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SUPPORTING INFORMATION

Sulfur Dioxide Prodrugs: Triggered Release of SO_2 via a Click Reaction

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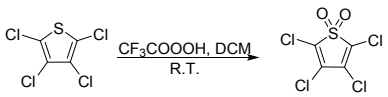
Materials and Methods

All reagents and chemicals were purchased from commercial suppliers as reagent grade or higher, and were used without further purification unless otherwise noted. NMR spectra were recorded on a Bruker Avance NMR spectrometer at 400 MHz for ^{1}H and 101 MHz for ^{13}C at room temperature unless otherwise specified. Solvent peaks were served as internal standard. Mass spectral analyses were performed by the GSU Mass Spectrometry Facilities. UV-Vis absorption spectra were recorded on a Shimadzu PharmaSpec UV-1700 UV-Visible spectrophotometer. Fluorescence spectra were recorded on a Shimadzu RF- 5301PC fluorimeter.

Synthesis of thiophene dioxides

General method for the oxidation of thiophenes:

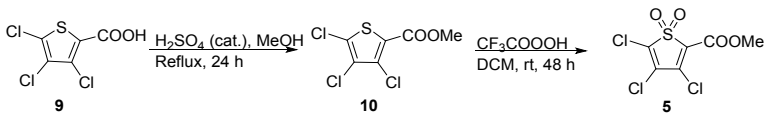
Thiophene S-dioxides were synthesized following a literature procedure using peroxytrifluoroacetic acid as the oxidant.[1]



2 **3**

Tetrachlorothiophene-1,1-dioxide (3)

Tetrachlorothiophene-1,1-dioxide was synthesized following the general procedure of thiophene oxidation. Off-white solid was obtained. Yield: 85% ^{13}C NMR ($CDCl_3$): δ 131.2, 127.4 ppm. IR: 1598 cm^{-1}, 1337cm^{-1}, 1166 cm^{-1}.



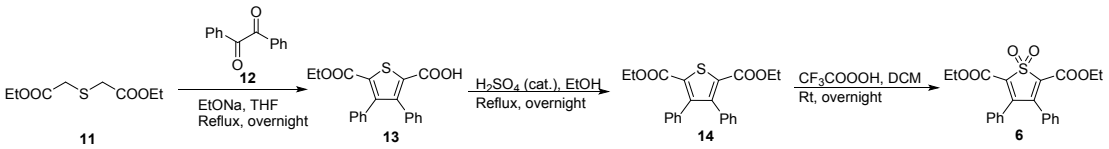
9 **10** **5**

Methyl 3,4,5-trichlorothiophene-2-carboxylate (10)

500 mg 3,4,5-trichlorothiophene-2-carboxylic acid (**9**, 2.16 mmol) was dissolved in 10 mL MeOH. 1 mL concentrated H_2SO_4 was added dropwise with stirring. The reaction mixture was stirred under reflux for 1 day. Then the reaction mixture was cooled down to room temperature and stirred for another 2 days. White precipitate was filtered off. Filter cake was kept. The filtrate was dried over vacuum, washed with sat. $NaHCO_3$ solution, and extracted with EtOAc. The organic phase was dried over Na_2SO_4 followed by solvent evaporation with rotavap. Solid was combined with filter cake and recrystallized in MeOH. 400 mg beige needle crystals were obtained. Yield: 75%. ^{1}H NMR ($CDCl_3$): δ 3.91 (s, 3H) ppm. HRMS (ESI): calcd for $C_6H_4Cl_3O_2S$ $[M+H]^+$ 244.8992, found 244.9002.

Methyl 3,4,5-trichlorothiophene-2-carboxylate 1,1-dioxide (5)

Methyl 3,4,5-trichlorothiophene-2-carboxylate 1,1-dioxide was synthesized following the general procedure of thiophene oxidation from methyl 3,4,5-trichlorothiophene-2-carboxylate (**10**). Pale yellow cylinder crystals were obtained. Yield: 60%. ^{1}H NMR ($CDCl_3$): δ 3.99 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$): δ 156.3, 145.0, 133.0, 130.7, 127.8, 53.7 ppm. IR: 1727, 1564, 1437, 1335, 1244, 1196, 1172 cm^{-1}. HRMS (ESI): calcd for $C_6H_3Cl_2O_4S$ $[M-Cl]^+$ 240.9124, found 240.9134.



5-(Ethoxycarbonyl)-3,4-diphenylthiophene-2-carboxylic acid (13)

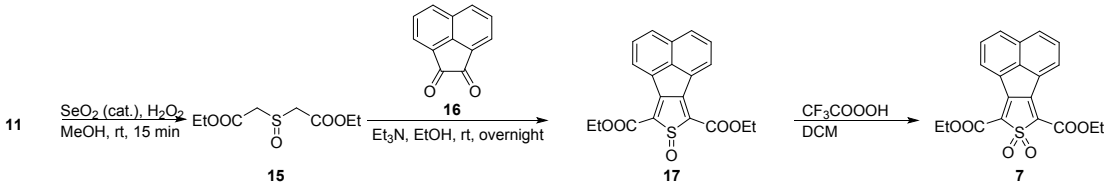
1500 mg diethyl 2,2'-thiodiacetate (**11**, 7.26 mmol) and 1270 mg benzil (**12**, 6.05 mmol) were dissolved in 30 mL dry THF. 850 mg EtONa (12.50 mmol) was then added. The reaction mixture was refluxed overnight under argon protection. Then the reaction was quenched by pouring onto ice followed by washing with EtOAc. The aqueous phase was acidified using 1N HCl. The solid was filtered off and kept. The filtrate was extracted with EtOAc. The organic phase was dried over Na_2SO_4. Solvent was removed by vacuum. The remaining solid was combined with filter cake and directly used for the next step as crude (1.42 g). Crude yield: 67%.

