SUPPORTING INFORMATION

Esterase-sensitive Sulfur Dioxide Prodrugs Inspired by Modified Julia Olefination

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Materials and Methods

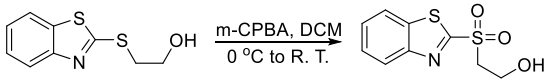
All chemicals, reagents, and solvents were purchased from commercial suppliers as reagent grade and were used without further purification. NMR spectra were recorded on a Bruker Avance NMR spectrometer at 400 MHz for ^{1}H spectra and 101 MHz for ^{13}C spectra at room temperature. Solvent peaks were used as internal standards. Mass spectral analyses were performed by the GSU Mass Spectrometry Facilities. HPLC analyses were performed on a Shimadzu HPLC equipped with UV detector. Column: Shimadzu C18 3μm 4.6×50 mm column.

Synthesis

General procedure of the oxidation of sulfide to sulfone:

Sulfone compounds were synthesized following similar procedure with necessary adjustments using 3-chloroperoxybenzoic acid (m-CPBA) as the oxidant.

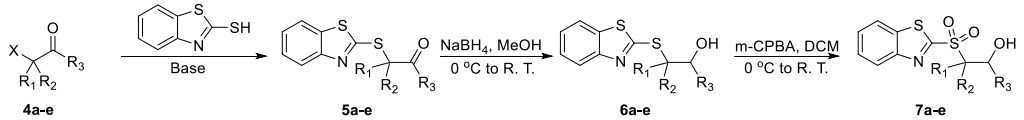
Generally, 1 equiv. of sulfide was dissolved in dichloromethane (DCM) at a concentration of ~0.5 mmol/10 mL. The resulting solution was treated with 2.5~3 equiv. of m-CPBA in small portions under stirring at room temperature (ice bath can be applied for large scale reaction). Reaction process was monitored by thin layer chromatography (TLC). Usual reaction time is between 1~4 hours. When reaction finished, the reaction was quenched with an equal volume of sat. $NaHCO_3$ aqueous solution. For small scale reactions, dilution with DCM was used to facilitate separation and washing. The aqueous phase was separated with separatory funnel and back-extracted with fresh DCM twice. Combined organic phase was washed with brine and dried over Na_2SO_4. Solvent was then removed under reduced pressure. Column chromatography was used for purification. Further washing with sat. $NaHCO_3$ solution was used to remove residual m-CPBA/m-CBA.



2-(2-Benzothiazolylthio)ethanol **1**

2-(Benzo[*d*]thiazol-2-ylsulfonyl)ethan-1-ol (**1**)

Synthesis followed the general procedure of oxidation of sulfide to sulfone. Product was obtained as white solid. Yield: 77%. ^{1}H NMR ($CDCl_3$) δ 8.21 (d, J = 7.9 Hz, 1H), 8.04 (d, J = 7.9 Hz, 1H), 7.72 – 7.56 (m, 2H), 4.21 (s, 2H), 3.81 – 3.73 (m, 2H), 2.97 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$) δ 166.2, 152.4, 136.6, 128.3, 127.9, 125.42, 122.4, 57.7, 56.5 ppm. IR: ν_{max}/cm^{-1} 3388 (O-H), 2929 (C-H), 1322 (S=O), 1301 (C-O), 1128 (S=O). HRMS (ESI): calcd. for $C_9H_{10}NO_3S_2^+$ $[M+H]^+$ 244.0097, found 244.0089.



4a-e **5a-e** **6a-e** **7a-e**

4a: X=Br, R_1=R_2=H, R_3=Ph
4b: X=Br, R_1=H, R_2=Me, R_3=Ph
4c: X=Br, R_1=R_2=Me, R_3=Ph
4d: X=Br, R_1=R_2=H, R_3=*i*-Pr
4e: X=Cl, R_1=H R_2, R_3=

5a: R_1=R_2=H, R_3=Ph
5b: R_1=H, R_2=Me, R_3=Ph
5c: R_1=R_2=Me, R_3=Ph
5d: R_1=R_2=H, R_3=*i*-Pr
5e: R_1=H R_2, R_3=

6-7a: R_1=R_2=H, R_3=Ph
6-7b: R_1=H, R_2=Me, R_3=Ph
6-7c: R_1=R_2=Me, R_3=Ph
6-7d: R_1=R_2=H, R_3=*i*-Pr
6-7e: R_1=H R_2, R_3=, *cis*-

2-(Benzo[d]thiazol-2-ylthio)-1-phenylethan-1-one (5a)

2-Mercaptobenzothiazole (1.0 g, 6.0 mmol) and 2-bromoacetophenone (1.2 g, 6.0 mmol) were dissolved in 50 mL acetone and treated with K_2CO_3 (0.83 g, 6.0 mmol). The reaction mixture was heated at reflux temperature for 0.5 h and then cooled down to room temperature. Solvent was evaporated under reduced pressure. The residue was re-dissolved in ethyl acetate (EA, ~50 mL) and

washed with brine (~50 mL). Organic phase was then dried over Na_2SO_4, and concentrated *in vacuo*. Residue was recrystallized in ethanol to yield 1.4 g of product as yellow needle crystals. Yield: 82%. ^{1}H NMR ($CDCl_3$) δ 8.13 – 8.05 (m, 2H), 7.81 (dd, *J* = 8.1, 0.4 Hz, 1H), 7.75 (dd, *J* = 8.0, 0.6 Hz, 1H), 7.68 – 7.58 (m, 1H), 7.56 – 7.47 (m, 2H), 7.43 – 7.37 (m, 1H), 7.33 – 7.27 (m, 1H), 4.98 (s, 2H) ppm. ^{13}C NMR ($CDCl_3$) δ 193.0, 165.2, 152.9, 135.6, 133.9, 128.8, 128.6, 126.1, 124.4, 121.5, 121.1, 41.0 ppm. HRMS (ESI): calcd. for $C_{15}H_{11}NOS_2Na^+$ $[M+Na]^+$ 308.0174, found 308.0189.

2-(Benzo[d]thiazol-2-ylthio)-1-phenylpropan-1-one (5b)

To a solution of 2-mercaptobenzothiazole (334 mg, 2 mmol) in 4 mL DCM was added triethylamine (TEA, 0.39 mL, 2.8 mmol). The 2-bromopropiophenone (0.30 mL, 2 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 1 h and then dried under vacuum. The residue was re-dissolved in 10 mL EA and washed with water (10 mL × 2). Water phase was back extracted with 15 mL EA. Combined organic phase was dried over Na_2SO_4, concentrated in vacuo, and purified by column chromatography (EA/Hex 1:20 to 1:10). Product (470 mg) was obtained as yellow oil. Yield: 78%. ^{1}H NMR ($CDCl_3$) δ 8.11 (dt, *J* = 8.5, 1.7 Hz, 2H), 7.83 (dd, *J* = 8.1, 0.4 Hz, 1H), 7.75 (dd, *J* = 8.0, 0.6 Hz, 1H), 7.64 – 7.54 (m, 1H), 7.53 – 7.45 (m, 2H), 7.42 (ddd, *J* = 8.3, 7.4, 1.2 Hz, 1H), 7.35 – 7.27 (m, 1H), 5.88 (q, *J* = 7.1 Hz, 1H), 1.77 (d, *J* = 7.1 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$) δ 197.3, 164.7, 152.9, 135.7, 135.0, 133.6, 128.9, 128.8, 126.1, 124.5, 121.6, 121.1, 47.1, 18.3 ppm. HRMS (ESI): calcd. for $C_{16}H_{13}NOS_2Na^+$ $[M+Na]^+$ 322.0331, found 322.0352.

2-(Benzo[d]thiazol-2-ylthio)-2-methyl-1-phenylpropan-1-one (5c)

A solution of 2-mercaptobenzothiazole (167 mg, 1 mmol) in 2 mL DCM was added with 0.20 mL TEA (1.4 mmol). To the solution was then added 2-bromo-2-methylpropiophenone (0.17 mL, 1 mmol) dropwise. The reaction mixture was stirred at room temperature for 0.5 h and then heated to 60 °C for 4 h. After cooling to room temperature, solvent was removed by reduced pressure. Residue was re-dissolved in 10 mL EA and washed with water (10 mL × 2). Aqueous phase was back extracted with 15 mL EA. Combined organic phase was dried over Na_2SO_4, concentrated *in vacuo*, and purified by column chromatography (EA/Hex 1:20 to 1:10). 175 mg product was obtained as yellow oil. Yield: 56%. ^{1}H NMR ($CDCl_3$) δ 8.13 – 8.05 (m, 2H), 7.89 (d, *J* = 8.1 Hz, 1H), 7.71 (d, *J* = 8.0 Hz, 1H), 7.50 – 7.43 (m, 1H), 7.43 – 7.35 (m, 3H), 7.33 – 7.27 (m, 1H), 1.86 (s, 6H) ppm. ^{13}C NMR ($CDCl_3$) δ 200.6, 161.4, 153.1, 136.7, 136.3, 131.8, 129.1, 127.9, 126.1, 125.0, 122.5, 121.0, 58.1, 27.4 ppm. HRMS (ESI): calcd. for $C_{17}H_{16}NOS_2^+$ $[M+H]^+$ 314.0668, found 314.0655.

