Supporting Information

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Regulation of Human Carbonic Anhydrase I (hCAI) Activity by a Photochromic Inhibitor

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Supporting Information

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1. Synthesis and characterization of new compounds

General. Thin layer chromatography (TLC) was performed on alumina plates coated with silica gel (Merck silica gel 60 F 254, layer thickness 0.2 mm). Column chromatography was performed on silica gel (70–230 mesh) from Merck. Starting materials were purchased from either Acros or Sigma-Aldrich and used without any further purification. Solvents were purchased from Aldrich and used without further purification except for dry THF, which was prepared by distillation from potassium. 1,2-Bis(5-carboxy-2-methylthien-3-yl)cyclopentene[1] and 2-(2-methoxyethoxy)-ethyamine[2] were prepared according to literature known procedures.

Techniques. Melting points (MP) were determined with a Büchi SMP 20. IR-spectra were recorded with a Bio-Rad FTS 2000 MX FT-IR spectrometer. NMR spectra were recorded on a Bruker Avance 400 (1H: 400.1 MHz, 13C: 100.6 MHz, T = 300 K) or a Bruker Avance 300 (1H: 300.1 MHz, 13C: 75.5 MHz, T = 300 K). The spectra are referenced against the NMR-solvent and chemical shifts are reported in ppm. The symbol “+” stands for an NMR signal with positive intensity in the DEPT 135 spectra indicating a CH or CH3 carbon. The symbol “−” stands for an NMR signal with negative intensity in the DEPT 135 spectra indicating a CH2 carbon. MS-Spectra were determined on a Varian CH-5 (EI), a Finnigan MAT 95 (Cl; FAB and FD) or a Finnigan MAT TSQ 7000 (ESI). UV–Vis absorption spectroscopy was performed using a Cary 50 Bio spectrophotometer.

Photochemistry. Standard hand-held lamps used for visualizing TLC plates (Herolab, 6 W) were used to carry out the ring-closing reactions at 312 nm. The ring-opening reactions were carried out using the light of a 200 W tungsten source that was passed through a 420 nm cut-off filter to eliminate higher energy light. The power of the light source is given based on the specifications supplied by the company when the lamps were purchased. A light detector was not used to measure the intensity during the irradiation experiments.
Scheme S1. Synthesis of starting material 3.

Synthesis of [(4-amino-benzyl)-tert-butoxycarbonylmethylamino]acetic acid tert-butyl ester (3). In a 250 mL round-bottom flask, a mixture of 4-aminobenzylamine (1.9 mL, 16.4 mmol), tert-butylbromoacetate (4.6 mL, 31.1 mmol, 1.9 equiv), K₂CO₃ (9.0 g, 65.5 mmol, 4.0 equiv), KI (5.4 g, 32.7 mmol, 2.0 equiv) and CH₃CN (100 mL) was heated at reflux for 25 h. After cooling to room temperature, the precipitate was filtered off washed with CH₃CN and the solvent was removed in vacuo. Purification by column chromatography (silica, EtOAc/petroleum ether 3:2, Rᵣ = 0.48) afforded 3.4 g (9.7 mmol, 59 %) of compound 3 as a pale yellow solid.

¹H-NMR (300 MHz, CDCl₃): δ [ppm] = 1.45 (s, 9H, CH₃), 3.38 (s, 4H, CH₂), 1.72 (br s, 2H, NH₂), 3.75 (s, 2H, CH₂), 6.62 (d, 3 J = 8.5 Hz, 2H, CH), 7.14 (d, 3 J = 8.2 Hz, 2H, CH); ¹³C-NMR (75 MHz, CDCl₃): δ [ppm] = 28.20 (+), 54.90 (–), 56.99 (–), 80.75 (Cₙ quat), 114.97 (+), 128.31 (Cₙ quat), 130.30 (+), 145.61 (Cₙ quat), 170.72 (Cₙ quat); CI-MS (NH₃): m/z (%) = 351.2 (100) [MH⁺]; PI-EI MS: calcd.: 350.2206, found: 350.2207; MP = 83 °C; FT-IR (ATR): ν [cm⁻¹] = 3462 (m), 3372 (m), 2976 (w), 2828 (w), 1731 (s), 1623 (m), 1518 (m), 1457 (w), 1423 (w), 1369 (m), 1283 (m), 1252 (m), 1213 (m), 1134 (s), 987 (m), 941 (w), 831 (m), 756 (w), 592 (m), 523 (m).
Scheme S2. Synthesis of photoresponsive DTE 1a.