Diethyl 3,4-diphenylthiophene-2,5-dicarboxylate (14)

1.42 g crude 5-(ethoxycarbonyl)-3,4-diphenylthiophene-2-carboxylic acid (**13**) was dissolved in 50 mL EtOH. 2 mL concentrated H_2SO_4 was added. The reaction mixture was refluxed with stirring for 2 days and then cooled to room temperature. Solid was filtered out and kept. The solution was then dried under vacuum. To the residue was added with sat. $NaHCO_3$ solution. The resulting solution was extracted with EtOAc. Organic phase was dried over Na_2SO_4 followed by solvent evaporation under vacuum. Solid was combined with the filter cake and recrystallized in EtOH to give 576 mg product. Combined yield of two steps: 25%. ^{1}H NMR ($CDCl_3$): δ 7.23 – 7.11 (m, 6H), 7.02 (dd, J = 6.5, 3.1 Hz, 4H), 4.20 (q, J = 7.1 Hz, 4H), 1.18 (t, J = 7.1 Hz, 6H) ppm.

Diethyl 3,4-diphenylthiophene-2,5-dicarboxylate 1,1-dioxide (6)

Diethyl 3,4-diphenylthiophene-2,5-dicarboxylate 1,1-dioxide was synthesized following the general procedure of thiophene oxidation from diethyl 3,4-diphenylthiophene-2,5-dicarboxylate (**14**). Product was obtained as yellow solid. Yield: 65%. ^{1}H NMR ($CDCl_3$): δ 7.32 (t, J = 7.5 Hz, 2H), 7.22 (t, J = 7.6 Hz, 4H), 6.93 (d, J = 7.3 Hz, 4H), 4.30 (q, J = 7.1 Hz, 4H), 1.26 (t, J = 7.1 Hz, 6H) ppm. ^{13}C NMR ($CDCl_3$): δ 157.7, 150.1, 130.0, 129.9, 129.5, 128.9, 127.8, 62.5, 13.8 ppm. IR: 3061, 1725, 1583, 1311, 1241, 1194 cm^{-1}. HRMS (ESI): calcd for $C_{22}H_{21}O_6S$ $[M+H]^+$ 413.1053, found 413.1045.



Diethyl 2,2'-sulfinyldiacetate (15)

1 g diethyl 2,2'-thiodiacetate (**11**, 4.84 mmol) and 30 mg SeO_2 (0.27 mmol) were dissolved in 10 mL MeOH and added with 1.22 mL H_2O_2 (~27% w/w in water, ~10 mmol) slowly. The reaction mixture was stirred under room temperature for 15 min. Then the reaction mixture was concentrated over a rotavap. The residue was dissolved in water (10 mL) and extracted with DCM (20 mL × 3). The organic phase was combined. Removal of solvent yielded 1.05 g crude oil, which was used directly in the next step. Crude yield: 98%. ^{1}H NMR ($CDCl_3$): δ 4.25 (q, J = 7.2 Hz, 4H), 4.05 (d, J = 14.3 Hz, 2H), 3.83 (d, J = 14.3 Hz, 2H), 1.30 (t, J = 7.1 Hz, 6H) ppm.

Diethyl acenaphtho[1,2-c]thiophene-7,9-dicarboxylate 8-oxide (17)

373 mg acenaphthylene-1,2-dione (**16**, 2.05 mmol) and 500 mg diethyl 2,2'-sulfinyldiacetate (**15**, 2.25 mmol) were dissolved in 2.5 mL ethanol. 456 mg trimethylamine (4.51 mmol) was added slowly to the reaction mixture. Then the reaction mixture was stirred at room temperature overnight. Then the precipitates were filtered out and recrystallized using DCM/hexane. 400 mg yellow crystals were obtained. Yield: 53%. ^{1}H NMR ($CDCl_3$): δ 8.75 (d, J = 7.2 Hz, 2H), 8.05 (d, J = 8.2 Hz, 2H), 7.76 (t, J = 7.7 Hz, 2H), 4.54 (m, 4H), 1.50 (t, J = 7.1 Hz, 6H) ppm. ^{13}C NMR ($CDCl_3$): δ 161.3, 151.2, 147.1, 133.2, 131.1, 130.4, 129.0, 128.6, 128.4, 62.6, 14.5 ppm.

