Supplementary Information

Mesotrione Conjugation Strategies to Create Proherbicides with Reduced Soil Mobility

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Number of tables: 2

EXPERIMENTAL

Materials. All reagents and solvents were purchased from Sigma Aldrich (St. Louis, MO) or Fisher Scientific (Hampton, NH) and used without further purification unless otherwise noted. Mesotrione was purchased from Jinan Boss Chemical Industry Company Ltd. (Shandong Province, China). Two soils were used for experiments. Soil with no prior exposure to mesotrione was collected from two sites. Soil A was collected in Los Angeles, CA; it was a sandy loam soil with a pH of 6.82. Soil B was collected from a rice field at the Rice Experiment Station (Biggs, CA) in February 2020; it had a pH of 5.86 and composition of 2.7 % organic matter, 21 % sand, 30 % silt and 49 % clay. Soil was dried and passed through a 2-mm sieve before use. *Chenopodium album* (common lambsquarters) seeds were purchased from Strictly Medicinal Seeds (Williams, Oregon).

Characterization. ^{1}H-NMR and ^{13}C-NMR spectroscopy were performed on a Bruker AV 400 or 500 MHz instrument. Infrared absorption spectra were obtained using a PerkinElmer FT-IR equipped with an attenuated total reflectance (ATR) accessory. ESI mass spectra were obtained using either a Waters Acquity LCT Premier XE equipped with an autosampler and direct injection port or an Agilent 6530 QTOF-ESI with a 1260 Infinity LC with an autosampler using a Poroshell 120 2.7-μm C18 120 Å column (analytical: 2.7 μm, 4.6 × 100 mm) with monitoring at $\lambda = 220$ and 254 nm and with a flow rate of 0.8 mL/min. A gradient of 10 – 95 % solvent B (solvent A: water, solvent B: acetonitrile, both in 0.1 % formic acid (vol/vol)) over 15 minutes was applied. Analytical reverse phase high performance liquid chromatography (HPLC) was carried out on an Agilent 1260 Infinity II HPLC system equipped with an autosampler and a UV detector using a Poroshell 120 2.7-μm C18 120 Å column (analytical: 2.7 μm, 4.6 × 100 mm) with monitoring at

λ = 220 and 254 nm and with a flow rate of 0.8 mL/min. A gradient of 10 - 95 % solvent B (solvent A: water, solvent B: acetonitrile, both in 0.1 % trifluoroacetic acid (vol/vol)) over 15 minutes was applied. Concentrations of mesotrione released in samples was determined by comparing values to a standard curve created in the same matrix. Mesotrione eluted after approximately 9.4 minutes using this method. TGA experiments were performed on a Perkin Elmer Diamond Thermogravimetric Differential Thermal Analyzer. The specific procedure was as follows: each sample was placed in an alumina crucible and heated from 50˚C to 700˚C under argon at a heating rate of 15˚C min^{-1}.

General synthesis of thioethers. Mesotrione (200 mg, 0.6 mmol, 1 equiv.) was dissolved in anhydrous dichloromethane (DCM, 1 mL). Oxalyl chloride (112 mg, 0.9 mmol, 1.5 equiv.) was then slowly added to the solution followed by a catalytic amount of dimethylformamide (DMF, 4.5 μL). After addition of all reagents, the reaction was refluxed at 40 °C for 2 hours. Solvent and excess oxalyl chloride were then removed *in vacuo*. The next step was immediately followed without purification. In another vial, thiol (1.8 mmol, 3 equiv.) was added to water (645 μL) on ice. To the thiol solution, tris(2-carboxyethyl)phosphine) (TCEP, 7.1 mg, 0.03 mmol, 30 mM) was added and the pH of the solution was adjusted to approximately 9-10 using sodium hydroxide (NaOH) (1 N). Dried chlorinated mesotrione was dissolved in acetonitrile (300 μL) then added dropwise to the thiol solution. The reaction was stirred for 6 hours before being neutralized. Reaction contents were then concentrated *in vacuo* and the product was purified by silica column chromatography (10-95 % ethyl acetate/methylene chloride gradient).

Phenyl thioether mesotrione derivative, 2-(4-(methylsulfonyl)-2-nitrobenzoyl)-3-(phenylthio)-cyclohex-2-en-1-one (**1**). Orange powder (193.1 mg, 76 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.73-8.69 (d, *J* = 1.6 Hz, 1H), 8.25-8.21 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.63 – 7.60 (m, 2H), 7.55 – 7.47 (m, 4H), 3.18-3.13 (s, 3H), 2.55-2.50 (t, *J* = 6.1 Hz, 2H), 2.37 – 2.32 (t, 2H), 1.94-1.86 (p, *J* = 6.3 Hz, 3H) ppm. ^{13}C NMR (500 MHz, $CDCl_3$) δ: 193.9, 189.6, 184.0, 145.3, 144.2. 141.2, 135.3, 132.6, 130.7, 129.9, 129.8, 128.5, 127.5, 123.3, 44.5, 37.3, 33.9, 21.6 ppm. (*m/z*) $[M + H]^+$ calculated = 432.06 ; found = 432.05. FTIR: ν 2926, 1736, 1709, 1662, 1532, 1472, 1349, 1318, 1231, 1161, 1145, 922, 755 cm^{-1}.

Ethyl thioether mesotrione derivative, 3-(ethylthio)-2-(4-(methylsulfonyl)-2-nitrobenzoyl)-cyclohex-2-en-1-one (**2**). Yellow powder (226.0 mg, 63 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.70-8.65 (d, *J* = 1.6 Hz, 1H), 8.21-8.16 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.42-7.38 (d, 1H), 3.16-3.11 (s, 3H), 3.08 – 3.00 (m, 4H), 2.44 – 2.36 (m, 2H), 2.14-2.02 (p, *J* = 6.3 Hz, 2H), 1.47-1.38 (t, *J* = 7.5 Hz, 3H) ppm. ^{13}C NMR (500 MHz, $CDCl_3$) δ: 194.2, 189.4, 185.6, 145.2, 144.4, 141.0, 132.6, 128.3, 127.5, 123.4, 44.5, 37.1, 32.1, 26.9, 21.5, 13.1 ppm. (*m/z*) $[M + H]^+$ calculated = 384.06; found = 384.05. FTIR: ν 2928, 1736, 1708, 1656, 1531, 1457, 1350, 1317, 1232, 1161, 1143, 922, 758 cm^{-1}

