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Synthesis of SMZ derivatives and investigation of effects on germination, root, and plant growth of *Arabidopsis thaliana* L.

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Abstract: A series of sulfonamide derivatives were synthesized by reactions with various functional groups containing benzenesulfonyl chlorides and aniline derivatives under different substitution reaction conditions. The structures of SMZ derivatives were confirmed with melting point, FT-IR, ^{1}H NMR, ^{13}C NMR, and LC-MS/MS techniques. In order to investigate the cytotoxic effects of these derivatives, we used a model plant species. The synthesized compounds (**S1**–**S5**) and sulfamethazine (SMZ) as a positive control were applied to *Arabidopsis thaliana* seeds. Our results indicated that **S3** and **S4** induced shorter roots and lower wet weight in plants. Plants treated with **S2** and **S5** showed no growth effects, similar to the untreated control group, while **S1** slightly reduced root length and wet weight. These results suggest that **S3** and the newly synthesized **S4** derivatives have potential for use as herbicides since they possess cytotoxic effects on *A. thaliana* plants.

Key words: SMZ derivatives, *Arabidopsis thaliana* L., herbicide, sulfonamide, synthesis

1. Introduction

Sulfonamides are well-known structures with various applications in medicinal chemistry and biological activity. The most important implementation of sulfa drugs (e.g., sulfamethoxazole and sulfisoxazole) is to inhibit the growth and multiplication of bacteria. Sulfonamides are also used as carbonic anhydrase inhibitors, matrix metalloproteinase inhibitors, and antiallergy, antiinflammatory, antiviral, antifungal, antimalarial, anticancer, antiarthritis, and antiemphysema drugs [1–5].

Sulfamethazine (SMZ) (4-amino-*N*-(4,6-dimethylpyrimidine-2-yl)benzenesulfonamide), known as sulfadimidine, sulfadimerazine, or sulfadimezine, is a broad-spectrum antibiotic used to treat bronchitis, prostate, and urinary infections. SMZ is also used as veterinary antibiotic. Its presence in animal excreta and manure, uptake by plants, and distribution in soil have been researched [6,7]. Interestingly, sulfamethazine can also suppress DNA methylation in plants by impairing folate synthesis [8]. This phenomenon decreases the levels of the universal methyl donor, S-adenosyl methionine, which is used by most methyltransferases [9]. DNA methyltransferase enzymes are responsible for the transfer of a methyl group of S-adenosylmethionine to the cytosine residues in DNA [10]. DNA methylation is a well-known epigenetic marker and regulates various developmental

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stages in plants such as vernalization, embryogenesis and seed viability, apical dominance, plant size, leaf size and shape, fertility, and root length [11–15]. Several studies indicated that root length and fresh weight were reduced when *Arabidopsis thaliana* plants were hypomethylated [8,13]. *A. thaliana* is a model organism for plant genetics and molecular biology studies. *A. thaliana* was chosen in this study since it has a short life span, grows easily in laboratory conditions, and has the potential to reveal main issues in plant biological structure and functions [16].

Therefore, in this study, we synthesized and characterized some SMZ derivatives (**S1**–**S5**) and investigated the cytotoxic effects of these compounds on *A. thaliana* plants (Figure 1). Our results indicated that **S3** and **S4** are toxic at higher concentrations and may reduce the root length and fresh weight if applied at lower concentrations. However, other SMZ derivatives (**S1**, **S2**, and **S5**) did not have a significant effect on plant growth.

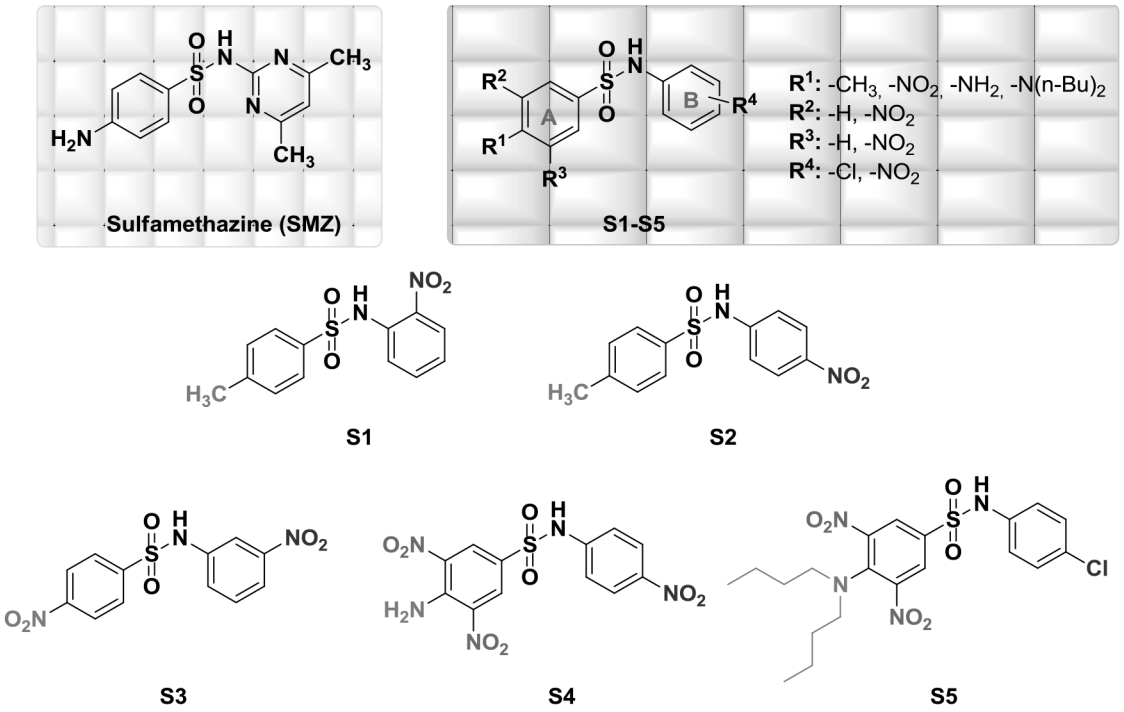

Figure 1. Structure of SMZ and synthesized sulfonamide derivatives (**S1-S5**).

2. Results and discussion

2.1. Chemistry

As shown in Figure 1, sulfamethazine (SMZ), which was used as a standard in our study, is a sulfonamide derivative consisting of two aromatic rings, 4-aminophenyl and dimethylpyrimidine. We aimed to synthesize some modified SMZ derivatives, investigate their herbicide properties on *A. thaliana* plants, and compare the results with the standard SMZ. For this purpose, compounds **S1**–**S5** were designed to have nitrophenyl or chlorophenyl instead of a pyrimidine ring. Also, methyl, nitro, amino, and dibutylamino groups were used in the 4-position of the phenyl ring. Unlike others, **S4** and **S5** have two nitro groups at 3,5-positions in the phenyl ring (Figure 1).

The **S1**–**S3** derivatives were prepared with 47%–72% yield by heating 4-methyl/4-nitro benzenesulfonyl chloride and corresponding nitroaniline derivatives in basic conditions (Figure 2). **S1** was synthesized according to the literature [17]. **S2** was obtained from TosCl and 4-nitroaniline using NaH as a base in DMF under modified literature conditions. Reaction of 3-nitroaniline and 4-nitrobenzenesulfonyl chloride in DMF containing pyridine at 40–50 °C for 3 h led to **S3** with 72% yield [18]. To obtain **S4**, first 4-amino-3,5-dinitrobenzenesulfonyl chloride as an intermediate was prepared from 2,6-dinitroaniline and chlorosulfonic acid at 100 °C for 2 h. Then, after stirring this intermediate and 4-nitroaniline in DMA as a solvent at room temperature for 48 h, target sulfonamide **S4** was obtained with 42% yield. **S5** was synthesized in five steps. The first two steps consisted of sulfonylation of chlorobenzene and nitration of 4-chloro benzenesulfonic acid with potassium nitrate, respectively [19]. The following two steps were the substitution of dibutylamine and potassium 4-chloro-3,5-dinitro benzenesulfonate and the chlorination reaction of sulfonate by using PCl_5 at room temperature conditions, leading to 4-(dibutylamino)-3,5-dinitrobenzenesulfonyl chloride [20]. Finally, **S5** was prepared by the substitution reaction of the obtained intermediate and 4-chloroaniline in pyridine as a solvent at 50 °C for 24 h as shown in Figure 2.