Diethyl acenaphtho[1,2-c]thiophene-7,9-dicarboxylate 8,8-dioxide (7)

Diethyl acenaphtho[1,2-c]thiophene-7,9-dicarboxylate 8,8-dioxide was synthesized following the general procedure of thiophene oxidation. 100 mg diethyl acenaphtho[1,2-c]thiophene-7,9-dicarboxylate 8-oxide (**17**, 0.27 mmol) was dissolved in 2 mL DCM and added with 1 mL freshly prepared peroxytrifluoroacetic acid. The reaction mixture was stirred at room temperature for 1 day. Then the reaction mixture was dried under vacuum and redissolved in DCM. The drying process was repeated several times till the residue is free of acid. Recrystallization from EtOAC/DCM yielded orange solid 46 mg. Yield: 45%. ^{1}H NMR ($CDCl_3$): δ 8.90 (d, J = 7.3 Hz, 2H), 8.11 (d, J = 8.2 Hz, 2H), 7.86-7.77 (m, 2H), 4.55 (q, J = 7.1 Hz, 4H), 1.51 (t, J = 7.1 Hz, 6H) ppm. ^{13}C NMR ($CDCl_3$): δ 158.9, 147.8, 145.2, 131.0, 130.9, 129.1, 129.1, 128.0, 124.3, 62.7, 14.2 ppm. IR: 1707, 1654, 1581, 1318, 1259, 1231, 1196 cm^{-1}. HRMS (ESI): calcd for $C_{20}H_{17}O_6S$ $[M+H]^+$ 385.0740, found 385.0729.

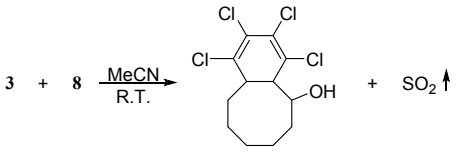
General procedure of the reaction between thiophene dioxides and strained alkyne/alkene

Thiophene dioxide was dissolved in a small amount of MeCN or MeOH to give a final concentration of around 50~100 mM. 1~1.5 equiv. of strained alkyne (or *trans*-alkene) was then added to the solution. TLC was used to monitor the procession of the reaction. After completion, the reaction mixture was directly dried on a rotavap and purified by column chromatography to yield the product after SO_2 release.

((1s,1aR,9aS)-4,5,6,7-tetrachloro-1a,2,3,8,9,9a-hexahydro-1H-benzo[a]cyclopropa[e][8]annulen-1-yl)methanol (4)

Product obtained as white solid. Yield: 53%. ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.35 (br, 1H), 3.52 (br, 2H), 3.26 (br, 2H), 3.00 (br, 2H), 2.18 (br, 2H), 1.78 (br, 2H), 0.82 (br, 1H), 0.32 (br, 2H) ppm. ^{13}C NMR ($CDCl_3$): δ 140.6, 132.4, 130.5, 59.7, 29.9, 21.1, 16.3 ppm.

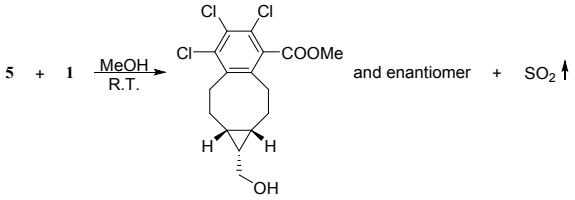
To allow HRMS characterization, compound **4** was acetylated by reacting with acetic anhydride (3 equiv.) and triethylamine (3 equiv.) in DCM in the presence of DMAP (0.1 equiv.) at room temperature overnight. Reaction mixture was dried over vacuum and purified by column chromatography (eluent: EtOAc/Hex 1:2). HRMS of acetyl derivative (ESI): calcd for $C_{16}H_{16}O_2Cl_4Na$ $[M+Na]^+$ 402.9797, found 402.9814.



18

1,2,3,4-Tetrachloro-4a,5,6,7,8,9,10,10a-octahydrobenzo[8]annulen-5-ol (18)

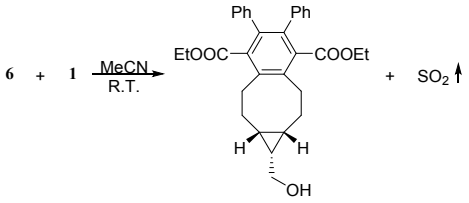
Product obtained as white solid. Yield: 77%.^{1}H NMR ($CDCl_3$): δ 4.29 – 4.15 (m, 1H), 2.94 – 2.78 (m, 1H), 2.73 – 2.63 (m, 1H), 2.15 (d, *J* = 2.9 Hz, 1H), 2.09 – 1.73 (m, 6H), 1.73 – 1.58 (m, 2H), 1.53 – 1.42 (m, 1H), 1.16 – 1.00 (m, 1H) ppm. ^{13}C NMR ($CDCl_3$): δ 133.6, 129.3, 125.2, 122.7, 74.3, 47.2, 43.0, 36.6, 30.6, 27.9, 26.2, 23.3 ppm. HRMS (ESI): calcd for $C_{12}H_{14}Cl_4NaO$ $[M+Na]^+$ 336.9691, found 336.9681.