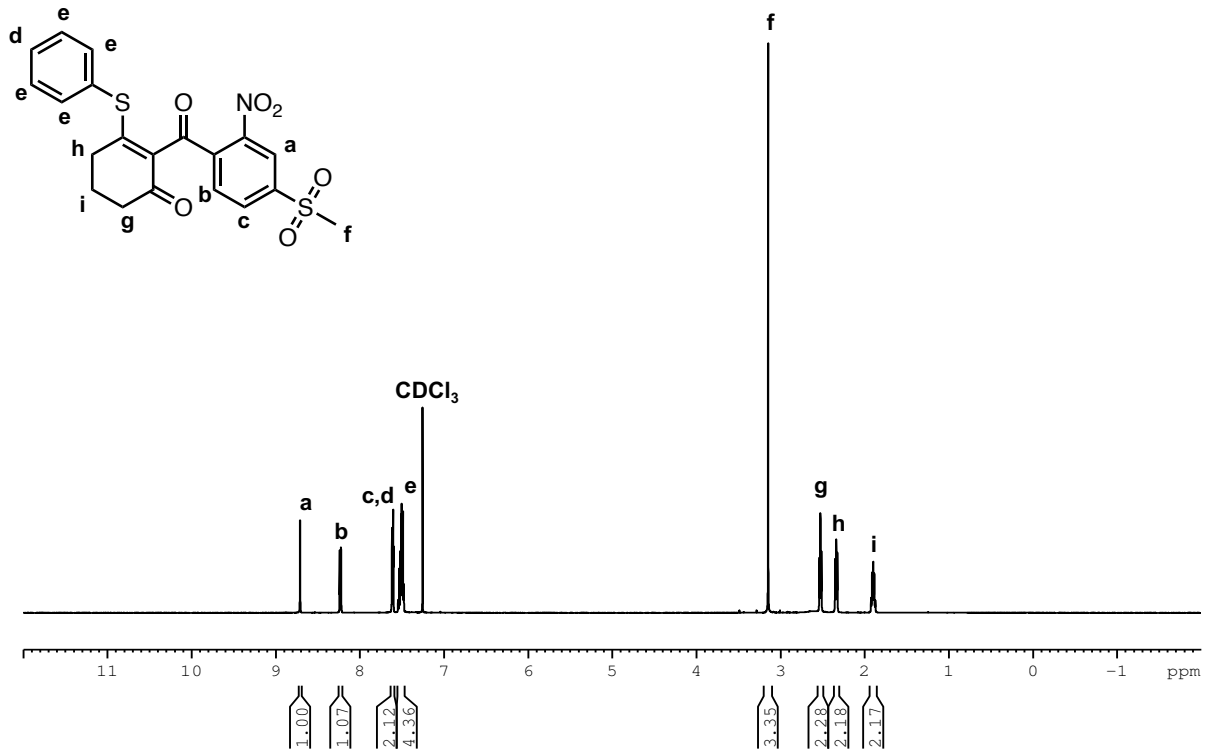


Figure S1. ^{1}H-NMR of phenyl thioether derivative (**1**) in deuterated chloroform ($CDCl_3$).

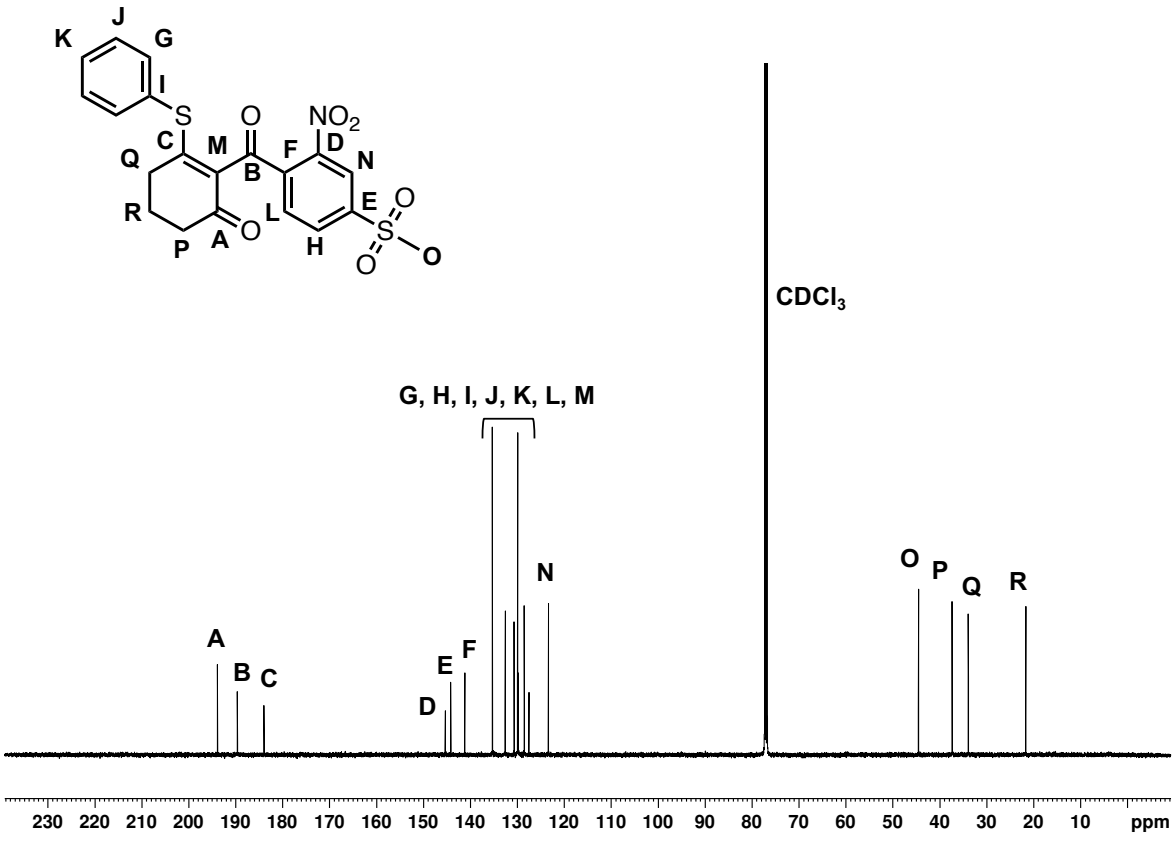


Figure S2. ^{13}C-NMR of phenyl thioether derivative (**1**) in $CDCl_3$.

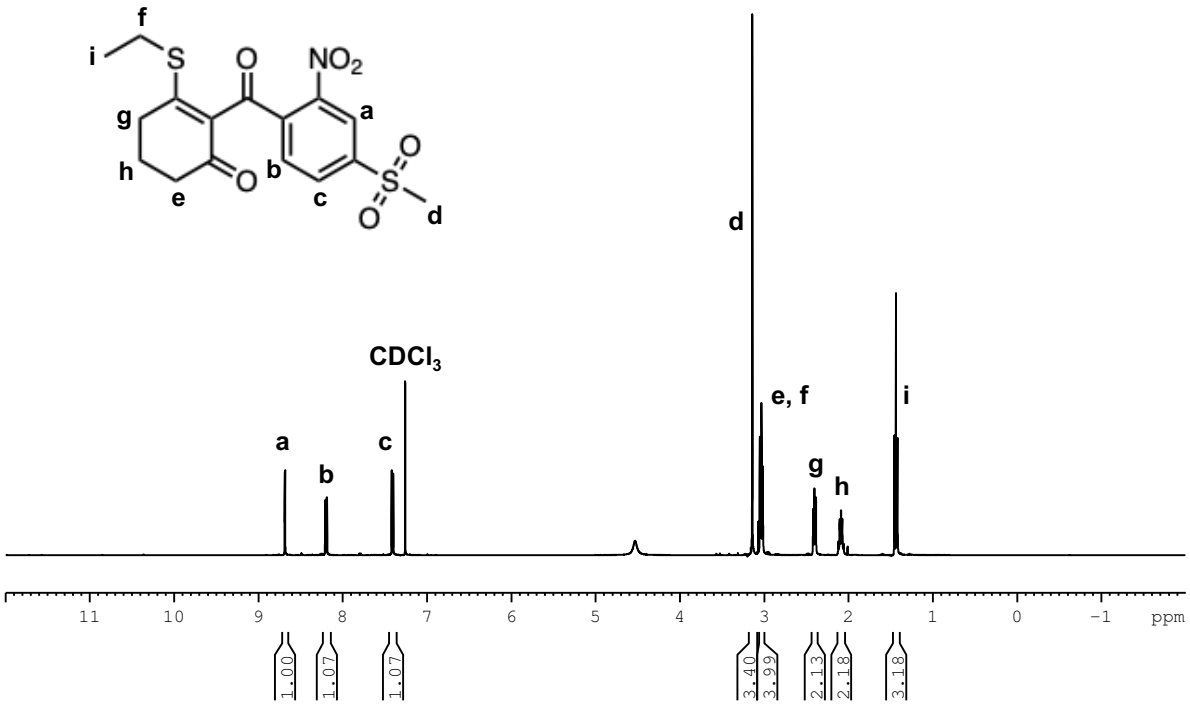


Figure S3. ^{1}H-NMR of ethyl thioether derivative (**2**) in $CDCl_3$.