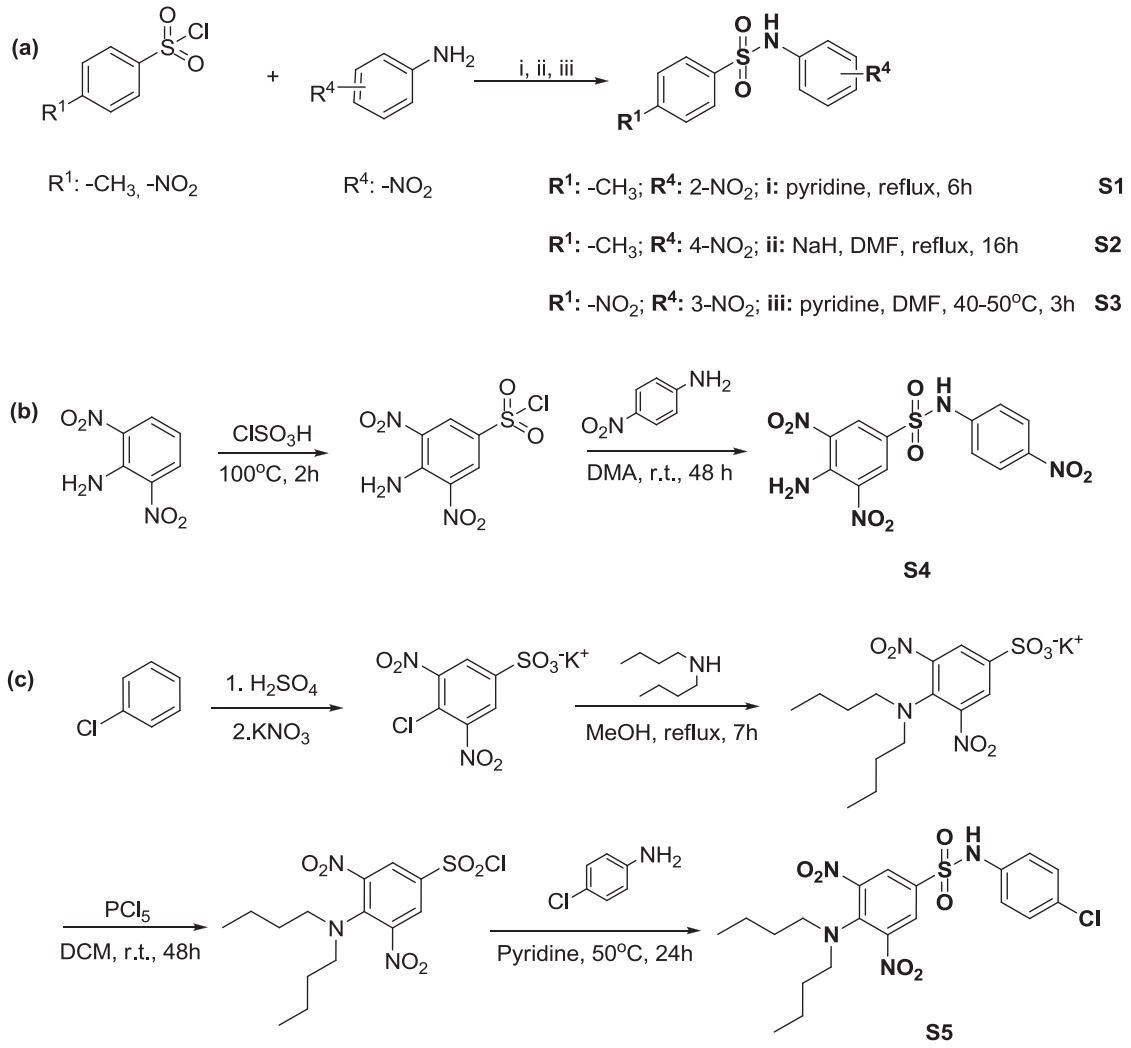

Figure 2. Synthesis of sulfonamide derivatives (**S1-S5**).

All compounds were characterized by melting point, FT-IR, ^{1}H NMR, ^{13}C NMR, and LC-MS/MS analyses (see Section 3 and Supplementary information). Observations of the characteristic N-H stretching peak of sulfonamide at 3251–3356 cm^{-1} in the FT-IR spectra and the –NH singlet peak of sulfonamide between 8.79 and 11.33 ppm in the ^{1}H NMR spectra are the greatest evidence of the formation of the final sulfonamide products. In the ^{1}H NMR spectrum of compound **S3**, six aromatic hydrogens were observed at 7.73–8.88 ppm and a singlet sulfonamide N-H peak was observed at 11.13 ppm. Ten different aromatic carbons were obtained in the 114.08–154.49 ppm range with ^{13}C NMR.

While three different aromatic hydrogen signals at 7.31–8.69 ppm and a NH_2 singlet signal on ring A at 8.74 ppm were observed in the ^{1}H NMR spectrum of compound **S4**, the NH proton of sulfonamide was found at higher chemical shifts at 11.33 ppm. Additionally, eight different aromatic carbons of **S4** were observed in the 119.13–143.82 ppm range in the ^{13}C NMR spectrum. In the ^{1}H NMR spectrum of compound **S5**, the CH_3 chemical shift of the butyl chain was observed at 0.90 ppm as a triplet peak. Also, two multiplet CH_2 peaks of butyl were determined at 1.36 and 1.59 ppm, respectively, and the N-CH_2 peak has a chemical shift at 3.44 ppm. Doublet and doublet of doublets peaks of H_e and H_f were observed at 7.18 and 8.20 ppm, respectively.

Aromatic hydrogen signals on ring A and the sulfonamide N-H signal overlapped at 8.80 ppm with 2 + 1 integrations. There are eight different aromatic carbons in the 115.66–148.55 ppm range and four different aliphatic carbons in the 14.06–43.00 ppm range in the ^{13}C NMR spectrum of compound **S5**. All peaks of other functional groups such as nitro, aromatic, or aliphatic on FT-IR and signals of proton/carbons in NMR spectra confirm the structures of the sulfonamide derivatives. Molecular weights of compounds **S3**–**S5** were confirmed with LC-MS/MS analysis in negative mode (see Supplementary information).

2.2. Cytotoxic effects of the SMZ derivatives

To compare the cytotoxic effects of the SMZ derivatives, we used SMZ as a positive control. First, we treated seeds with 50, 25, and 1 μM SMZ and cultured them in half-strength Murashige and Skoog medium for 15 days. These treatments promoted a high frequency of seed germination within 7 days in culture. Seedlings initially appeared healthy and produced green cotyledons and roots. During the following days, the leaves did not appear, and seedlings became white. We analyzed the root lengths and the fresh weight of seedlings after 15 days of culture.

Our results showed that SMZ treatment decreased the root length compared with untreated control seeds. The control seeds (without SMZ) produced 18.66 $\pm$ 3.57 mm roots. Meanwhile, seedlings treated with 50 μM SMZ produced 2.80 $\pm$ 0.34 mm roots, 25 μM SMZ induced 3.10 $\pm$ 0.28, and 1 μM SMZ produced 5.04 $\pm$ 0.94 mm roots. The mean weight was measured as 0.6569 mg per untreated control plant. However, plants treated with 50, 25, or 1 μM SMZ produced 0.25, 0.33, and 0.184 mg fresh weight, respectively. Therefore, these results indicated that higher levels of SMZ (50 μM) suppressed growth due to some cytotoxic effects (Figure 3) [8]. However, the same concentrations (1, 25, and 50 μM) of SMZ derivatives produced different root growth and biomass production patterns.

Derivative **S1** promoted a high frequency of seed germination for all treatments. Even though the seedlings treated with 1 μM **S1** were pale green after 2 weeks of culture, higher levels of **S1** (25 μM) produced healthy plants with many root hairs (Figure 3). While seedlings treated with 50 μM **S1** produced 9.37 mm roots, 25 μM **S1** induced 15.6 $\pm$ 2.6 mm and 1 μM **S1** produced 20.5 $\pm$ 5.5 mm root lengths (Figure 4). Furthermore, seedlings treated with 50, 25, or 1 μM **S1** produced 0.512, 0.4, and 0.672 mg fresh weight, respectively (Figure 5).

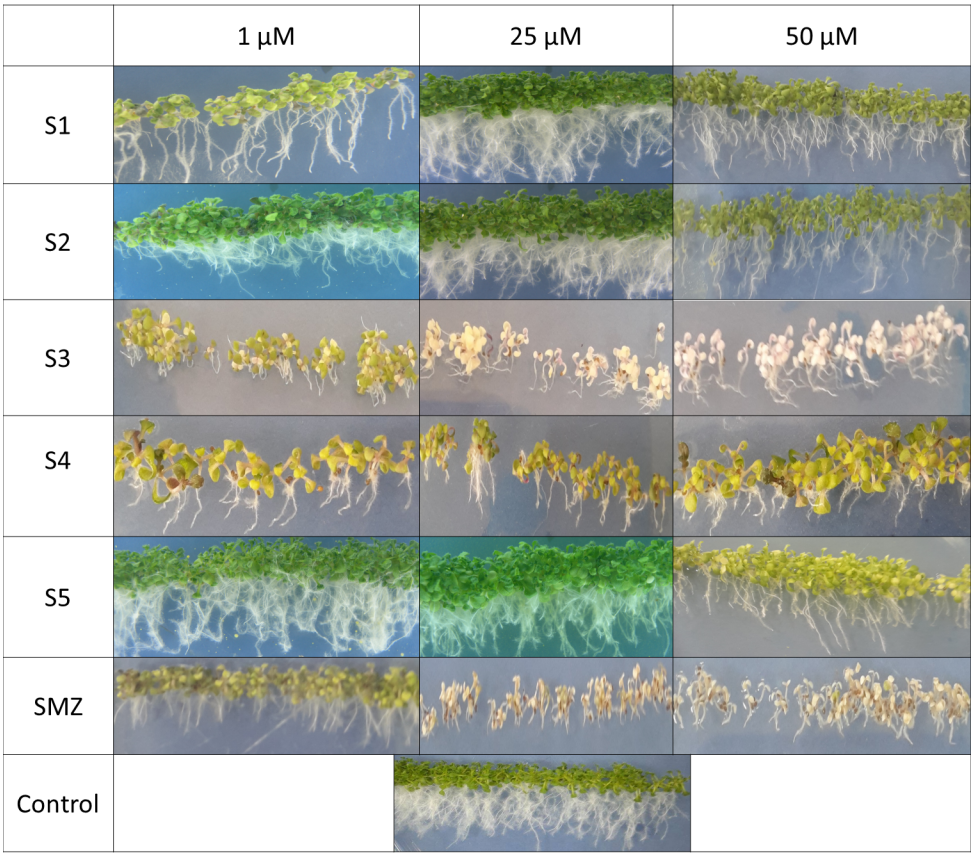

Figure 3. A. thaliana seedlings 15 days after treatments.