19

Methyl (1S,1aS,9aR)-5,6,7-trichloro-1-(hydroxymethyl)-1a,2,3,8,9,9a-hexahydro-1H-benzo[a] cyclopropa[e][8]annulene-4-carboxylate and enantiomer (19)

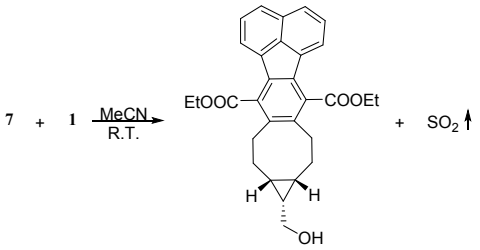
Product obtained as white solid. Yield: 61%. ^{1}H NMR ($CDCl_3$): δ 3.98 (s, 3H), 3.75 (br, 2H), 3.28 (br, 1H), 2.96 (br, 2H), 2.80-2.65 (m, 1H), 2.50-2.35 (m, 1H), 2.24 (br, 1H), 1.75 (br, 1H), 1.38-1.00 (br, 3H), 0.95-0.44 (br, 2H). ^{13}C NMR ($CDCl_3$): δ 167.5, 140.8, 138.8, 134.7, 129.9, 127.5, 59.7, 53.0 30.5, 28.8, 22.3, 21.0, 20.4, 16.9, 15.7. HRMS (ESI): calcd for $C_{16}H_{17}O_3NaCl_3$ $[M+Na]^+$ 385.0141, found 385.0128.



20

Diethyl (1s,1aR,9aS)-1-(hydroxymethyl)-5,6-diphenyl-1a,2,3,8,9,9a-hexahydro-1H-benzo[a] cyclopropa[e][8]annulene-4,7-dicarboxylate (20)

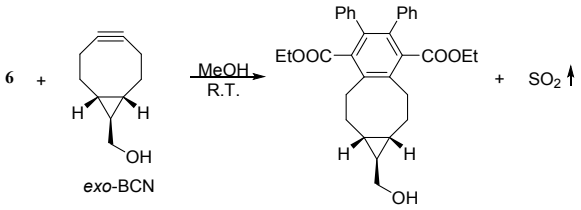
Product obtained as yellowish solid. Yield: 46%. ^{1}H NMR ($CDCl_3$): δ 7.16 – 7.05 (m, 6H), 7.02 (br, 4H), 3.94 (q, *J* = 7.1 Hz, 4H), 3.75 (br, 2H), 2.98 (br, 2H), 2.90 – 2.74 (m, 2H), 2.31 (br, 2H), 1.87 – 1.64 (br, 1H), 1.55 – 1.29 (br, 2H), 1.21 – 0.98 (br, 3H), 0.89 (t, *J* = 7.1 Hz, 6H) ppm. ^{13}C NMR ($CDCl_3$): δ 169.6, 138.3, 136.5, 130.4, 127.2, 126.8, 61.0, 59.8, 13.7 ppm. HRMS (ESI): calcd for $C_{32}H_{35}O_5$ $[M+H]^+$ 499.2479, found: 499.2479.



21

Diethyl (9aR,10s,10aS)-10-(hydroxymethyl)-9,9a,10,10a,11,12-hexahydro-8H-cyclopropa[5,6] cycloocta[1,2-k]fluoranthene-7,13-dicarboxylate (21)

Product obtained as yellow solid. Yield: 60%. ^{1}H NMR ($CDCl_3$): δ 7.85 (d, *J* = 8.2 Hz, 2H), 7.77 (d, *J* = 7.1 Hz, 2H), 7.65 – 7.55 (m, 2H), 4.60 (q, *J* = 7.1 Hz, 4H), 3.81 – 3.57 (m, 2H), 3.17 – 2.95 (m, 2H), 2.95 – 2.78 (m, 2H), 2.33 (br, 2H), 1.83 – 1.52 (m, 2H), 1.51 – 1.37 (m, 6H), 1.17 – 0.62 (m, 4H) ppm. ^{13}C NMR ($CDCl_3$): δ 169.8, 134.2, 133.7, 132.7, 129.9, 127.9, 127.4, 121.9, 61.7, 59.8, 14.3 ppm. HRMS (ESI): calcd for $C_{30}H_{30}O_5Na$ $[M+Na]^+$ 493.1991, found: 493.1987.



22

Diethyl (1r,1aR,9aS)-1-(hydroxymethyl)-5,6-diphenyl-1a,2,3,8,9,9a-hexahydro-1H-benzo[a] cyclopropa[e][8]annulene-4,7-dicarboxylate (22)

Product obtained as yellowish solid. Yield: 58%. ^{1}H NMR ($CDCl_3$) δ 7.08 (s, 6H), 7.01 (s, 4H), 4.07 – 3.78 (m, 4H), 3.44 (d, *J* = 6.7 Hz, 2H), 3.03 – 2.88 (m, 2H), 2.88 – 2.68 (m, 2H), 2.62 – 2.38 (br, 2H), 1.49 – 1.31 (br, 3H), 0.89 (t, *J* = 7.1 Hz, 6H), 0.85 – 0.72 (br, 2H) ppm. ^{13}C NMR ($CDCl_3$) δ 169.6, 138.5, 138.3, 136.4, 136.3, 130.4, 130.2, 127.2, 126.8, 66.5, 61.0, 30.4, 29.7, 29.5, 21.6, 13.7 ppm. HRMS (ESI): calcd for $C_{32}H_{35}O_5$ $[M+H]^+$ 499.2479, found: 499.2462.

Confirmation of SO_2 release by the DTNB test

3 was prepared as 500 μM stock solution in 5% DMSO/PBS. **1** was prepared as 5 mM stock solution in 5% DMSO/PBS. DTNB was prepared as 1.5 mM stock solution in 10% EtOH/PBS stock solution. $NaHSO_3$ and Na_2SO_3 as positive controls were prepared as 500 μM stock solution in 5% DMSO/PBS, respectively.

