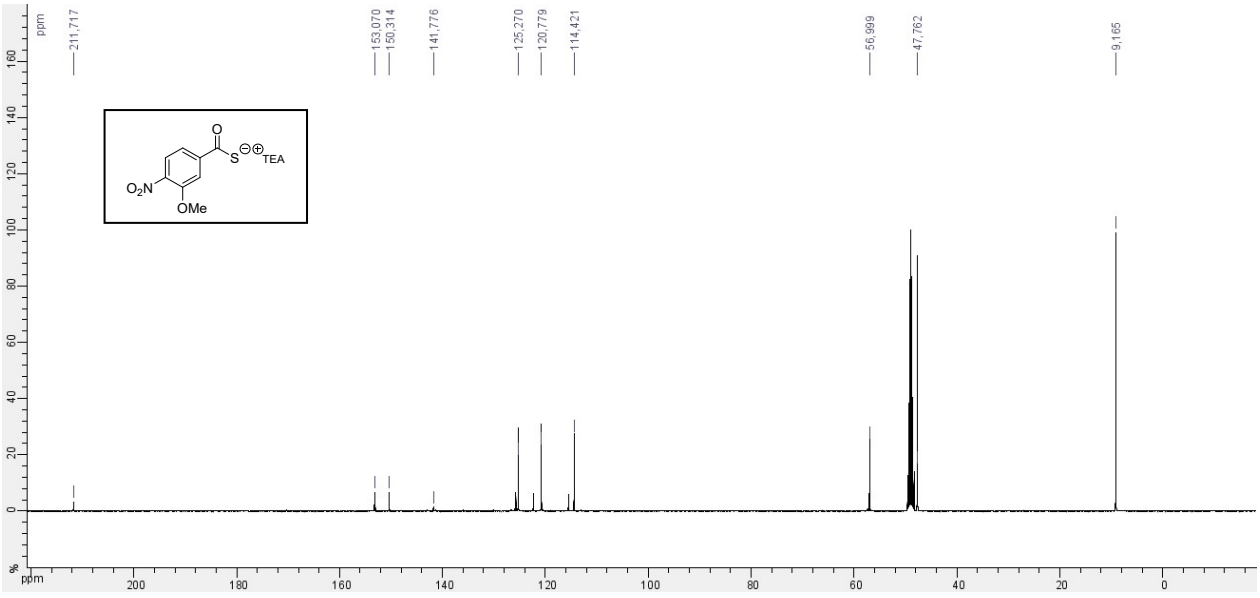
Triethylammonium 3-Methoxy-4-nitrobenzothioate (3d)



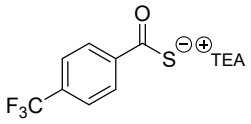
3-Methoxy-4-nitrobenzoic acid (100 mg, 500 μmol) was dissolved in dry DMF (2 mL). Thereafter, potassium thioacetate (171 mg, 1.5 mmol) and acetyl sulfide (10 μL, 100 µmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system B, conversion about 69%). Thereafter, aq. 50 mM TEAA (pH 7.0, 18 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-30%) in aq. 50 mM TEAA as the mobile phase, giving the triethylammonium 3-Methoxy-4-nitrobenzothioate **3d** as a red amorphous powder after lyophilization (100 mg, 318 μmol, 92% after recovery of starting material). Noteworthy, the product is likely to degrade at RT in solution preventing the obtaining of clean spectra. ^{1}H NMR (400 MHz, MeOD): δ = 1.31 (t, *J* = 7.3 Hz, 9H), 3.23 (q, *J* = 7.3 Hz, 6H), 3.99 (s, 3H), 7.74 (s, 2H), 7.99 (s, 1H) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 9.2, 47.8, 57.0, 114.4, 120.8, 125.3, 141.8, 150.3, 153.1, 211.7 ppm. HRMS (ESI) *m/z*: $[M-H]^-$ Calcd for $C_8H_6NO_4S$: 212.0018; found 212.0017.

^{1}H and ^{13}C NMR Spectra (MeOD)



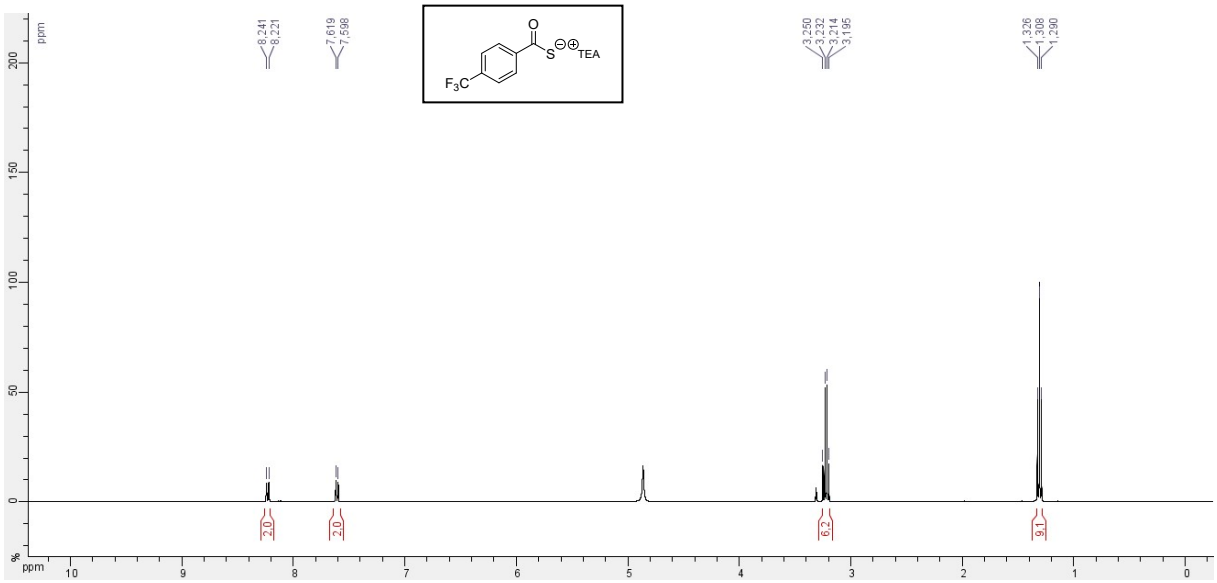


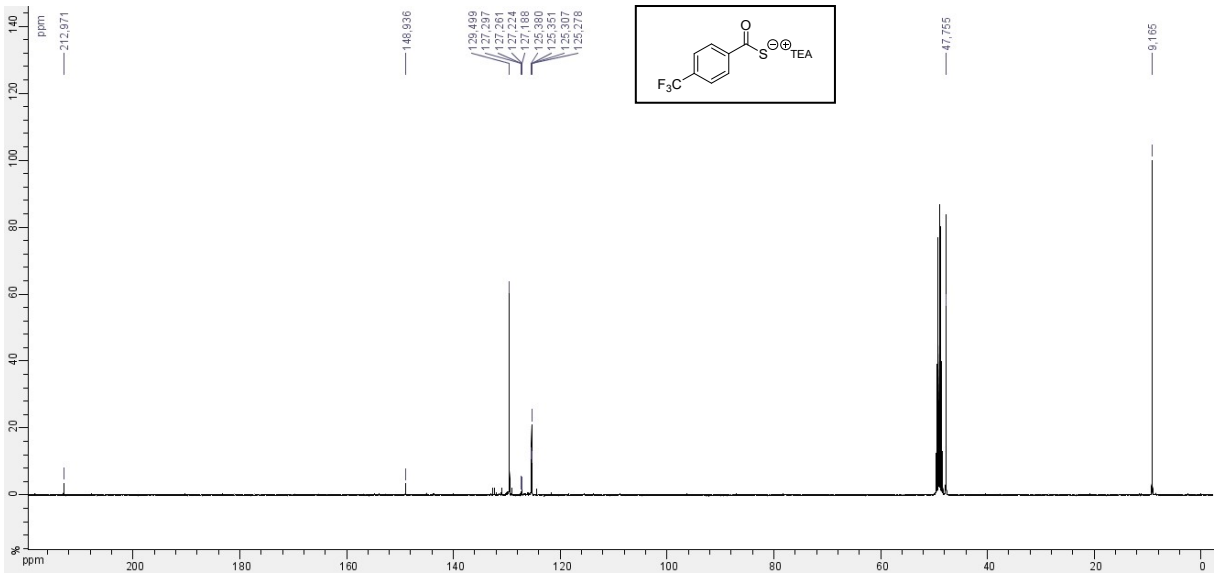
Triethylammonium 4-(trifluoromethyl)benzothioate (3e)



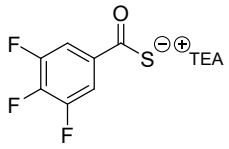
4-(trifluoromethyl)benzoic acid (285 mg, 1.5 mmol) was dissolved in dry DMF (6 mL). Thereafter, potassium thioacetate (513 mg, 4.5 mmol) and acetyl sulfide (30 μL, 300 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system B, conversion about 85%). Thereafter, aq. 50 mM TEAA (pH 7.0, 54 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-30%) in aq. 50 mM TEAA as the mobile phase, giving the triethylammonium 4-(trifluoromethyl)benzothioate **3e** as a red amorphous powder after lyophilization (317 mg, 1.03 mmol, 81% after recovery of starting material). ^{1}H NMR (400 MHz, MeOD): δ = 1.31 (t, *J* = 7.3 Hz, 9H), 3.22 (q, *J* = 7.3 Hz, 6H), 7.60-8.24 (m, 4H) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 9.2, 47.8, 125.3 (q, $J_{C\text{-}F}$ = 3.7 Hz), 127.2 (q, $J_{C\text{-}F}$ = 3.7 Hz), 149.0, 213.0 ppm. HRMS (ESI) *m/z*: $[M\text{-}H]^-$ Calcd for $C_8H_4F_3OS$: 204.9940; found 204.9944.

^{1}H and ^{13}C NMR Spectra (MeOD)



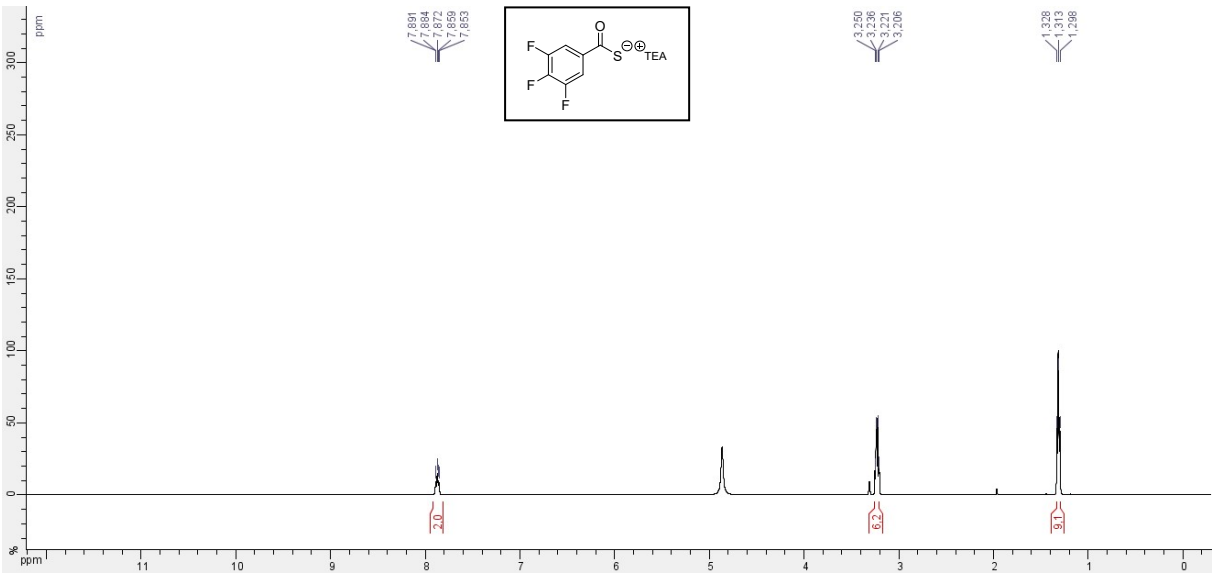


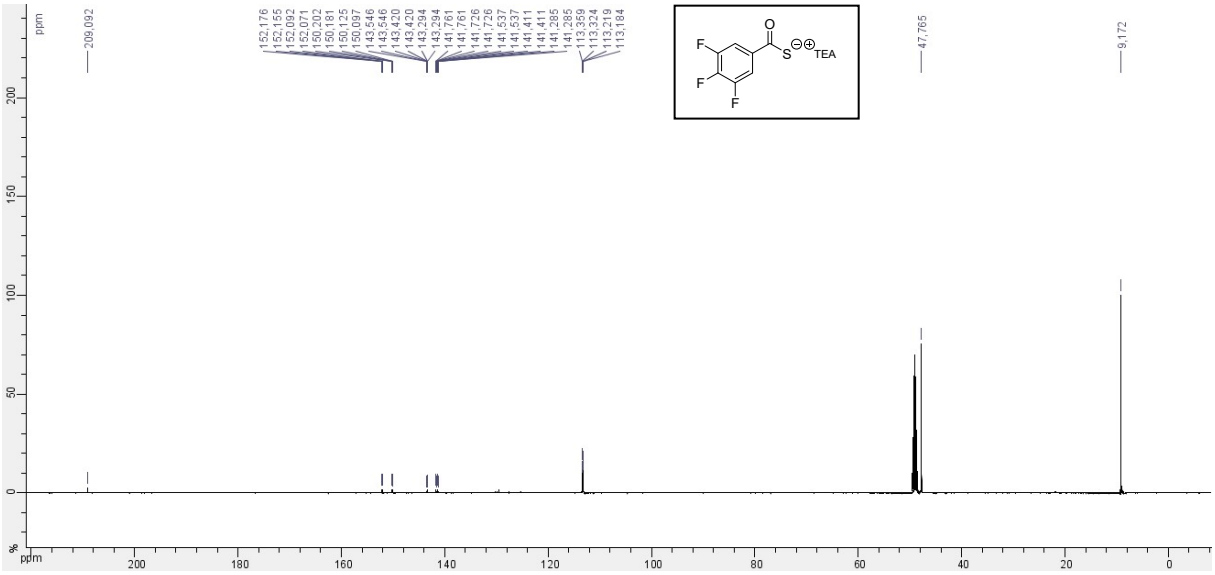
Triethylammonium 3,4,5-trifluorobenzothioate (3f)



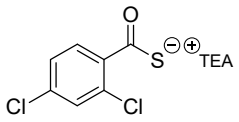
3,4,5-trifluorobenzoic acid (264 mg, 1.5 mmol) was dissolved in dry DMF (6 mL). Thereafter, potassium thioacetate (513 mg, 4.5 mmol) and acetyl sulfide (30 μL, 300 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system B, conversion about 60%). Thereafter, aq. 50 mM TEAA (pH 7.0, 54 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-30%) in aq. 50 mM TEAA as the mobile phase, giving the triethylammonium 3,4,5-trifluorobenzothioate **3f** as a red amorphous powder after lyophilization (160 mg, 641 μmol, 71% after recovery of starting material). ^{1}H NMR (500 MHz, MeOD): δ = 1.31 (t, *J* = 7.3 Hz, 9H), 3.23 (q, *J* = 7.3 Hz, 6H), 7.85-7.89 (m, 2H) ppm. ^{13}C NMR (125 MHz, MeOD): δ = 9.2, 47.8, 113.2-113.4 (m), 141.3-141.8 (m), 143.3-143.5 (m), 150.1-150.2 (m), 152.1-152.2 (m), 209.1 ppm. HRMS (ESI) *m/z*: $[M-H]^-$ Calcd for $C_7H_2F_3OS$: 190.9784; found 190.9793.