1-(Benzo[d]thiazol-2-ylthio)-3-methylbutan-2-one (5d)

1-Bromo-3-methyl-2-butanone (0.12 mL, 1 mmol) and 2-mercaptobenzothiazole (167 mg, 1 mmol) were dissolved in 5 mL acetone. To the solution was added K_2CO_3 (138 mg, 1 mmol). The reaction mixture was stirred at room temperature for 1 h then dried under reduced pressure. The residue was re-dissolved in 10 mL EA and washed with water (10 mL × 2). The aqueous phase was back extracted with EA (15mL × 2). Combined organic phase was dried over Na_2SO_4 and concentrated over vacuum. The crude product was purified by column chromatography (EA/Hex 1:20 to 1:10). 204 mg product was obtained as white solid. Yield: 81%. ^{1}H NMR ($CDCl_3$) δ 7.81 (d, J = 8.1 Hz, 1H), 7.74 (d, J = 8.0 Hz, 1H), 7.44 – 7.36 (m, 1H), 7.32 – 7.27 (m, 1H), 4.35 (s, 2H), 2.97 (dt, J = 13.9, 6.9 Hz, 1H), 1.22 (d, J = 6.9 Hz, 6H) ppm. ^{13}C NMR ($CDCl_3$) δ 207.3, 165.3, 152.9, 135.6, 126.1, 124.4, 121.4, 121.1, 41.4, 40.1, 18.4 ppm. HRMS (ESI): calcd. for $C_{12}H_{14}NOS_2^+$ $[M+H]^+$ 252.0511, found 252.0505.

2-(Benzo[d]thiazol-2-ylthio)cyclohexan-1-one (5e)

To a solution of 2-mercaptobenzothiazole (500 mg, 3.0 mmol) and trimethylamine (0.58 mL, 4.2

mmol) in 6 mL DCM was added 2-chlorocyclohexan-1-one (0.36 mL, 3.1 mmol) slowly. The solution was heated at reflux for 4.5 h, then cooled to room temperature, and stirred overnight. The reaction mixture was diluted with DCM to 15 mL and washed with water (10 mL × 2). The combined aqueous phase was back extracted with DCM (15 mL × 2). Combined organic phase was dried over Na_2SO_4, concentrated in vacuo, and purified by column chromatography (EA/Hex 1:10 to 1:5) to yield the product (454 mg) as yellow solid. Yield: 57%. ^{1}H NMR ($CDCl_3$) δ 7.89 (d, *J* = 8.2 Hz, 1H), 7.77 (dd, *J* = 8.0, 0.6 Hz, 1H), 7.50 – 7.39 (m, 1H), 7.38 – 7.30 (m, 1H), 4.93 (dd, *J* = 10.2, 5.7 Hz, 1H), 2.82 – 2.65 (m, 2H), 2.65 – 2.48 (m, 1H), 2.25 – 2.09 (m, 1H), 2.05 – 1.74 (m, 4H) ppm. ^{13}C NMR ($CDCl_3$) δ 205.5, 164.8, 153.1, 135.5, 126.0, 124.4, 121.6, 121.0, 57.4, 41.6, 35.5, 27.6, 25.1 ppm. HRMS (ESI): calcd. for $C_{13}H_{13}NOS_2Na^+$ $[M+Na]^+$ 286.0331, found 286.0339.

General procedure of reducing β-ketosulfides to β-alkoxysulfides:

Compounds **6a-e** were prepared by reducing compounds **5a-e** with $NaBH_4$. In general, 1 equiv. of compounds **5a-e** was dissolved in MeOH (10~15 mL/mmol) and cooled to 0 °C in an ice bath. $NaBH_4$ (4 equiv.) was added to the solution in small portions. The reaction was then allowed to warm up to room temperature. The reaction process was monitored by TLC. After completion, the reaction was quenched with sat. NH_4Cl aqueous solution. The mixture was then extracted with EA and washed with water and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Crude product was purified by column chromatography.

2-(Benzo[d]thiazol-2-ylthio)-1-phenylethan-1-ol (6a)

Synthesis followed the general procedure of reducing β-ketosulfides to β-alkoxysulfides from **5a**. Product was obtained as yellow solid. Yield: 99%. ^{1}H NMR ($CDCl_3$) δ 7.92 (d, *J* = 8.1 Hz, 1H), 7.76 (d, *J* = 7.9 Hz, 1H), 7.57 – 7.25 (m, 7H), 5.31 – 5.05 (m, 2H), 3.74 (dd, *J* = 14.3, 2.5 Hz, 1H), 3.58 (dd, *J* = 14.3, 7.9 Hz, 1H) ppm. ^{13}C NMR ($CDCl_3$) δ 167.9, 152.5, 142.9, 135.5, 128.6, 127.9, 126.3, 125.9, 124.7, 121.4, 121.1, 73.6, 42.8 ppm. HRMS (ESI): calcd. for $C_{15}H_{13}NOS_2Na^+$ $[M+Na]^+$ 310.0331, found 310.0340.

2-(Benzo[d]thiazol-2-ylthio)-1-phenylpropan-1-ol (6b)

Synthesis followed the general procedure of reducing β-ketosulfides to β-alkoxysulfides from **5b**. Two isomers were obtained in (Ration of 35:65 between the compounds with a high and low R_f) with a total yield of 100 %. Both isomers were obtained as clear oil. Isomer with the lower R_f on TLC was characterized as below and used for the next step. ^{1}H NMR ($CDCl_3$) δ 7.95 (d, *J* = 8.1 Hz, 1H), 7.79 (d, *J* = 7.7 Hz, 1H), 7.51 – 7.29 (m, 7H), 4.88 (d, *J* = 7.2 Hz, 1H), 4.12 – 4.02 (m, 1H), 1.45 (d, *J* = 7.2 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$) δ 166.9, 152.5, 142.5, 135.6, 128.4, 127.9, 126.7, 126.3, 124.8, 121.5, 121.1, 78.5, 52.3, 18.3 ppm. HRMS (ESI): calcd. for $C_{16}H_{16}NOS_2^+$ $[M+H]^+$ 302.0668, found 302.0661.

2-(Benzo[d]thiazol-2-ylthio)-2-methyl-1-phenylpropan-1-ol (6c)

Synthesis followed the general procedure of reducing β-ketosulfides to β-alkoxysulfides from **5c**. Product obtained as white solid. Yield: 87%. ^{1}H NMR ($CDCl_3$) δ 7.99 (d, *J* = 8.1 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.56 – 7.44 (m, 1H), 7.44 – 7.35 (m, 3H), 7.35 – 7.27 (m, 3H), 4.95 (s, 1H), 1.58 (s, 3H), 1.41 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$) δ 165.3, 152.6, 141.0, 136.1, 128.1, 127.7, 127.7, 126.5, 125.2, 121.9, 121.1, 81.7, 60.1, 27.1, 24.1 ppm. HRMS (ESI): calcd. for $C_{17}H_{17}NOS_2Na^+$ $[M+Na]^+$ 338.0644, found 338.0663.

1-(Benzo[d]thiazol-2-ylthio)-3-methylbutan-2-ol (6d)

Synthesis followed the general procedure of reducing β-ketosulfides to β-alkoxysulfides from **5d**. Product was obtained as clear oil. Yield: 90%. ^{1}H NMR ($CDCl_3$) δ 7.84 (d, *J* = 8.1 Hz, 1H), 7.74 (d,

J = 8.0 Hz, 1H), 7.48 – 7.37 (m, 1H), 7.37 – 7.27 (m, 1H), 4.37 (s, br, 1H), 3.76 (ddd, *J* = 8.2, 6.0, 2.5 Hz, 1H), 3.52 (dd, *J* = 14.4, 2.5 Hz, 1H), 3.38 (dd, *J* = 14.4, 7.9 Hz, 1H), 1.88 (dq, *J* = 13.4, 6.7 Hz, 1H), 1.05 (d, *J* = 6.8 Hz, 3H), 1.00 (d, *J* = 6.8 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$) δ 168.0, 152.5, 135.4, 126.2, 124.6, 121.3, 121.0, 76.6, 38.8, 33.6, 18.7, 17.8 ppm. HRMS (ESI): calcd. for $C_{12}H_{16}NOS_2^+$ $[M+H]^+$ 254.0668, found 254.0660.

***Cis*-2-(benzo[d]thiazol-2-ylthio)cyclohexan-1-ol (6e)**

Synthesis followed the general procedure of reducing β-ketosulfides to β-alkoxysulfides from **5e**. Both *cis*- and *trans*-products were obtained in about 5:4 ratio (according to crude NMR). Total yield: 91%. *cis*-product was characterized as below and used for further synthesis. ^{1}H NMR ($CDCl_3$) δ 7.84 (d, *J* = 8.1 Hz, 1H), 7.73 (d, *J* = 7.9 Hz, 1H), 7.47 – 7.35 (m, 1H), 7.35 – 7.27 (m, 1H), 4.38 – 4.23 (m, 1H), 4.23 – 4.08 (m, 1H), 2.15 – 2.01 (m, 1H), 1.92 (dtd, *J* = 10.3, 6.4, 4.1 Hz, 1H), 1.85 – 1.67 (m, 3H), 1.64 – 1.49 (m, 2H), 1.49 – 1.33 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 167.4, 152.7, 135.4, 126.2, 124.5, 121.4, 121.0, 70.2, 53.9, 31.5, 29.2, 23.9, 21.8 ppm. HRMS (ESI): calcd. for $C_{13}H_{15}NOS_2Na^+$ $[M+Na]^+$ 288.0487, found 288.0497.