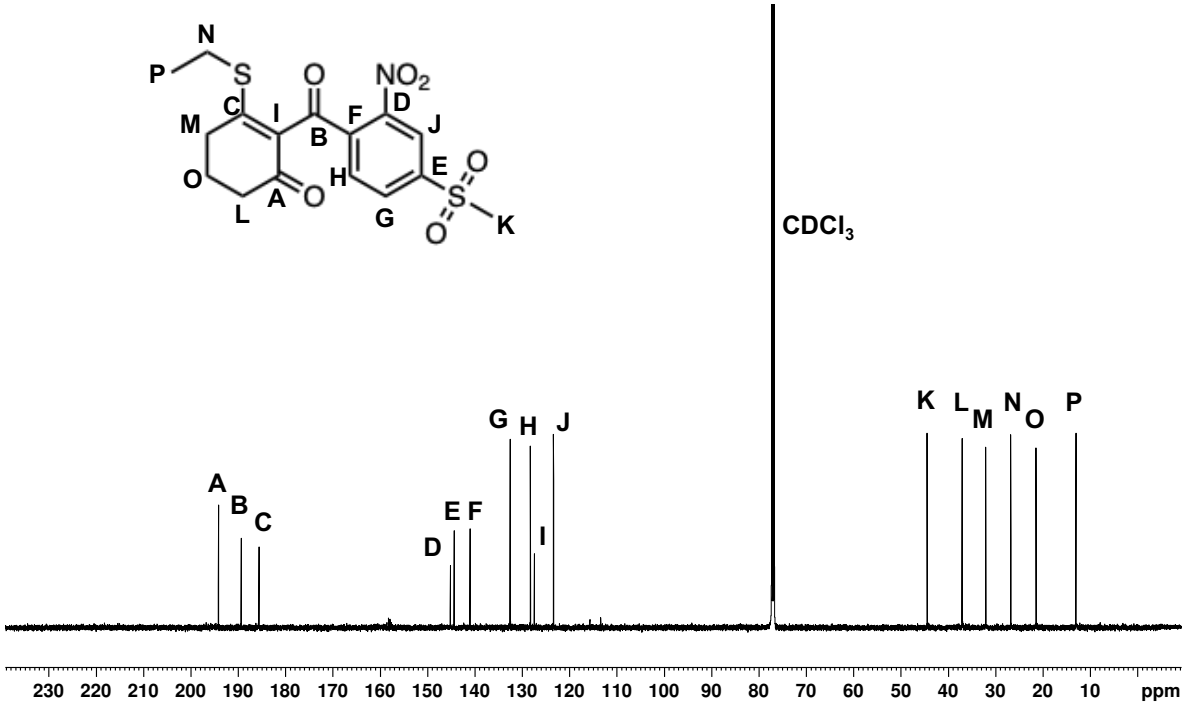
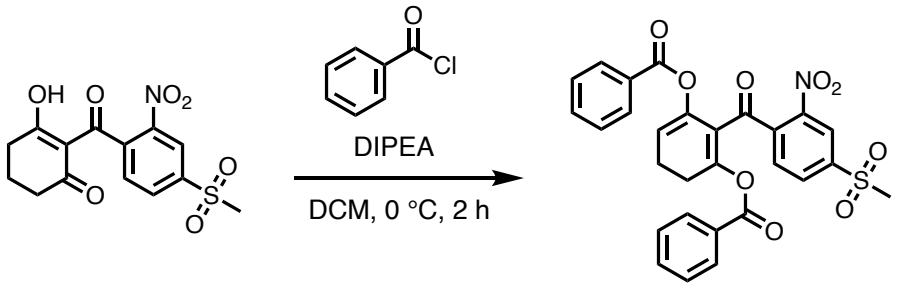


Figure S4. ^{13}C-NMR of ethyl thioether derivative (**2**) in $CDCl_3$.

Scheme S1. Synthesis of disubstituted phenyl ester mesotrione derivative, **4**.



4
12.8 % yield

Synthesis of disubstituted phenyl ester mesotrione derivative, 4. Mesotrione (50.0 mg, 0.15 mmol, 1 equiv.) was dissolved in dry DCM (2 mL). DIPEA (77.0 μL, 0.44 mmol, 3 equiv.) was added dropwise and the mixture was stirred for 60 minutes, after which the reaction was then cooled to 0 °C. Finally, a solution of benzoyl chloride (51.4 μL, 0.44 mmol, 3 equiv.) in dry DCM (1 mL) was added dropwise to the cooled reaction. The reaction was stirred for 2 hours at 0 °C. The reaction was then extracted twice with DCM and then washed with brine. The organic layers were collected and dried over $MgSO_4$. The mixture of isomers was evaluated by LC/MS, and one isomer was isolated by preparatory HPLC (10-95 % acetonitrile in water with 1 % TFA) (10.3 mg, 12.8 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.66-8.60 (d, J = 1.6 Hz, 1H), 8.16-8.09 (dd, J = 8.1, 1.7 Hz, 1H), 7.87-7.79 (d, J = 8.1 Hz, 1H), 7.79 – 7.73 (m, 2H), 7.57-7.50 (d, J = 7.3 Hz, 2H), 7.36-7.27 (m, 2H), 7.10-7.00 (m, 6H), 6.09-6.02 (t, J = 4.8 Hz, 1 H), 3.17-3.06 (s, 3H), 2.77-2.70 (m, 3H), 2.70-2.63 (m, 3H) ppm. ^{13}C NMR (500 MHz, $CDCl_3$) δ: 197.8, 164.5, 163.1, 147.5, 145.2, 143.6, 142.0, 136.6, 133.9, 133.8, 133.3, 131.4, 130.1, 130.0, 128.4, 128.2, 128.1, 127.4, 124.0, 123.5, 123.3, 44.4, 38.2, 21.1 ppm. (*m/z*) $[M + NH_4]^+$ calculated = 565.13; found = 565.12. FTIR: ν 2996, 2670, 1783, 1740, 1678, 1599, 1537, 1451, 1351, 1316, 1246, 1218, 1060, 1021, 778, 703 cm^{-1}.

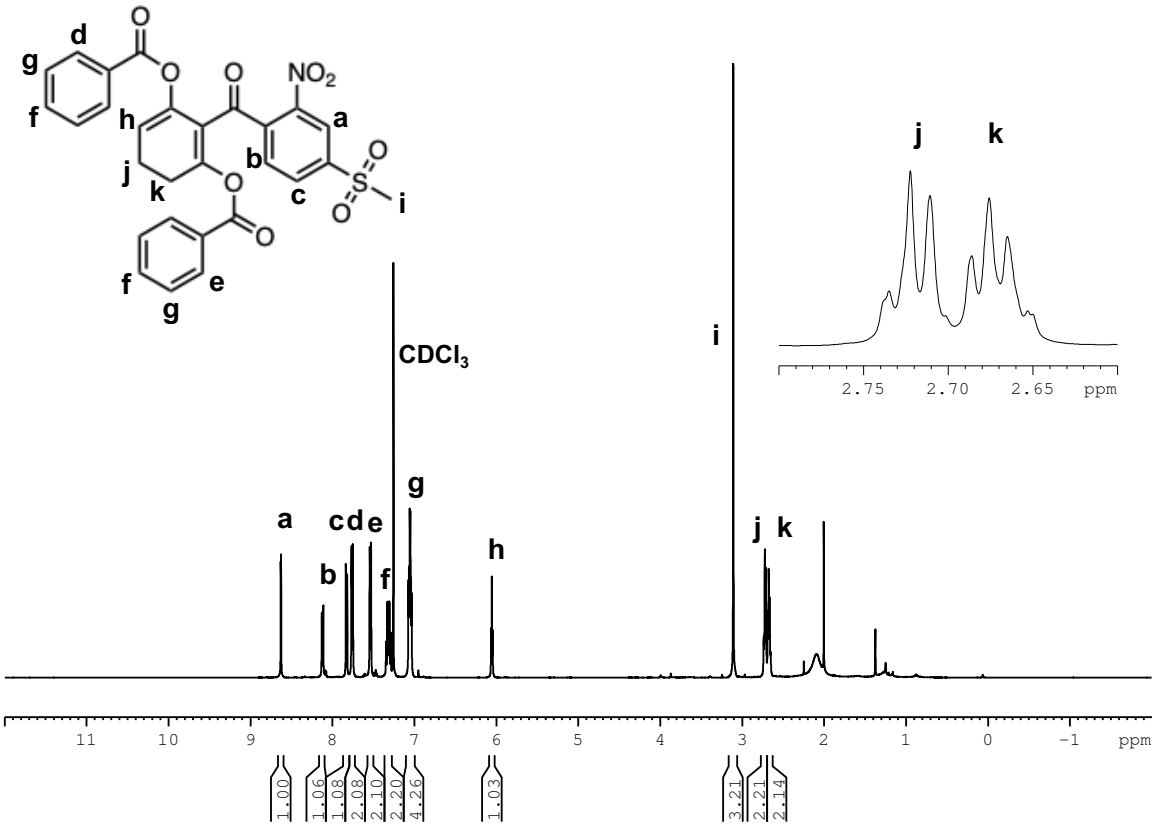


Figure S5. ^{1}H-NMR of disubstituted phenyl ester derivative (**4**) in $CDCl_3$.