^{1}H and ^{13}C NMR Spectra (MeOD)



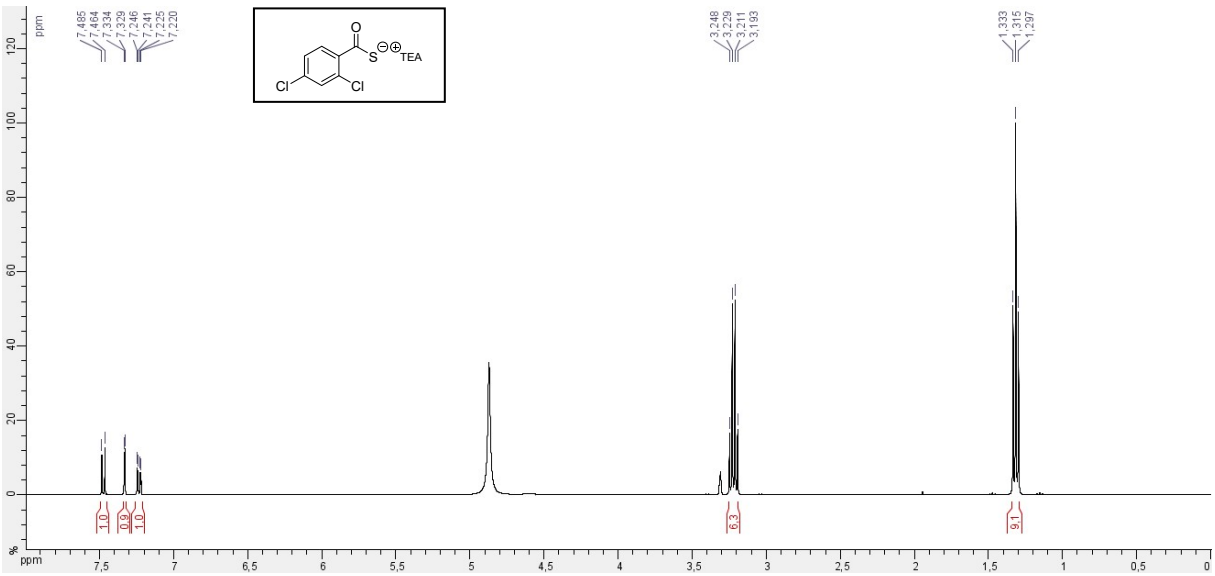


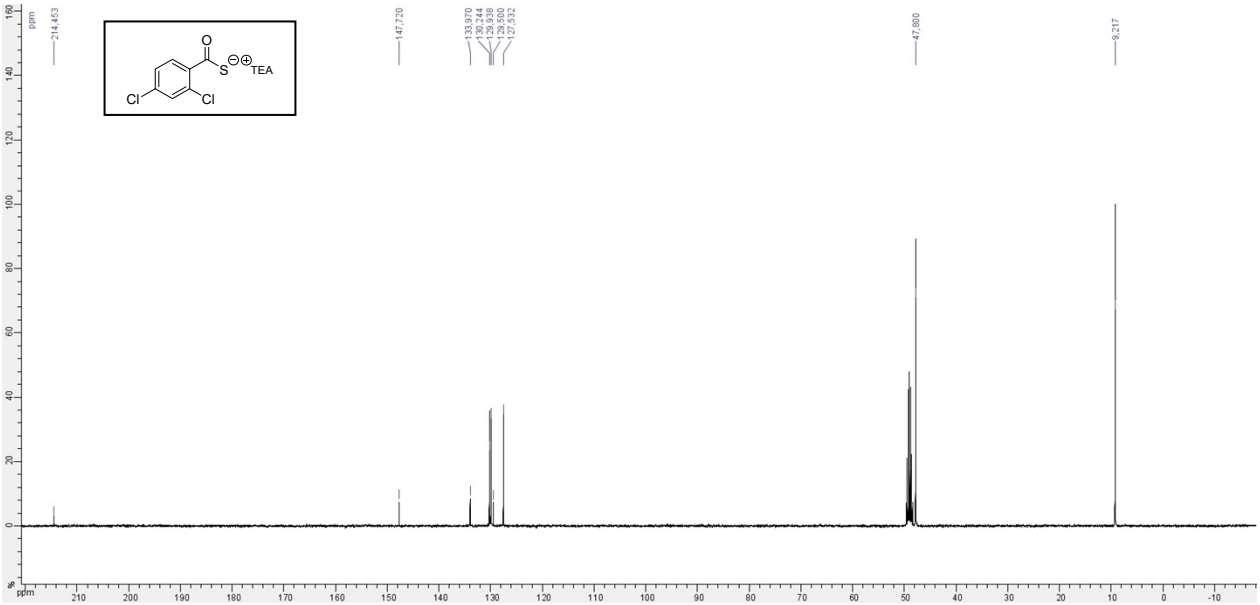
Triethylammonium 2,4-dichlorobenzothioate (3g)



2,4-dichlorobenzoic acid (285 mg, 1.5 mmol) was dissolved in dry DMF (6 mL). Thereafter, potassium thioacetate (513 mg, 4.5 mmol) and acetyl sulfide (30 μL, 300 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system B, conversion about 60%). Thereafter, aq. 50 mM TEAA (pH 7.0, 54 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-30%) in aq. 50 mM TEAA as the mobile phase, giving the triethylammonium 2,4-dichlorobenzothioate **3g** as a red amorphous powder after lyophilization (215 mg, 702 μmol, 78% after recovery of starting material). ^{1}H NMR (400 MHz, MeOD): δ = 1.32 (t, *J* = 7.3 Hz, 9H), 3.22 (q, *J* = 7.3 Hz, 6H), 7.22-7.49 (m, 3H) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 9.2, 47.8, 127.5, 129.5, 129.9, 130.2, 134.0, 147.7, 214.5 ppm. HRMS (ESI) *m/z*: $[M-H]^-$ Calcd for $C_7H_3OSCl_2$: 204.9282; found 204.9278.

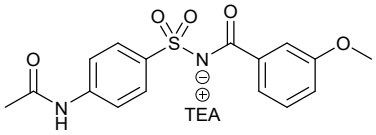
^{1}H and ^{13}C NMR Spectra (MeOD)





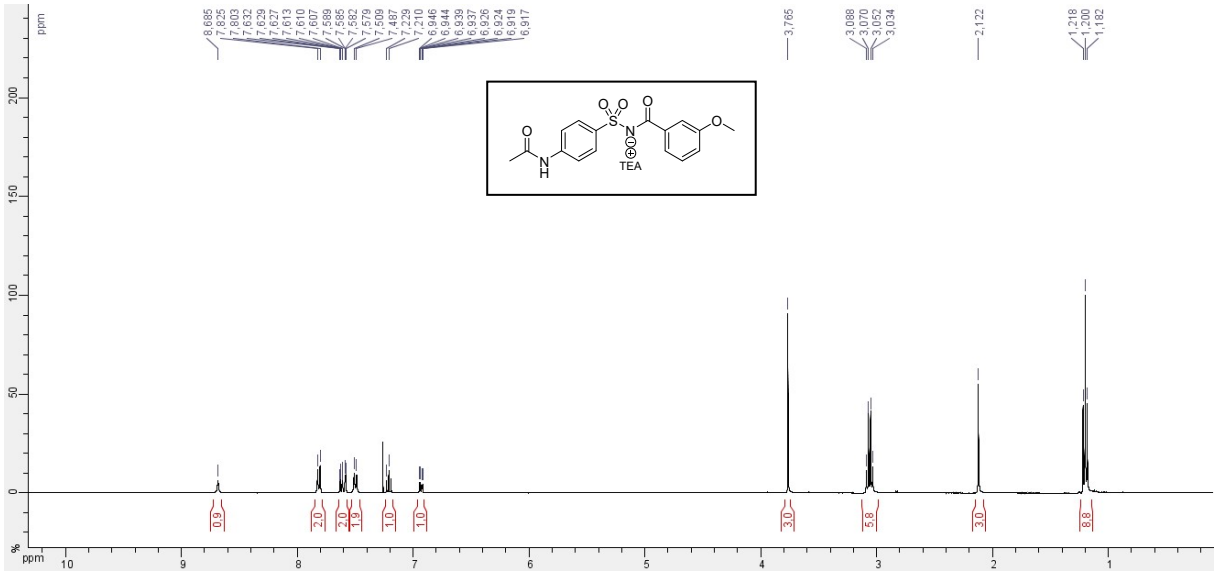
Synthesis of sulfadrug analogues

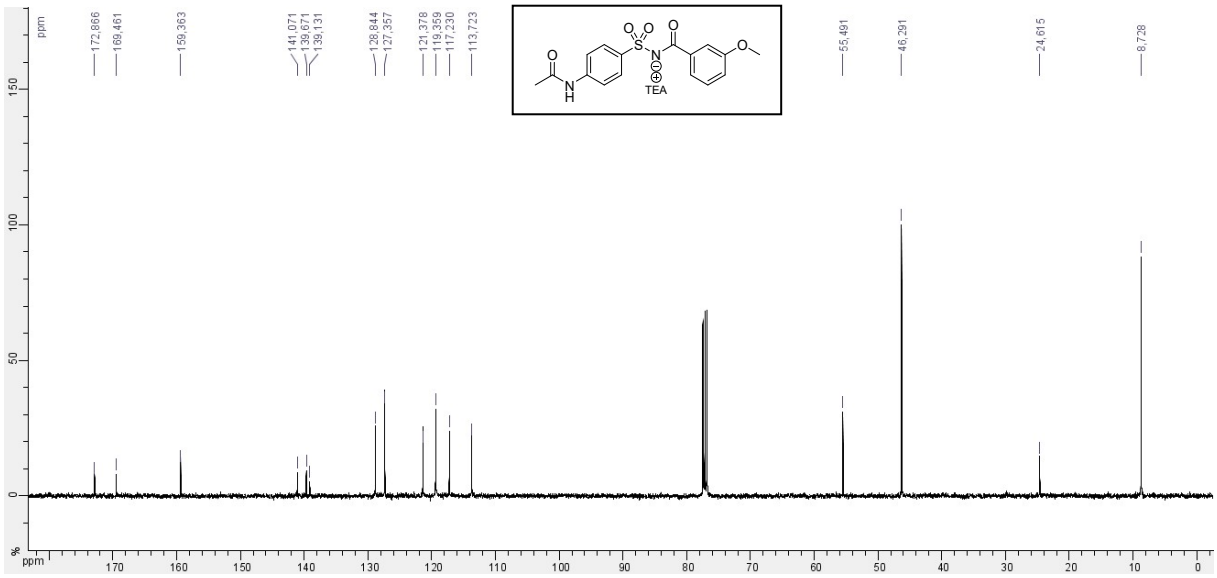
Triethylammonium ((4-acetamidophenyl)sulfonyl)(3-methoxybenzoyl)amide (4a)



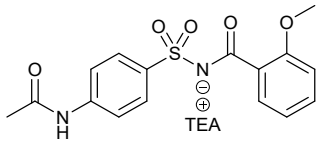
4-acetamidobenzenesulfonyl azide **1** (26 mg, 108 μmol) was dissolved in dry NMP (1.5 mL). Thereafter, triethylammonium 3-methoxybenzothioate **3a** (35 mg, 130 μmol), water (500 μL) and $NaHCO_3$ (22 mg, 260 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system B). Thereafter, aq. 50 mM TEAA (pH 7.0, 13.5 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-30%) in H_2O as the mobile phase, giving the triethylammonium ((4-acetamidophenyl)sulfonyl)(3-methoxybenzoyl)amide **4a** as a white amorphous powder after lyophilization (45 mg, 99 μmol, 92%, 96% purity). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.20 (t, *J* = 7.3 Hz, 9H), 2.12 (s, 3H), 3.06 (q, *J* = 7.3 Hz, 6H), 3.77 (s, 3H), 6.92-7.83 (m, 8H), 8.69 (bs, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 8.7, 24.6, 46.3, 55.5, 113.7, 117.2, 119.4, 121.4, 127.4, 128.8, 139.1, 139.7, 141.1, 159.4, 169.5, 172.9 ppm. HRMS (ESI) *m/z*: $[M-H]^-$ Calcd for $C_{16}H_{15}N_2O_5S$ 347.0707; found 347.0715.

^{1}H and ^{13}C NMR Spectra ($CDCl_3$)



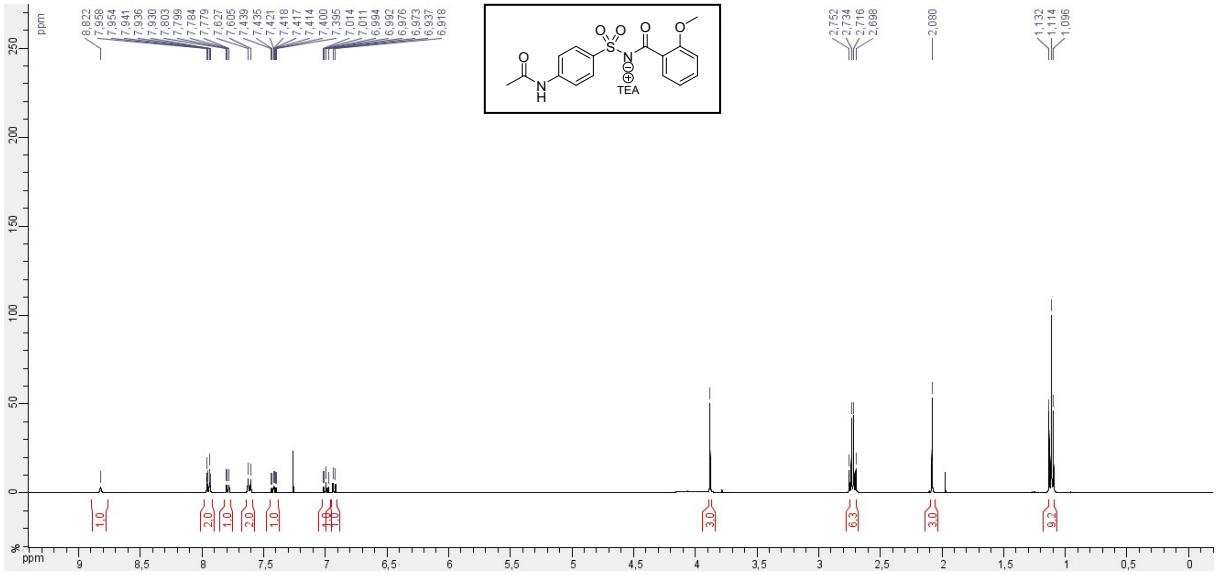


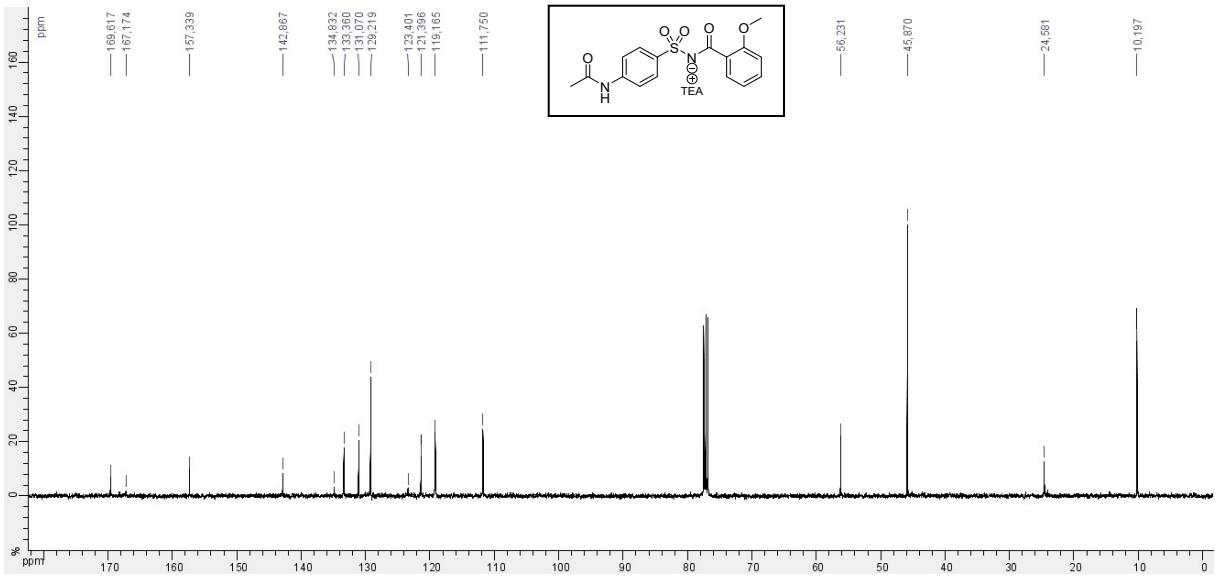
Triethylammonium ((4-acetamidophenyl)sulfonyl)(2-methoxybenzoyl)amide (4b)



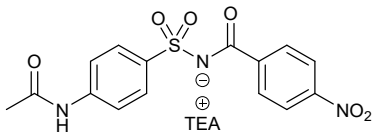
4-acetamidobenzenesulfonyl azide **1** (26 mg, 108 μmol) was dissolved in dry NMP (1.5 mL). Thereafter, triethylammonium 2-methoxybenzothioate **3b** (35 mg, 130 μmol), water (500 μL) and $NaHCO_3$ (22 mg, 260 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system B). Thereafter, aq. 50 mM TEAA (pH 7.0, 13.5 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-30%) in H_2O as the mobile phase, giving the triethylammonium ((4-acetamidophenyl)sulfonyl)(2-methoxybenzoyl)amide **4b** as a white amorphous powder after lyophilization (44 mg, 97 μmol, 90%, 96% purity). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.11 (t, *J* = 7.3 Hz, 9H), 2.08 (s, 3H), 2.73 (q, *J* = 7.3 Hz, 6H), 3.88 (s, 3H), 6.92-7.96 (m, 8H), 8.82 (bs, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.2, 24.6, 45.9, 56.2, 111.8, 119.2, 121.4, 123.4, 129.2, 131.1, 133.4, 134.8, 142.9, 157.4, 167.2, 169.6 ppm. HRMS (ESI) *m/z*: $[M-H]^-$ Calcd for $C_{16}H_{15}N_2O_5S$ 347.0707; found 347.0707.