Trans-product characterization: ^{1}H NMR ($CDCl_3$) δ 7.86 (d, *J* = 8.1 Hz, 1H), 7.74 (d, *J* = 7.9 Hz, 1H), 7.48 – 7.35 (m, 1H), 7.35 – 7.27 (m, 1H), 4.67 (s, 1H), 3.73 – 3.56 (m, 2H), 2.33 – 2.14 (m, 2H), 1.84 – 1.72 (m, 2H), 1.66 – 1.29 (m, 4H) ppm. ^{13}C NMR ($CDCl_3$) δ 167.4, 152.5, 135.6, 126.3, 124.7, 121.6, 121.0, 75.0, 55.6, 35.6, 32.2, 26.1, 24.1 ppm. HRMS (ESI): calcd. for $C_{13}H_{16}NOS_2^+$ $[M+H]^+$ 266.0668, found 266.0672.

2-(Benzo[d]thiazol-2-ylsulfonyl)-1-phenylethan-1-ol (7a)

Synthesis followed the general procedure of the oxidation of sulfide to sulfone from **6a**. Product was obtained as white solid. Yield: 78%. ^{1}H NMR ($CDCl_3$) δ 8.28 – 8.17 (m, 1H), 8.09 – 7.97 (m, 1H), 7.75 – 7.54 (m, 2H), 7.42 – 7.27 (m, 5H), 5.51 (dd, *J* = 9.7, 1.8 Hz, 1H), 3.94 (dd, *J* = 14.9, 9.7 Hz, 1H), 3.82 (dd, *J* = 14.9, 2.1 Hz, 1H), 3.59 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$) δ 166.3, 152.4, 140.3, 136.7, 128. 9, 128.6, 128.3, 127.9, 125.7, 125.5, 122.4, 68.7, 63.0 ppm. IR: ν_{max}/cm^{-1} 3347 (O-H), 2930 (C-H), 1334 (S=O) and 1141 (S=O). HRMS (ESI): calcd. for $C_{15}H_{13}NO_3S_2^+$ $[M+H]^+$ 320.0410, found 320.0400.

2-(Benzo[d]thiazol-2-ylsulfonyl)-1-phenylpropan-1-ol (7b)

Synthesis followed the general procedure of the oxidation of sulfide to sulfone from **6b**.

Product was obtained as white solid. Yield: 23% ^{1}H NMR ($CDCl_3$) δ 8.33 – 8.18 (m, 1H), 8.09 – 7.95 (m, 1H), 7.73 – 7.63 (m, 1H), 7.63 – 7.57 (m, 1H), 7.40 – 7.28 (m, 5H), 5.18 (d, *J* = 9.1 Hz, 1H), 4.08 – 3.94 (m, 1H), 3.59 (d, *J* = 1.9 Hz, 1H), 1.13 (d, *J* = 7.2 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$) δ 166.0, 152.7, 139.5, 136.9, 128.9, 128.8, 128.1, 127.7, 127.1, 125.6, 122.3, 74.0, 66.2, 11.9 ppm. IR: ν_{max}/cm^{-1} 3484 (O-H), 2851 (C-H), 1313 (S=O), and 1137(S=O). HRMS (ESI): calcd. for $C_{16}H_{15}NO_3S_2Na^+$ $[M+Na]^+$ 356.0386, found 356.0381.

2-(Benzo[d]thiazol-2-ylsulfonyl)-2-methyl-1-phenylpropan-1-ol (7c)

Synthesis followed the general procedure of the oxidation of sulfide to sulfone from **6c**. Product was obtained as white solid. Yield: 35%. ^{1}H NMR ($CDCl_3$) δ 8.33 – 8.22 (m, 1H), 8.09 – 7.99 (m, 1H), 7.72 – 7.56 (m, 2H), 7.39 – 7.27 (m, 5H), 5.54 (s, 1H), 1.54 (s, 3H), 1.22 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$) δ 164.9, 152.8, 138.0, 137.3, 128.5, 128.2, 128.1, 128.0, 127.8, 125.7, 122.2, 74.8, 70.4, 20.8, 14.4 ppm. IR: ν_{max}/cm^{-1} 3335 (O-H), 1314 (S=O), and 1158 (S=O). HRMS (ESI): calcd. for $C_{17}H_{18}NO_3S_2^+$ $[M+H]^+$ 348.0723, found 348.0718.

1-(Benzo[d]thiazol-2-ylsulfonyl)-3-methylbutan-2-ol (7d)

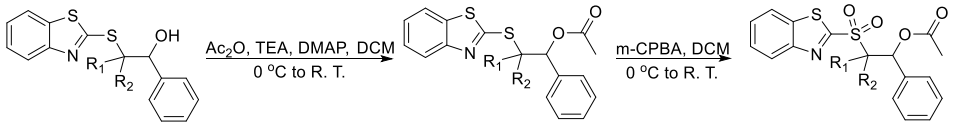
Synthesis followed the general procedure of the oxidation of sulfide to sulfone from **6d**. Product

was obtained as white solid. Yield: 70%. ^{1}H NMR ($CDCl_3$) δ 8.21 (dd, *J* = 7.5, 1.3 Hz, 1H), 8.02 (dd, *J* = 7.1, 1.1 Hz, 1H), 7.71 – 7.51 (m, 2H), 4.19 (ddd, *J* = 9.0, 5.1, 2.1 Hz, 1H), 3.68 (dd, *J* = 14.7, 2.1 Hz, 1H), 3.61 (dd, *J* = 14.7, 9.0 Hz, 1H), 1.91 – 1.73 (m, 1H), 0.96 (t, *J* = 6.7 Hz, 6H) ppm. ^{13}C NMR ($CDCl_3$) δ 166.4, 152.4, 136.6, 128.2, 127.8, 125.4, 122.4, 70.5, 59.5, 33.5, 18.1, 17.1 ppm. IR: ν_{max}/cm^{-1} 3540 (O-H), 2969 (C-H), 1304 (S=O), and 1126(S=O). HRMS (ESI): calcd. for $C_{12}H_{16}NO_3S_2^+$ $[M+H]^+$ 286.0566, found 286.0561.

***Cis*-2-(benzo[d]thiazol-2-ylsulfonyl)cyclohexan-1-ol (7e)**

Synthesis followed the general procedure of the oxidation of sulfide to sulfone from **6e**.

Product was obtained as white solid. Yield: 79%. ^{1}H NMR ($CDCl_3$) δ 8.29 – 8.13 (m, 1H), 8.09 – 7.93 (m, 1H), 7.72 – 7.51 (m, 2H), 4.59 (s, 1H), 3.59 – 3.43 (m, 1H), 3.29 (s, 1H), 2.20 – 1.85 (m, 4H), 1.85 – 1.63 (m, 1H), 1.55 – 1.20 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 165.5, 152.7, 136.9, 128.2, 127.8, 125.5, 122.4, 67.2, 63.8, 32.5, 25.1, 19.5, 18.6 ppm. IR: ν_{max}/cm^{-1} 3548 (OH) 2925 (C-H), 1318 (S=O) and 1141 (S=O). HRMS (ESI): calcd. for $C_{13}H_{16}NO_3S_2^+$ $[M+H]^+$ 298.0566, found 298.0565.



6b: R_1=H, R_2=Me
6c: R_1=R_2=Me

8b: R_1=H, R_2=Me
8c: R_1=R_2=Me

9: R_1=H, R_2=Me
10: R_1=R_2=Me

2-(Benzo[d]thiazol-2-ylthio)-1-phenylpropyl acetate (8b)

A solution of **6b** (100 mg, 0.33 mmol) in 2 mL DCM was treated with Ac_2O (0.09 mL, 1 mmol), TEA (0.14 mL, 1 mmol), and *N,N*-dimethylaminopyridine (DMAP, 4 mg, 0.03 mmol) under stirring at room temperature. After 1 hour, the reaction mixture was directly dried under reduced pressure and purified by column chromatography (EA/Hex 1:20). Product was obtained as clear oil. Yield: 98%. ^{1}H NMR ($CDCl_3$) δ 7.89 (d, *J* = 8.1 Hz, 1H), 7.75 (dd, *J* = 7.9, 0.5 Hz, 1H), 7.42 (dd, *J* = 11.2, 4.1 Hz, 3H), 7.38 – 7.27 (m, 4H), 5.98 (d, *J* = 7.1 Hz, 1H), 4.50 (p, *J* = 7.1 Hz, 1H), 2.03 (s, 3H), 1.42 (d, *J* = 7.0 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$) δ 169.8, 165.6, 153.2, 137.5, 135.4, 128.6, 128.4, 127.3, 126.1, 124.4, 121.7, 121.0, 77.8, 48.3, 21.0, 18.1 ppm. HRMS (ESI): calcd. for $C_{18}H_{18}NO_2S_2^+$ $[M+H]^+$ 344.0773, found 344.0768.