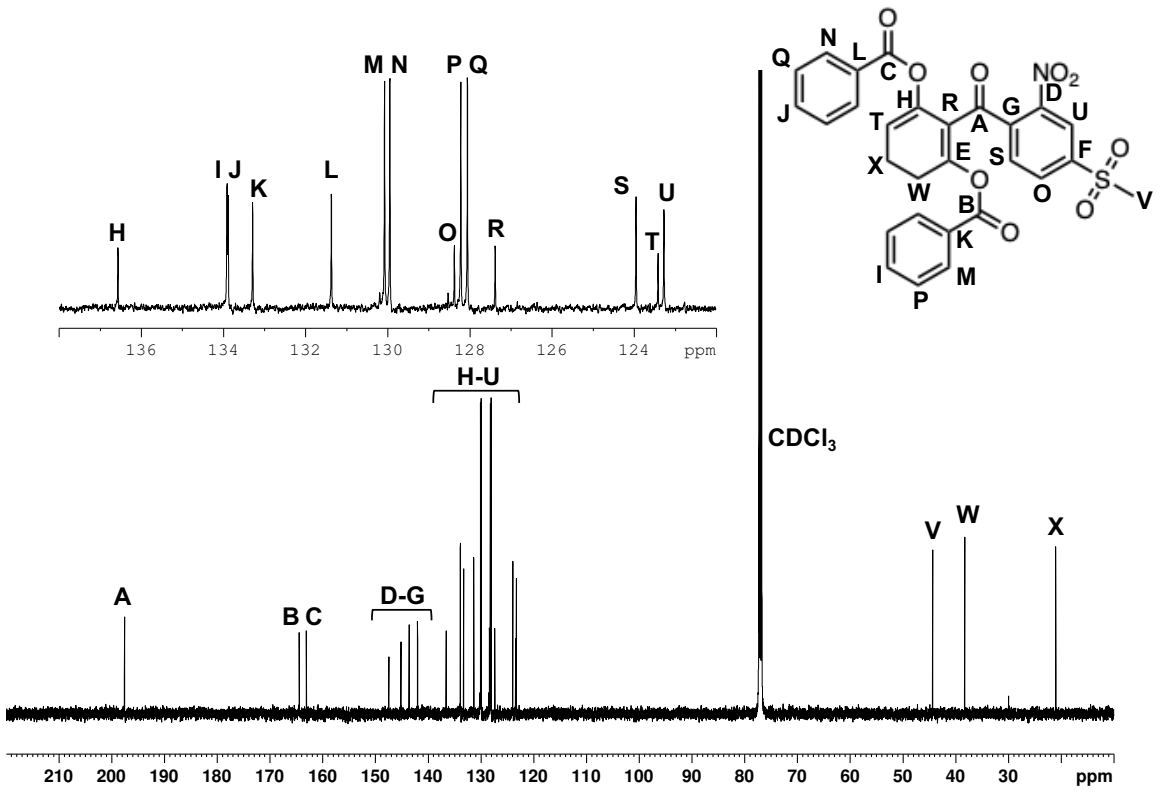


Figure S6. ^{13}C-NMR of disubstituted phenyl ester derivative (**4**) in $CDCl_3$.

Synthesis of mesotrione-TEA salt. Mesotrione was dissolved in DCM. Triethylamine (TEA) was then added, and the solution was stirred for 120 minutes at 22 °C. Following this, the solvent and excess TEA were removed *in vacuo* to produce an orange powder. The powder was then recrystallized using 25 mL of a 15% DCM in diethyl ether solution. Bright yellow crystals were formed when the super-saturated solution was let stand at 22 °C overnight (94.9% yield). ^{1}H NMR (500 MHz, CD_2Cl_2) δ: 8.61 (d, J = 1.7 Hz, 1H), 8.0 (dd, J = 8.0, 1.8 Hz, 1H), 7.3 (d, J = 8.0 Hz, 1H), 3.2 (q, J = 7.3 Hz, 6H), 2.3 (t, J = 6.4 Hz, 4H), 1.9 (m, 2H), 1.3 (t, J = 7.3 Hz, 10H) ppm. ^{13}C NMR (500 MHz, CD_3CN) δ: 197.2, 188.3, 148.4, 145.1, 138.6, 132.1, 127.8, 123.6, 114.0, 53.5,

45.8, 44.6, 38.7, 20.1, 8.5 ppm. IR: ν = 3001, 2915, 2843, 2746, 1640, 1579, 1519, 1459, 1350, 1310, 1158, 1143, 1066, 995, 973, 927, 807, 779, 737 cm^{-1}.

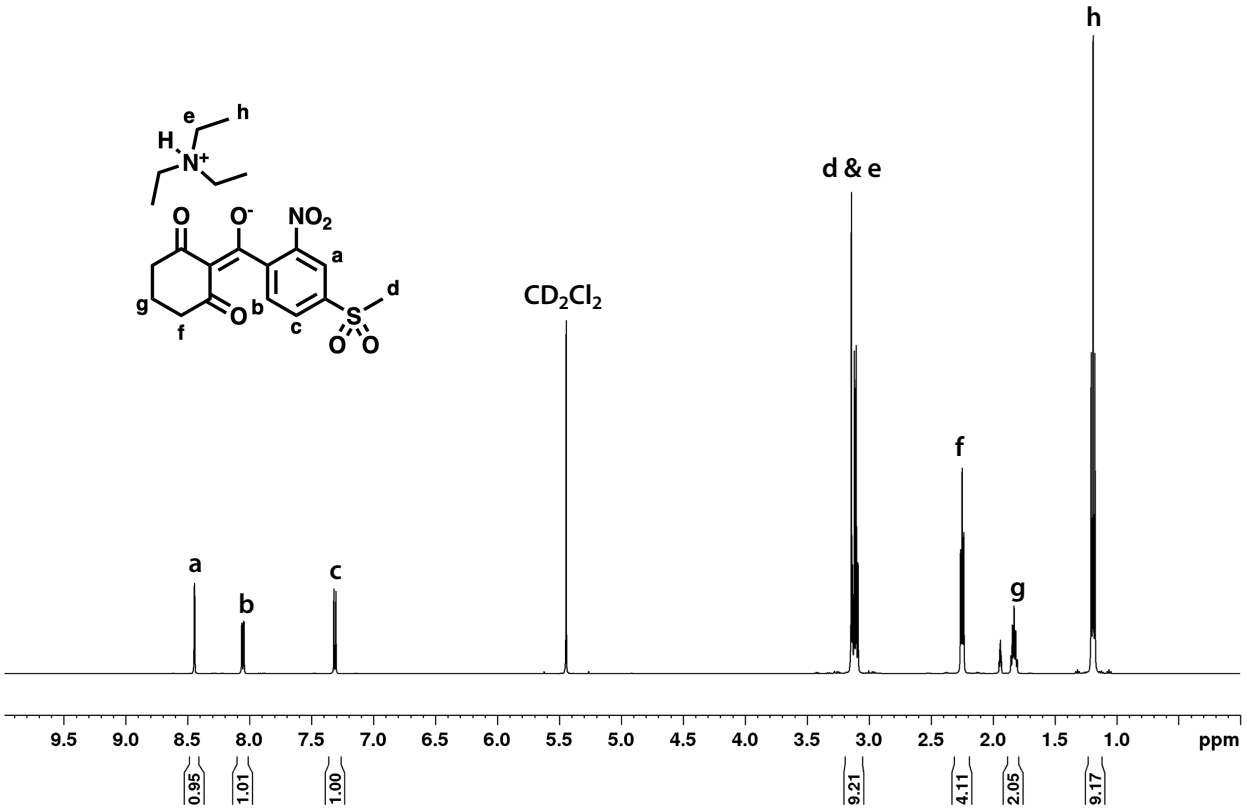


Figure S7. ^{1}H-NMR of mesotrione-TEA salt in deuterated dichloromethane (CD_2Cl_2).