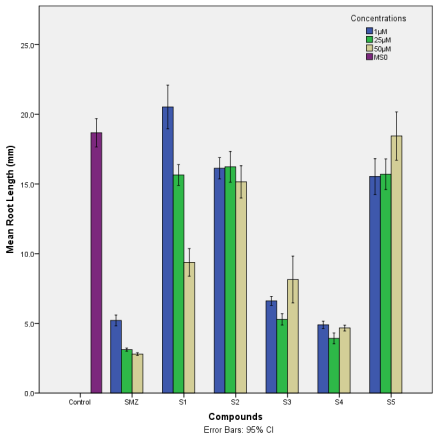

Figure 4. Root lengths of *A. thaliana* plants after 15 days of treatment with SMZ derivatives.

S2 caused high germination rates for all treatments. Although the seedlings treated with 50 μM **S2** were pale green after 2 weeks in culture, 25 μM and 1 μM **S2** produced healthy plants with many root hairs (Figure 3). Seedlings treated with 50 μM **S2** produced 16.13 $\pm$ 2.30 mm roots, 25 μM **S2** induced 16.23 $\pm$ 3.88 mm, and 1 μM **S2** produced 15.16 $\pm$ 4.08 mm root lengths (Figure 4). Also, seedlings treated with 50, 25, or 1 μM **S2** produced 0.253, 0.440, and 0.398 mg fresh weight, respectively (Figure 5).

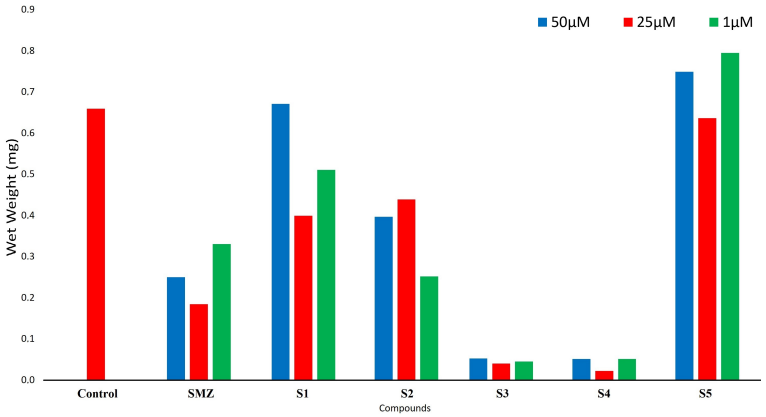

Figure 5. Mean weight of one plant 15 days after transfer to germination medium.

Although plants treated with 1 μM compound **S3** were yellow in color and died after 2 weeks of culture, at 25 μM and 50 μM plants were white and died earlier in culture (Figure 3). Mean root lengths of **S3**-treated plants were 5.63 $\pm$ 1.03 at 1 μM, 5.28 $\pm$ 1.42 at 25 μM, and 6.61 $\pm$ 3.14 at 50 μM (Figure 4). Also, seedlings treated with 50, 25, or 1 μM S3 produced 0.052, 0.04, and 0.045 mg fresh weight, respectively (Figure 5).

Compound **S4**-treated seedlings died after 2 weeks in culture at all concentrations (Figure 3). Meanwhile, seedlings treated with 50 μM **S4** produced 4.89 $\pm$ 0.92 mm roots, 25 μM **S4** induced 3.92 $\pm$ 1.36 mm, and 1 μM **S4** produced 4.67 $\pm$ 0.67 mm roots (Figure 4). Also, seedlings treated with 50, 25, or 1 μM **S4** produced 0.051, 0.051, and 0.219 mg fresh weight, respectively (Figure 5).

The SMZ derivative **S5** also promoted a high frequency of seed germination for all treatments. Although the seedlings treated with 50 μM **S5** were yellow-green after 2 weeks of culture, 25 μM and 1 μM **S5** produced healthy plants with many root hairs (Figure 3). **S5** showed similar root growth pattern to the control group with means of 15.53 $\pm$ 4.61 mm at 1 μM, 15.70 $\pm$ 3.86 mm at 25 μM, and 18.44 $\pm$ 5.97 mm at 50 μM (Figure 4). **S5** treatment resulted in 0.749 mg at 50 μM, 0.636 mg at 25 μM, and 0.795 mg at 1 μM fresh weight, which is similar to the control group (Figure 5).

2.3. Conclusions

A series of sulfonamide derivatives (**S1**–**S5**) were synthesized under different reaction conditions and characterized by various spectral methods. With the results obtained from this study, we conclude that among all SMZ derivatives, only **S3** and **S4** that have a 4-nitro (**S3**) or 4-amino-3,5-dinitro (**S4**) on phenyl ring A and contain an *m*- or *p*-nitro group on ring B showed similar results to SMZ. Plants grown in Murashige and Skoog medium supplemented with **S3** and **S4** were bleached. Both root length and fresh weight of plants treated with **S3** and **S4** derivatives showed statistically significant decreases compared to the control group without treatment. **S1** treatment results showed dose-dependent effects on plants, with higher concentrations reducing the root lengths. Also, at the highest concentration **S1** was not as effective as SMZ, **S3**, or **S4**. **S2** and **S5** did not affect root growth or plant biomass. When the experimental results were interpreted structurally, it was determined that both **S1** and **S2** have a methyl group at the *p*-position of ring A and positions (*o*- or *p*-) of the nitro group on ring B causing effectiveness at higher concentrations for compound **S1** (*o*-nitro) and ineffectiveness for compound **S2** (*p*-nitro). Also, compound **S5** with bulky dibutyl groups on the nitrogen atom on ring A and the chloro atom on ring B had no effect on *A. thaliana* plants.

In our study, we aimed to analyze cytotoxic effects of synthesized SMZ derivatives on *A. thaliana* plants as a model. Several SMZ derivatives were synthesized with various effects on plants, including **S3** and **S4**, which are promising. SMZ is also routinely used as a veterinary drug and was found to accumulate in animal tissues such as muscles and also urine, blood, and milk [21–26]. Therefore, there are concerns about SMZ pollution in soil and plants, as well. The removal of SMZ from soil is another concern and a subject of several studies [27–29]. The SMZ derivatives synthesized in this study may overcome these problems. However, further studies on these compounds are required to assess their potential.

3. Experimental

3.1. Chemistry

3.1.1. General Information

All chemicals were purchased from Sigma-Aldrich and Merck and used as supplied without further purification. SMZ was also obtained from Sigma-Aldrich (S6256). Synthesis of the compounds was monitored by TLC on 0.25-mm silica gel plates ($60F_{254}$) and visualized with UV light and/or $KMnO_4$ stain. Melting points were uncorrected with an X-4 melting-point apparatus. Infrared spectra were obtained with a PerkinElmer Spectrum 100 FTIR spectrophotometer using ATR techniques. Frequencies are given in cm^{-1} and only selected absorbances are provided. ^1H NMR and ^{13}C NMR spectra were recorded at 500 and 125 MHz or 400 and 100 MHz in deuterated DMSO. Chemical shifts (δ) are expressed in parts per million (ppm), the coupling constants (J) are expressed in hertz (Hz), and tetramethylsilane (TMS) was used as an internal standard. The following abbreviations were used to specify peaks: s, singlet; d, doublet; t, triplet; q, quadruplet; quint, quintuplet; sext, sextuplet; m, multiplet; and dd, double doublet.

3.1.2. Preparation of sulfonamide derivatives (S1–S5)

3.1.2.1. 4-Methyl-*N*-(2-nitrophenyl)benzenesulfonamide (S1)

Compound **S1** was synthesized from 2-nitroaniline and 4-methylbenzenesulfonyl chloride (TosCl) according to the described procedure [17]. Yellow crystal; yield: 63%; mp 112–113 °C (lit. mp 112–113 °C) [17]; IR (ATR; ϑ/cm^{-1}): 3275, 3123, 3104, 3084, 1609, 1599, 1522, 1484, 1382, 1343, 1273, 1213, 1163, 1143, 1089, 1039, 914, 847, 815, 779, 739, 704, 661; ^1H NMR (500 MHz, DMSO-d_6, ppm): δ 10.21 (s, 1H, -NH), 7.92 (d, 1H, j =8.2 Hz), 7.61 (m, 2H + 1H), 7.36 (m, 2H + 1H), 7.27 (d, 1H, j =8.24 Hz), 2.36 (s, 3H); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm): δ 144.24, 143.48, 136.79, 134.65, 130.82, 130.24, 127.29, 126.62, 125.93, 125.72, 21.46.