2-(Benzo[d]thiazol-2-ylthio)-2-methyl-1-phenylpropyl acetate (8c)

A solution of **6c** (63 mg, 0.20 mmol) in 2 mL DCM was treated with Ac_2O (0.06 mL, 0.60 mmol), TEA (0.08 mL, 0.6 mmol), and DMAP (2 mg, 0.02 mmol) under stirring at room temperature. After 1 hour, the reaction mixture was directly dried under reduced pressure and purified by column chromatography (EA/Hex 1:20). Product was obtained as clear oil. Yield: 90%. ^{1}H NMR ($CDCl_3$) δ 8.03 (d, *J* = 7.9 Hz, 1H), 7.80 (dd, *J* = 8.0, 0.5 Hz, 1H), 7.50 – 7.44 (m, 1H), 7.44 – 7.27 (m, 6H), 6.36 (s, 1H), 2.08 (s, 3H), 1.60 (s, 3H), 1.51 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$) δ 169.6, 162. 6, 153.7, 136.9, 136.4, 128.3, 128.2, 127.9, 126.2, 125.1, 122.8, 120.9, 79.7, 56.4, 25.7, 24.3, 21.1 ppm. HRMS (ESI): calcd. for $C_{19}H_{19}NO_2S_2Na^+$ $[M+Na]^+$ 380.0794, found 380.0761.

2-(Benzo[d]thiazol-2-ylsulfonyl)-1-phenylpropyl acetate (9)

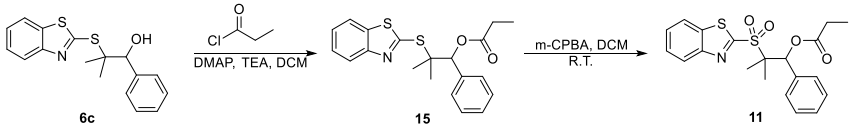
Synthesis procedure followed the general procedure of the oxidation of sulfide to sulfone from **8b**.

Product obtained as pale white oil. Yield: 83%. ^{1}H NMR ($CDCl_3$) δ 8.27 (dd, *J* = 8.1, 0.5 Hz, 1H), 8.03 (dd, *J* = 8.0, 0.6 Hz, 1H), 7.72 – 7.63 (m, 1H), 7.63 – 7.55 (m, 1H), 7.39 – 7.28 (m, 5H), 6.05 (d, *J* = 9.7 Hz, 1H), 4.35 (dq, *J* = 9.7, 7.3 Hz, 1H), 1.53 (s, 3H), 1.26 (d, *J* = 7.3 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$) δ 168. 7, 166.9, 152. 8, 136.7, 136.6, 129.2, 128.9, 128.1, 127.8, 127.6, 125.5, 122.27, 74.9, 62.8, 20. 6, 10.6 ppm. IR: ν_{max}/cm^{-1} 2941 (C-H), 1745 (C=O), 1317 (S=O), 1221 (C-O), and

1145 (S=O). HRMS (ESI): calcd. for $C_{18}H_{18}NO_4S_2^+$ $[M+H]^+$ 376.0672, found 376.0666.

2-(Benzo[d]thiazol-2-ylsulfonyl)-2-methyl-1-phenylpropyl acetate (10)

Synthesis followed the general procedure of the oxidation of sulfide to sulfone from **8c**. Product was obtained as white solids. Yield: 85%. ^{1}H NMR ($CDCl_3$) δ 8.27 (d, *J* = 7.7 Hz, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.69 – 7.62 (m, 1H), 7.60 (td, *J* = 7.7, 1.3 Hz, 1H), 7.36 – 7.24 (m, 5H), 6.29 (s, 1H), 1.76 (s, 3H), 1.66 (s, 3H), 1.31 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$) δ 168.7, 165.3, 153.1, 137.3, 135.8, 128.8, 128.4, 128.1, 127.8, 127.7, 125.7, 122.2, 75.7, 69.1, 20.7, 20.3, 16.5 ppm. IR: ν_{max}/cm^{-1} 2992 (C-H), 1748 (C=O), 1312 (S=O) 1225 (C-O), 1153 (S=O). HRMS (ESI): calcd. for $C_{19}H_{19}NO_4S_2Na^+$ $[M+Na]^+$ 412.0648, found 412.0651.

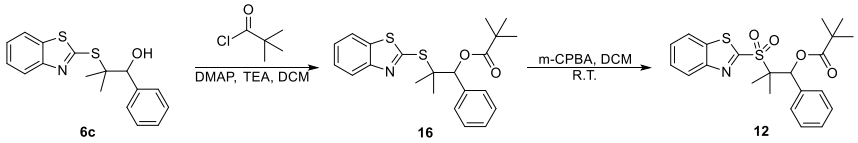


2-(Benzo[d]thiazol-2-ylthio)-2-methyl-1-phenylpropyl propionate (15)

A solution of **6c** (150 mg, 0.48 mmol) was dissolved in 1 mL DCM. To the solution was added DMAP (6 mg, 0.05 mmol), TEA (0.13 mL, 0.96 mmol), and propionyl chloride (0.05 mL, 0.58 mmol) sequentially under stirring. The reaction mixture was heated to 60 °C for 8 hours and then stirred at room temperature overnight. The reaction mixture was directly dried over reduced pressure and purified by column chromatography (EA/Hex 1:20). Product was obtained as clear oil. Yield: 52%. ^{1}H NMR ($CDCl_3$) δ 8.04 (d, *J* = 7.8 Hz, 1H), 7.80 (dd, *J* = 8.0, 0.5 Hz, 1H), 7.53 – 7.27 (m, 7H), 6.38 (s, 1H), 2.48 – 2.27 (m, 2H), 1.61 (s, 3H), 1.52 (s, 3H), 1.15 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$) δ 172.9, 162.6, 153.7, 137.1, 136.4, 128.2, 128.2, 127.9, 126.2, 125.1, 122.8, 120.9, 79.4, 56.5, 27. 8, 25.6, 24.4, 9.1 ppm. HRMS (ESI): calcd. for $C_{20}H_{21}NO_2S_2Na^+$ $[M+Na]^+$ 394.0906, found 394.0905.

2-(Benzo[d]thiazol-2-ylsulfonyl)-2-methyl-1-phenylpropyl propionate (11)

Synthesis followed the general procedure of the oxidation of sulfide to sulfone from **11**. Product was obtained as clear oil. Yield: 81%. ^{1}H NMR ($CDCl_3$) δ 8.26 (d, *J* = 7.7 Hz, 1H), 8.01 (d, *J* = 7.6 Hz, 1H), 7.70 – 7.61 (m, 1H), 7.61 – 7.54 (m, 1H), 7.36 – 7.23 (m, 5H), 6.31 (s, 1H), 2.02 – 1.79 (m, 2H), 1.76 (s, 3H), 1.30 (s, 3H), 0.89 (t, *J* = 7.5 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$) δ 172.0, 165.3, 153.1, 137.2, 135.9, 128.8, 128.4, 128.1, 127.8, 127.7, 125.7, 122.1, 75.4, 69.1, 27.3, 20.4, 16.5, 8.5 ppm. IR: ν_{max}/cm^{-1} 2985 (C-H), 1746 (C=O), 1319 (S=O), 1151 (S=O). HRMS (ESI): calcd. for $C_{20}H_{21}NO_4S_2Na^+$ $[M+Na]^+$ 426.0804, found 426.0821.



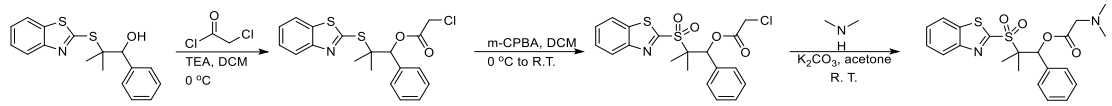
2-(Benzo[d]thiazol-2-ylthio)-2-methyl-1-phenylpropyl pivalate (16)

Compound **6c** (200 mg, 0.63 mmol) and DMAP (7 mg, 0.06 mmol) were dissolved in 2 mL DCM. To this solution was added TEA (0.18 mL, 1.26 mmol) and pivaloyl chloride (0.09 mL, 0.73 mmol) slowly under stirring. The reaction mixture was heated at 60 °C for 8 hours, then cooled to room temperature, and stirred overnight. The reaction mixture was diluted with 10 mL DCM, and washed with 8 mL water. The aqueous phase was back extracted with DCM (10 mL × 2). Combined organic phase was dried over Na_2SO_4, concentrated in vacuo, and purified by column chromatography (DCM/Hex 1:2 to 1:1 to pure DCM). Product was obtained as clear oil. Yield: 63%. ^{1}H NMR ($CDCl_3$)

δ 8.03 (d, *J* = 7.8 Hz, 1H), 7.80 (dd, *J* = 8.0, 0.5 Hz, 1H), 7.52 – 7.28 (m, 7H), 6.35 (s, 1H), 1.61 (s, 3H), 1.52 (s, 3H), 1.26 (s, 9H) ppm. ^{13}C NMR ($CDCl_3$) δ 176.6, 162.6, 153.8, 137.1, 136.3, 128.2, 128.1, 127.8, 126.1, 125.0, 122.8, 120.9, 79.09, 56.7, 39.0, 27.2, 25.5, 24.4 ppm. HRMS (ESI): calcd. for $C_{22}H_{26}NO_2S_2^+$ $[M+H]^+$ 400.1399, found 400.1384.