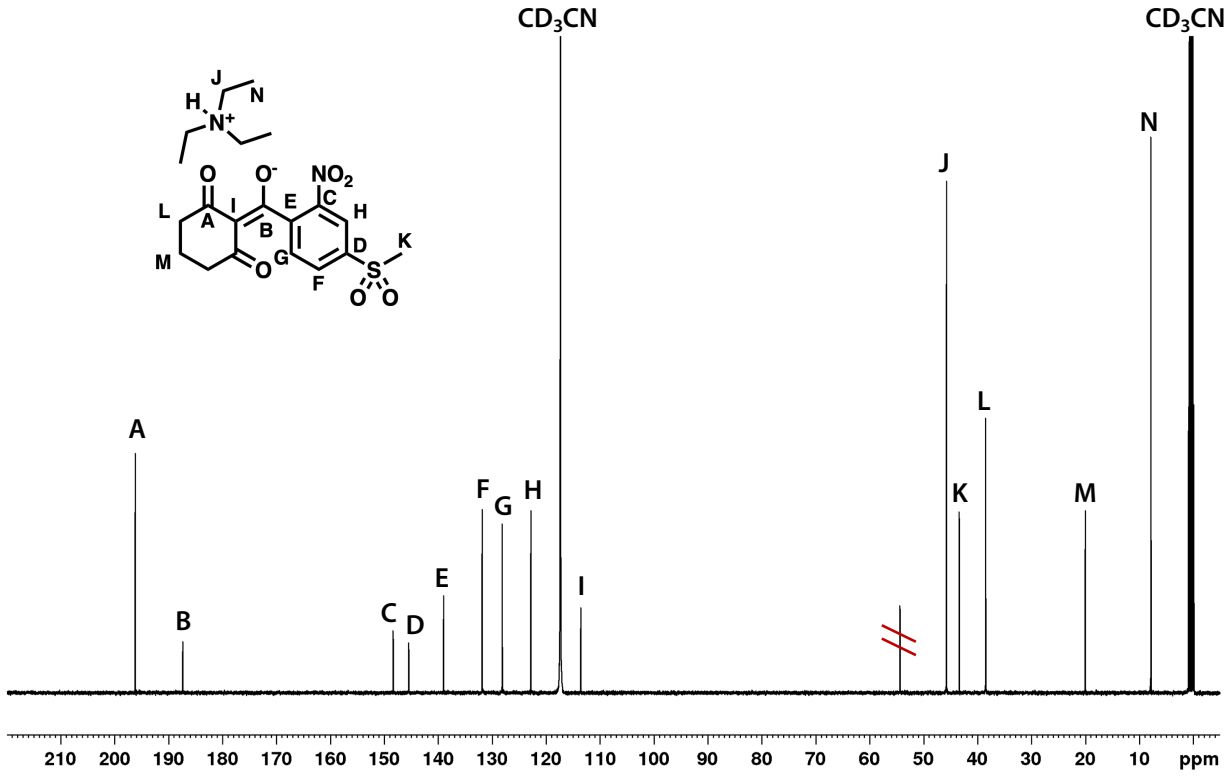


Figure S8. ^{13}C-NMR of mesotrione-TEA salt in deuterated acetonitrile (CD_3CN).

Synthesis of phenylester mesotrione derivative, 2-(4-(methylsulfonyl)-2-nitrobenzoyl)-3-oxocyclohex-1-en-1-yl benzoate **(3).** Mesotrione-TEA salt (1.00 g, 2.3 mmol, 1 equiv.) was placed in a dry dram vial under an argon atmosphere and was dissolved in dry DCM (3 mL) and cooled to 0 °C. Benzoyl chloride (336 mg, 278 µL, 2.4 mmol, 1.05 equiv.) was then added and the solution was stirred at 0 °C for 60 minutes and then room temperature for an additional 2 hours. The reaction was monitored via thin layer chromatography (TLC) and the reaction was stopped after 120 minutes. The crude produce was purified by silica column chromatography with a 10-80% ethyl acetate gradient against DCM. The product cleanly eluted around 30% ethyl acetate and the corresponding fractions were pooled, concentrated under vacuum, and subsequently characterized

using ^{1}H and ^{13}C NMR. (648 mg, 64.2%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.5 (d, J = 1.6 Hz, 1H), 8.1 (dd, J = 8.0, 1.7 Hz, 1H), 8.0 (dd, J = 8.4, 1.3 Hz, 2H), 7.7 (d, J = 8.0 Hz, 1H), 7.6 (m, 1H), 7.5 (t, J = 7.9 Hz, 2H), 3.1 (s, 3H), 2.9 (t, J = 6.2 Hz, 2H), 2.5 (t, J = 6.7 Hz, 2H), 2.2 (m, 2H) ppm. ^{13}C NMR (500 MHz, $CDCl_3$) δ: 195.6, 187.7, 175.3, 162.7, 146.2, 142.5, 141.8, 134.6, 132.2, 130.5, 129.8, 128.9, 127.7, 125.7, 123.4, 44.3, 37.3, 30.7, 19.8 ppm. IR: ν = 3085, 2927, 1743, 1673, 1532, 1351, 1315, 1241, 1152, 1143, 1061, 1018, 927, 705 cm^{-1}. (*m/z*) $[M + Na]^+$ calculated = 466.059; found = 466.0673.