Room temperature incubation: Each group (2 mL) was prepared in 4 mL glass vial, sealed with Parafilm® M, and incubated at room temperature (21 °C) for 45 min. 2 mL DTNB stock solution was then added to each group. The solutions were allowed to stand at room temperature for another 15 min. Then the solution was filtered through 0.45 μm micron filters and its UV-vis absorption measured at 412 nm in a quartz cuvette.

37 °C incubation: Each group (2 mL) was prepared in 4 mL glass vial, added with 0.3 mL silicon fluid (to minimize SO_2 escape), and sealed with Parafilm® M. Then all the groups were incubated in a 37 °C water bath for 45 min. Then the solutions were taken out and allowed 15 min to cool to room temperature. 2 mL DTNB was then added to each group. The solution was allowed to stand at room temperature for another 15 min. Then the water phase of each solution was taken out by syringe and

filtered through 0.45 μm micron filters. The UV-vis absorption of the filtrate was measured at 412 nm in a quartz cuvette.
All DTNB tests were in triplicates.

Kinetics studies of cycloaddition reactions

Second order reaction rate constants (k_2) were obtained as slopes of pseudo-first reaction rate constants (k_1) plotted linearly against concentrations of strained alkyne/alkene.
UV-vis kinetic measurements: Separate stock solutions of thiophene dioxide (**3**, **5**, or **6**) and BCN (**1**) or *trans*-cyclooctene (**8**) (concentration 10~18 times of respective thiophene dioxide) were prepared in HPLC-grade solvent (MeOH, MeCN) at room temperature. The "click-release" reaction led to significant changes in the UV-vis spectrum of thiophene dioxide. Thiophene dioxide stock solution (200 μL), BCN (or *trans*-alkene) (300, 400, 500, 600 μL), and solvent (300, 200, 100, 0 μL) were added into 10 mm quartz cuvettes, thoroughly mixed and sealed with a PTFE cap and monitored for time-dependent UV-vis absorption changes. All kinetic runs were in triplicates. Curve fitting was operated using the SigmaPlot® 10 software (Figure S1).