2-(Benzo[d]thiazol-2-ylsulfonyl)-2-methyl-1-phenylpropyl pivalate (12)

Synthesis followed the general procedure of the oxidation of sulfide to sulfone from **15**. Product yielded as white solid. Yield: 86%. ^{1}H NMR ($CDCl_3$) δ 8.32 – 8.20 (m, 1H), 8.09 – 7.93 (m, 1H), 7.72 – 7.53 (m, 2H), 7.37 – 7.27 (m, 5H), 6.33 (s, 1H), 1.77 (s, 3H), 1.23 (s, 3H), 1.15 (s, 9H) ppm. ^{13}C NMR ($CDCl_3$) δ 176.1, 164.6, 153.2, 137.3, 136.0, 128.8, 128.4, 128.1, 127.9, 127.6, 125.8, 122.2, 74.9, 68.6, 38.8, 26.9, 21.6, 16.4 ppm. IR: ν_{max}/cm^{-1} 2975 (C-H), 1734 (C=O), 1321 (S=O), 1139 (S=O). HRMS (ESI): calcd. for $C_{22}H_{26}NO_4S_2^+$ $[M+H]^+$ 432.1298, found 432.1284.



6c 17 18 13

2-(Benzo[d]thiazol-2-ylthio)-2-methyl-1-phenylpropyl 2-chloroacetate (17)

Compound **6c** (140 mg, 0.44 mmol) was dissolved in 5 mL DCM. To this solution was added TEA (0.11 mL, 0.80 mmol). The solution was cooled to 0 °C in ice bath. 2-Chloroacetyl chloride (0.04 mL, 0.50 mmol) was then added slowly to the solution. The reaction mixture was stirred at 0 °C for 90 min. Then 5 mL water was added into the reaction mixture. The aqueous layer was extracted with DCM (10 mL × 3). Combined organic phase was washed with brine, dried over Na_2SO_4, concentrated, and purified by column chromatography (EA/Hex 1:40 to 1:20). Product was obtained as clear oil. Yield: 50%. ^{1}H NMR ($CDCl_3$) δ 8.03 (dd, *J* = 8.1, 0.4 Hz, 1H), 7.81 (dd, *J* = 8.0, 0.6 Hz, 1H), 7.53 – 7.45 (m, 1H), 7.45 – 7.28 (m, 6H), 6.54 (s, 1H), 4.10 (s, 2H), 1.61 (s, 3H), 1.51 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$) δ 165.8, 162.3, 153.7, 136.2, 136.1, 128.6, 128.1, 128.0, 126.2, 125.2, 122.8, 121.0, 81.2, 56.1, 41.0, 25.4, 24.1 ppm. HRMS (ESI): calcd. for $C_{19}H_{18}ClNO_2S_2Na^+$ $[M+Na]^+$ 414.0360, found 414.0383.

2-(Benzo[d]thiazol-2-ylsulfonyl)-2-methyl-1-phenylpropyl 2-chloroacetate (18)

Synthesis followed the general procedure of the oxidation of sulfide to sulfone from **16**. Product was obtained as white solid. Yield: 88%. ^{1}H NMR ($CDCl_3$) δ 8.35 – 8.17 (m, 1H), 8.10 – 7.91 (m, 1H), 7.76 – 7.56 (m, 2H), 7.42 – 7.27 (m, 5H), 6.41 (s, 1H), 3.88 – 3.68 (m, 2H), 1.73 (s, 3H), 1.31 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$) δ 165.2, 164.7, 153.0, 137.3, 134.8, 129.2, 128.6, 128.3, 127.9, 127.8, 125.7, 122.3, 77.1, 69.0, 40.8, 20.4, 16.3 ppm. IR: ν_{max}/cm^{-1} 2989 (C-H), 1743 (C=O), 1313 (S=O), 1152 (S=O). HRMS (ESI): calcd. for $C_{19}H_{18}ClNO_4S_2Na^+$ $[M+Na]^+$ 446.0258, found 446.0271.

2-(Benzo[d]thiazol-2-ylsulfonyl)-2-methyl-1-phenylpropyl dimethylglycinate (13)

Compound **17** (100 mg, 0.24 mmol) was dissolved in 5 mL acetone and added with K_2CO_3 (66 mg, 0.48 mmol). Dimethylamine (2.0 M in methanol, 0.24 mL, 0.48 mmol) was added into this solution and then the reaction mixture was stirred overnight. Subsequently, the reaction mixture was filtered. The filtrate was concentrated under reduced pressure. Residue was purified by column chromatography (EA/Hex 1:4 to 1:1). Product was obtained as yellowish solid. Yield: 62%. ^{1}H NMR ($CDCl_3$) δ 8.27 (d, *J* = 7.8 Hz, 1H), 8.01 (d, *J* = 7.5 Hz, 1H), 7.72 – 7.62 (m, 1H), 7.62 – 7.50 (m, 1H), 7.36 – 7.26 (m, 5H), 6.33 (s, 1H), 2.86 (d, *J* = 16.9 Hz, 1H), 2.71 (d, *J* = 16.9 Hz, 1H), 2.14 (s, 6H), 1.76 (s, 3H), 1.30 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$) δ 168.5, 165.2, 153.1, 137.2, 135.6, 128.9

128.4 128.1, 127.85, 127.7 125.8 122.2, 75.5, 69.0, 59.6, 44.9, 20.5, 16.4 ppm. IR: ν_{max}/cm^{-1} 2934 (C-H), 1742 (C=O), 1320 (S=O), 1110 (S=O). HRMS (ESI): calcd. for $C_{21}H_{25}N_2O_4S_2^+$ $[M+H]^+$ 433.1250, found 433.1244.

Confirmation of SO_2 release by DTNB test

DTNB test was adopted from a published method.[1] **7b** and **7c** were prepared as 500-μM DMSO stock solutions. DTNB was prepared as 15-mM EtOH stock solution. $NaHSO_3$ as positive control was prepared as a 1-mM PBS (pH 7.4) stock solution and stored on ice before use. 2-OHBT and 2-methyl-1-phenyl-propene as negative controls were prepared as 500-μM DMSO stock solutions respectively.

Each group (200 μL total volume) was prepared by adding 20 μL stock solution into the wells of a 96-well plate followed by addition of 180 μL PBS. Each final solution contained 10% DMSO. The plate was incubated at 37 °C for 0.5 h with gentle shaking on a Barnstead shaker. Then 20 μL DTNB was added into each well. After incubating at room temperature for another 15 min, the UV absorption was read by a PerkinElmer multiplate reader at 405 nm (n=3).

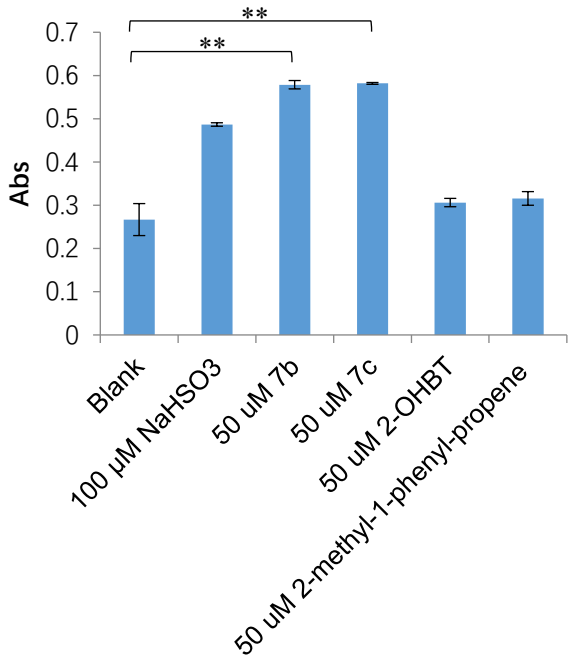


Figure S1. DTNB test results. **: $p < 0.01$.

Kinetics study of compound 7a-e by HPLC

Compounds **7a-d** were prepared as 500-μM DMSO stock solution at room temperature. Test solution (50 μM) was prepared in 8 mL glass vial by adding 200 μL of a DMSO stock solution into 1800 μL PBS and the resulting solution was incubated immediately in a water bath at 37 °C. 100 μL of samples for analysis were taken at random time points (n = 10) and were mixed with 300 μL ACN in a 1.5-mL eppendorf tube and stored at -80 °C. Frozen samples were thawed and centrifuged with benchtop microcentrifuge shortly to allow salt precipitation. Supernatant was subjected to HPLC analysis. Standards of 2-OHBT, styrene, and substituted styrenes were purchased from

commercial vendors. k_{obs} was calculated using 2-OHBT peak areas. All kinetic runs were in triplicates. Curve fitting was conducted using the SigmaPlot 10 software (Figure S2).