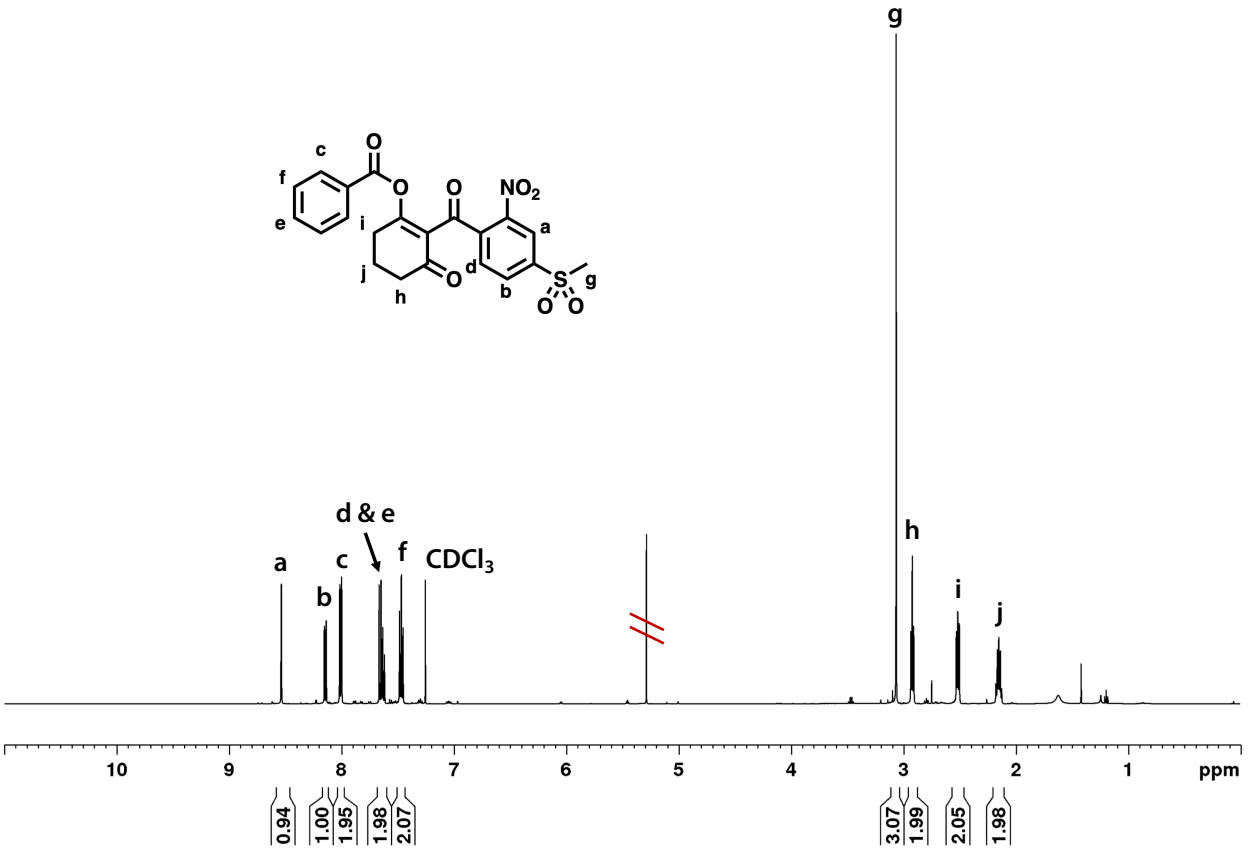


Figure S9. ^{1}H-NMR of phenyl ester derivative (**3**) in $CDCl_3$.

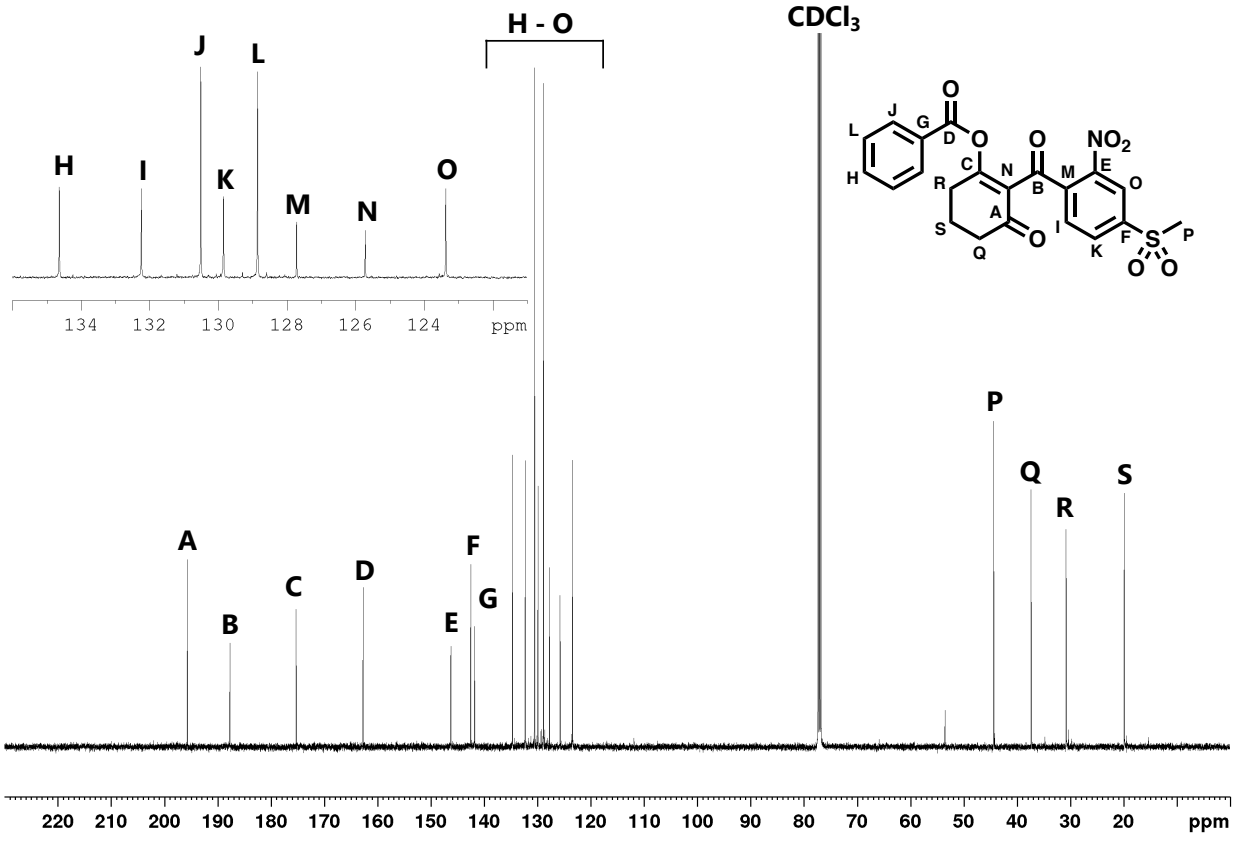


Figure S10. ^{13}C-NMR of phenyl ester derivative (**3**) in $CDCl_3$.

Preparation of Buffer Solutions. Three buffers were prepared at pH 4, 7, or 9 at 50 mM buffer strength. The pH 4 and 7 buffers were prepared by modifying guidelines from the Organization for Economic Co-operation and Development (OECD).[38] The pH 4 buffer was prepared with potassium biphthalate and NaOH; pH 7 buffer was prepared with monopotassium phosphate and NaOH; pH 9 buffer was prepared with sodium carbonate/bicarbonate. All buffers were titrated to within ± 0.05 of the intended pH using 1 N hydrochloric acid (HCl) or 1 N NaOH aqueous solutions.

Aqueous Release Studies. Solutions of derivatives were prepared at a concentration of 0.2 mg/mL (with respect to mesotrione) in a 1:1 mixture of acetonitrile and the pH 4, 7, and 9 buffers. All samples were filtered (0.2 μm) before being analyzed by HPLC at pre-determined time points where mesotrione eluted at 9.2 - 9.3 minutes. Studies were all conducted at 22 °C. Experiments were run in triplicate. To monitor the degradation of the phenyl thioether derivative at 9, the same method was used, but degradation products were monitored using quadrupole time-of-flight liquid chromatography mass spectrometry (QTOF LC/MS). The release studies of the disubstituted phenyl ester derivative were conducted in a 1:1 mixture of pH 5.0 Dulbecco's phosphate-buffered saline (DPBS) and acetonitrile. The release studies of the enamine derivative were done in a 1:1 mixture of pH 5.5 or 6.8 DPBS buffer and acetonitrile.