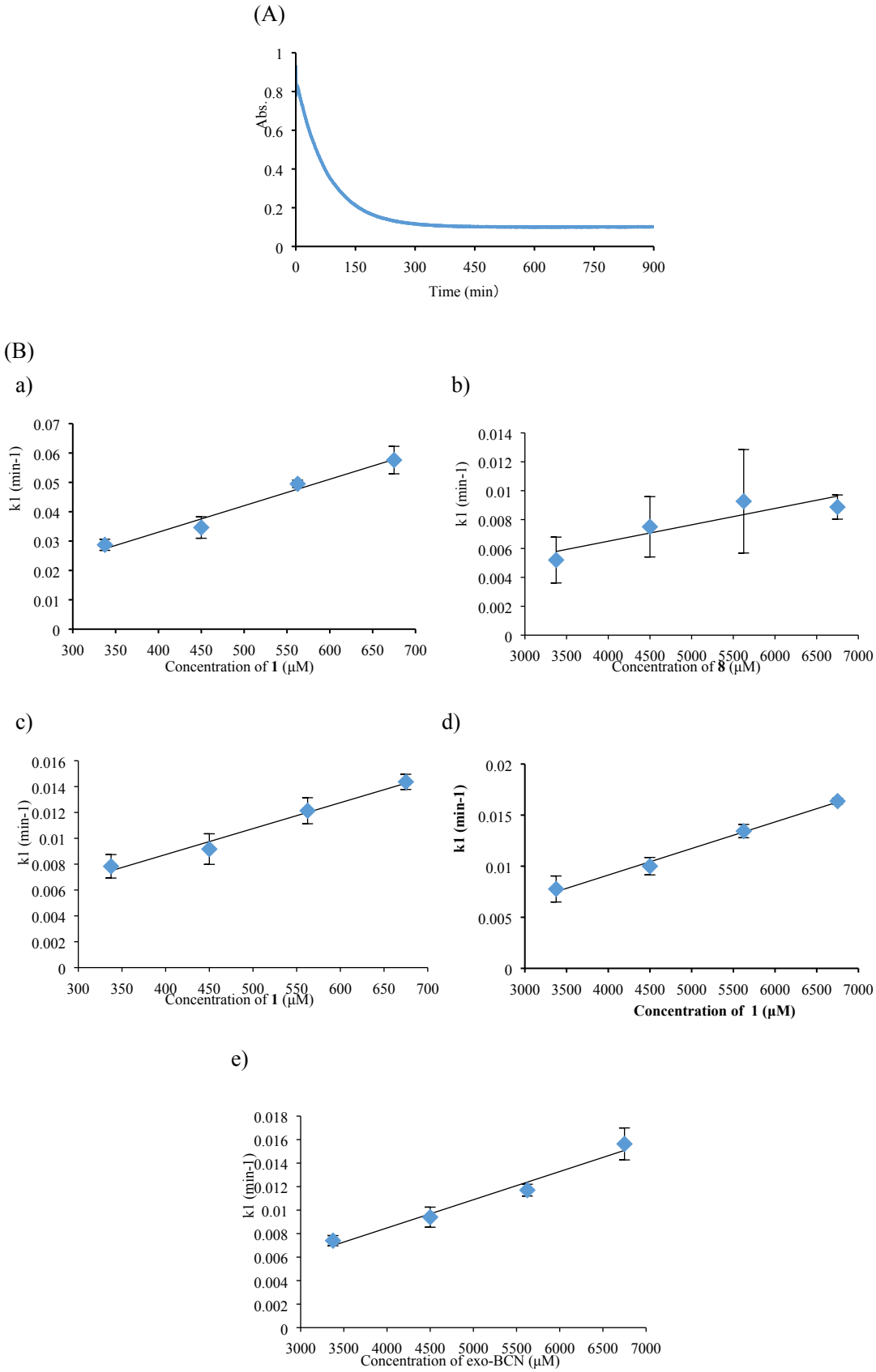


Figure S1 Kinetics studies of cycloaddition reactions by UV-vis absorbance change. (A) Example of pseudo-first reaction monitored by UV absorbance decrease. A typical curve shows reaction between

125 μM **6** and 5.625 mM **1** in MeOH at room temperature. λ =324 nm. (B) Linear plot of k_1 at different concentrations of **1** or **8**. SO_2 donor pairs and solvents: a) **3** + **1**, MeOH; b) **3** + **8**, MeOH; c) **5** + **1**, MeCN; d) **6** + **1**, MeOH; e) **6** + exo-BCN.

Fluorescence kinetics measurements: Separate solutions of thiophene dioxide (**7**, 500 μM) and **1** (5 mM) were prepared in HPLC-grade solvent (DMSO/PBS 4:1) at room temperature. The "click-release" reaction gives a fluorescent product that can be monitored by fluorimeter. The solutions containing thiophene dioxide (200 μL), BCN (300, 400, 500, 600 μL), and solvent (300, 200, 100, 0 μL) were added into quartz cuvettes, thoroughly mixed and sealed with a PTFE cap, kept at 37 °C in water bath, and tested for fluorescence emission change (λ_{ex}=350 nm, λ_{em}=470 nm). All kinetics runs were in triplicates. Curve fitting was done using the SigmaPlot® 10 software (Figure S2).