3.1.2.2. 4-Methyl-*N*-(4-nitrophenyl)benzenesulfonamide (S2)

DMF solution (10 mL) of 4-nitroaniline (1.0 g, 7.24 mmol) was added dropwise to NaH solution (0.35 g, 7.96 mmol, 1.1 eq., 55% oil dispersion) in 5 mL of DMF and stirred at room temperature for 3 h. 4-Methylbenzenesulfonyl chloride (1.37 g, 7.24 mmol) was added to the reaction mixture and refluxed for 16 h. After completion of the reaction, the mixture was cooled to room temperature and poured into cold water (200 mL). The resulting precipitate was filtered, washed with water, and dried at room temperature. The crude product was purified by crystallization with ethanol. Yellow crystal; 1.0 g; yield: 47%; mp 190–191 °C (lit. mp 191 °C) [30,31]; IR (ATR; ϑ/cm^{-1}): 3333, 3119, 3086, 3041, 1594, 1521, 1495, 1464, 1338, 1291, 1235, 1152, 1089, 906, 854, 750, 692, 663; ^1H NMR (500 MHz, DMSO-d_6, ppm): δ 11.22 (s, 1H), 8.13 (d, 2H, j =9.27

Hz), 7.765 (d, 2H, j =8.36 Hz), 7.395 (d, 2H,j =8.40 Hz), 7.31 (d, 2H, j =9.23 Hz), 2.35 (s, 3H); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm): δ 144.90, 144.46, 142.83, 136.63, 130.43, 127.19, 125.79, 118.32, 21.41.

3.1.2.3. 4-Nitro-*N*-(3-nitrophenyl)benzenesulfonamide (S3)

DMF solution (10 mL) of 3-nitroaniline (0.3 g; 2.17 mmol) was added dropwise to a mixture of 5 mL of DMF solution of 4-nitrobenzenesulfonyl chloride (0.48 g; 2.17 mmol) and pyridine (0.21 g; 2.65 mmol; 1.2 eq.). The reaction mixture was heated at 40–50 °C for 3 h. After this time, the mixture was poured into cold water (100 mL). The obtained solid was filtered and dried. The crude product was purified by crystallization with ethanol to obtain the corresponding sulfonamide. Cream solid; 0.5 g; yield: 72%; mp 268 °C. IR (ATR; ϑ/cm^{-1}): 3251, 3160, 3110, 3094, 3043, 1603, 1518, 1473, 1428, 1343, 1241, 1176, 1118, 1026, 1003, 890, 863, 821, 739, 678; ^{1}H NMR (600 MHz, DMSO-d_6, ppm): δ 11.13 (s, 1H), 8.88 (m, broad, 1H), 8.31 (s, 1H), 8.15 (m, broad, 2H), 8.08 (m, broad, 1H), 7.81 (m, broad, 2H), 7.73 (m, broad, 1H); ^{13}C NMR (DMSO-d_6, 150 MHz, ppm): δ 154.49, 148.84, 147.68, 139.22, 131.53, 127.32, 125.63, 123.75, 120.72, 114.08; LC-MS/MS (negative mode): m/z calcd. for $C_{12}H_9N_3O_6S$: 323.28; found: 322.00.

3.1.2.4. 4-Amino-3,5-dinitro-*N*-(4-nitrophenyl)benzenesulfonamide (S4)

The starting material for **S4** (4-amino-3,5-dinitrobenzenesulfonyl chloride) was prepared from 2,6-dinitroaniline and chlorosulfonic acid according to the reported method [32]. Yellow solid; mp 154–157 °C. IR (ATR; ϑ/cm^{-1}): 3443, 3334, 3097, 1632, 1555, 1530, 1448, 1419, 1367, 1275, 1172, 1139, 1095, 1045, 918, 898, 775, 726. This solid (0.14 g; 0.49 mmol) was dissolved in 3 mL of DMA and 4-nitroaniline (69 mg; 0.49 mmol) was added to the reaction medium. The resulting mixture was stirred at room temperature for 48 h. After completion of the reaction, 30 mL of ethyl acetate was added to the medium. The organic phase was washed with water (2 × 50 mL), brined, and dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The residue was purified by washing with diethyl ether to give **S4** as an orange solid. 80 mg; yield: 42%; mp 274–275 °C. IR (ATR; ϑ/cm^{-1}): 3437, 3329, 3196, 3092, 3077, 1624, 1596, 1511, 1494, 1339, 1249, 1168, 1133, 1104, 1045, 896, 849, 824, 776, 729, 693; ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 11.33 (s, 1H), 8.74 (s, 2H), 8.69 (s, 2H), 8.135 (d, 2H, j =9.05 Hz), 7.315 (d, 2H,j =9.10 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm): δ 143.82, 143.48, 143.22, 135.29, 131.48, 125.91, 123.09, 119.13; LC-MS/MS (negative mode): m/z calcd. for $C_{12}H_9N_5O_8S$: 383.29; found: 381.00.

3.1.2.5. 4-(*N*^{i},*N*^{i}-Dibutylamino)-3,5-dinitro-*N*-(4-chlorophenyl)benzenesulfonamide (S5)

Potassium 4-chloro-3,5-dinitrobenzenesulfonate was synthesized from chlorobenzene by the reactions of sulfonylation and then nitration [19]. This compound was converted to 4-(dibutylamino)-3,5-dinitrobenzenesulfonyl chloride in two steps according to the reported method [20]. The obtained intermediate (0.6 g; 1.52 mmol) and 4-chloroaniline (0.68 g; 5.33 mmol; 3.5 eq.) were dissolved in 30 mL of pyridine and heated at 50 °C for 24 h. After completion of the reaction, excess pyridine was distilled at reduced pressure and the residue was poured into cold water (50 mL). The mixture was extracted with ethyl acetate (3 × 40 mL) and the organic layer was washed with water (50 mL). The ethyl acetate phase was dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by column chromatography over silica gel (0% to 30% methanol in a chloroform gradient) and preparative thin-layer chromatography $CHCl_3$:hexane (100:1) to give **S5** as bright

yellow crystal. 0.3 g; yield: 41%; mp 83–84 °C. IR (ATR; ϑ/cm^{-1}): 3356, 3109, 2960, 2932, 2872, 1618, 1586, 1520, 1494, 1419, 1330, 1311, 1267, 1225, 1111, 1071, 1049, 922, 831, 744, 714; ^{1}H NMR (600 MHz, DMSO-d_6, ppm): δ 8,805 (d, 2H, j =2.71 Hz), 8.79 (s, 1H), 8.20 (dd, 2H, j =9.59 and 2.25 Hz), 7.185 (d, 2H, j =9.65 Hz), 3.44 (q, 4H, j =6.78 Hz), 1.58 (quint, 4H, j =7.32 Hz), 1.36 (sext, 4H, j =7.46 Hz), 0.90 (t, 6H, j =7.40 Hz); ^{13}C NMR (DMSO-d_6, 150 MHz, ppm): δ 148.55, 135.02, 130.35, 129.98, 124.07, 115.66, 43.00, 30.60, 19.93, 14.06; LC-MS/MS (negative mode): m/z calcd. for $C_{20}H_{25}ClN_4O_6S$: 484.95; found: 483.00.

3.2. Plant material

In this study, *Arabidopsis thaliana* Columbia ecotype seeds were kindly provided by the University of Bath, Department of Biology and Biochemistry, and used to test the cytotoxic effects of the synthesized and characterized SMZ derivatives (**S1**–**S5**) on plants. For this purpose, first of all, the seeds were surface-sterilized with 6% sodium hypochlorite for 10 min and washed 3 times with sterile distilled water. Secondly, half-strength Murashige and Skoog plant growth medium was prepared with 30 g/L sucrose and 8 g/L agar. Then **S1**–**S5** and SMZ were added to the media respectively. Applications were performed with 1 μM, 25 μM, and 50 μM concentrations for each group. A control group, which did not include any plant growth regulators, was also used. Afterwards, seeds were transferred to the media in sterile petri dishes and incubated in the dark at 4 °C for 2 days to break the seed dormancy. At the end of the second day, these petri dishes were transferred to a plant growth chamber. Germinating seeds were kept in 16:8 light conditions at 21 °C and 70% relative humidity for 15 days.

3.3. Phenotypic and statistical analysis

A. thaliana Columbia ecotype seeds were germinated on Murashige and Skoog media containing **S1**–**S5** and SMZ and a plant growth regulator-free Murashige and Skoog medium to determine and compare the cytotoxic effects of the synthesized SMZ derivatives. Fifteen days after the germination, the root length and the fresh weight of the seedlings in all groups were measured. A minimum of 37 and a maximum of 50 plant roots were measured for different groups. One-way analysis of variance (ANOVA) tests and Tukey's honestly significant differences tests were used to assess significant differences within each group of root length data. Mean fresh weight values were calculated for each group and used to prepare graphs for fresh weight/application.