Synthesis of (tert-butoxycarbonylmethyl-{4-[(5-methyl-4-{2-[2-methyl-5-(4-sulfamoyl-phenyl)carbamoyl]-thiophen-3-yl]-cyclopent-1-enyl}-thiophene-2-carbonyl)-amino]benzyl}-amino)acetic acid tert-butyl ester (5). *Diester (5).* In a 100 mL round-bottom flask, a solution of 1,2-bis(5'-carboxy-2'-methylthien-3'-yl)cyclopentene 2 (1.5 g, 4.3 mmol) in dry THF (20 mL) was treated with 4 drops of DMF followed by SOCl₂ (3.7 mL, 51.6 mmol, 4 equiv) drop-wise. The resulting solution was stirred at room temperature for 15 h, at which time the solvent was evaporated *in vacuo* and the solid residue was dried under high vacuum. This residue was dissolved in dry THF (50 mL) under an N₂ atmosphere and treated with Et₃N (2 mL, 14.3 mmol) in one portion, followed by a solution of [(4-aminobenzyl)-tert-butoxycarbonylmethylamino]acetic acid tert-butyl ester 3 (0.5 g, 1.4 mmol) in dry THF (20 mL) drop-wise over 4 h. After stirring at room temperature for 1.5 h, solid sulphanilamide 4 (1.5 g, 8.58 mmol) was added in one portion. After stirring at room temperature for an additional 42 h, the insoluble materials were filtered off, washed with THF and the filtrate was evaporated to dryness. The residue purified by column chromatography (flash silica, EtOAc/petroleum ether 3:2, Rₛ = 0.48) yielding 0.6 g (0.7 mmol, 49 %, according to the consumption of 3) of compound 5 as a brown solid.
$^{1}$H-NMR (300 MHz, CD$_2$Cl$_2$): $\delta$ [ppm] = 1.33–1.52 (m, 26H), 1.97–1.98 (m, 4H), 3.37 (s, 4H), 3.80 (s, 2H), 7.26 (d, $^{3}J$ = Hz, 2H), 7.47–7.77 (m, 10H), 8.54 (br s, 1H), 9.13 (br s, 1H); $^{13}$C-NMR (75 MHz, CD$_2$Cl$_2$): $\delta$ [ppm] = 13.06 (+), 13.77 (+), 22.98 (–), 38.00 (–), 54.28 (–), 56.41 (–), 120.79 (+), 121.31 (+), 128.13 (+), 130.49 (+), 134.05 (C$_{quat}$), 134.37 (C$_{quat}$), 134.66 (C$_{quat}$), 134.70 (C$_{quat}$), 135.28 (C$_{quat}$), 136.24 (C$_{quat}$), 136.47 (C$_{quat}$), 137.23 (C$_{quat}$), 137.50 (C$_{quat}$), 140.68 (C$_{quat}$), 141.54 (C$_{quat}$), 141.86 (C$_{quat}$), 159.61 (C$_{quat}$), 159.87 (C$_{quat}$), 166.86 (C$_{quat}$), 170.60 (C$_{quat}$); MP = 160–165 °C; ES-MS (DCM/MeOH + 10 mmol/L NH$_4$Ac): m/z (%) = 835.3 (100) [M+H]$^+${; IR (KBr-pellet): $\nu$ [cm$^{-1}$] = 3350 (m), 2958 (w), 2919 (m), 2849 (m), 2361 (m), 1735 (s), 1647 (s), 1594 (m), 1522 (s), 1449 (w), 1400 (w), 1315 (s), 1247 (m), 1199 (w), 1157 (s), 1098 (w), 1024 (m), 911 (w), 807 (w), 669 (s), 574 (w), 542 (m), 429 (w); PL LIMS (CH$_2$Cl$_2$/NBA): calcd.: 835.2869 found: 835.2885.

Synthesis of (carboxymethyl-{4-[5-methyl-4-{2-[2-methyl-5-(4-sulfamoylphenyl)-carbamoyl]-thiophen-3-yl]-cyclopent-1-enyl}thiophene-2-carbonyl)amino]benzyl)amino)-acetic acid hydrochloride (10). Diacid (10). In a 50 mL round-bottom flask, a solution of diester 5 (150 mg, 0.18 mmol) in CH$_2$Cl$_2$ (5 mL) was cooled to 0 °C and treated with a saturated solution of HCl in Et$_2$O (2 mL). After stirring the mixture at room temperature for 140 min, the precipitate was collected by vacuum filtration and washed several times with CH$_2$Cl$_2$ and dried yielding 92 mg (0.12 mmol, 67 %) of diester 10 as a colorless solid, which was used without further purification.

$^{1}$H-NMR (300 MHz, MeOD): $\delta$ [ppm] = 1.97 (s, 3H), 1.99 (s, 3H), 2.15 (quintet, $^{3}J$ = 7.4 Hz, 2H), 2.90 (t, $^{3}J$ = 7.3 Hz, 4H), 4.16 (s, 4H), 4.53 (s, 2H), 7.49 (d, $^{3}J$ = 8.5 Hz, 2H), 7.75–7.85 (m, 8H); ES-MS (DCM/MeOH + 10 mmol/L NH$_4$Ac): m/z (%) = 721.1 (100) [M–H]$^+${; MP = 163–167 °C.