^{1}H and ^{13}C NMR Spectra ($CDCl_3$)



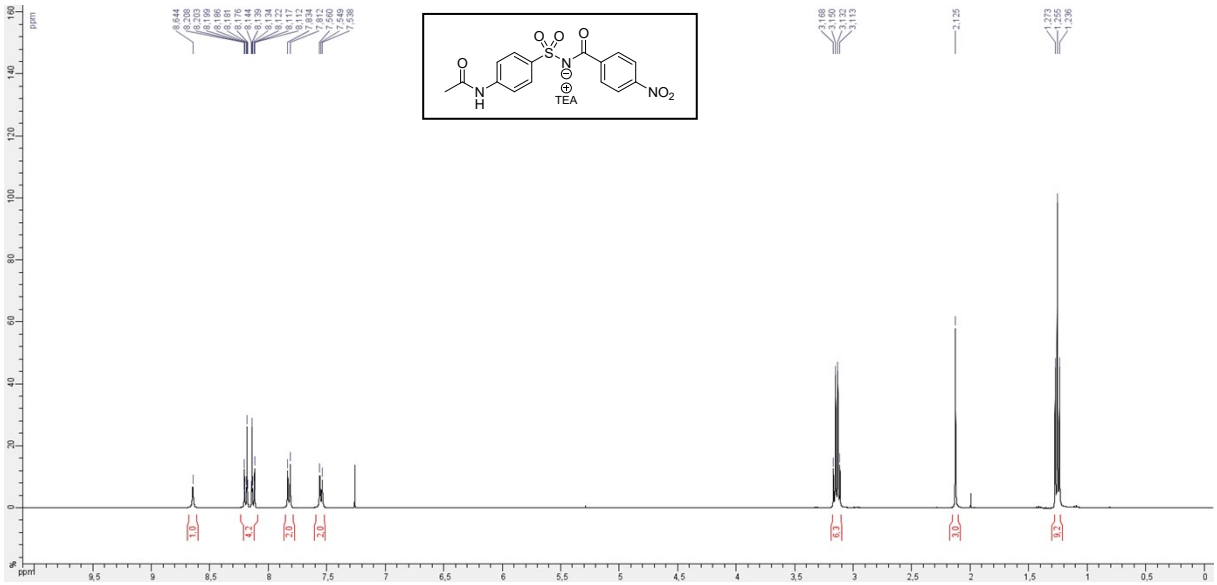


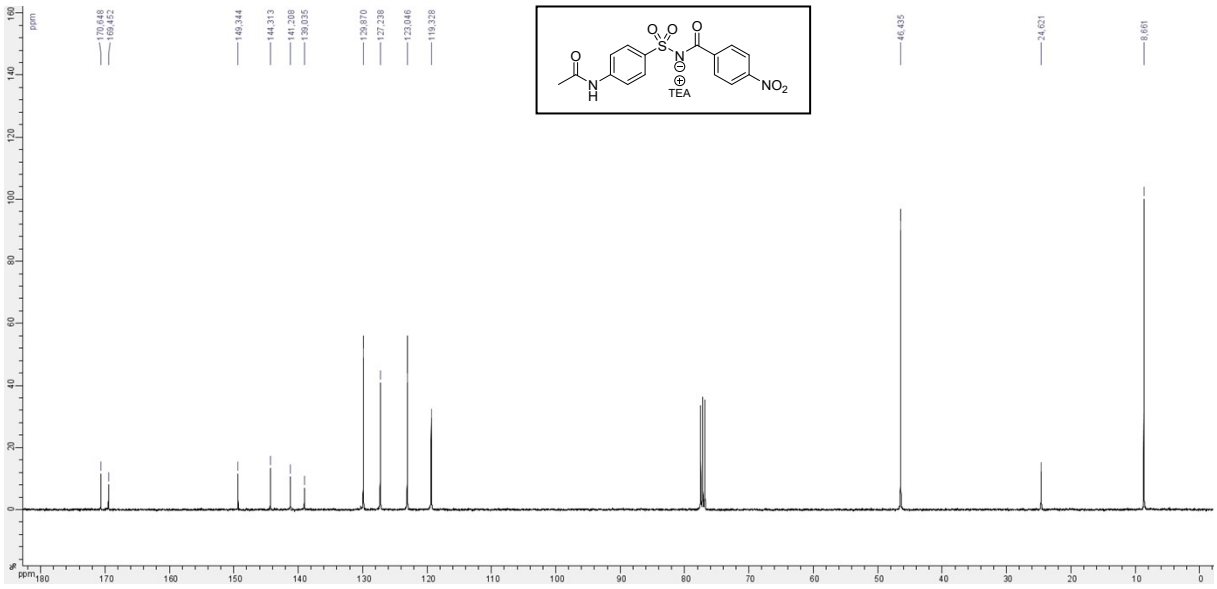
Triethylammonium ((4-aminophenyl)sulfonyl)(4-nitrobenzoyl)amide (4c)



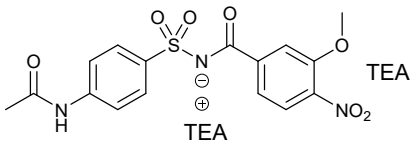
4-acetamidobenzenesulfonyl azide **1** (20 mg, 83 μmol) was dissolved in dry NMP (750 μL). Thereafter, triethylammonium 4-nitrobenzothioate **3c** (35 mg, 125 μmol), water (250 μL) and $NaHCO_3$ (11 mg, 125 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system B). Thereafter, aq. 50 mM TEAA (pH 7.0, 9 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-30%) in H_2O as the mobile phase, giving the triethylammonium ((4-aminophenyl)sulfonyl)(4-nitrobenzoyl)amide **4c** as a yellow amorphous powder after lyophilization (29 mg, 69 μmol, 84%, 97% purity). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.26 (t, *J* = 7.3 Hz, 9H), 2.13 (s, 3H), 3.14 (q, *J* = 7.3 Hz, 6H), 7.54-8.21 (m, 8H), 8.64 (s, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 8.7, 24.6, 46.4, 119.3, 123.0, 127.2, 129.9, 139.0, 141.2, 144.3, 149.3, 169.5, 170.6 ppm. HRMS (ESI) *m/z*: $[M-H]^-$ Calcd for $C_{15}H_{12}N_3O_6S$: 362.0447; found 362.0453.

^{1}H and ^{13}C NMR Spectra ($CDCl_3$)



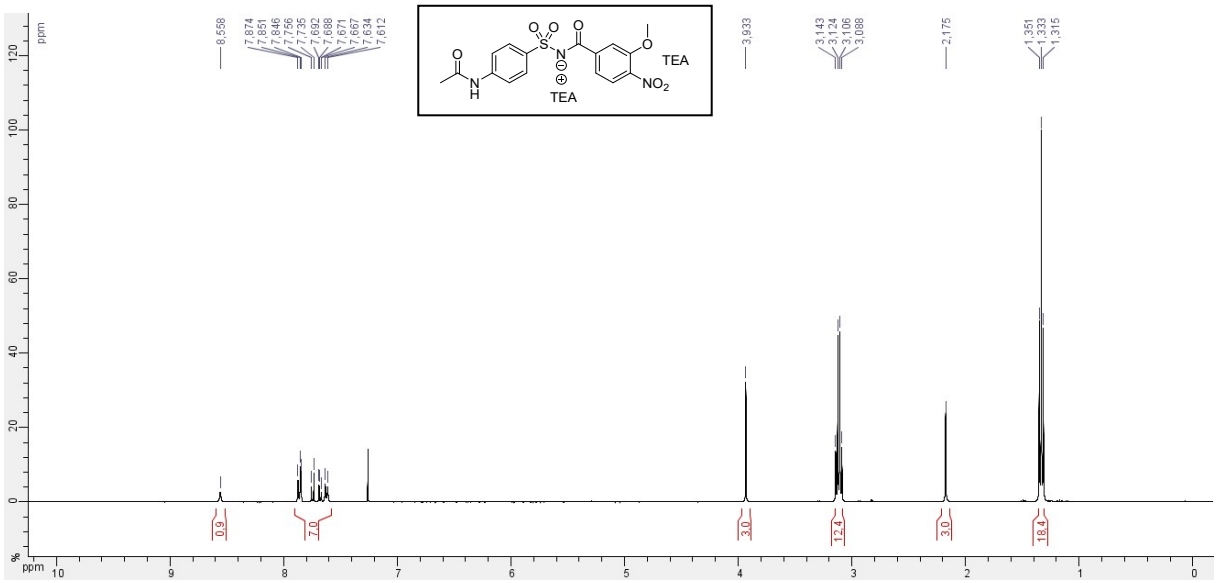


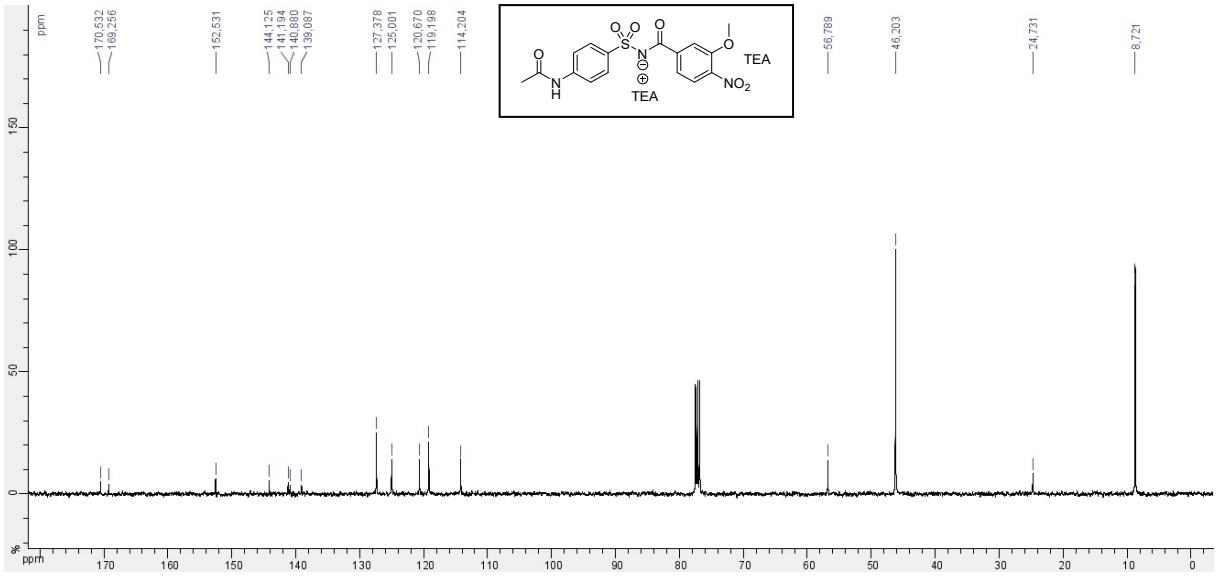
Triethylammonium ((4-aminophenyl)sulfonyl)(3-methoxy-4-nitrobenzoyl)amide (4d)



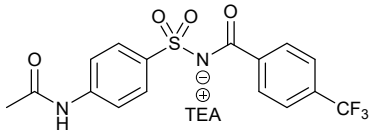
4-acetamidobenzenesulfonyl azide **1** (20 mg, 83 μmol) was dissolved in dry NMP (750 μL). Thereafter, triethylammonium 3-methoxy-4-nitrobenzothioate **3d** (39 mg, 125 μmol), water (250 μL) and $NaHCO_3$ (7 mg, 83 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system B). Thereafter, aq. 50 mM TEAA (pH 7.0, 9 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-30%) in H_2O as the mobile phase, giving the triethylammonium ((4-aminophenyl)sulfonyl)(3-methoxy-4-nitrobenzoyl)amide **4d** as a white amorphous powder after lyophilization (38 mg, 77 μmol, 93%, 97% purity). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.33 (t, *J* = 7.3 Hz, 18H), 2.18 (s, 3H), 3.12 (q, *J* = 7.3 Hz, 12H), 3.93 (s, 3H), 7.61-7.87 (m, 7H), 8.56 (s, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 8.7, 24.7, 46.2, 56.8, 114.2, 119.2, 120.7, 125.0, 127.4, 139.1, 140.9, 141.2, 144.1, 152.5, 169.3, 170.5 ppm. HRMS (ESI) *m/z*: $[M-H]^-$ Calcd for $C_{16}H_{14}N_3O_7S$: 392.0558; found 392.0567.

^{1}H and ^{13}C NMR Spectra ($CDCl_3$)



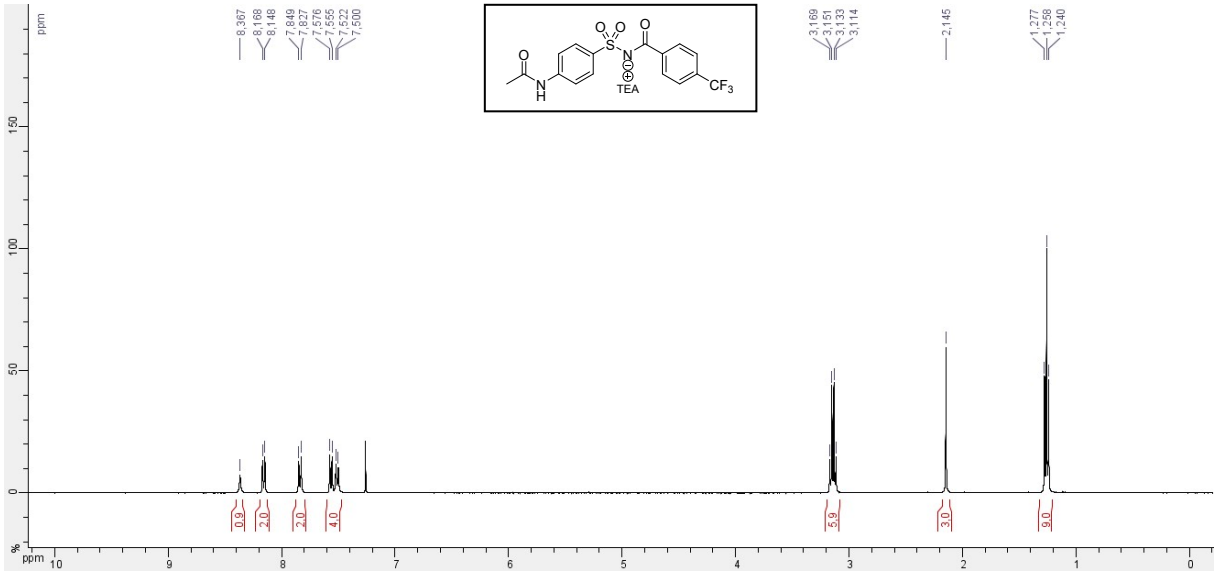


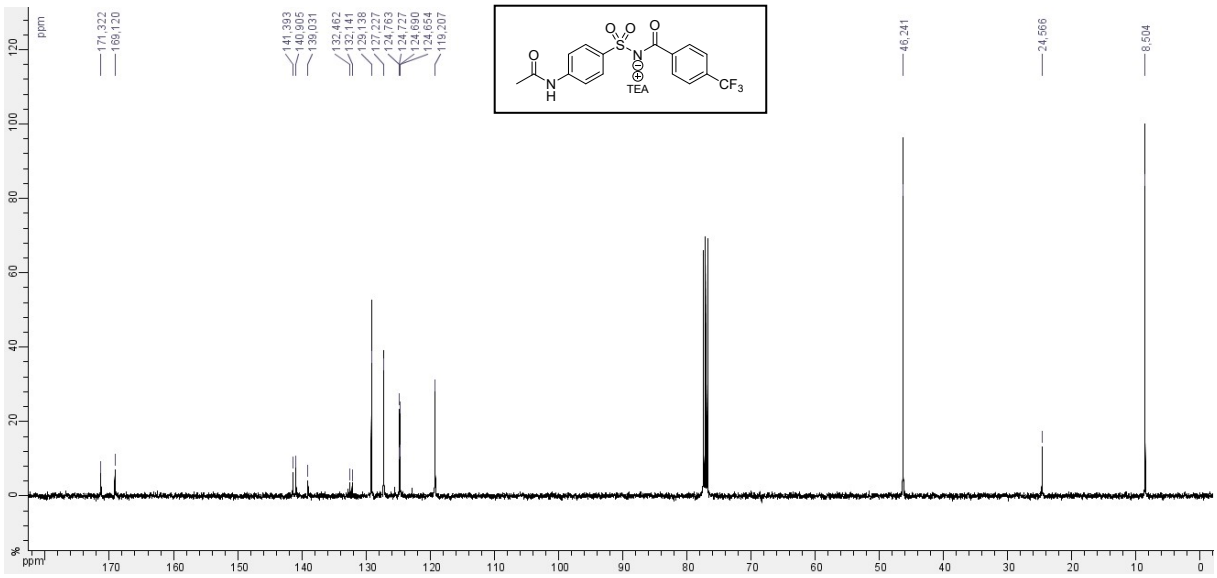
Triethylammonium ((4-acetamidophenyl)sulfonyl)(4-(trifluoromethyl)benzoyl)amide (4e)



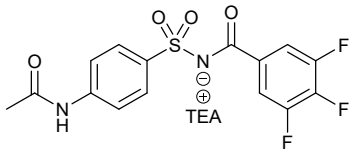
4-acetamidobenzenesulfonyl azide **1** (25 mg, 102 μmol) was dissolved in dry NMP (1.5 mL). Thereafter, triethylammonium 4-(trifluoromethyl)benzothioate **3e** (47 mg, 153 μmol), water (500 μL) and $NaHCO_3$ (13 mg, 65 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system B). Thereafter, aq. 50 mM TEAA (pH 7.0, 13.5 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-30%) in H_2O as the mobile phase, giving the triethylammonium ((4-acetamidophenyl)sulfonyl)(4-(trifluoromethyl)benzoyl)amide **4e** as an orange amorphous powder after lyophilization (49 mg, 100 μmol, quant., 97% purity). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.26 (t, *J* = 7.3 Hz, 9H), 2.15 (s, 3H), 3.14 (q, *J* = 7.3 Hz, 6H), 7.50-8.17 (m, 8H), 8.37 (bs, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 8.5, 24.6, 48.2, 119.2, 124.7 (q, $J_{C\text{-}F}$ = 3.7 Hz) 127.2, 129.1, 132.1, 132.5, 139.0, 140.9, 141.4, 169.1, 171.3 ppm. HRMS (ESI) *m/z*: $[M\text{-}H]^-$ Calcd for $C_{16}H_{12}F_3N_2O_4S$ 385.0475; found 385.0478.