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Supplementary Information (SI)

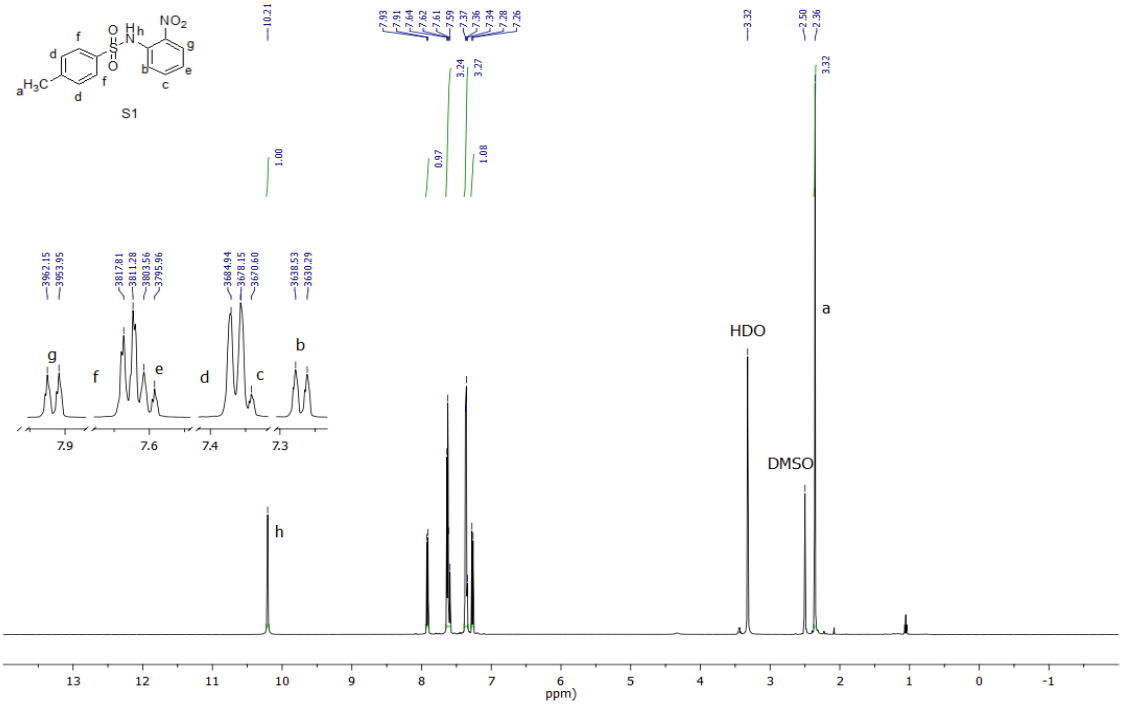

Figure S1. ^{1}H NMR spectra of **S1.**

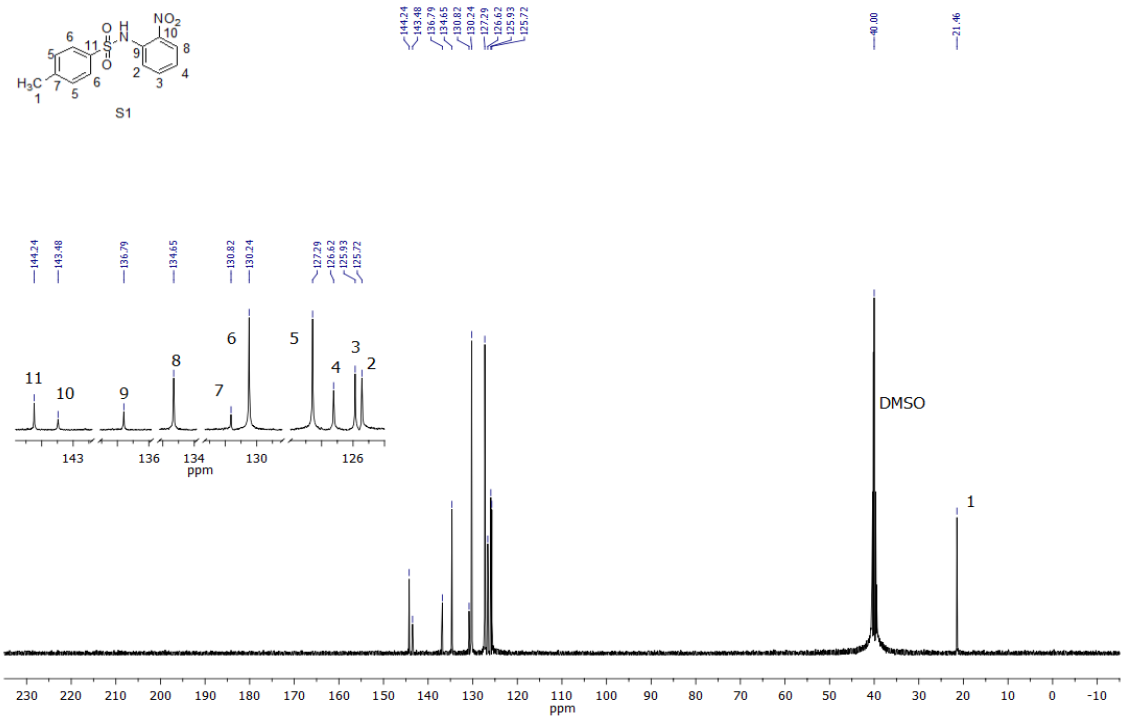

Figure S2. ^{13}C NMR spectra of **S1.**

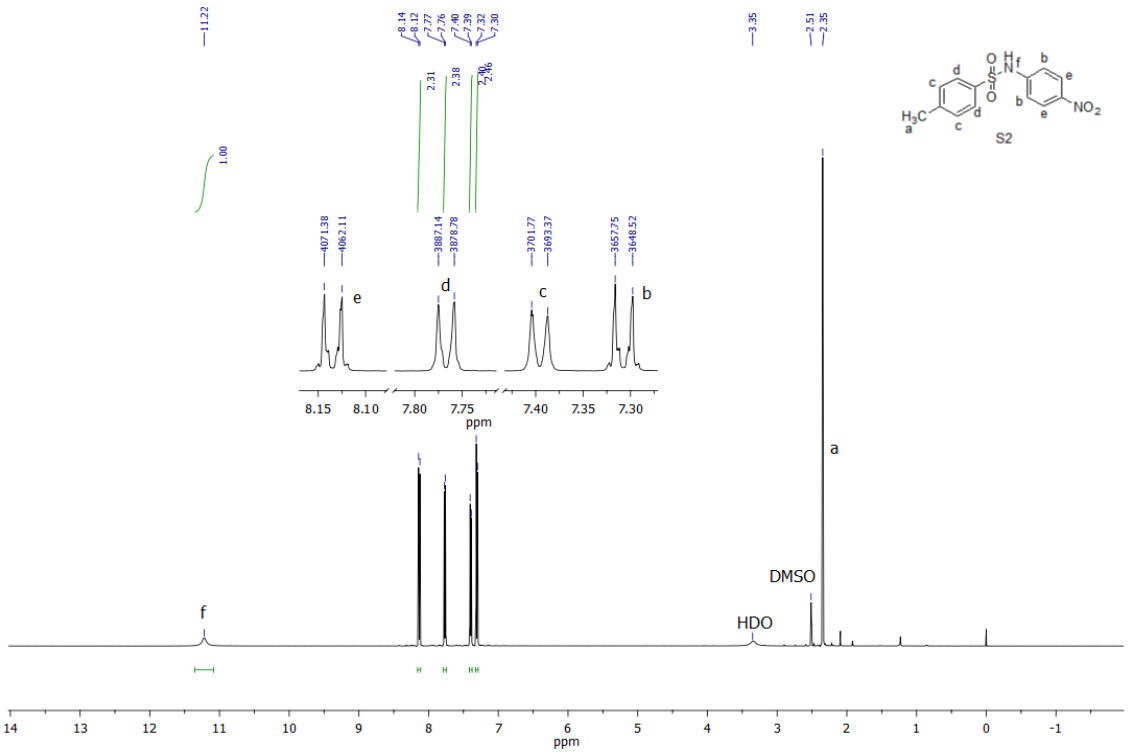