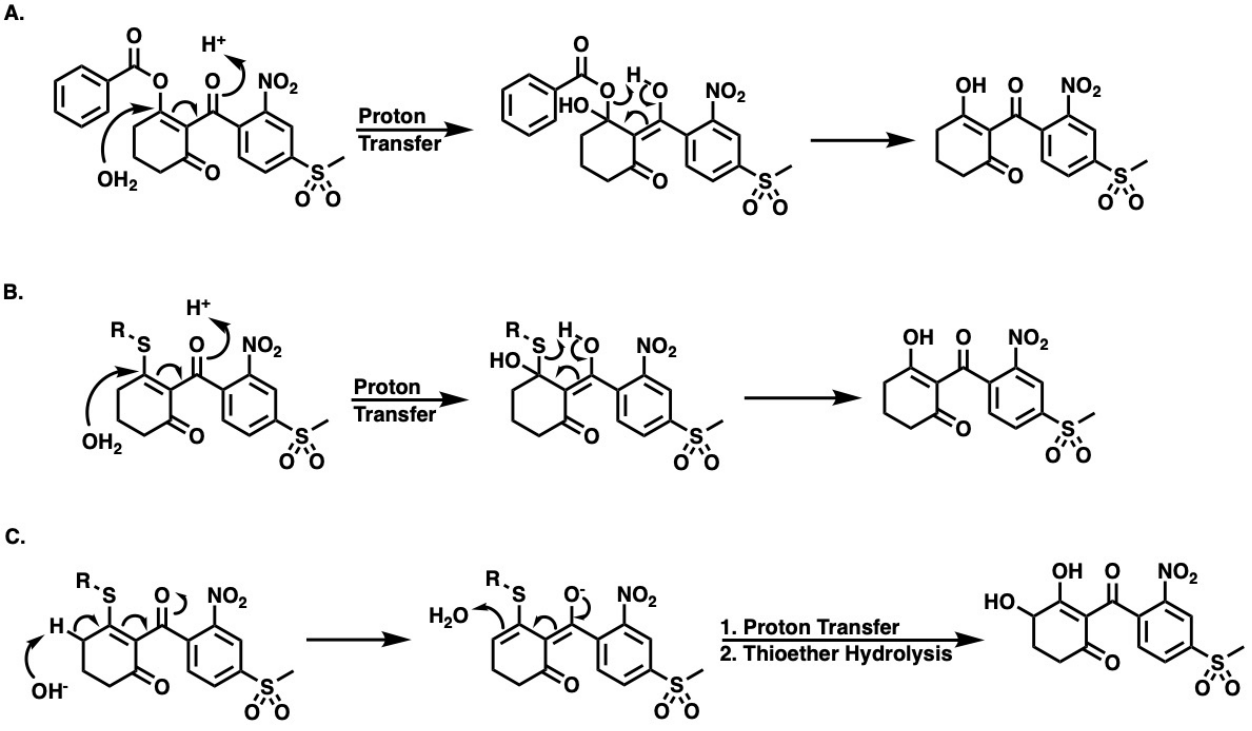


Figure S11. Proposed mechanism of (A) the hydrolysis of the mesotrione phenyl ester derivative (B) the hydrolysis of the mesotrione thioether derivatives (C) the side product formation during hydrolysis of thioether mesotrione derivatives in basic or neutral conditions.

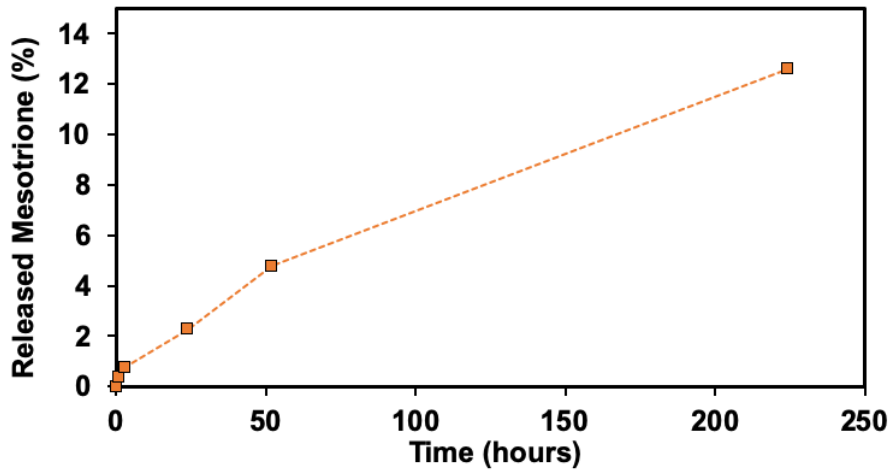


Figure S12. Release of mesotrione from disubstituted phenyl ester derivative in pH 5.0 DPBS solution. Released mesotrione (%) was determined using HPLC and by comparing the amount of free mesotrione at each time point to the total amount of mesotrione in the proherbicide. Note this experiment was only performed once.

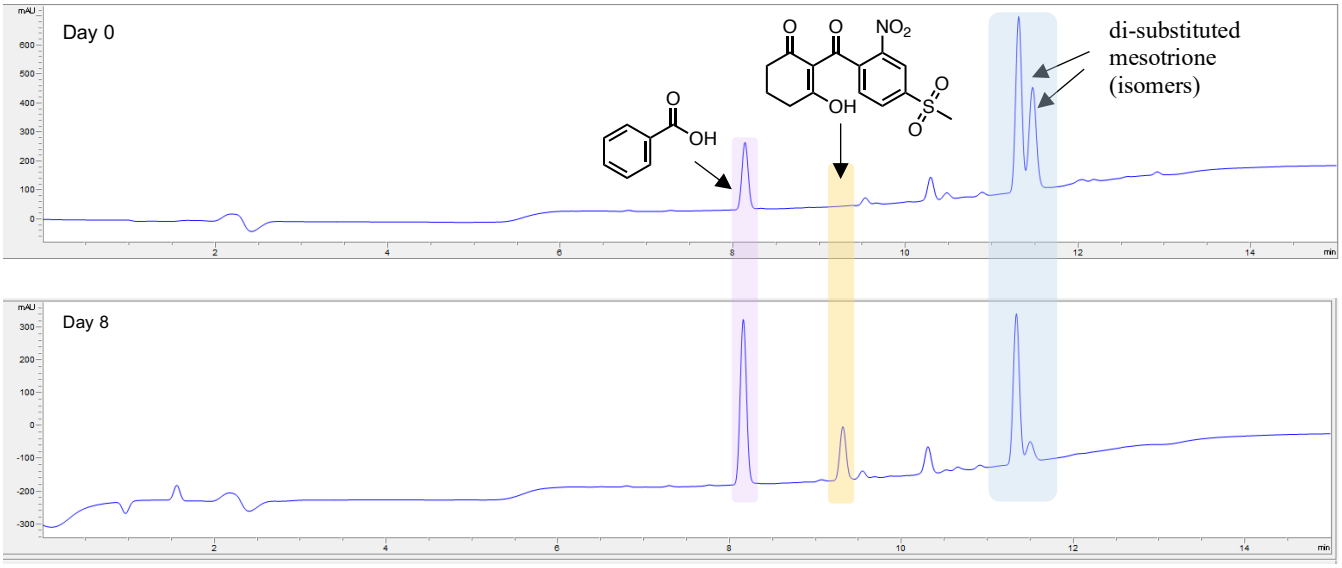


Figure S13. HPLC traces of disubstituted phenyl ester derivative (**4**) in pH 5.0 DPBS solution after 0 and 8 days. Mesotrione elutes at approximately 9.4 minutes, demonstrating no release on Day 0 and some release on Day 8. The disubstituted derivatives (elution at 11.3 and 11.5 minutes) and benzoic acid byproduct (elution at 8.1 minutes) are observed as well.

Table S1. Thermal stabilities of mesotrione proherbicides determined by TGA analysis.

Sample	$T_{onset5\%}$ (˚C)
Mesotrione	185.1
Phenyl Ester	160.4
Ethyl Thioether	196.3
Phenyl Thioether	140.8

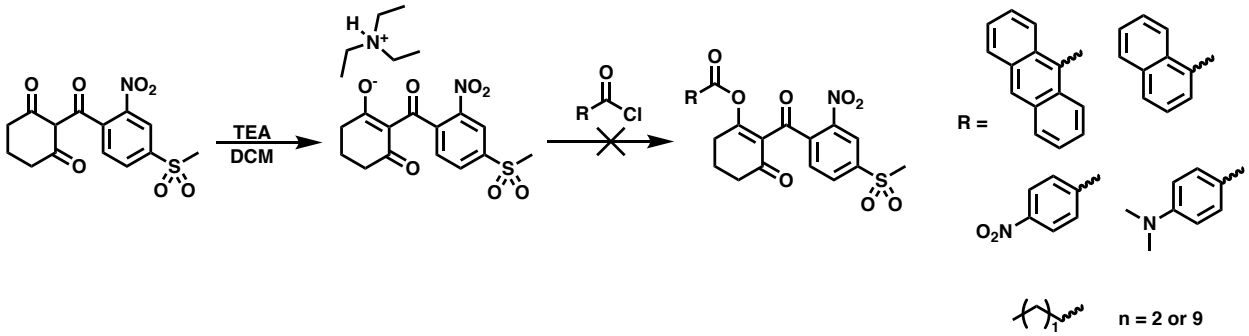


Figure S14. Alternative mesotrione ester proherbicides that were synthetically attempted, but were unsuccessful due to the reactivity and structure of mesotrione.