HPLC conditions:

Binary solvent system: Solvent A: H_2O with 0.05% TFA; Solvent B: ACN

Gradient elution: Solvent B 15% to 60%

UV detector wavelength: 254 nm

Injection volume: 20 µL

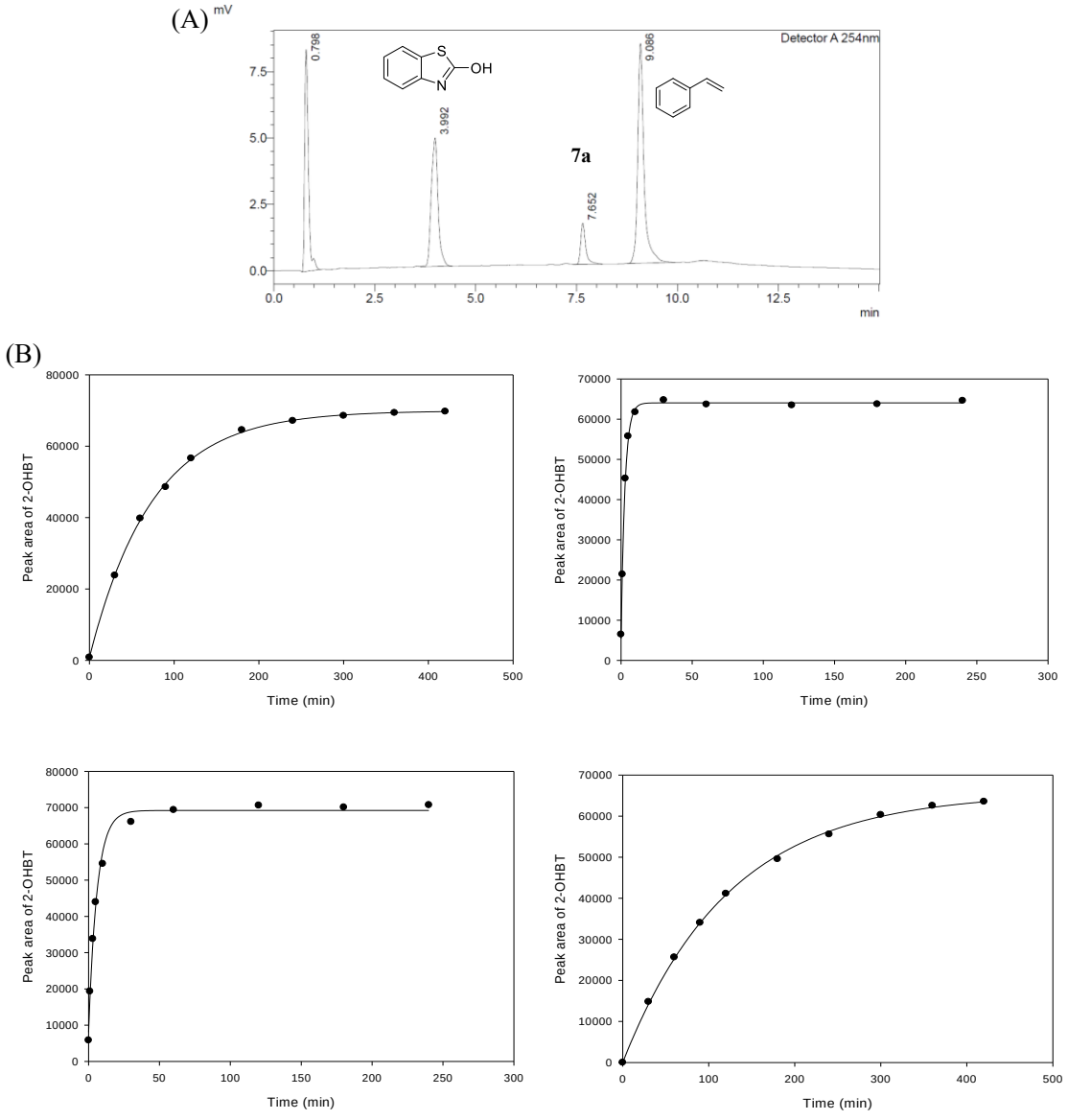


Figure S2 Kinetics studies of compounds **7a-e** by measuring 2-OHBT peak areas using HPLC. (A) An example of HPLC chromatogram obtained by HPLC. Chromatogram showing 50 µM **7a** in 10% DMSO/PBS (pH 7.4) at 37 °C at the 120-min time point. Peak at 3.992 min: 2-OHBT; peak at 7.652 min: compound **7a**; peak at 9.086 min: styrene. (B) Examples of first-order reaction monitored by measuring 2-OHBT peak areas. Upper left: **7a**; upper right: **7b**; lower left: **7c**; lower right: **7d**.

Compound **7e** was prepared as a 20-mM DMSO stock solution at room temperature. Test solution

(200 μM) was prepared in 1.5 mL eppendorf tube by adding 10 μL of the DMSO stock solution into 990 μL PBS followed by incubation in a water bath at 37 °C. Samples for HPLC analyses were taken at random time points and subjected to HPLC analysis directly. 2-OHBT standard was pretested for retention time (~3.8 min). Kinetic runs were in triplicates (Figure S3).

HPLC conditions:

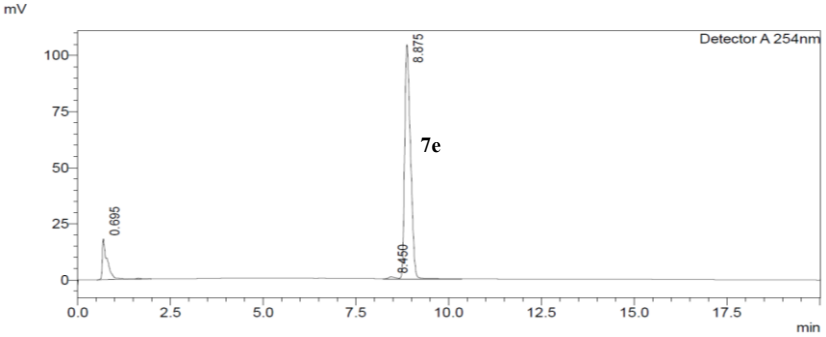
Binary solvent system: Solvent A: H_2O with 0.05% TFA; Solvent B: ACN

Gradient elution: Solvent B 15% to 40%

UV detector wavelength: 254 nm

Injection volume: 20 μL

0 min:



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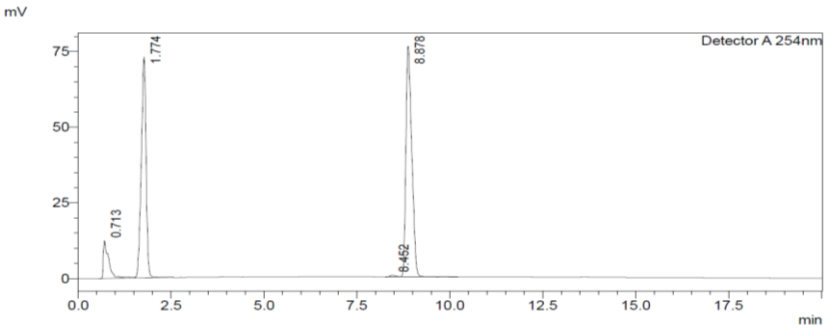

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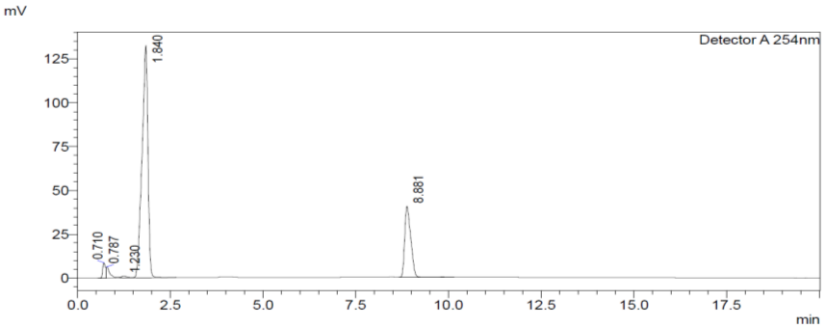

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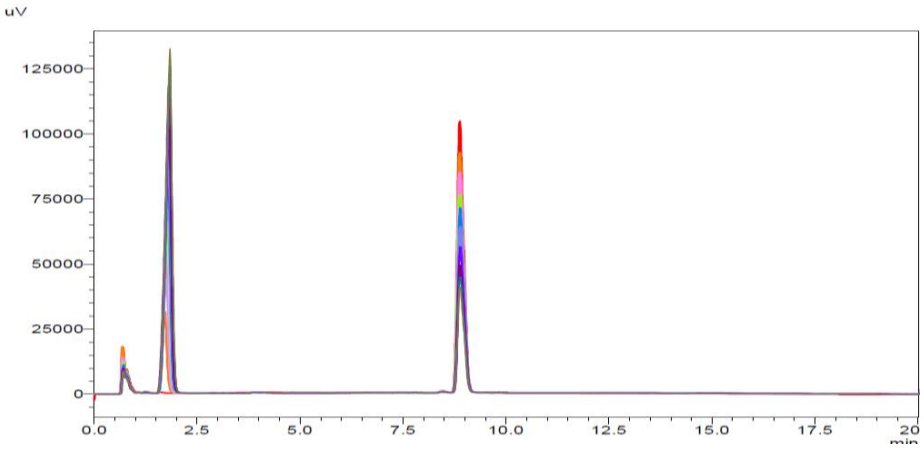


Figure S3. An example of reaction of **7e** monitored by HPLC. Peaks at 8.87~8.88 min: compound **7e**.

Stability study of compound 9, 10, and 13 by HPLC

Compound **9** was prepared as a 5-mM DMSO stock solution at room temperature. Test solution (50 μM) was prepared in a 1.5 mL eppendorf tube by adding 10 μL of the DMSO stock solution into 990 μL PBS followed by incubation at 37 °C in a water bath. Samples for HPLC analysis were taken at random time points (n = 10) and subjected to HPLC analysis directly. Stability tests were in duplicates (Figure S4).