^{1}H and ^{13}C NMR Spectra ($CDCl_3$)



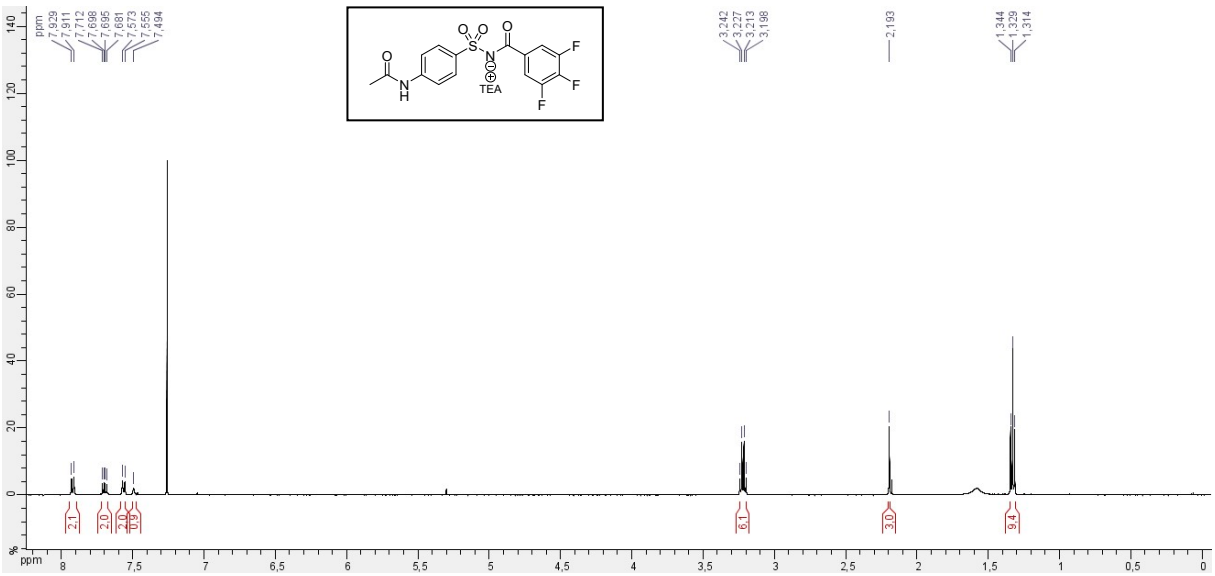


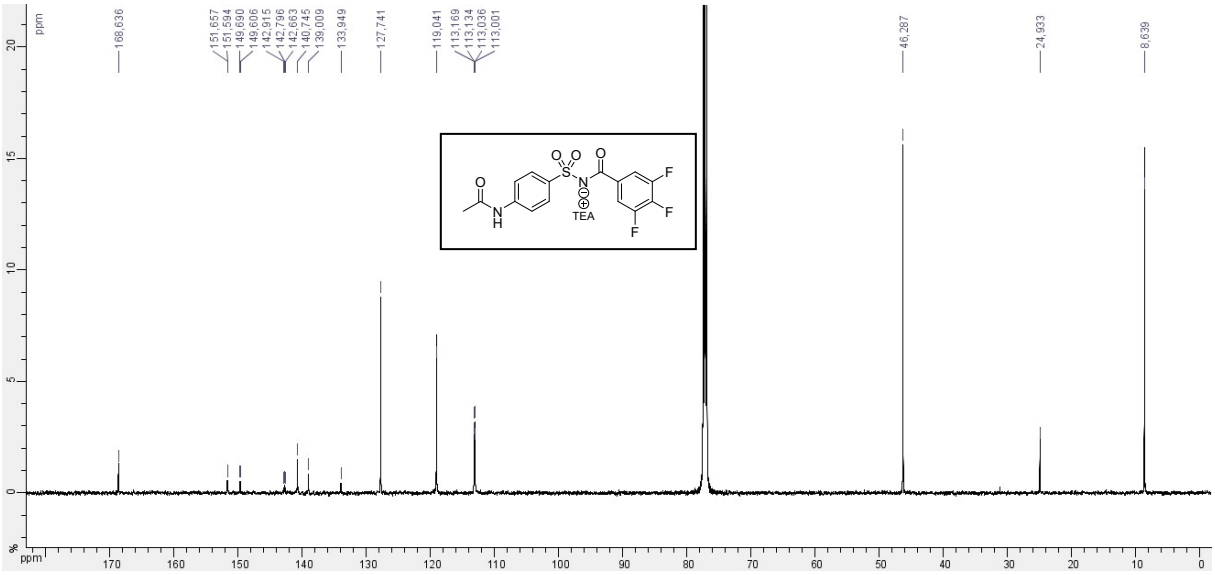
Triethylammonium ((4-acetamidophenyl)sulfonyl)(3,4,5-trifluorobenzoyl)amide (4f)



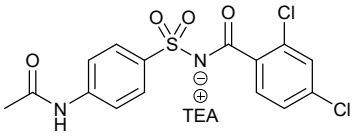
4-acetamidobenzenesulfonyl azide **1** (41 mg, 171 μmol) was dissolved in dry NMP (2 mL). Thereafter, triethylammonium 3,4,5-trifluorobenzothioate **3f** (75 mg, 256 μmol), water (650 μL) and $NaHCO_3$ (14 mg, 171 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system B). Thereafter, aq. 50 mM TEAA (pH 7.0, 18 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-30%) in H_2O as the mobile phase, giving the triethylammonium ((4-acetamidophenyl)sulfonyl)(3,4,5-trifluorobenzoyl)amide **4f** as a white amorphous powder after lyophilization (76 mg, 160 μmol, 94%, 95% purity). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.33 (t, J = 7.3 Hz, 9H), 2.19 (s, 3H), 3.22 (q, J = 7.3 Hz, 6H), 7.49 (bs, 1H), 7.56-7.93 (m, 6H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 8.6, 24.9, 46.3, 113.0-113.2 (m), 119.0, 127.7, 133.9, 139.0, 140.7, 141.8 (dt, $J_{1C\text{-}F}$ = 256 Hz, $J_{2C\text{-}F}$ =16 Hz), 150.1 (dd, $J_{1C\text{-}F}$ = 250 Hz, $J_{2C\text{-}F}$ = 10.9 Hz), 168.6 ppm. HRMS (ESI) *m/z*: $[M\text{-}H]^-$ Calcd for $C_{15}H_{10}F_3N_2O_4S$: 371.0319; found 371.0319.

^{1}H and ^{13}C NMR Spectra ($CDCl_3$)



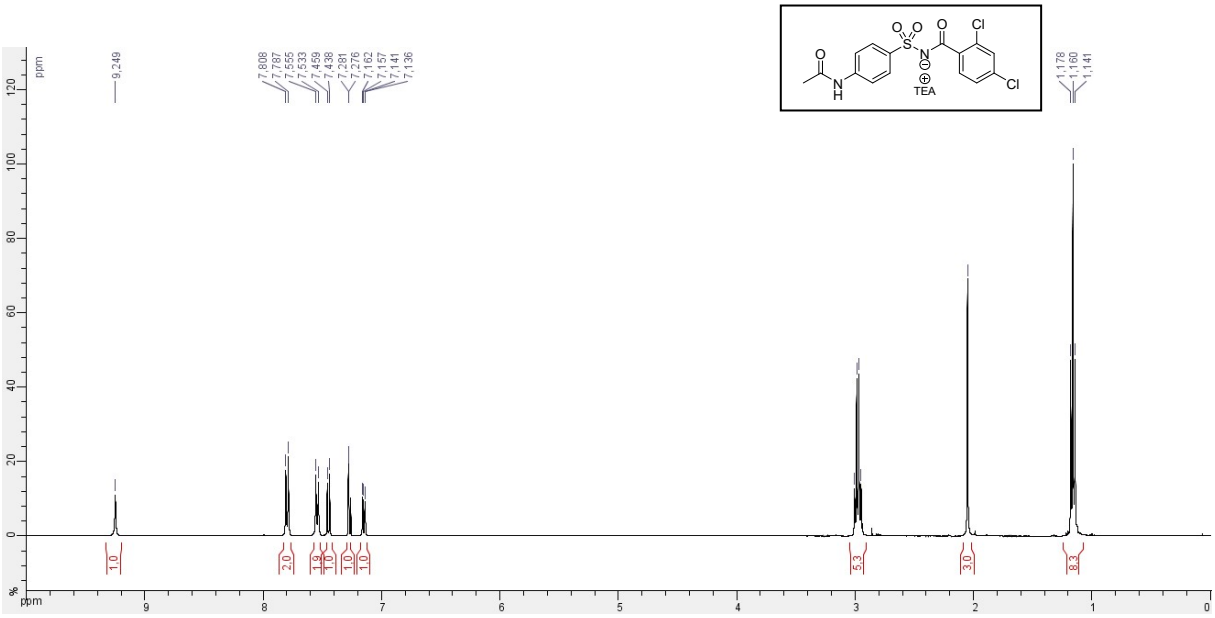


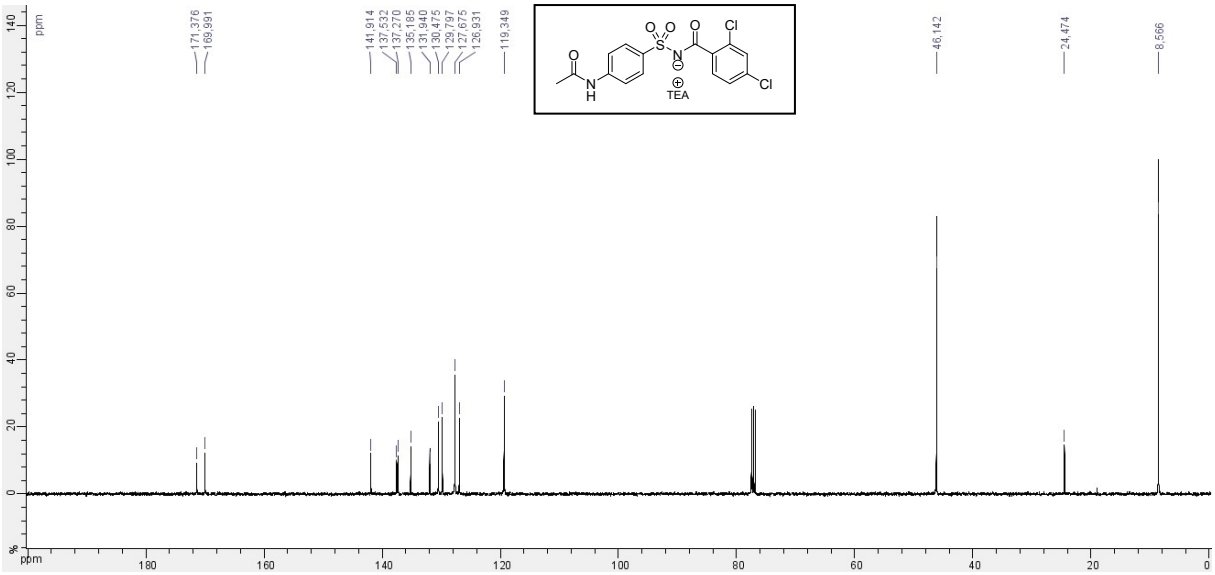
Triethylammonium ((4-acetamidophenyl)sulfonyl)(2,4-dichlorobenzoyl)amide (4g)



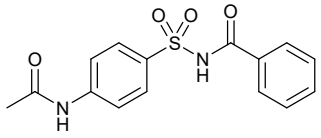
4-acetamidobenzenesulfonyl azide **1** (16 mg, 65 μmol) was dissolved in dry NMP (750 μL). Thereafter, triethylammonium 2,4-dichlorobenzothioate **3g** (30 mg, 97 μmol), water (250 μL) and $NaHCO_3$ (6 mg, 65 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system B). Thereafter, aq. 50 mM TEAA (pH 7.0, 9 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-30%) in H_2O as the mobile phase, giving the triethylammonium ((4-acetamidophenyl)sulfonyl)(2,4-dichlorobenzoyl)amide **4g** as a white amorphous powder after lyophilization (30 mg, 61 μmol, 93%, 99% purity). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.16 (t, *J* = 7.3 Hz, 9H), 2.98 (q, *J* = 7.3 Hz, 6H), 7.14-7.81 (m, 7H), 9.25 (s, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 8.6, 24.5, 46.1, 119.3, 126.9, 127.7, 129.8, 130.5, 131.9, 135.2, 137.3, 137.5, 141.9, 170.0, 171.4 ppm. HRMS (ESI) *m/z*: $[M-H]^-$ Calcd for $C_{15}H_{11}Cl_2N_2O_4S$: 384.9822; found 384.9827.

^{1}H and ^{13}C NMR Spectra ($CDCl_3$)



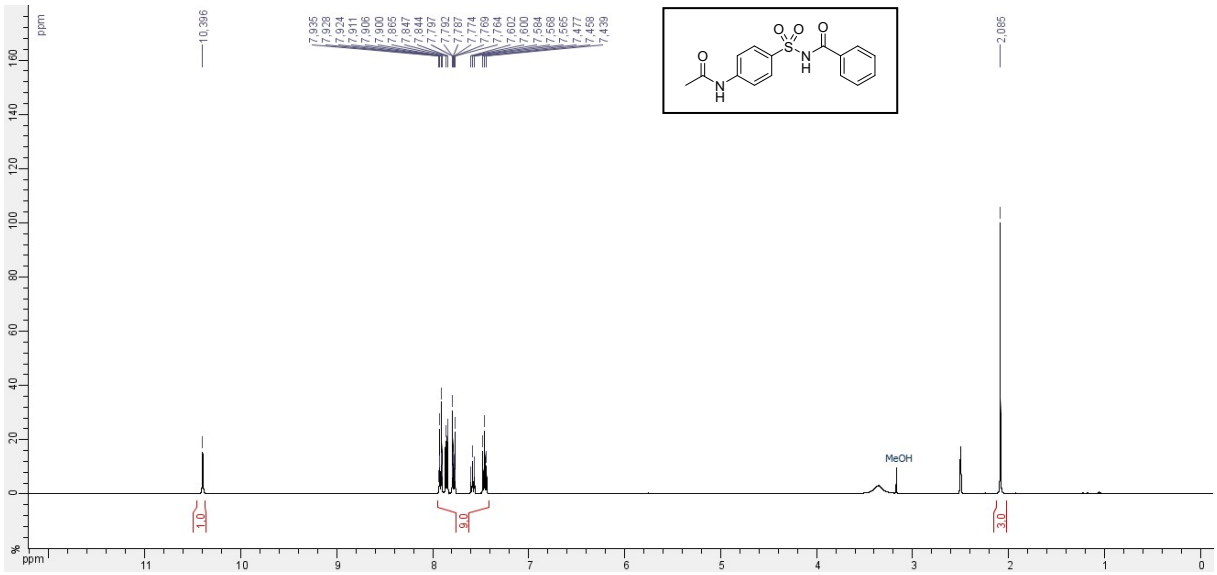


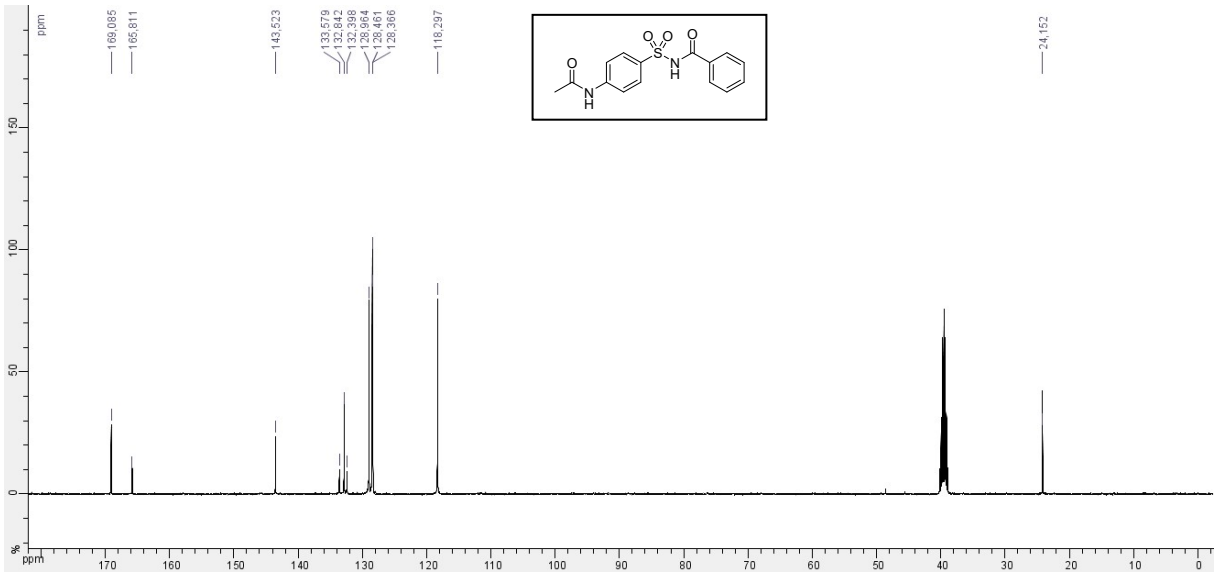
***N*-((4-acetamidophenyl)sulfonyl)benzamide (4h)**



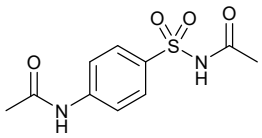
4-acetamidobenzenesulfonyl azide **1** (127 mg, 530 μmol) was dissolved in dry MeCN (12 mL). Thereafter, thiobenzoic acid **3h** (93 μL, 795 μmol), water (4 mL) and $NaHCO_3$ (134 mg, 1.6 mmol) were sequentially added. The resulting mixture was stirred at room temperature for 16 h. The reaction was checked for completion by TLC (DCM-MeOH, 96 : 4, v/v) and evaporated to dryness. The resulting residue was purified by chromatography on a silica gel column with a linear gradient of MeOH (0-20%) in DCM as the mobile phase, giving N-((4-acetamidophenyl)sulfonyl)benzamide **4h** as a white powder (152 mg, 477 μmol, 90%, 97% purity). ^{1}H NMR (400 MHz, DMSO): δ = 2.09 (s, 3H), 7.44-7.94 (m, 9H), 10.40 (s, 1H) ppm. ^{13}C NMR (100 MHz, DMSO): δ = 24.2, 118.3, 128.4, 128.5, 129.0, 132.4, 132.8, 133.6, 143.5, 165.8, 169.1 ppm. HRMS (ESI) *m/z*: $[M-H]^-$ Calcd for $C_{16}H_{14}N_3O_7S$: 392.0558; found 392.0567.