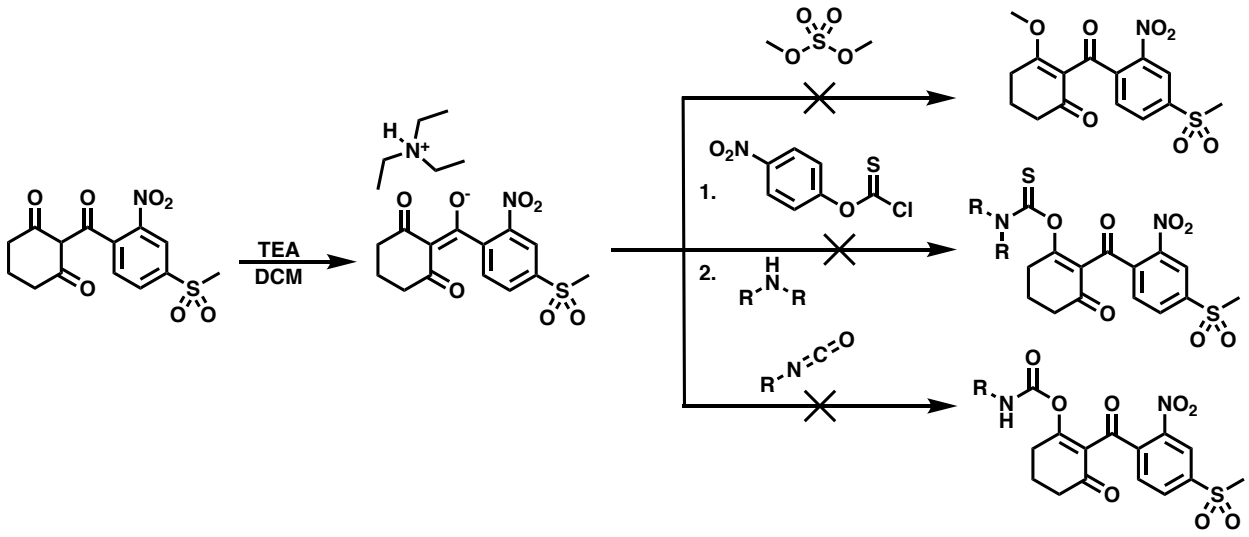


Figure S15. Additional attempted proherbicide synthetic routes that were unsuccessful due to the reactivity and structure of mesotrione.

Soil Leaching Studies. A plastic cartridge with a 20 µm frit on the bottom was packed with 4.0 grams of sand followed by 12.0 grams of soil (1:1 mixture of Soil A and Soil B). After equilibrating the column with DI water for 20 minutes, a 200 µL solution of mesotrione or analogue was added to top of the soil layer at a concentration of 15 mg/mL of mesotrione. DI water flowed through the system at a rate of 0.5 mL/min and the leachate was collected in separate fractions at pre-determined time points, then filtered through a 10 kDa MWCO membrane and analyzed by HPLC. Studies were all conducted at 22 °C. The amount of residual mesotrione (%) was calculated indirectly by subtracting the amount of mesotrione eluted from the soil column (as determined by HPLC) by the initial amount of mesotrione added to the soil. Experiments were run in triplicate.

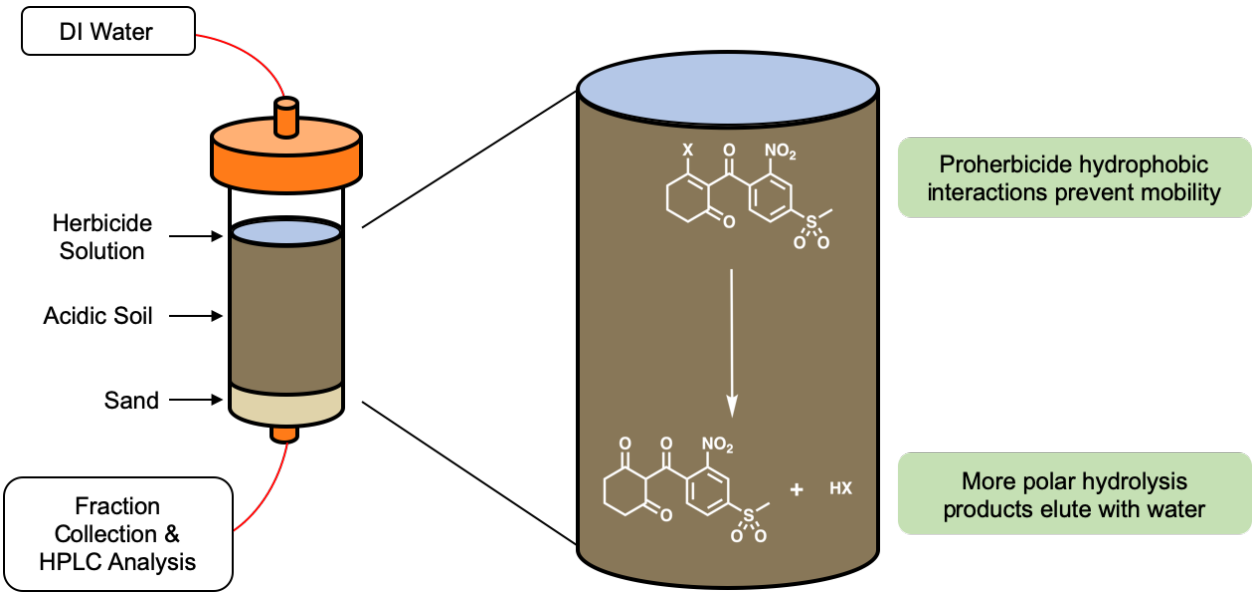


Figure S16. Proposed interactions of the proherbicides and their hydrolysis products in soil column.

Weed Efficacy Experiments. To a plastic pot with drainage holes, 100 grams of soil (1:4 mixture of Soil A and Soil B) was added. Solutions of mesotrione or the phenyl ester were applied at a concentration of 150 g AI ha^{-1} or 50 g AI ha^{-1} (with respect to free mesotrione active ingredient)

and 235 L ha^{-1} to the top of the soil. A control was evaluated by not adding any herbicide. Seeds (*Chenopodium album,* 300 mg, ~400 seeds) were then distributed in each pot. Finally, tap water was added at a rate of 10 mL per day for each condition. The control efficacy was observed visually by blinding the samples and having an outside evaluator assess them 21 days after treatment. The number of leaves per sample were counted, and then each leaf was evaluated on a scale where no visible decrease in green pigment (compared to the control) was described as 0 % control efficacy and complete bleaching 100 %. The individual leaves' control efficacies were then averaged across each sample. Experiments were run in triplicate.

Table S2. Representative data demonstrating the pre-emergent efficacies of various herbicides on lambsquaters (*Chenopodium album*).

Herbicide Treatment	Dose (g ha^{-1})	Days after Treatment	Efficacy	References
Metribuzin	85	21	50 %	(1)
Trifluralin	538	21	50 %	(1)
Isoxaflutole	140	30	83-100 %	(2)
Flumetsulam	140	30	53-100 %	(2)
Metolachlor	1,400	30	50-75 %	(2)

Statistical analysis. One-tailed Student *t*-test assuming unequal variance was implemented to compare control weed growth to conditions with herbicide. ** = $p < 0.01$ and * = $p < 0.05$ relative to the control with no herbicide.

References

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(2) Chomas, A. J.; Kells, J. J. *Weed Technology* **2004**, *18*, 551-554. DOI: 10.1614/WT-03-077R.