HPLC conditions:

Binary solvent system: Solvent A: H_2O with 0.05% TFA; Solvent B: ACN

Gradient elution: Solvent B 15% to 60%

UV detector wavelength: 254 nm

Injection volume: 20 μL

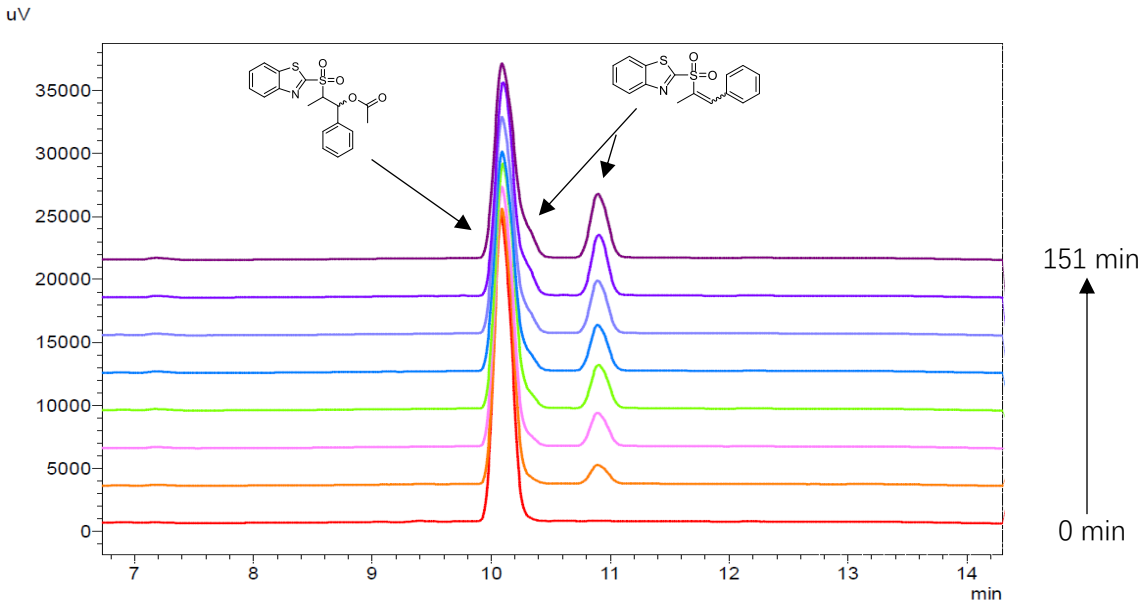


Figure S4 An example of stability tests of compound **9** monitored by HPLC. Peaks at 10.07~10.08 min: compound **9**; shoulder peaks and peaks at 10.87~10.89 min: elimination product.

Compound **10** was prepared as 100-μM DMSO stock solution at room temperature. Test solution (10 μM) was prepared in an 8-mL glass vial by adding 500 μL of the DMSO stock solution into 4.5 mL PBS followed by incubation at 37 °C in a water bath with stirring. Samples for HPLC analysis were taken at random time points (n = 5) and subjected to HPLC analysis directly. Stability tests were in duplicates (Figure S5).

HPLC conditions:

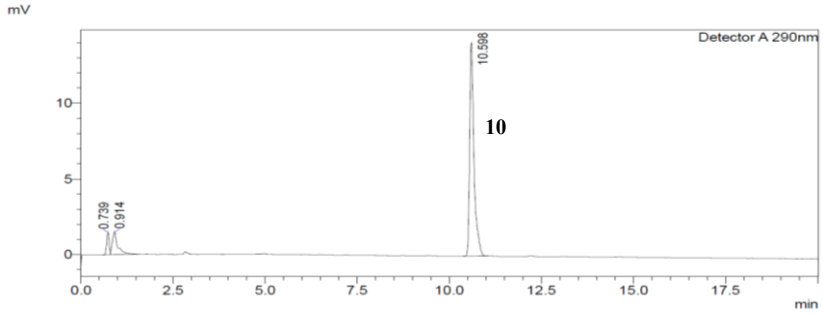
Binary solvent system: Solvent A: H_2O with 0.05% TFA; Solvent B: ACN

Gradient elution: Solvent B 15% to 60%

UV detector wavelength: 254 nm

Injection volume: 20 μL

0 min:



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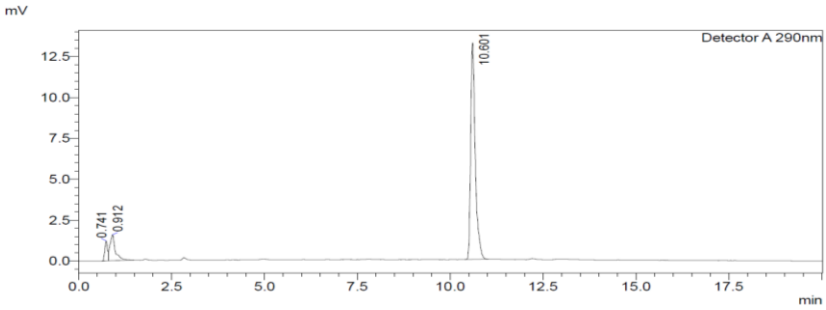

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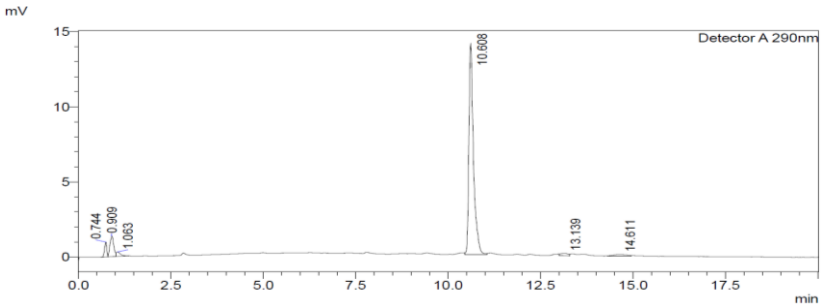

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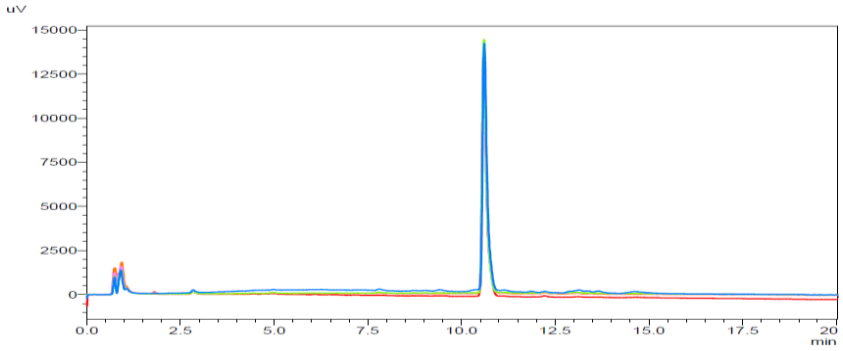


Figure S5 An example of stability tests of compound **10** monitored by HPLC. Peaks at 10.60~10.61 min: compound **10**

A 5-mM stock solution of Compound **13** was prepared at room temperature in DMSO. Test solution (50 μM) was prepared in an 8-mL glass vial by adding 20 μL of the DMSO stock solution into 1980 μL of PBS followed by incubation at 37 °C in a water bath. Samples for HPLC analysis were taken at random time points (n = 5) and subjected to HPLC analysis directly. Stability tests were in duplicates (Figure S6).

HPLC conditions:

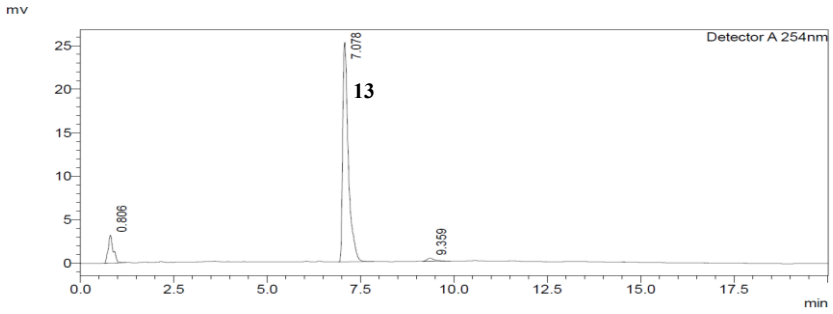
Binary solvent system: Solvent A: H_2O with 0.05% TFA; Solvent B: ACN

Gradient elution: Solvent B 15% to 60%

UV detector wavelength: 254 nm

Injection volume: 20 μL

0 h:



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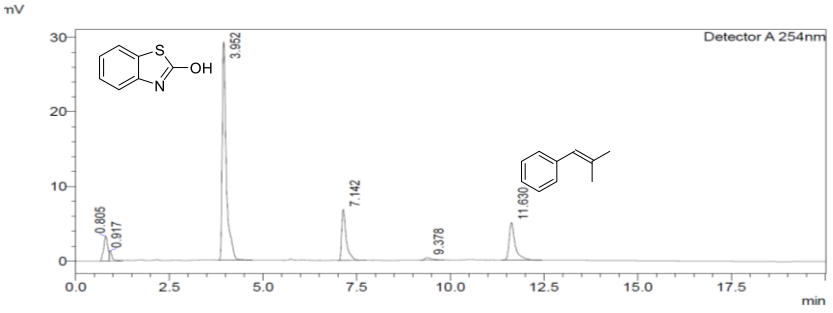