^{1}H and ^{13}C NMR Spectra (DMSO)



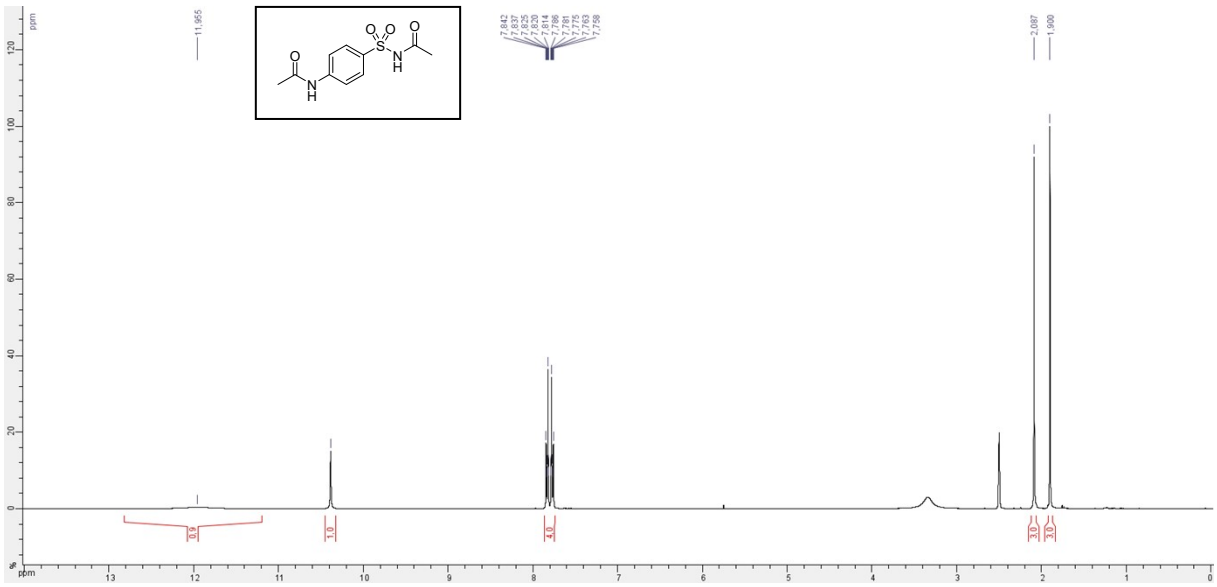


***N*-((4-acetamidophenyl)sulfonyl)acetamide (4i)**

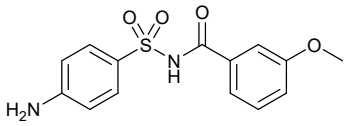


4-acetamidobenzenesulfonyl azide **1** (100 mg, 417 μmol) was dissolved in dry MeCN (7.5 mL). Thereafter, potassium thioacetate **3i** (71 mg, 625 μmol), water (2.5 mL) and $NaHCO_3$ (53 mg, 625 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 16 h. The reaction was checked for completion by TLC (DCM-MeOH, 96 : 4, v/v) and evaporated to dryness. The resulting residue was purified by chromatography on a silica gel column with a linear gradient of MeOH (0-20%) in DCM as the mobile phase, giving *N*-((4-acetamidophenyl)sulfonyl)acetamide **4i** as a white powder (97 mg, 379 μmol, 91%, 97% purity). ^{1}H NMR (400 MHz, DMSO): δ = 1.90 (s, 3H), 2.09 (s, 3H), 7.76-7.84 (m, 4H), 10.39 (s, 1H), 11.96 (bs, 1H) ppm. ^{13}C NMR (100 MHz, DMSO): δ = 23.2, 24.1, 118.4, 128.8, 132.8, 143.7, 168.7, 169.1 ppm. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{10}H_{13}N_2O_4S$: 257.0591; found 257.0601.

^{1}H and ^{13}C NMR Spectra (DMSO)

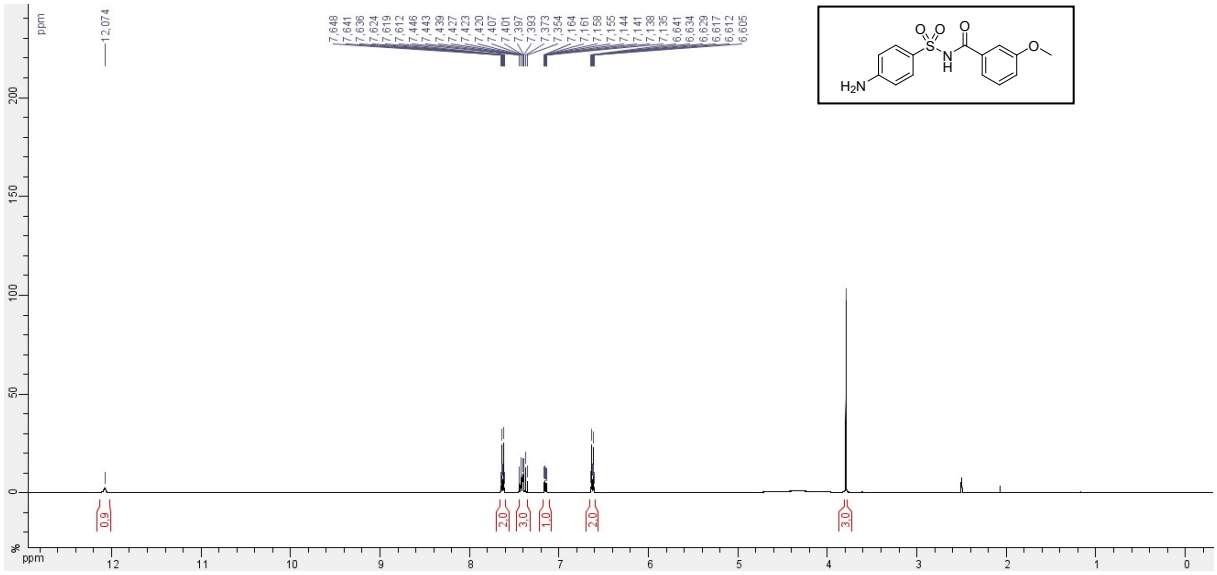


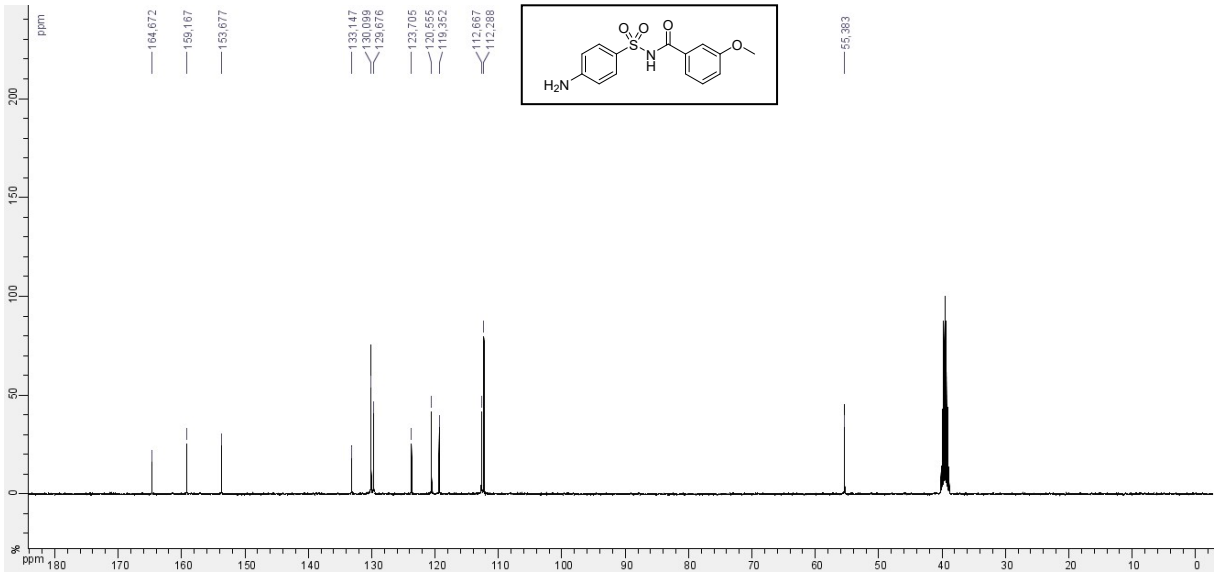
***N*-((4-aminophenyl)sulfonyl)-3-methoxybenzamide (5a)**



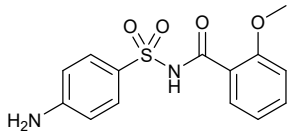
4-aminobenzenesulfonyl azide **2** (14 mg, 58 μmol) was dissolved in dry NMP (1.5 mL). Thereafter, triethylammonium 3-methoxybenzothioate **3a** (22 mg, 81 μmol), water (500 μL) and $NaHCO_3$ (14 mg, 162 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system A). 0.1% aq. TFA (pH 2.0, 15 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-50%) in 0.1% aq. TFA as the mobile phase, giving the *N*-((4-aminophenyl)sulfonyl)-3-methoxybenzamide **5a** as a white amorphous powder after lyophilization (19 mg, 46 μmol, 80%, 97% purity). ^{1}H NMR (400 MHz, DMSO): δ = 3.79 (s, 3H), 6.61-6.64 (m, 2H), 7.14-7.16 (m, 1H), 7.35-7.45 (m, 3H), 7.61-7.65 (m, 2H), 12.01 (bs, 1H) ppm. ^{13}C NMR (100 MHz, DMSO): δ = 55.4, 112.3, 112.7, 119.4, 120.6, 123.7, 129.7, 130.1, 133.1, 153.7, 159.2, 164.7 ppm. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{14}H_{15}N_2O_4S$: 307.0747; found 307.0753.

^{1}H and ^{13}C NMR Spectra (DMSO)



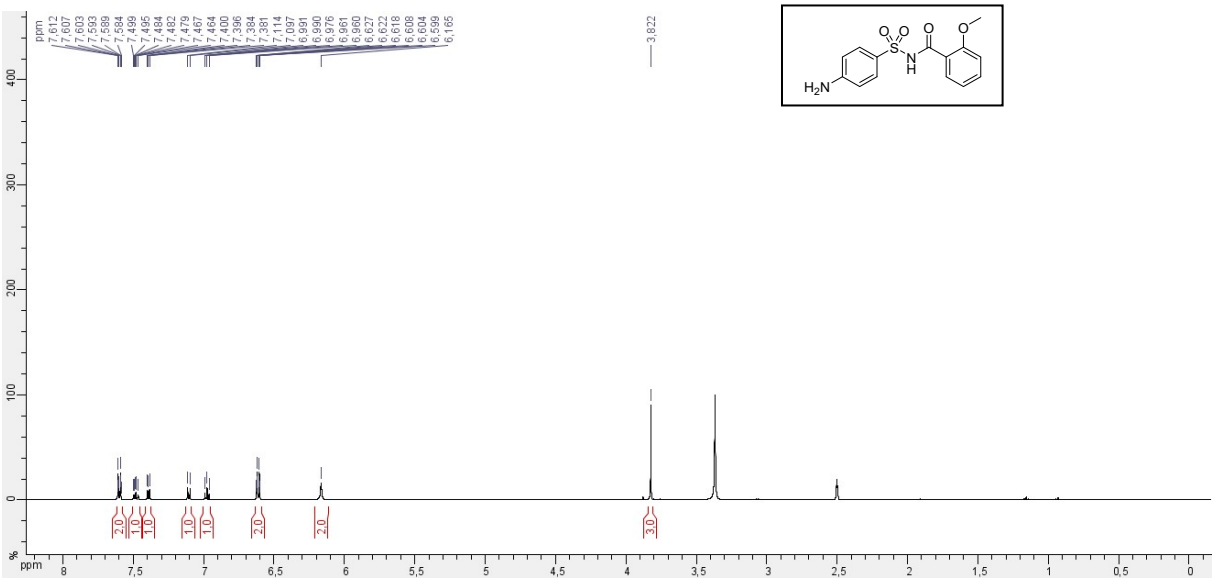


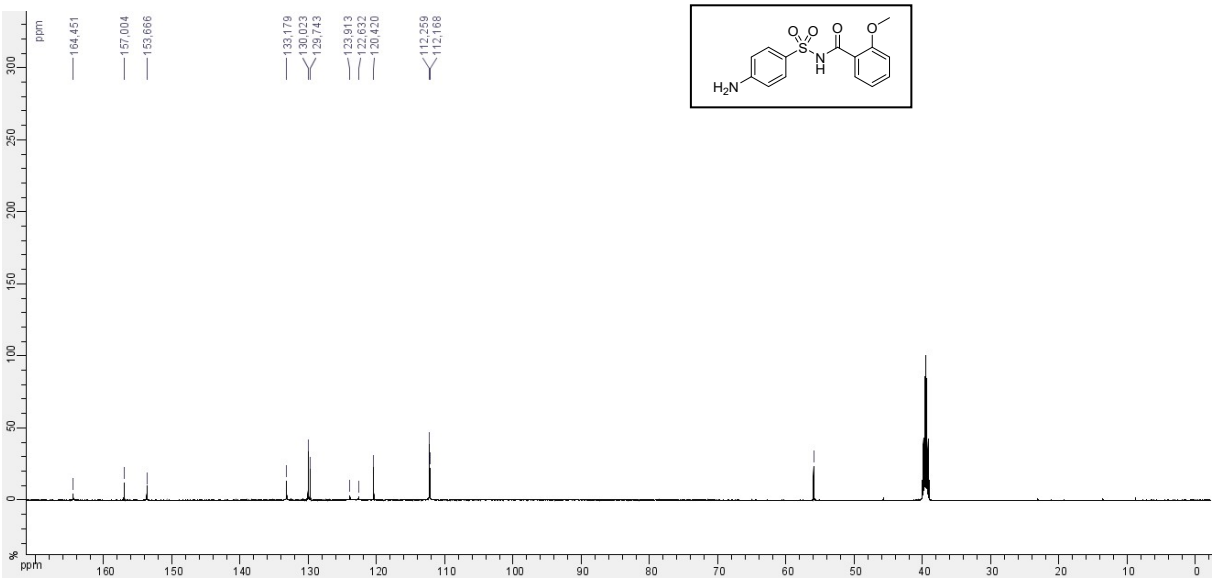
***N*-((4-aminophenyl)sulfonyl)-2-methoxybenzamide (5b)**



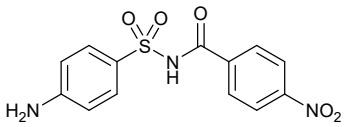
4-aminobenzenesulfonyl azide **2** (24 mg, 100 μmol) was dissolved in dry NMP (1.5 mL). Thereafter, triethylammonium 2-methoxybenzothioate **3b** (15 mg, 67 μmol), water (500 μL) and $NaHCO_3$ (17 mg, 200 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system B). Thereafter, aq. 50 mM TEAA (pH 7.0, 13.5 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-40%) in aq. 50 mM TEAA as the mobile phase, giving the *N*-((4-aminophenyl)sulfonyl)-2-methoxybenzamide **5b** as a white amorphous powder after lyophilization (26 mg, 86 μmol, 78%, 95% purity). ^{1}H NMR (500 MHz, DMSO): δ = 3.82 (s, 3H), 6.17 (s, 2H), 6.60-6.63 (m, 2H), 6.96-6.99 (m, 1H), 7.11 (d, *J* = 8.4 Hz, 1H), 7.38-7.40 (m, 1H), 7.46-7.50 (m, 1H), 7.58-7.61 (m, 2H) ppm. ^{13}C NMR (125 MHz, DMSO): δ = 56.0, 112.2, 112.3, 120.4, 122.6, 123.9, 129.7, 130.0, 133.2, 153.7, 157.0, 164.5 ppm. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{14}H_{15}N_2O_4S$: 307.0747; found 307.0749.