Figure S3. ^{1}H NMR spectra of **S2.**

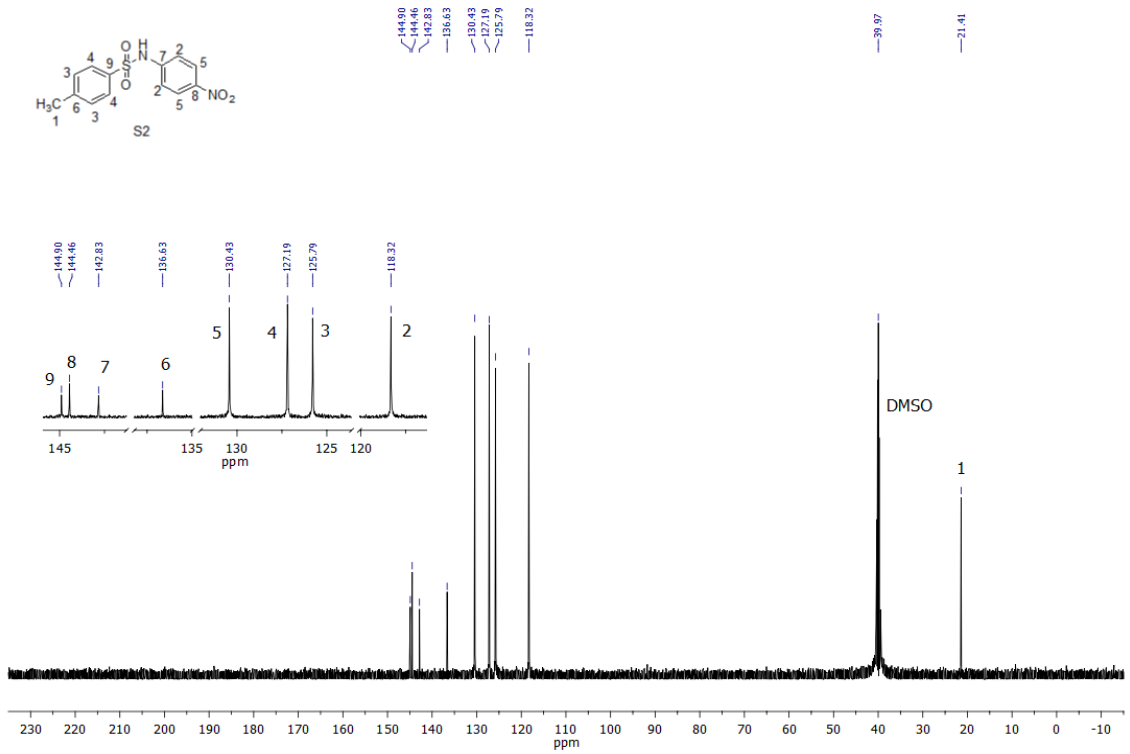

Figure S4. ^{13}C NMR spectra of **S2.**

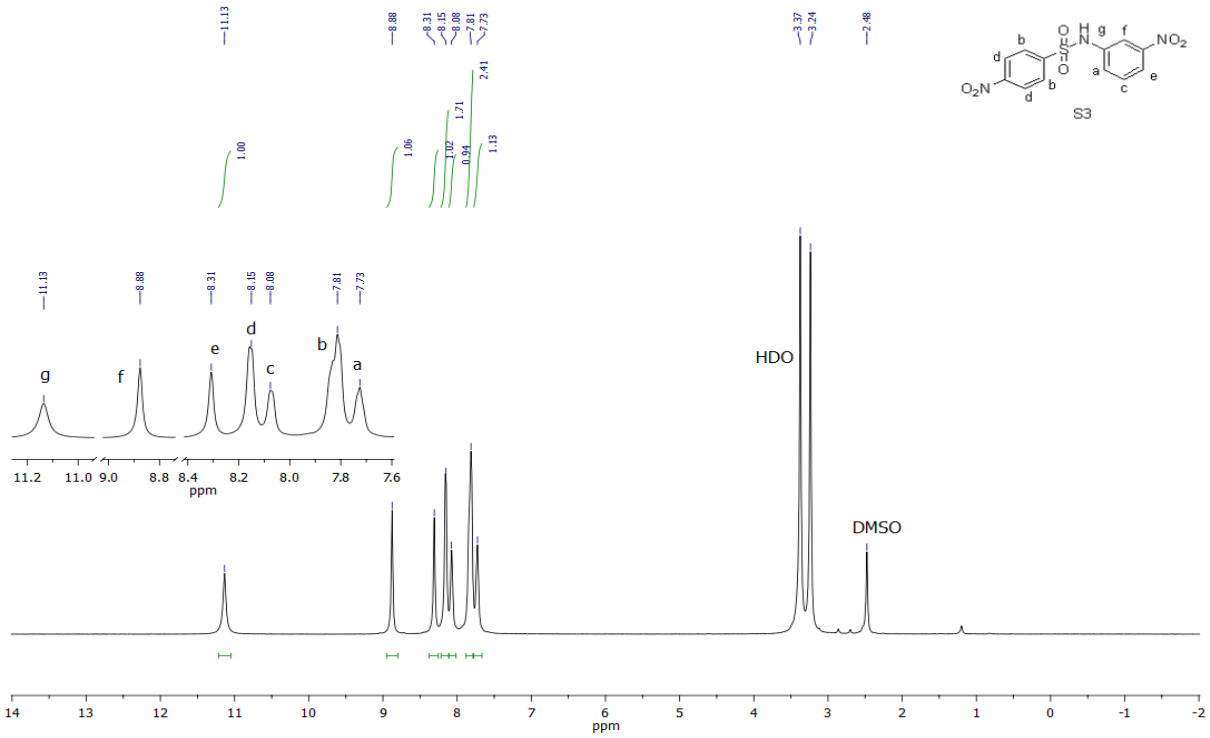

Figure S5. ^{1}H NMR spectra of **S3.**

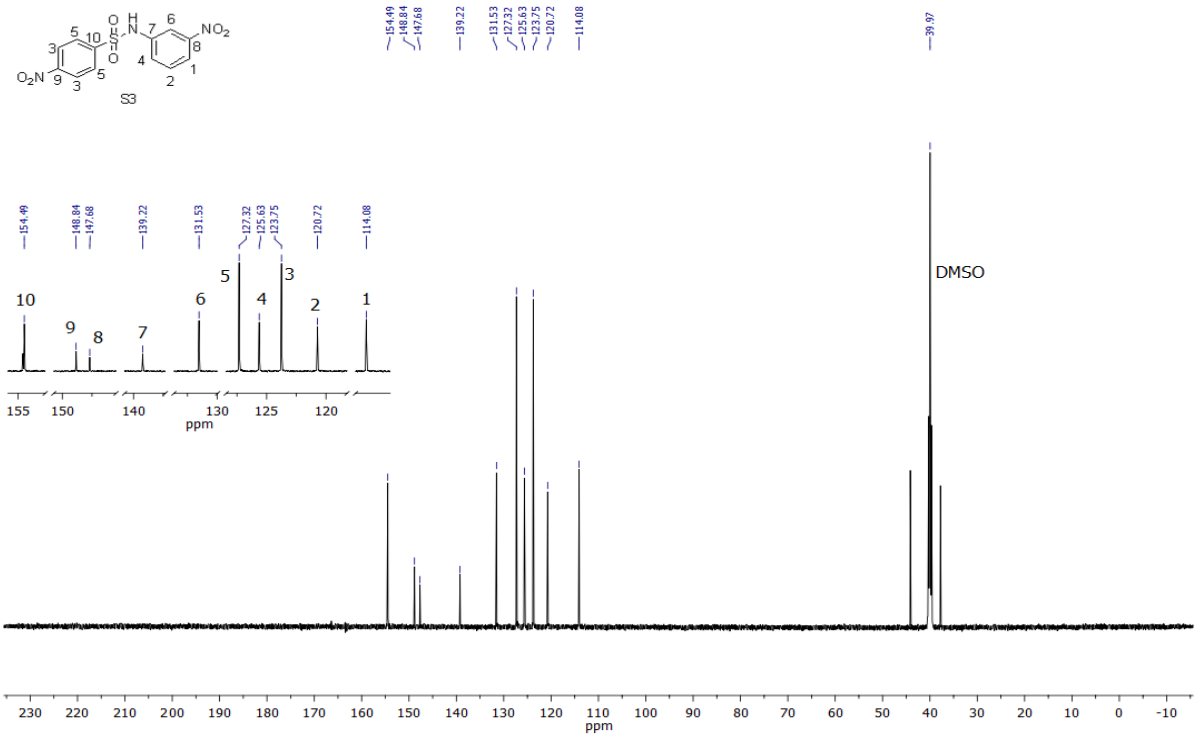

Figure S6. ^{13}C NMR spectra of **S3.**