97 h:

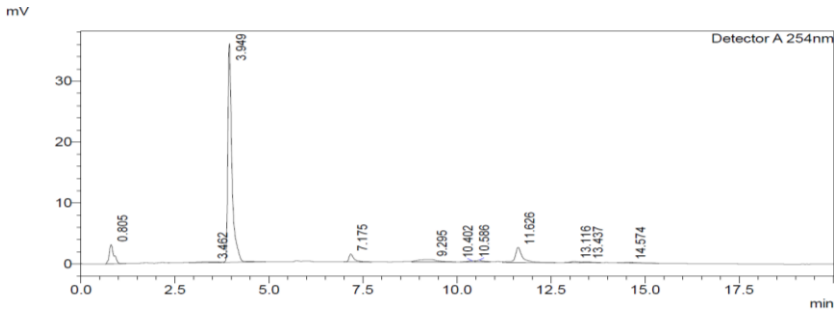

Figure S6 An example of stability studies of compound **13** monitored by HPLC. Peaks at 3.94~3.95min: 2-OHBT; peaks at 7.07~7.17 min: compound **13**; peaks at 11.62~11.63 min: 2-methyl-1-phenyl-propene.

Kinetics study of esterase-triggered SO_2 release of compound 10-13 by HPLC

Stock solutions (500 μM) of compounds **10-13** were prepared in DMSO at room temperature. Porcine liver esterase (PLE, 18 unit/mg) was prepared as 0.1 unit/μL PBS stock solution. Test solution (50 μM with 5 unit/mL PLE) was prepared in an 8-mL glass vial by first adding 200 μL of the DMSO stock solution into 1700 μL of PBS and thoroughly mixed. Then to the solution was added 100 μL of the PLE stock solution followed by incubation at 37 °C in a water bath. 100 μL samples for analysis were taken every 10 minutes (n=12) and mixed with 300 μL ACN in a 1.5-mL eppendorf tube and stored at -80 °C. Frozen samples were thawed and centrifuged with benchtop micro-centrifuge at 14.5×10^3 rpm for 4 min to allow esterase and salt to precipitate. Supernatant was subjected to HPLC analysis. 2-OHBT, styrene, and substituted styrenes were used as standard and pre-tested for retention time. All kinetic runs were in triplicates. Microsoft Excel was used for plotting. Kinetics studies of the esterase-triggered SO_2 release from compound **13** in 1% DMSO/PBS with 1 unit/mL PLE used the same method. Stock solutions were prepared accordingly (Figure 1).

HPLC conditions:

Binary solvent system: Solvent A: H_2O with 0.05% TFA; Solvent B: ACN

Gradient elution: Solvent B 15% to 60%

UV detector wavelength: 254 nm

Injection volume: 20 μL

Kinetics studies of esterase-triggered SO_2 release from compound 10-13 as examined with fluorescent probe 14

Standard curve:

500 μM probe **14** DMSO stock solution was prepared at room temperature. $NaHSO_3$ solutions of different concentrations were prepared in PBS. The test solution was prepared in a 4 mL (10 mm×10 mm) quartz cuvette by adding 300 μL probe DMSO stock solution into 2700 μL $NaHSO_3$ solution. Final solution contains 50 μM probe **14** and 10% DMSO. The test solution was sealed with a cap, thoroughly mixed, and incubated at room temperature for 2 min. Fluorescent emission at 465 nm was then recorded on a Shimadzu RF- 5301PC fluorimeter. (λ_{ex}=400 nm; slit width: ex: 5nm, em: 3 nm) Experiments were conducted in triplicates. Standard curves were fitted with Microsoft Excel 2016 (Figure S7(A)).

Studies of reaction kinetics:

Stock solutions (1 mM) of compounds **10-13** were prepared in DMSO at room temperature. PLE (18 unit/mg) was prepared as 0.1 unit/μL PBS stock solution. Probe **14** was prepared as 1 mM DMSO stock solution. Test solution (50 μM prodrug with 50 μM probe and 5 unit/mL PLE) was prepared by adding 150 μL of the prodrug DMSO stock solution, 150 μL of the probe **14** DMSO stock solution, 2550 μL of PBS, and 150 μL PLE stock solution sequentially into a 4 mL (10 mm×10 mm) quartz cuvette. The test solution was sealed with a cap, and thoroughly mixed; and fluorescent emission at 465 nm was recorded every 10 minutes (n=13) (λ_{ex}=400 nm; slit width: ex: 5nm, em: 3 nm). Fluorescence intensities obtained were converted to $NaHSO_3$ concentrations using the standard curve described above. All runs were in triplicates (Figure S7(B)).

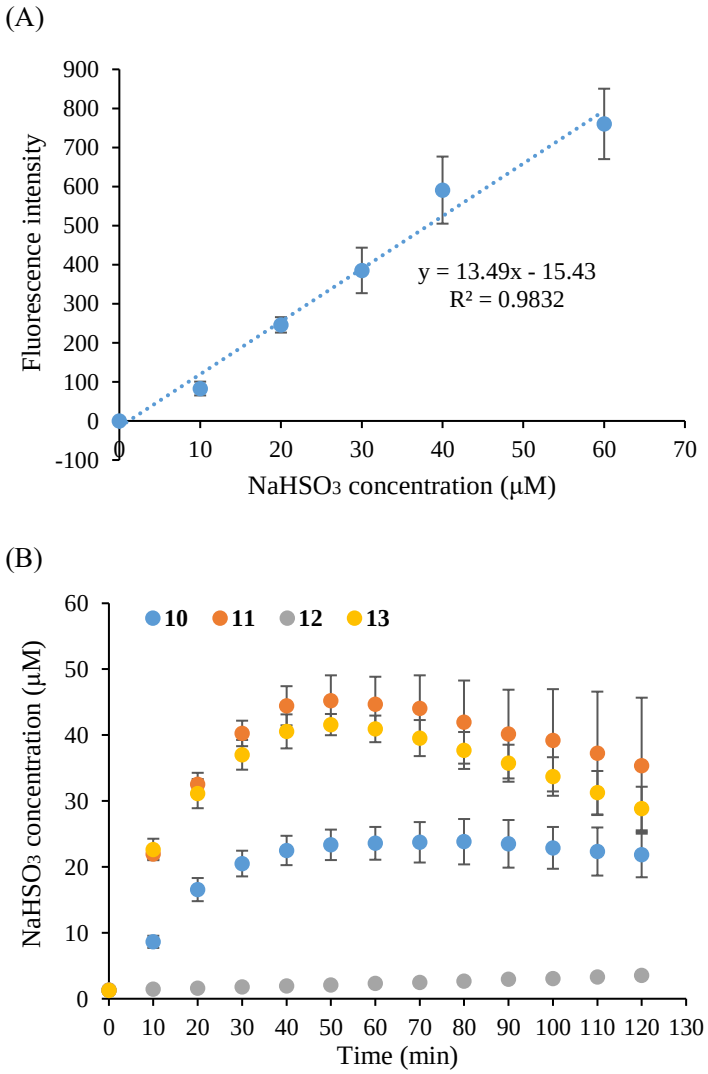

Figure S7 Kinetics study of esterase-triggered SO2 release from compound **10-13** by fluorescent probe **14**. (A) Standard curve. (B) Kinetics of esterase-triggered SO2 release from compounds **10-13** monitored with fluorescent probe **14**.

Cell imaging study

Cell culture:

HeLa cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with

10 % fetal bovine serum and 1% penicillin-streptomycin at 37 °C with 5 % CO_2. The media was changed every other day. Experiment was done within 10 passages of Hela cells.

Cell imaging:

HeLa cells were pre-seeded onto coverslips in 6-well plate a day before experiment. A 2-mM DMSO stock solution of probe **14** was prepared. Compound **13** was prepared as 20-mM DMSO stock solution. Cells were first treated with compound **13** to make a final concentration of 100 μM (0.5% DMSO). 400 μM $NaHSO_3$ was used as a positive control. A mixture containing 100 μM 2-OHBT and 100 μM 2-methyl-1-phenyl-propene was used as the negative control. Cell only group was also used as negative controls. All final media contained 0.5% DMSO. The cells were incubated at 37 °C for 2 hours and washed with PBS once. 2 mL of fresh DMEM media was added into each well. The cells were then treated with probe **14** to give a final concentration of 10 μM. Cells were incubated for another 0.5 h at 37 °C. After washing twice with PBS, the cells were fixed with 4% paraformaldehyde for 30 min at room temperature. The cells were then washed once with PBS. Then 25 mM glycine in PBS was added to quench the extra formaldehyde. After storing at 4 °C overnight. The glycine solution was discarded. All coverslips were immersed in DI water and mounted onto glass slides using DAPI-free mounting media (ProLong Live Antifade Reagent; P36974). Fluorescent imaging was performed on a Zeiss fluorescent microscope using the DAPI channel (λ_{ex}: 358 nm, λ_{em}: 461 nm).

References

1. Li, Y.; Zhao, M., Simple methods for rapid determination of sulfite in food products. *Food Control* **2006,** *17* (12), 975-980.