^{1}H and ^{13}C NMR Spectra (DMSO)



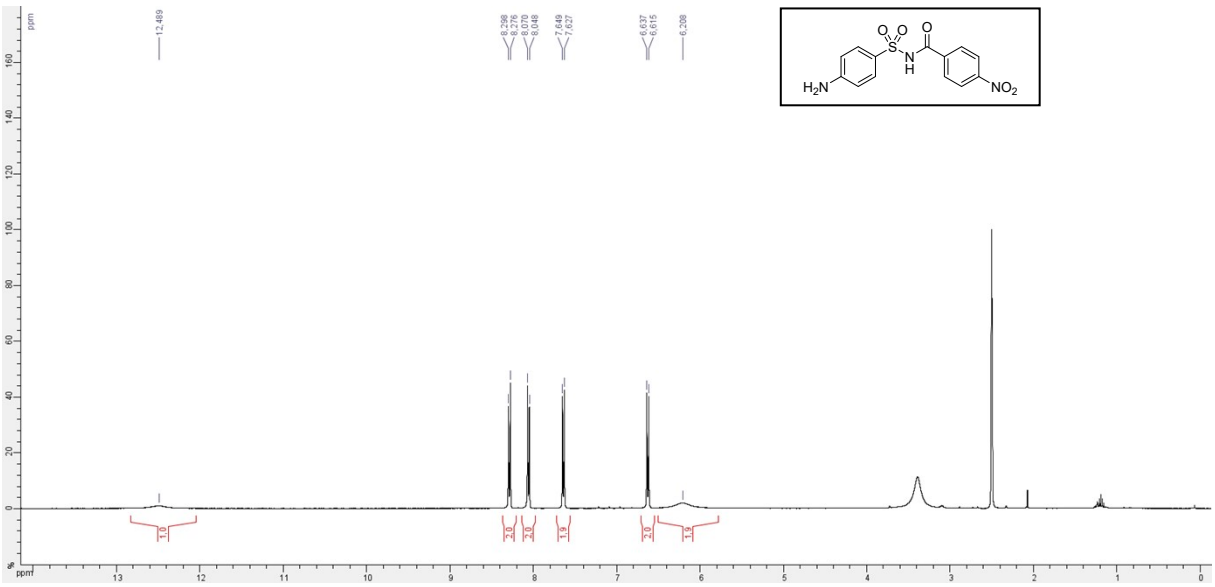


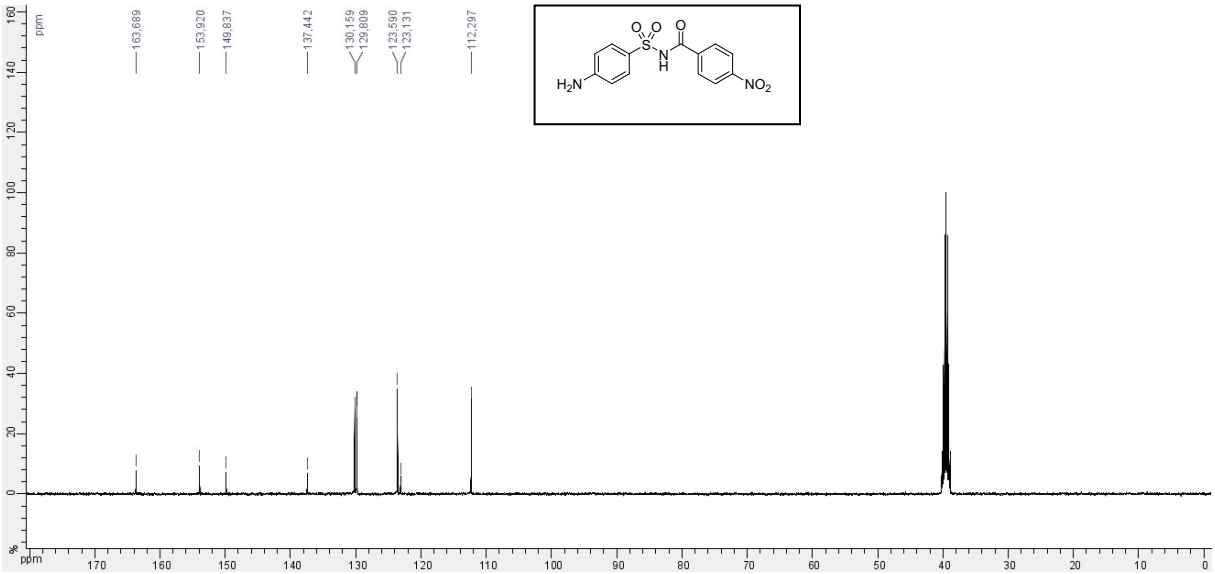
***N*-((4-aminophenyl)sulfonyl)-4-nitrobenzamide (5c)**



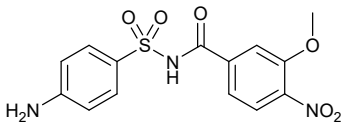
4-aminobenzenesulfonyl azide **2** (34 mg, 145 μmol) was dissolved in dry NMP (750 μL). Thereafter, triethylammonium 4-nitrobenzothioate **3c** (27 mg, 97 μmol), water (250 μL) and $NaHCO_3$ (24 mg, 290 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system A). 0.1% aq. TFA (pH 2.0, 9 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-50%) in 0.1% aq. TFA as the mobile phase, giving the *N*-((4-aminophenyl)sulfonyl)-4-nitrobenzamide **5c** as an orange amorphous powder after lyophilization (39 mg, 122 μmol, 84%, 95% purity). ^{1}H NMR (400 MHz, DMSO): δ = 6.21 (bs, 1H), 6,63(d, *J* = 8.8 Hz, 1H), 7.64 (d, *J* = 8.8 Hz, 1H), 8.06 (d, *J* = 8.8 Hz, 1H), 8.29 (d, *J* = 8.8 Hz, 1H), 12.49 (bs, 1H) ppm. ^{13}C NMR (100 MHz, DMSO): δ = 112.3, 123.1, 123.6, 129.8, 130.2, 137.4, 149.8, 153.9, 163.7 ppm. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for C_{13} $H_{12}N_3O_5S$: 322.0498; found 322.0511.

^{1}H and ^{13}C NMR Spectra (DMSO)



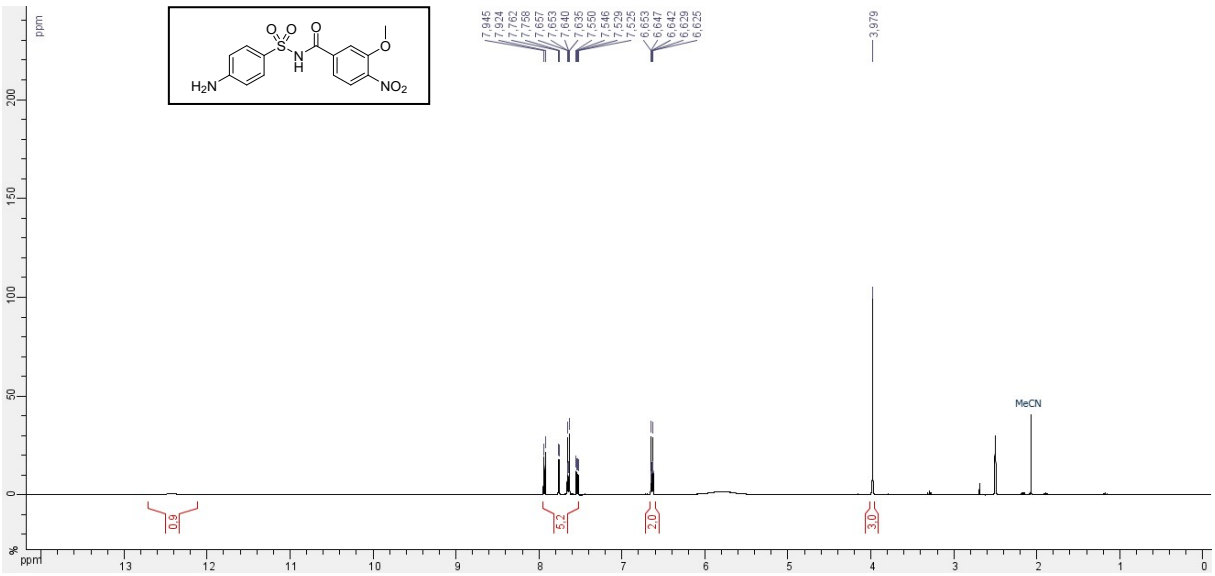


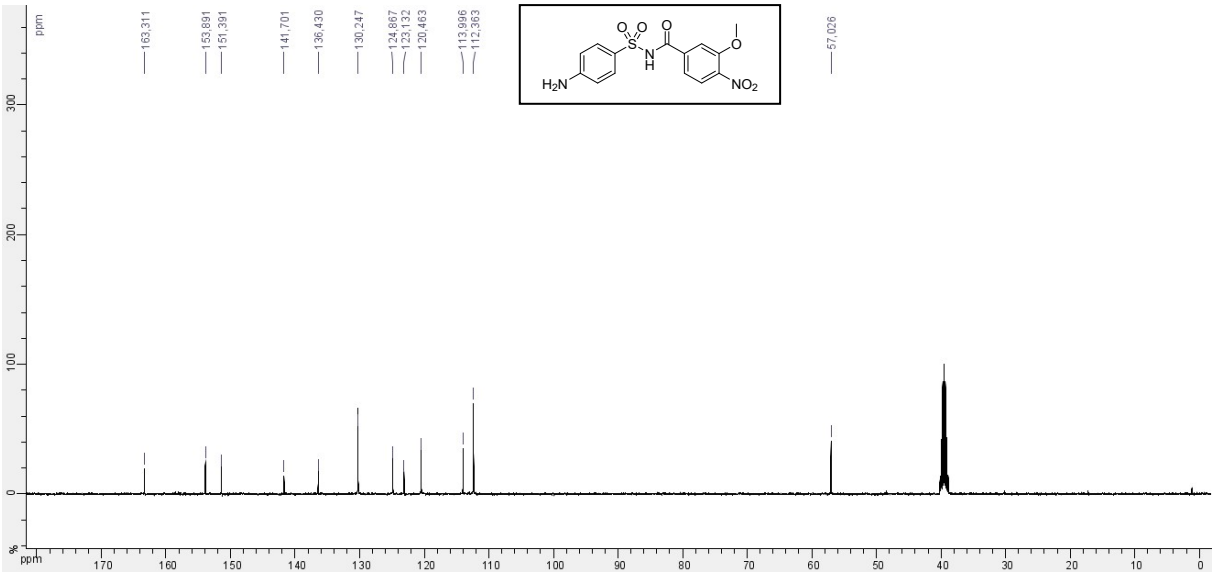
***N*-((4-aminophenyl)sulfonyl)-3-methoxy-4-nitrobenzamide (5d)**



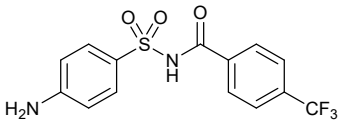
4-aminobenzenesulfonyl azide **2** (124 mg, 535 μmol) was dissolved in dry NMP (2.1 mL). Thereafter, triethylammonium 3-methoxy-4-nitrobenzothioate **3d** (112 mg, 356 μmol), water (700 μL) and $NaHCO_3$ (90 mg, 1.07 mmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system A). 0.1% aq. TFA (pH 2.0, 18 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-50%) in 0.1% aq. TFA as the mobile phase, giving the *N*-((4-aminophenyl)sulfonyl)-3-methoxy-4-nitrobenzamide **5d** as a yellow amorphous powder after lyophilization (134 mg, 300 μmol, 85%, 96% purity). ^{1}H NMR (400 MHz, DMSO): δ = 3.98 (s, 3H), 6.63-6.65 (m, 2H), 7.53-7.95 (m, 5H) ppm. ^{13}C NMR (100 MHz, DMSO): δ = 57.0, 112.4, 114.0, 120.5, 123.1, 124.9, 130.2, 136.4, 141.7, 151.4, 153.9, 163.3 ppm. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{14}H_{14}N_3O_6S$: 352.0603; found 352.0618.

^{1}H and ^{13}C NMR Spectra (DMSO)



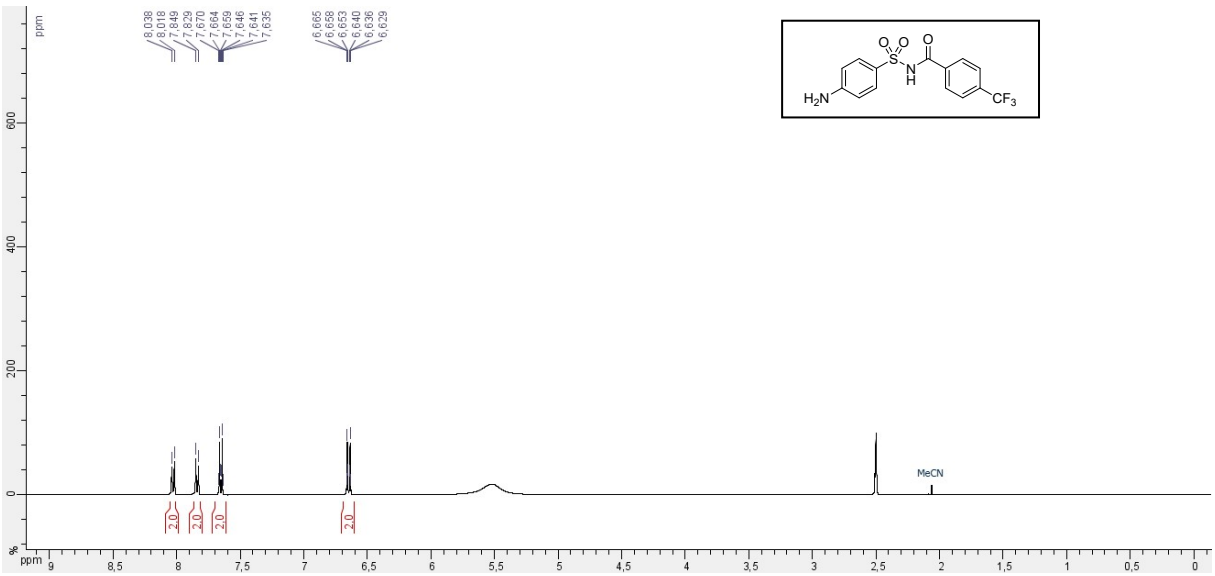


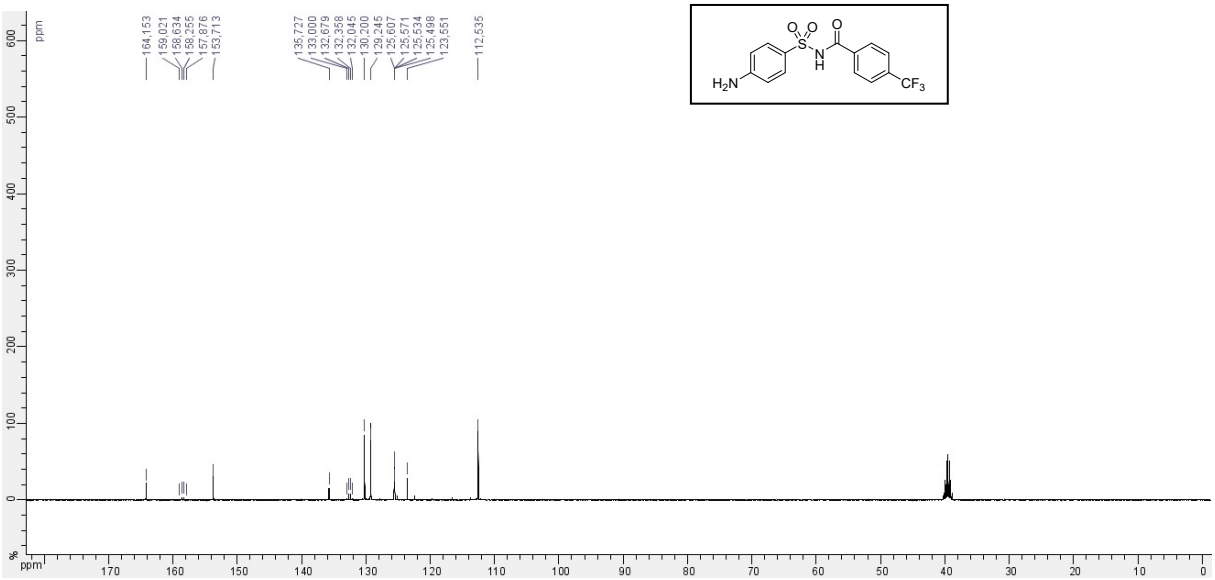
***N*-((4-aminophenyl)sulfonyl)-4-(trifluoromethyl)benzamide (5e)**



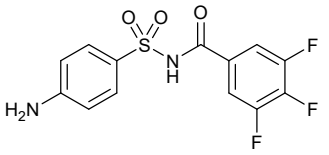
4-aminobenzenesulfonyl azide **2** (54 mg, 229 μmol) was dissolved in dry NMP (1.5 mL). Thereafter, triethylammonium 4-(trifluoromethyl)benzothioate **3e** (47 mg, 153 μmol), water (500 μL) and $NaHCO_3$ (38 mg, 458 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system A). 0.1% aq. TFA (pH 2.0, 15 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-50%) in 0.1% aq. TFA as the mobile phase, giving the *N*-((4-aminophenyl)sulfonyl)-4-(trifluoromethyl)benzamide **5e** as a white amorphous powder after lyophilization (66 mg, 150 μmol, quant. , 99% purity). ^{1}H NMR (400 MHz, DMSO): δ = 6.63-6.67 (m, 2H), 7.64-7.67 (m, 2H), 7.83-8.04 (m, 4H) ppm. ^{13}C NMR (100 MHz, DMSO): δ = 112.6, 123.6, 125.6 (q, $J_{C\text{-}F}$ = 3.7 Hz), 129.2, 130.2, 132.5 (q, $J_{C\text{-}F}$ = 32.1), 135.7, 153.7, 158.5 (q, $J_{C\text{-}F}$ = 38.2), 164.2 ppm. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{14}H_{12}F_3N_2O_3S$: 345.0515; found 345.0521.