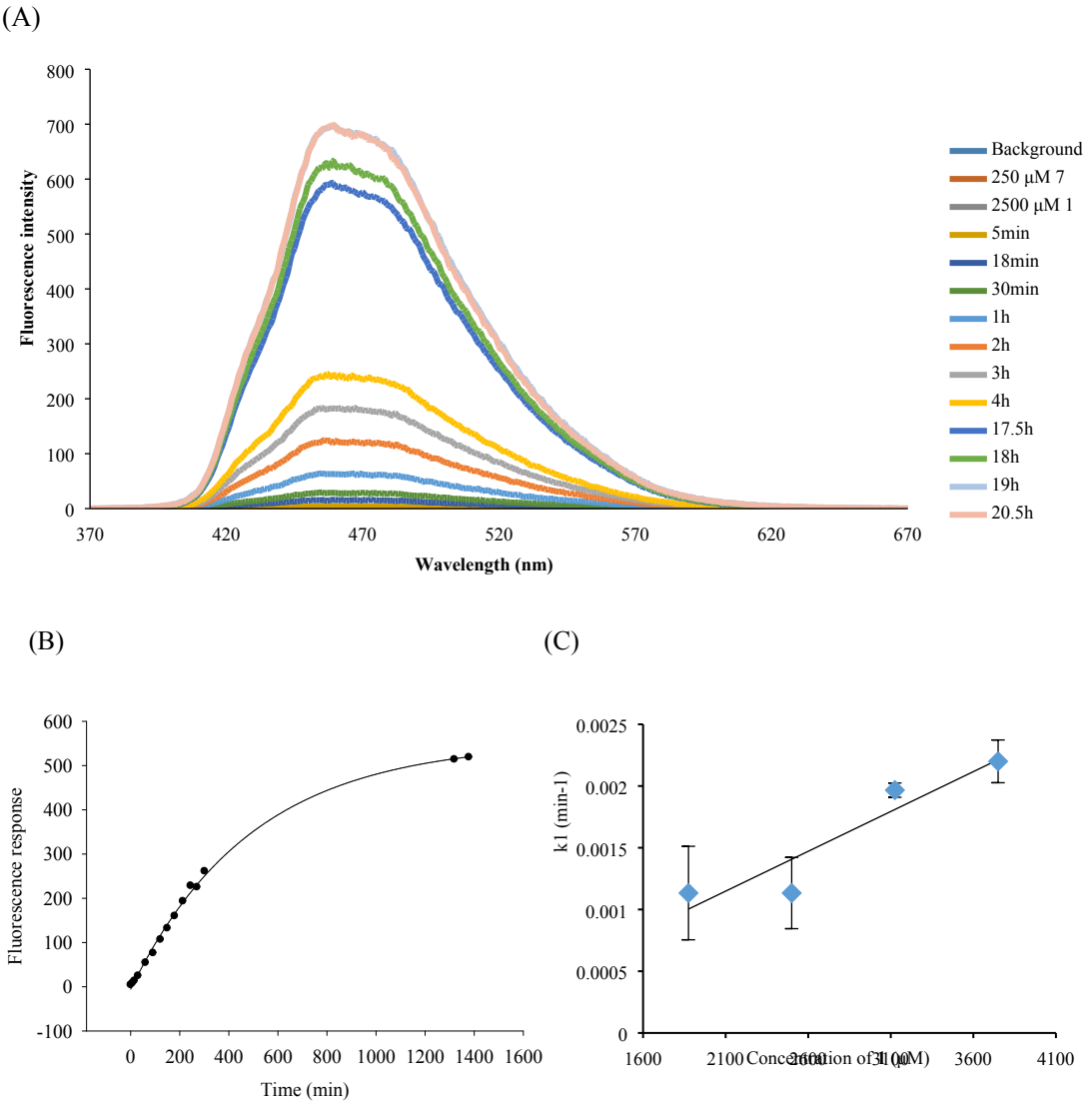


Figure S2 Kinetics studies of cycloaddition reaction between **7** and **1** by monitoring fluorescence changes. (A) Time-dependent fluorescence increases. Conditions: 250 μM **7** + 2500 μM **1** in 4:1 DMSO/PBS at 37 °C. (B) Example of pseudo-first reaction monitored by fluorescence increase. Conditions: 125 μM **7** + 3.125 mM **1** in 4:1 DMSO/PBS at 37 °C. λ_{ex}=350 nm, λ_{em}=470 nm; slit width: ex 3 nm, em 1.5 nm. (C) Linear plot of k_1 at different concentrations of **1**.

Determination of fluorescence quantum yields

Quinine sulfate (QS) in 0.1M H_2SO_4 water solution was chosen as the standard (ex: 350 nm, em: 400-600 nm). Literature[2] quantum yield: 0.54. The quantum yields of compound **7** and compound **21** were determined according to the equation:

$$\Phi_X = \Phi_{ST}\left(\frac{Grad_X}{Grad_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right)$$

Where Φ is the fluorescence quantum yield, the subscripts ST and X denote standard and test compounds respectively, *Grad* is the gradient from the plot of integrated fluorescence intensity versus UV-vis absorbance, and η is the refractive index of the solvent (1.3330 for water, 1.3284 for methanol). Excitation wavelength were chosen as UV-vis absorbance < 0.1 to avoid reabsorption effect.

Quantum yield determination of **7**:

Solutions of compound **7** at 0, 2, 4, 6, 8, 10 μM in methanol were prepared at room temperature. Each solution was tested for absorbance at 345 nm and fluorescence emission at 380-680 nm under 345 nm excitation (slit width: ex: 5, em: 3; fluorescence cuvette: 10 mm). Fluorescence responses were integrated in the same range to obtain AUC readings. Then AUC readings were plotted against absorbance readings to obtain $Grad_X$ as the slope of linear regression equation. $Grad_{ST}$ of QS were determined in the same manner using 0, 0, 1, 1, 2, 2 μM solutions instead (Figure S3). Calculated quantum yield for **7** was 0.006.

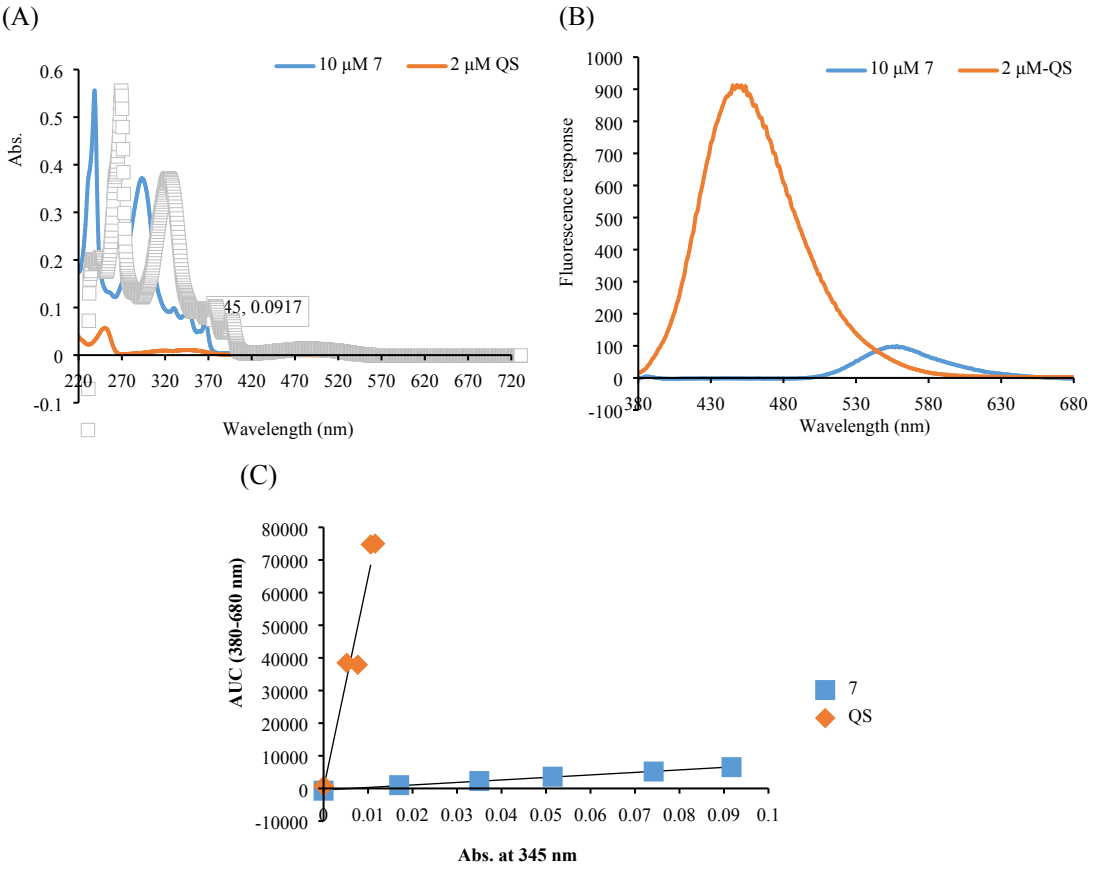


Figure S3 Quantum yield determination of **7** using QS as the reference. (A) UV-vis absorbance