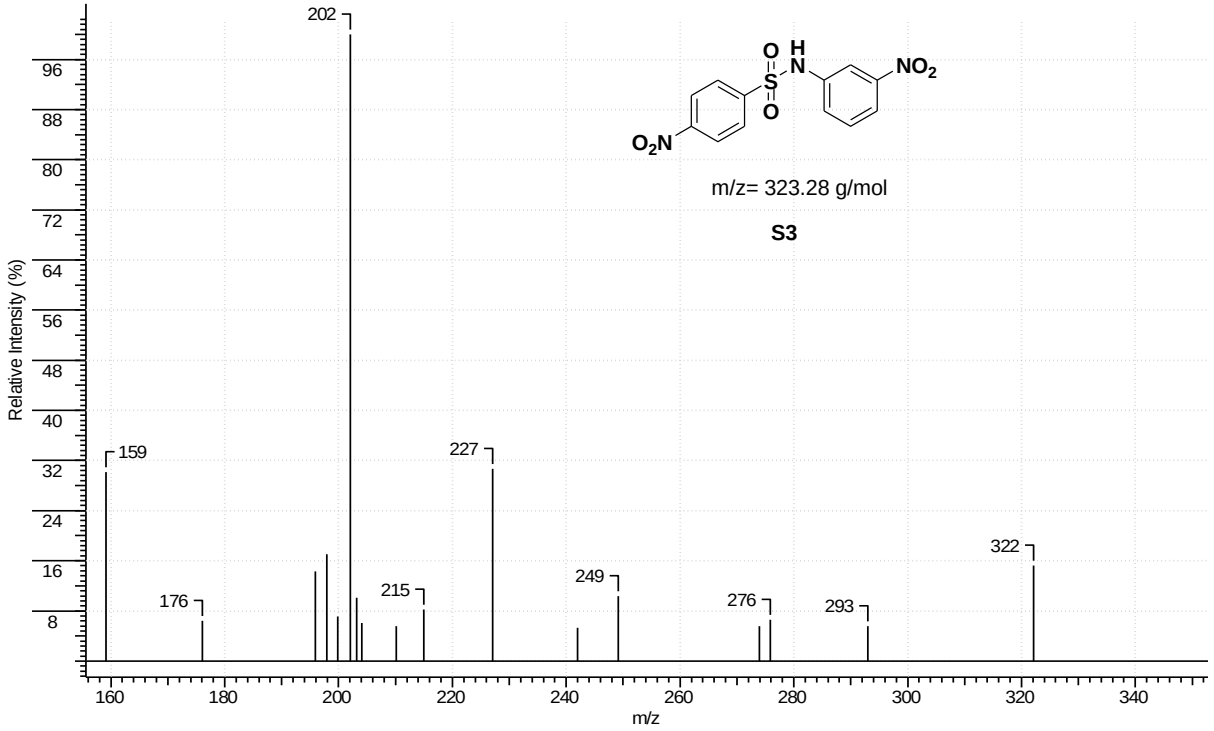

Figure S7. MS spectra of **S3.**

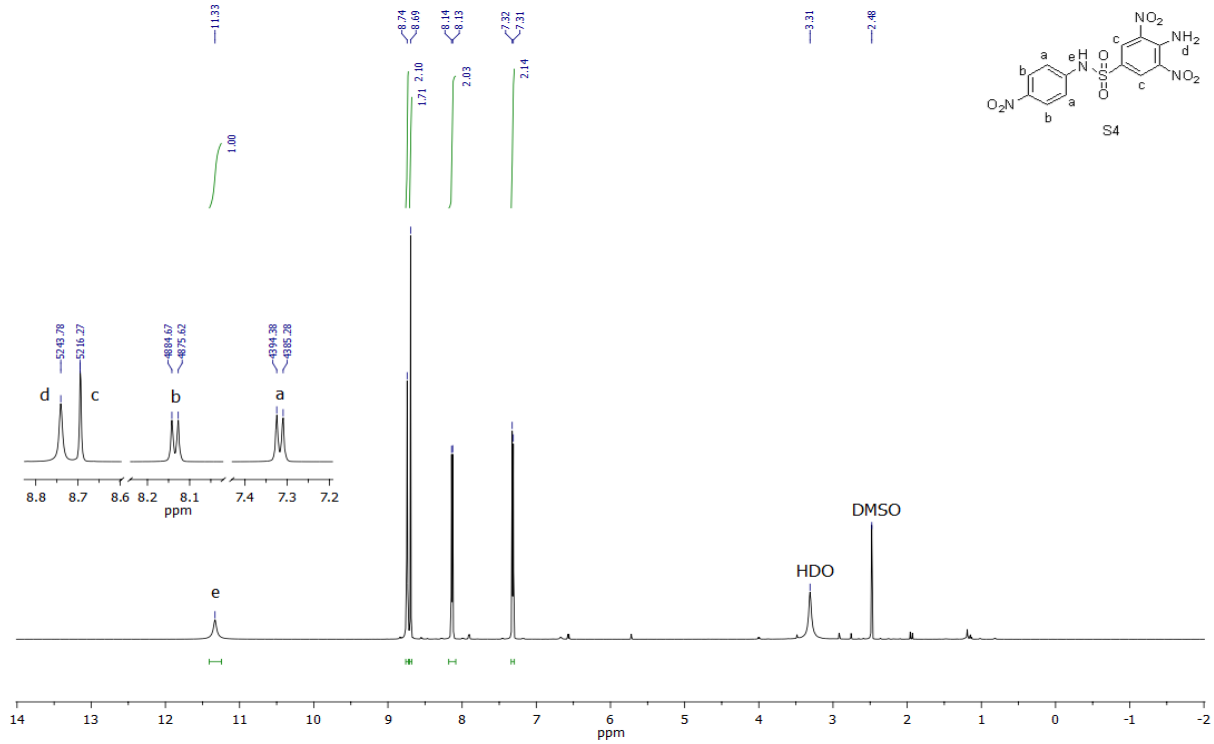

Figure S8. ^{1}H NMR spectra of **S4.**

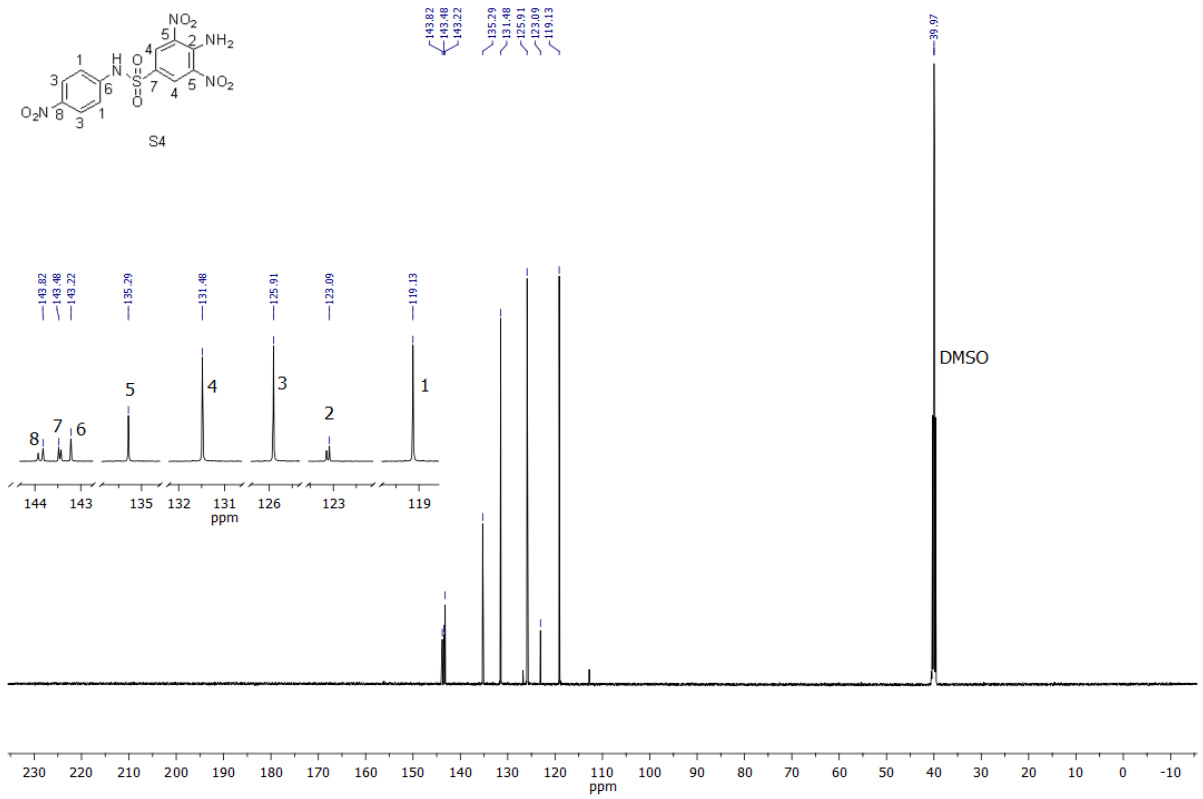

Figure S9. ^{13}C NMR spectra of **S4.**

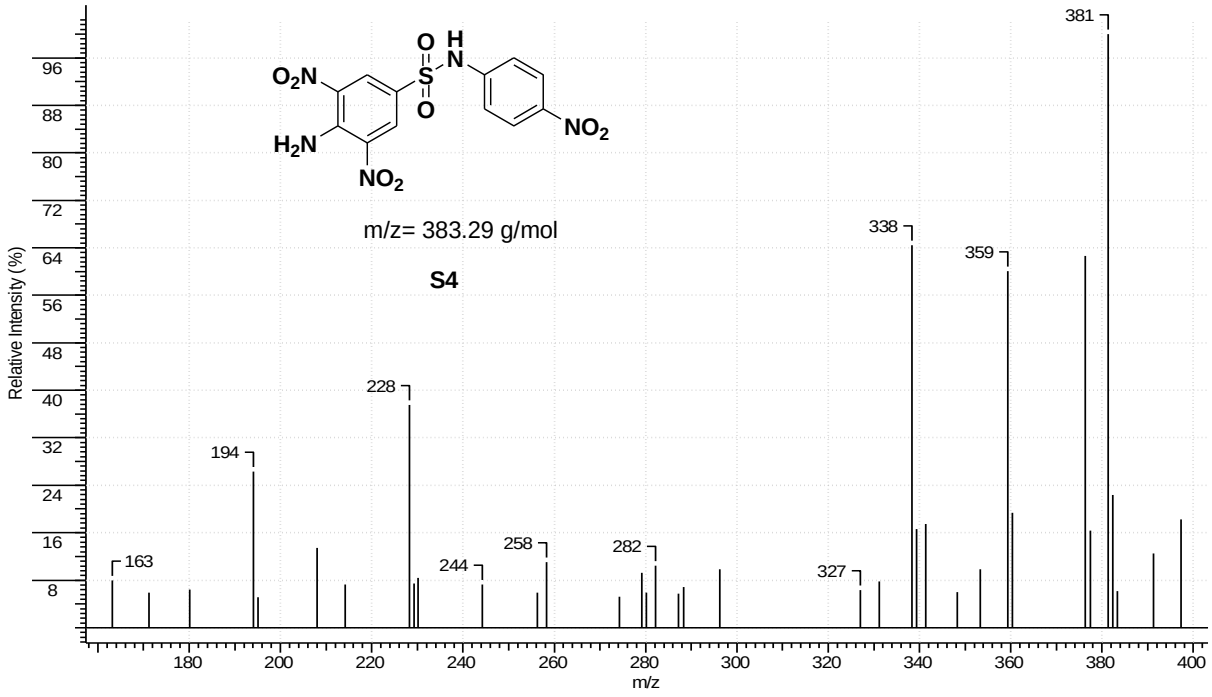