^{1}H and ^{13}C NMR Spectra (DMSO)



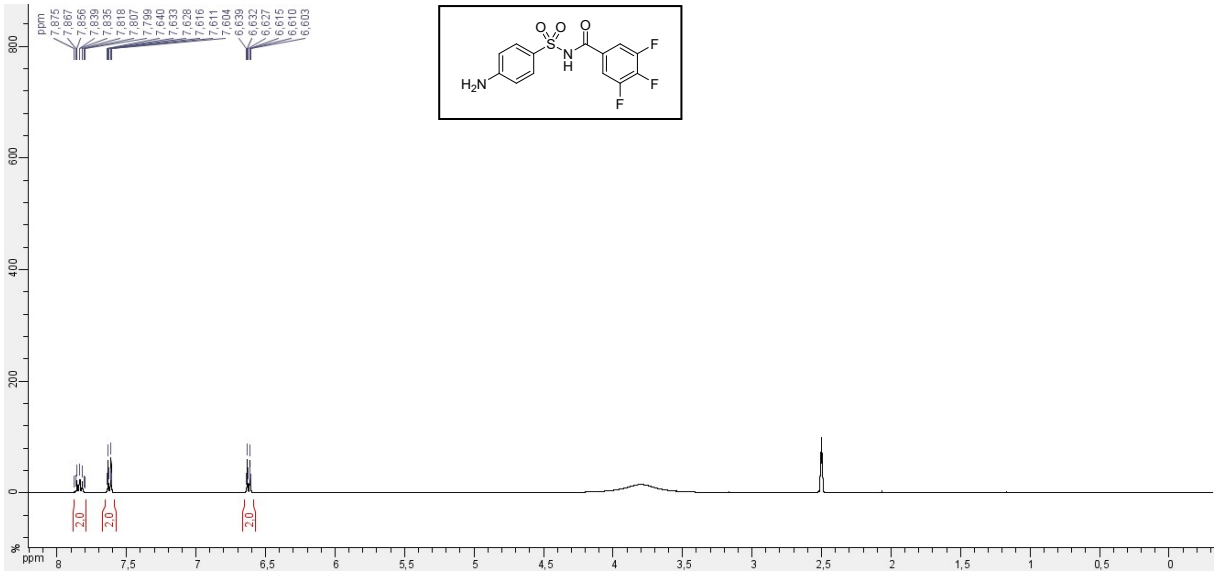


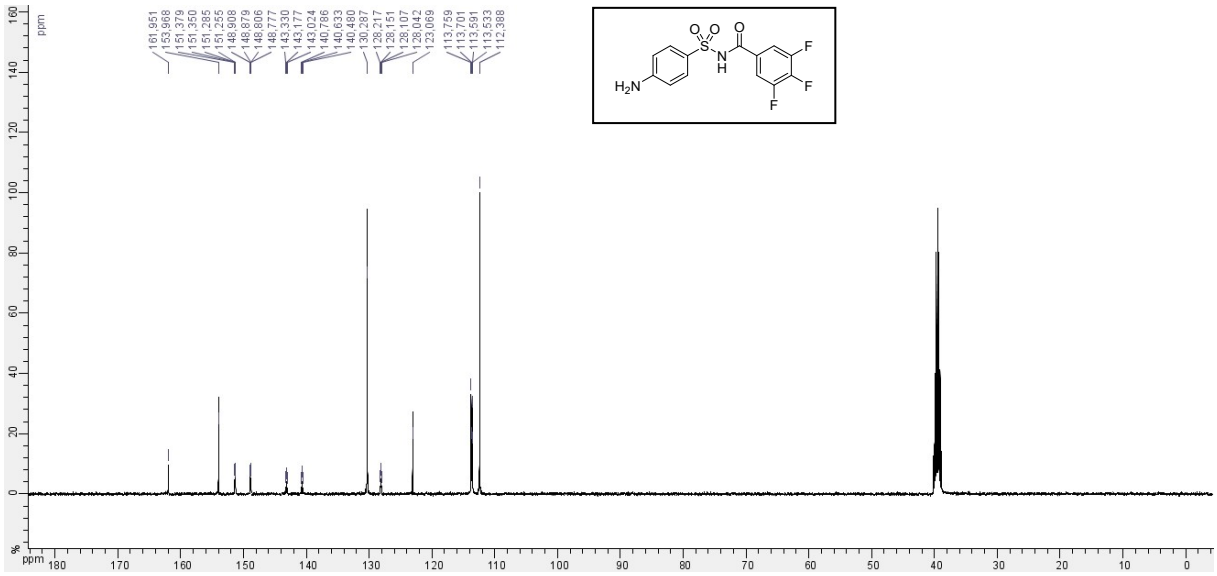
***N*-((4-aminophenyl)sulfonyl)-3,4,5-trifluorobenzamide (5f)**



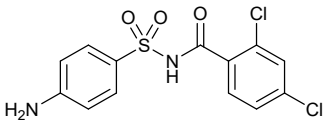
4-aminobenzenesulfonyl azide **2** (89 mg, 382 μmol) was dissolved in dry NMP (2 mL). Thereafter, triethylammonium 3,4,5-trifluorobenzothioate **3f** (75 mg, 256 μmol), water (650 μL) and $NaHCO_3$ (65 mg, 768 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system A). 0.1% aq. TFA (pH 2.0, 18 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-50%) in 0.1% aq. TFA as the mobile phase, giving the *N*-((4-aminophenyl)sulfonyl)-3,4,5-trifluorobenzamide **5f** as a white amorphous powder after lyophilization (86 mg, 202 μmol, 82%, 99% purity). ^{1}H NMR (400 MHz, DMSO): δ = 6.60-6.64 (m, 2H), 7.60-7.64 (m, 2H), 7.80-7.88 (m, 2H) ppm. ^{13}C NMR (100 MHz, DMSO): δ = 112.4, 113.5-113.8 (m), 123.1, 128.0-128.2 (m), 130.3, 141.9 (dt, $J_{1C\text{-}F}$ = 255 Hz, $J_{2C\text{-}F}$ =15.4 Hz), 150.1 (ddd, $J_{1C\text{-}F}$ = 249 Hz, $J_{2C\text{-}F}$ = 10.2 Hz, $J_{3C\text{-}F}$ = 2.9 Hz), 154.0, 162.0 ppm. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{13}H_{10}F_3N_2O_3S$: 331.0359; found 331.0365.

^{1}H and ^{13}C NMR Spectra (DMSO)



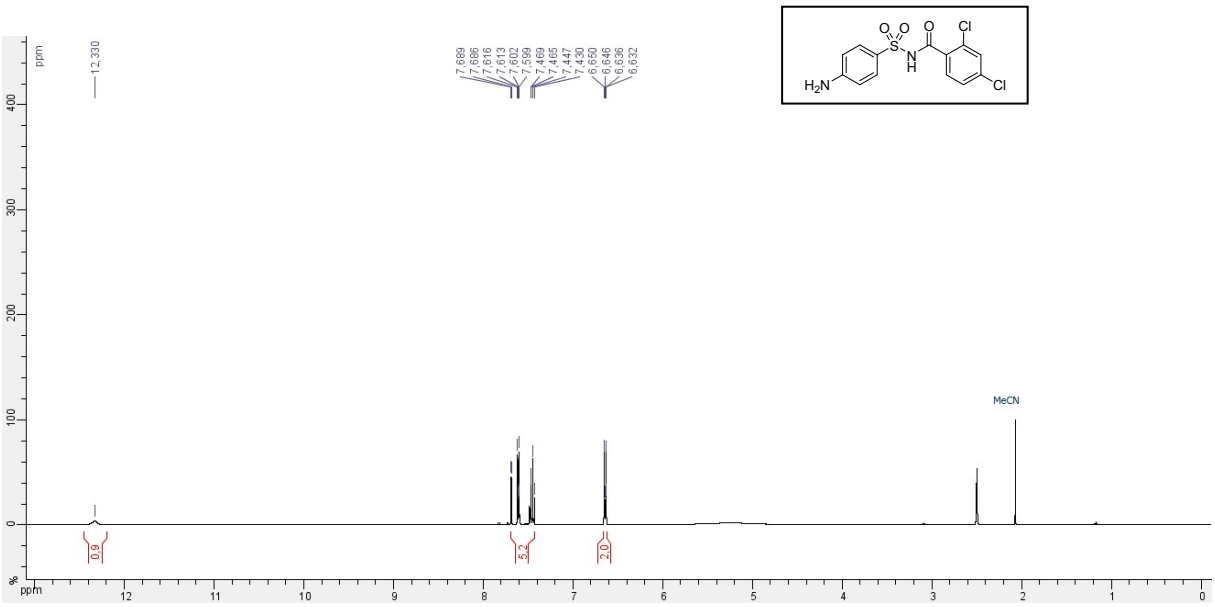


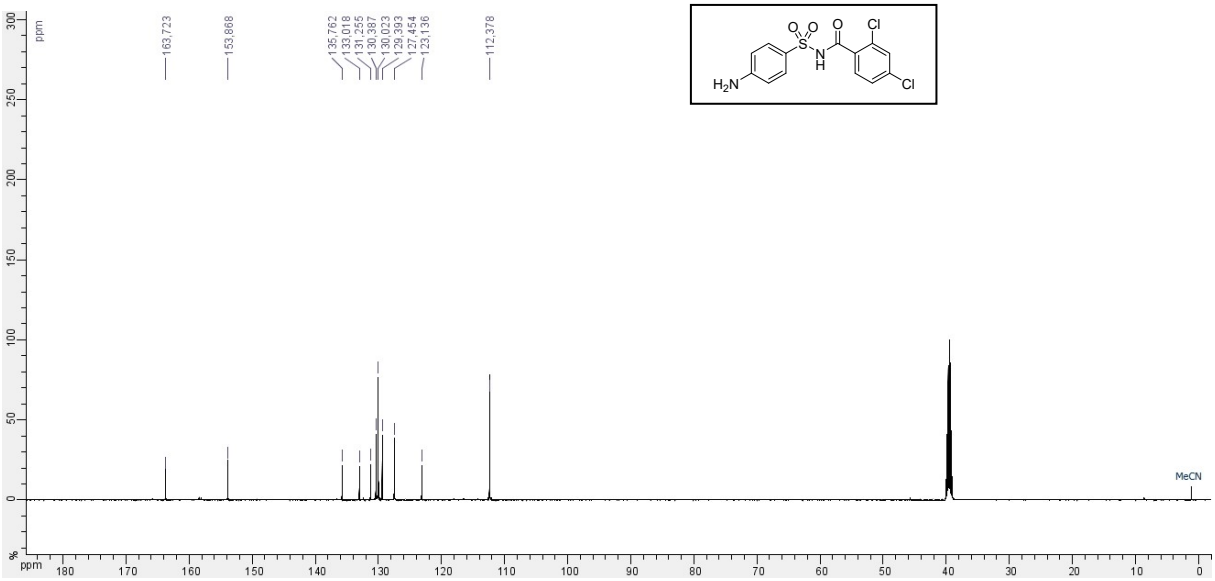
***N*-((4-aminophenyl)sulfonyl)-2,4-dichlorobenzamide (5g)**



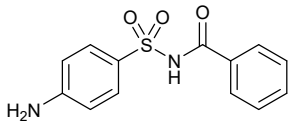
4-aminobenzenesulfonyl azide **2** (69 mg, 291 μmol) was dissolved in dry NMP (4.5 mL). Thereafter, triethylammonium 2,4-dichlorobenzothioate **3g** (60 mg, 195 μmol), water (1.5 mL) and $NaHCO_3$ (49 mg, 582 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 4 h. The reaction was checked for completion by RP-HPLC (system A). 0.1% aq. TFA (pH 2.0, 50 mL) was added and the resulting mixture was purified by RP-chromatography with a linear gradient of MeCN (0-50%) in 0.1% aq. TFA as the mobile phase, giving the *N*-((4-aminophenyl)sulfonyl)-2,4-dichlorobenzamide **5g** as a white amorphous powder after lyophilization (62 mg, 140 μmol, 72%, 95% purity). ^{1}H NMR (500 MHz, DMSO): δ = 6.63-7.69 (m, 7H), 12.33 (bs, 1H) ppm. ^{13}C NMR (125 MHz, DMSO): δ = 112.4, 123.1, 127.5, 129.4, 130.0130.4, 131.3, 133.0, 135.8, 153.9, 163.7 ppm. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{13}H_{11}Cl_2N_2O_3S$: 344.9862; found 344.9858.