spectrum. (B) Fluorescence response spectrum (λ_{ex}: 345 nm; slit width: ex: 5 nm, em: 3 nm; fluorescence cuvette: 10 mm). (C) Plot of AUC versus UV-vis absorbance (integration range: 380 nm to 680 nm).

Quantum yield determination of **21**:

Solutions of compound **21** at 0, 2, 4, 6, 8, 10, 12 μM in methanol were prepared at room temperature. Each solution was tested for absorbance at 364 nm and fluorescence emission at 380-620 nm under 364 nm excitation (slit width: ex: 3 nm, em: 1.5 nm; fluorescence cuvette: 10 mm). Fluorescence responses were integrated at the same range to obtain AUC readings. Then AUC readings were plotted against absorbance readings to get $Grad_X$ as the slope of linear regression equation. $Grad_{ST}$ of QS were determined in the same manner using 0, 5, 10, 15, 20, 25 μM solution instead (Figure S4). Calculated quantum yield for **21** is 0.138.

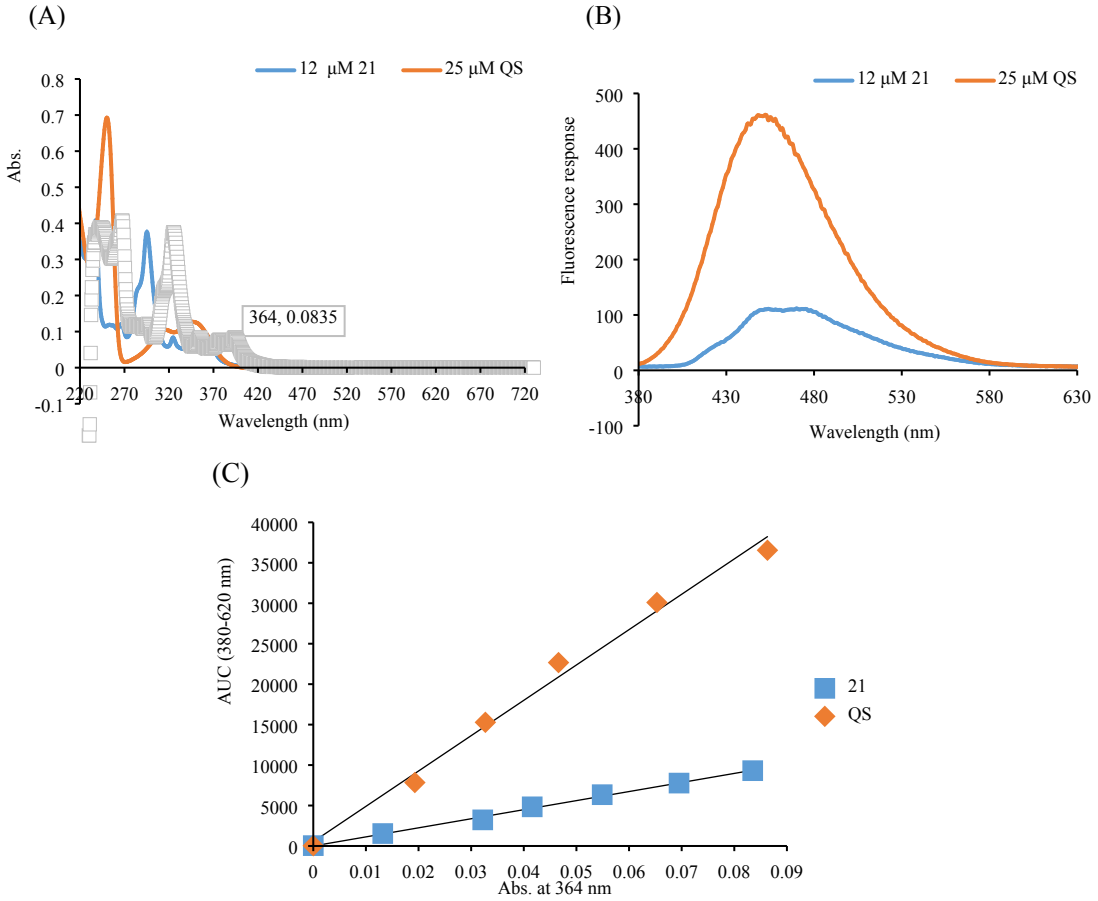


Figure S4. Quantum yield determination of **21** using QS as the reference. (A) UV-vis absorbance spectrum. (B) Fluorescence response spectrum (λ_{ex}: 364 nm; slit width: ex: 3 nm, em: 1.5 nm; fluorescence cuvette: 10 mm). (C) Plot of AUC versus UV-vis absorbance (integration range: 380 nm to 620 nm).

References

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