Figure S10. MS spectra of **S4.**

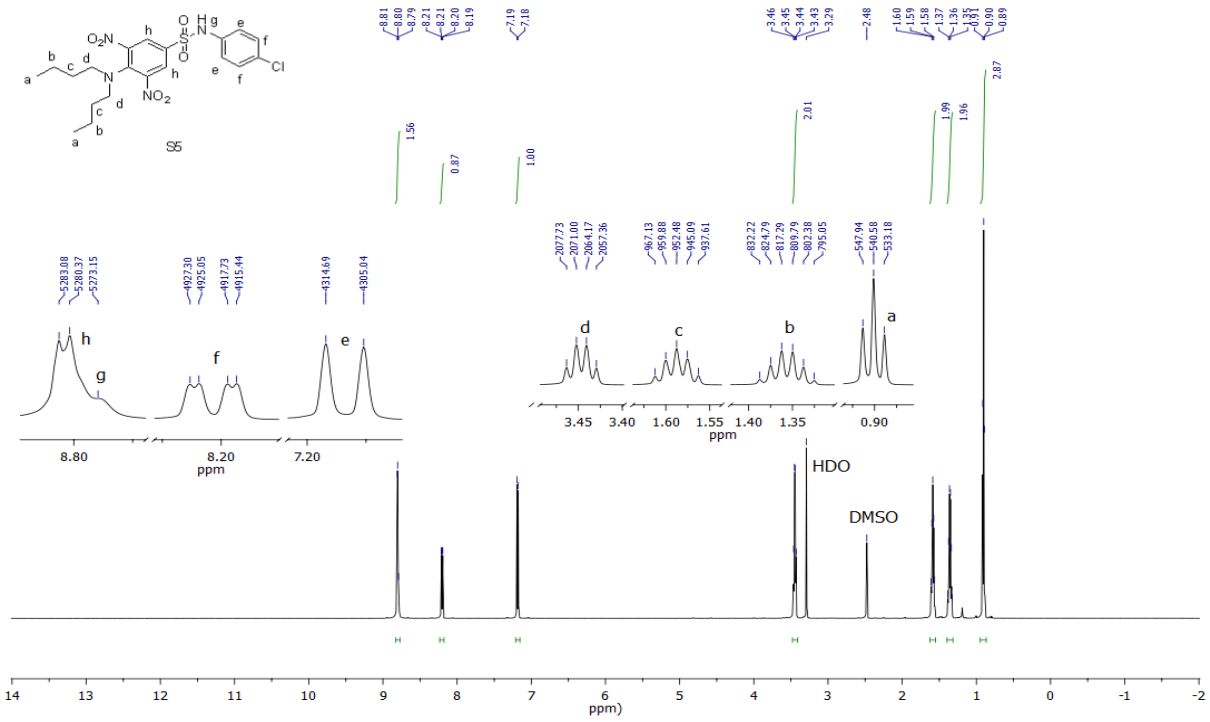

Figure S11. ^{1}H NMR spectra of **S5.**

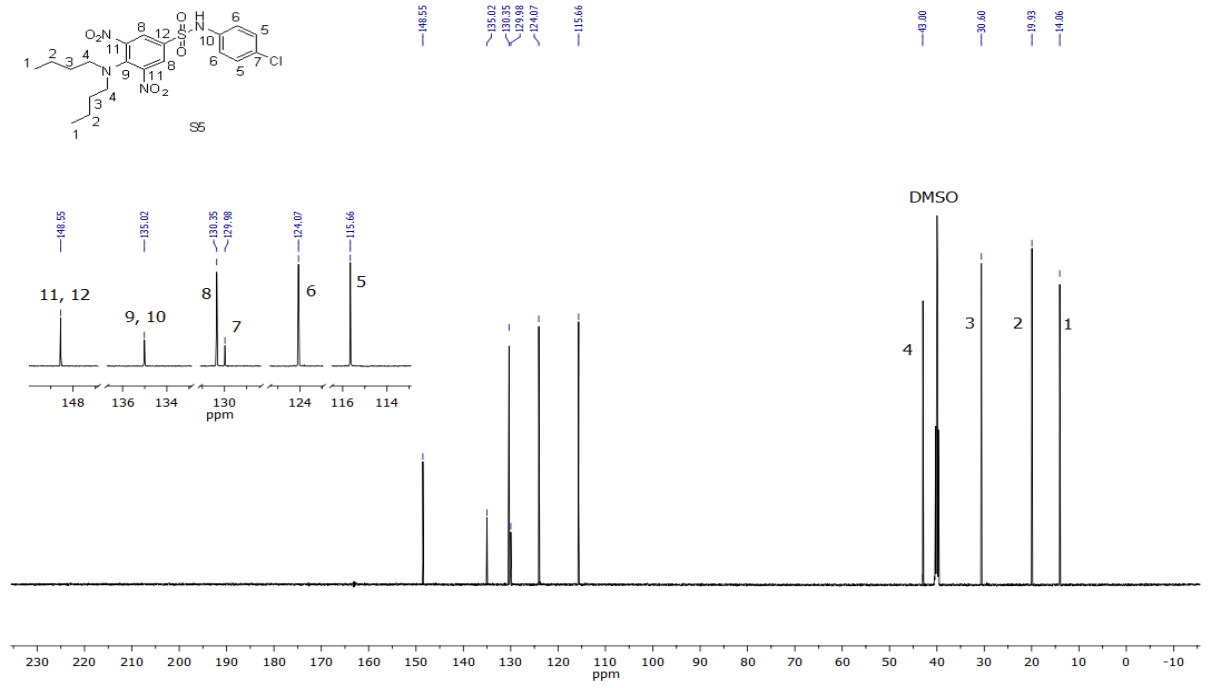

Figure S12. ^{13}C NMR spectra of **S5.**

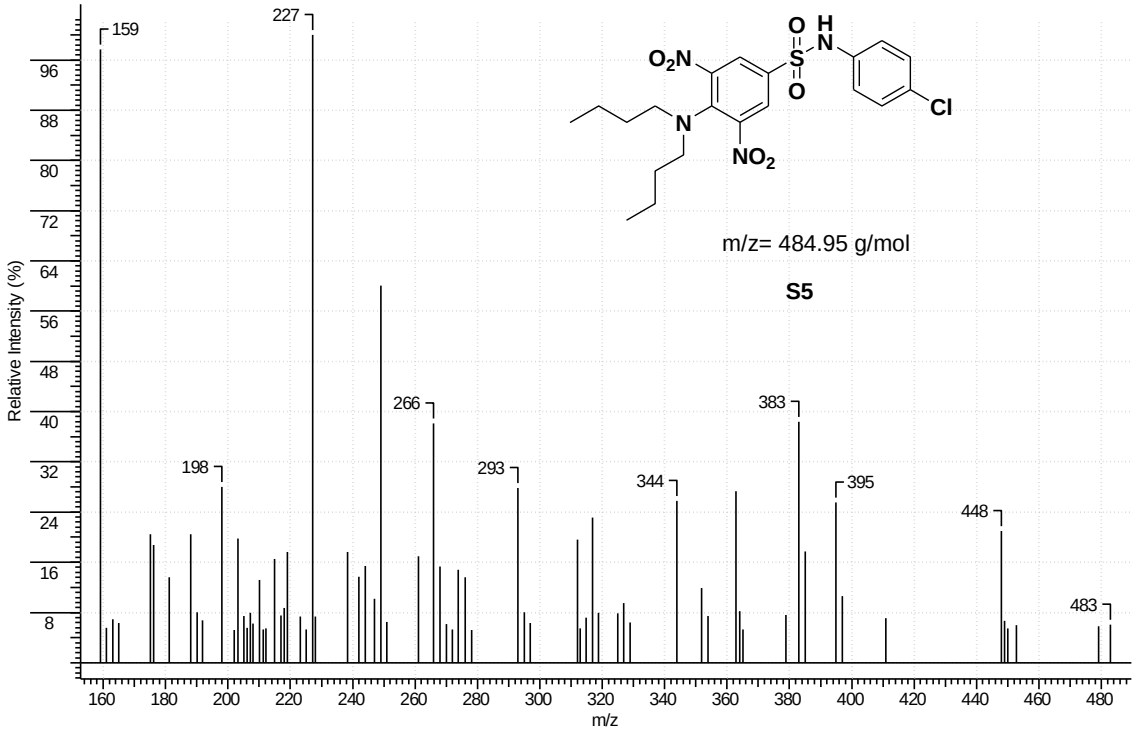

Figure S13. MS spectra of **S5.**