^{1}H and ^{13}C NMR Spectra (DMSO)



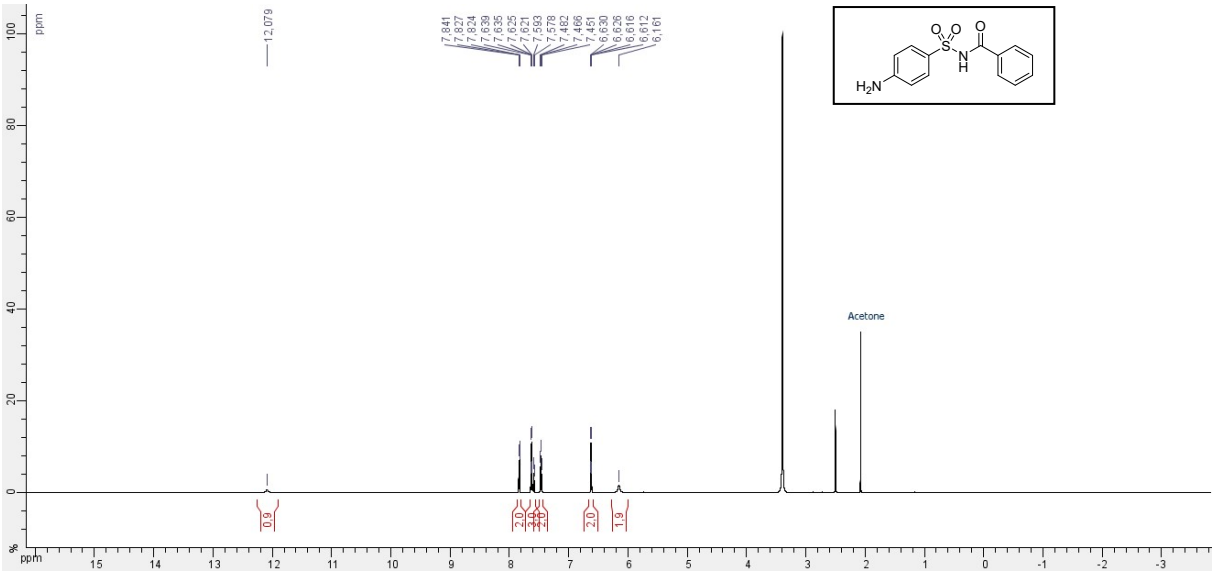


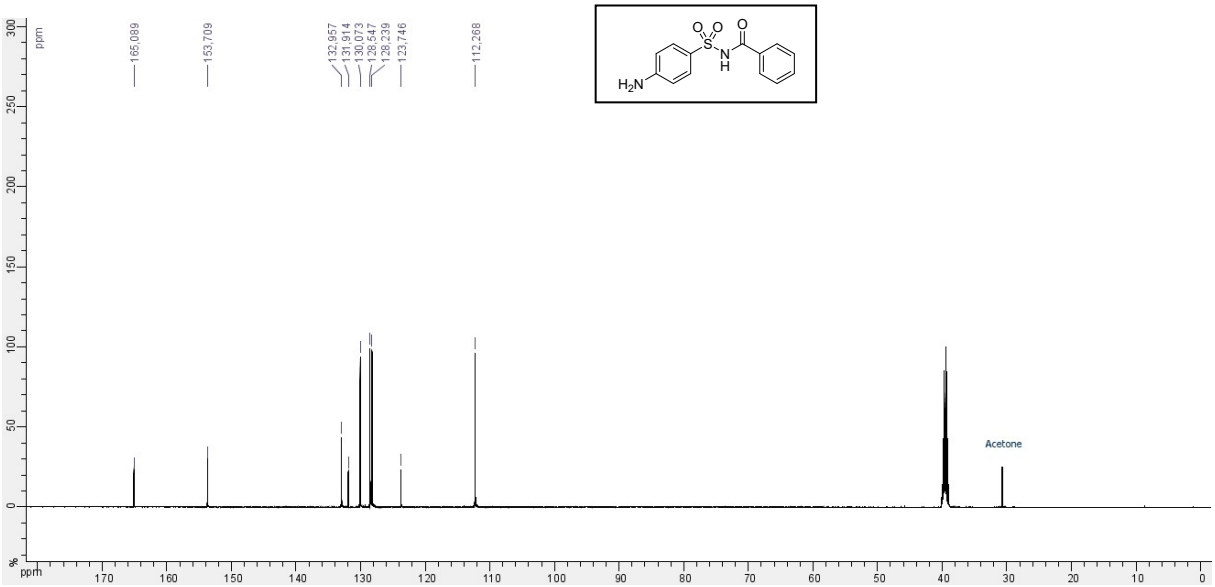
***N*-((4-aminophenyl)sulfonyl)benzamide (5h)**



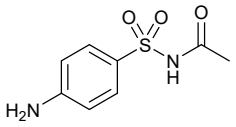
4-aminobenzenesulfonyl azide **2** (120 mg, 530 μmol) was dissolved in dry MeCN (12 mL). Thereafter, thiobenzoic acid **3h** (93 μL, 795 μmol), water (4 mL) and $NaHCO_3$ (134 mg, 1.6 mmol) were sequentially added. The resulting mixture was stirred at room temperature for 16 h. The reaction was checked for completion by TLC (DCM-MeOH, 96 : 4, v/v) and evaporated to dryness. The resulting residue was purified by chromatography on a silica gel column with a linear gradient of MeOH (0-10%) in DCM as the mobile phase, giving *N*-((4-aminophenyl)sulfonyl)benzamide **5h** as a white powder (108 mg, 390 μmol, 74%, 98% purity). ^{1}H NMR (500 MHz, DMSO): δ = 6.16 (bs, 2H), 6.16-8.84 (m, 9H), 12.08 (bs, 1H) ppm. ^{13}C NMR (125 MHz, DMSO): δ = 112.3, 123.7, 128.2, 128.5, 130.1, 131.9, 133.0, 153.7, 165.1, 206.6 ppm. HRMS (ESI) *m/z*: $[M-H]^-$ Calcd for $C_{13}H_{13}N_2O_3S$: 277.0647; found 277.0664.

^{1}H and ^{13}C NMR Spectra (DMSO)



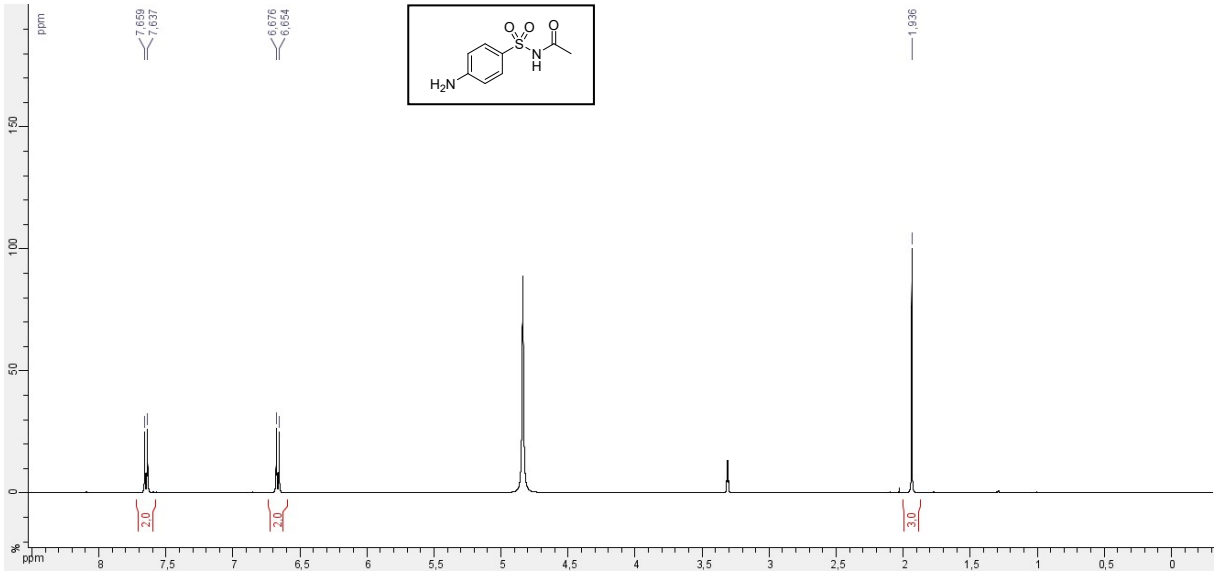


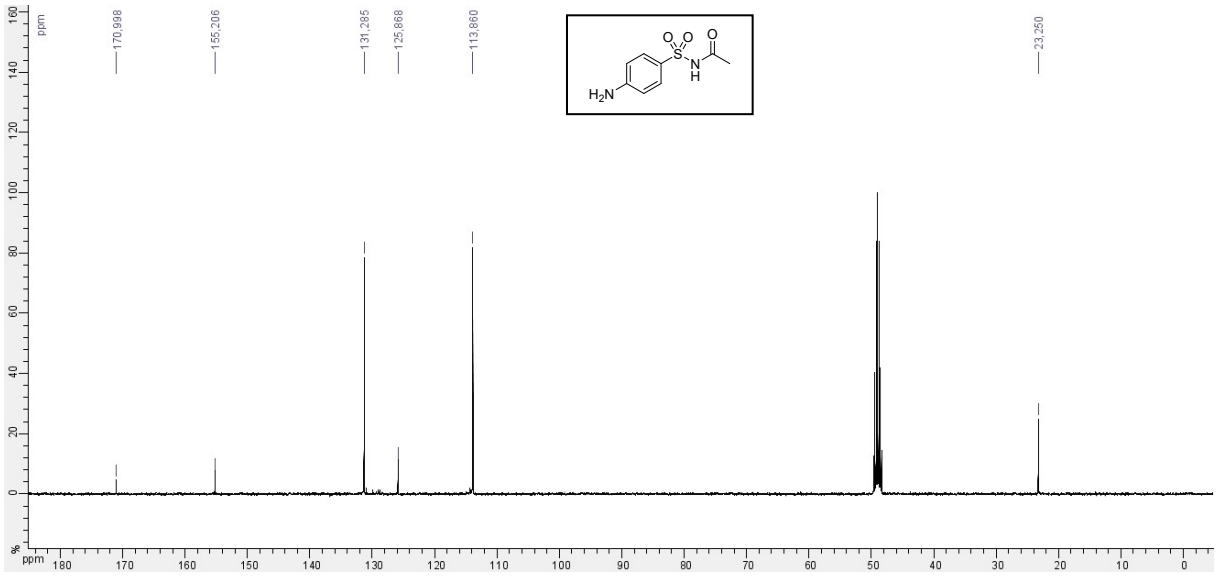
***N*-((4-aminophenyl)sulfonyl)acetamide (5i)**



4-aminobenzenesulfonyl azide **2** (24 mg, 106 μmol) was dissolved in dry MeCN (1.5 mL). Thereafter, potassium thioacetate **3i** (18 mg, 160 μmol), water (500 μL) and $NaHCO_3$ (18 mg, 212 μmol) were sequentially added. The resulting mixture was stirred at room temperature for 16 h. The reaction was checked for completion by TLC (DCM-MeOH, 96 : 4, v/v) and evaporated to dryness. The resulting residue was purified by chromatography on a silica gel column with a linear gradient of MeOH (0-10%) in DCM as the mobile phase, giving N-((4-aminophenyl)sulfonyl)acetamide **5i** as a white powder (20 mg, 47 μmol, 90%, 99% purity). ^{1}H NMR (400 MHz, MeOD): δ = 1.94 (s, 3H), 6.65-7.66 (m, 4H) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 23.3, 113.9, 125.9, 131.3, 155.2, 171.0 ppm. HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_8H_{11}N_2O_3S$: 215.0485; found 215.0488.

^{1}H and ^{13}C NMR Spectra (MeOD)





Antibacterial and antibiofilm activities of *N*-acylsulfonamide analogues

Determination of minimum inhibitory concentration (MIC)

All synthesized *N*-acylsulfonamide derivative analogues were evaluated to determine their minimum inhibitory concentrations (MICs) against representative Gram-negative (*Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 27853) and Gram-positive (*Bacillus subtilis* ATCC 6051 and *Staphylococcus aureus* CIP 107093) bacterial pathogens according to CLSI guidelines using broth microdilution method.[3] Briefly, *N*-acylsulfonamide derivative analogues were dissolved in DMSO (Sigma-Aldrich-D8418) at 25.6 mg mL^{-1} and diluted in cation-adjusted Mueller Hinton broth (BD DifcoTM-212322) adjusted to pH 7.4 (±0.2) to obtain concentration ranging from 1 to 256 µg mL^{-1}. Sulfacetamide was used as a reference drug. The inoculum with the test culture to give a final concentration of 5 × 105 CFU mL^{-1} was used. The MIC values were determined after 24 h of incubation at 37°C without shaking. After incubation, resazurin (Acros-organics- 418900050) at 0.015% was added to all wells (50 µL per well), and further incubated for 2–4 h for the observation of colour change. On completion of the incubation, wells with no colour change (blue resazurin color remained unchanged) were scored as above the MIC value.

Biofilm quantification using a crystal violet assay

To assess the propensity of *E. coli* ATCC 8739 and *P. aeruginosa* ATCC 27853 strains to form biofilms in the presence of *N*-acylsulfonamide derivative analogues (5a, 5b, 5h and 5i) at a ½ MIC, we performed crystal-violet staining assays as described by O'Toole.[4] Briefly, overnight cultures were inoculated into a fresh LB broth and grown at 37 ºC for 24 h in a 96-well microtiter plate under static conditions. Cell growth was determined at 580 nm. The biofilm was measured by discarding the medium, rinsing the wells with distilled water and staining any bound cells with 0.1% crystal violet (Sigma-Aldrich-V5265). The dye was dissolved in 30% (v/v) acetic acid (Supelco-1.00063.1011) and optical density was determined at 595 nm using the Spark 20M multimode microplate reader controlled by SparkControlTM software Version

2.1 (Tecan Group Ltd., Männedorf, Switzerland). In each experiment, the background staining was adjusted by subtracting the crystal violet bound to uninoculated controls.

Statistical Analyses

To assess the significance of the differences between groups, ordinary one-way ANOVA followed by Dunnett multiple-comparison test were performed to calculate the *P* values using Prism GraphPad (GraphPad, San Diego, CA, USA). All experiments were conducted independently with at least three replicates. The results were displayed as the mean ± standard error of the mean. Asterisks indicate values that are significantly different as follows: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ****, $P<0.0001$; ns, not significantly different.

Cytotoxicity assays

The potential cytotoxicity of *N*-acylsulfonamide derivative analogues (**5a**, **5b**, **5h** and **5i**) at a MIC and ½ MIC was determined by measurement of the lactate dehydrogenase (LDH) release by HaCaT and Caco 2 cell lines. Cells were grown at 37 °C in 5% CO_2 atmosphere in Dubelcco's modified Eagle medium (DMEM, Gibco, Thermo Scientific) with 10% of fetal calf serum (FCS, Biowest, VWR, Fontenay-sous-bois, France) for HaCaT cells and 20% of FCS for Caco 2 cells and 1% of antibiotics cocktail (penicillin–streptomycin, Corning, USA) for both. Cells were then seeded in 24-well plates (Nunc, Thermo Scientific). They were grown 72h before use. Cells were rinsed with phosphate buffer saline (PBS, Corning, USA) and incubated for 24h with the compounds which were diluted in fresh DMEM. LDH release was measured using the Invitrogen CyQuant LDH cytotoxicity assay.

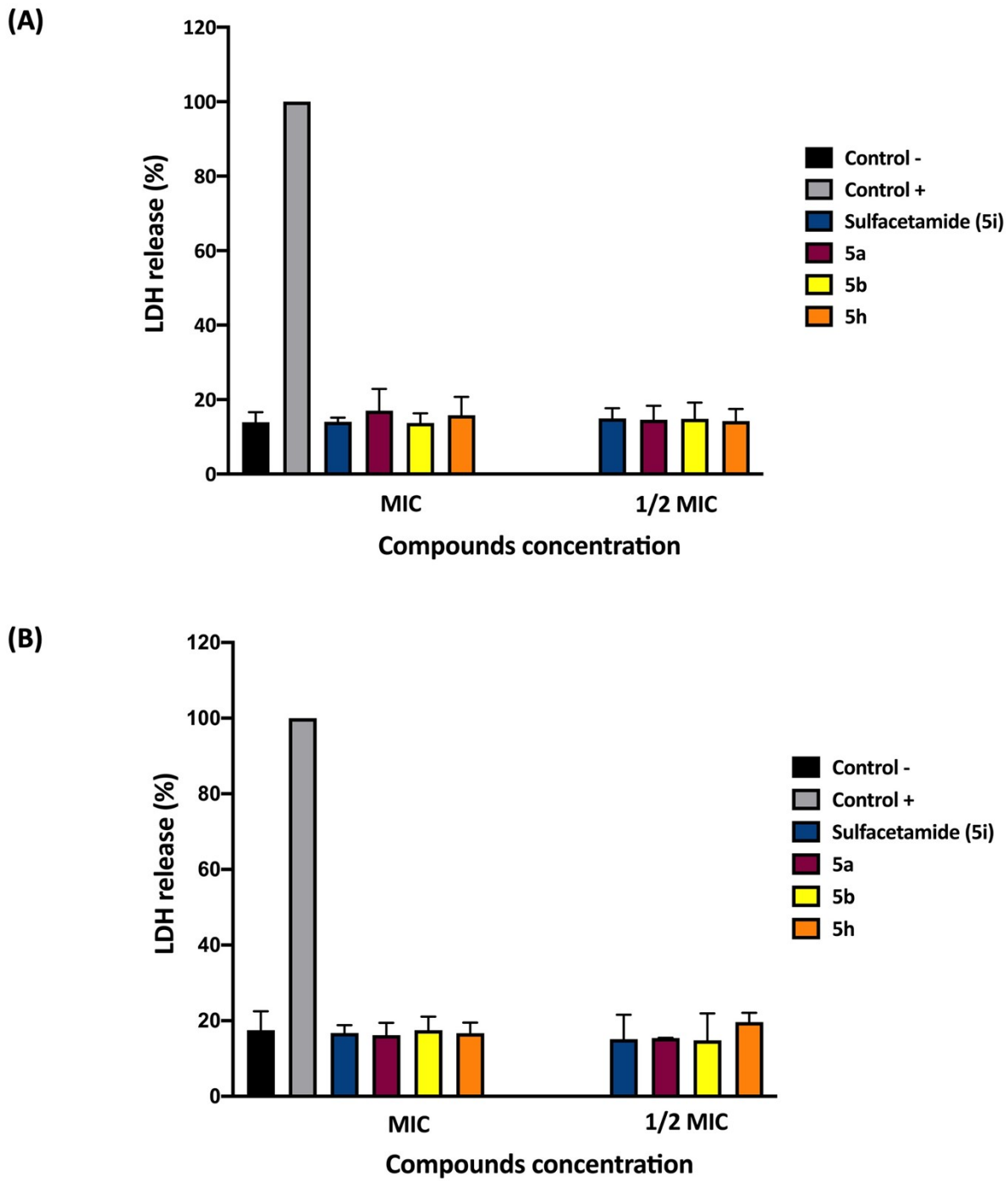


Fig. S1 Cytotoxicity of compounds **5a**, **5b**, **5h** and **5i** against HaCaT **(A)** and Caco-2 **(B)** cell lines. Control -, untreated cells; control +, damaged cells. The results were displayed as the mean ± standard error of the mean of two biological independent assays with at least 2 replicates.

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3. M. A. Wikler, *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard*, 2006.
4. G. A. O'Toole and R. Kolter, *Mol. Microbiol.*, 1998, **28**, 449-461.