Synthesis of copper complex 1a. In a 10 mL round-bottom flask, a solution of compound 10 (133 mg, 0.17 mmol) in a mixture of CH$_3$OH (5 mL) and CH$_3$CN (2 mL) was treated with NaHCO$_3$ (44 mg, 0.52 mmol, 3 equiv) and stirred at 50 °C for 10 min. A solution of CuCl$_2$ (29
mg, 0.17 mmol, 1 equiv) in CH$_3$OH (0.5 mL) was added at 50 °C and the resulting mixture was stirred at this temperature for 24 h. The precipitate that formed was collected by centrifugation and purified by washing the solid pellet in CH$_3$OH. The solid was again collected by centrifugation. This purification step was repeated 3 times yielding 92 mg (0.12 mmol, 71 %) of compound 1a as a light blue solid. **ES-MS (H$_2$O/THF 1:1):** m/z (%) = 782.2 (100) [M–H]$^+$; **UV (DMSO):** $\lambda_{\text{max}}$ = 294 nm (35000); **MP > 200 °C.**

**Scheme S3.** Synthesis of bis(sulphonamide) 6a, bis(IDA) 7a and bis(glycol) 8a.

**Synthesis of 4,4’-(cyclopentene-1,2-diyl)bis(5-methyl-N-(4-sulfamoylphenyl)thiophene-2-carboxamide) (6).** **Bis(sulphonamide) 6.** In a 100 mL round-bottom flask, a solution of 1,2-bis(5’-carboxy-2’-methylthien-3’-yl)cyclopentene 2 (0.5 g, 1.4 mmol) in dry THF (10 mL) was treated with SOCl$_2$ (1.9 mL, 26.6 mmol) dropwise, followed by DMF (4 drops). The solution was
stirred for 1 h at which time the solvent was evaporated and the residue dried in vacuo. The solid residue was dissolved in dry THF (10 mL) under an N₂ atmosphere and treated with Et₃N (1.8 mL, 4.5 mmol) in one portion, followed by a solution of sulphanilamide 4 in dry THF (20 mL), which was added drop-wise over 10 min. After stirring the mixture for 48 h, the solvent was removed in vacuo and the resulting residue was suspended in a mixture of H₂O and EtOH (3:56 mL:mL). The mixture was heated at reflux for 1 h, at which time the solid was collected by vacuum filtration, washed with water and air-dried yielding 0.4 g (0.6 mmol, 43 %) compound 6 as a colorless solid. 

**¹H-NMR (300 MHz, DMSO):** δ [ppm] = 1.90 (s, 6H), 2.07–2.14 (m, 2H), 2.86 (t, ³J = 7.5 Hz, 4H), 7.27–7.28 (m, 4H), 7.28 (br s, 4H), 7.79 (d, ³J = 8.8 Hz, 4H), 7.88 (d, ³J = 9.0 Hz, 4H), 7.91 (s, 2H), 10.38 (br s, 2H); 

**¹³C-NMR (75 MHz, DMSO):** δ [ppm] = 14.29 (+), 30.29 (–), 46.66 (–), 119.62 (+), 126.50 (+), 130.34 (+), 134.22 (Cquat), 135.15 (Cquat), 136.33 (Cquat), 138.52 (Cquat), 140.92 (Cquat), 141.64 (Cquat), 159.78 (Cquat); 

**FT-IR (ATR):** ν [cm⁻¹] = 3331 (m), 3107 (w), 1650 (m), 1591 (m), 1524 (s), 1456 (w), 1400 (w), 1315 (s), 1249 (m), 1148 (s), 1098 (w), 883 (w), 836 (w), 665 (m), 576 (m), 540 (m); 

**ES-MS (DCM/MeOH + 10 mmol/L NH₄Ac):** m/z (%) = 657.1 (46) [M+H]⁺, 674.2 (100) [M+H+NH₄]⁺, 655.2 (100) [M–H]⁻, 691.2 (30) [M+Cl]⁺, 715.3 (22) [M+CH₃COO]⁺; 

**PI LSIMS (MeOH/ CH₂Cl₂/ NBA):** calcd.: 656.0891 found: 656.0997; MP > 200 °C; 

**UV (DMSO):** λmax = 285 nm (21900).

**Synthesis of** [(4-{4-[2-(5-{4-[bis-tert-butoxycarbonylmethylamino)methyl]phenyl-carbamoyl}-2-methylthiophen-3-yl)cyclopent-1-enyl]-5-methylthiophene-2-carbonyl]-amino)benzyl]-tert-butoxycarbonylmethylamino)acetic acid tert-butyl ester (11).**

**Tetraester 11.** In a 100 mL round-bottom flask, a solution of 1,2-bis(5'-carboxy-2'-methylthien-3'-yl)cyclopentene 2 (0.5 g, 1.43 mmol) in dry THF (10 mL) was treated with SOCl₂ (1.9 mL, 26.6 mmol) drop-wise, followed by 4 drops DMF. The resulting solution was stirred for 1 h at room temperature, at which time the solvent was evaporated and the residue dried in vacuo. The residue was redissolved in dry THF (10 mL) under an N₂ atmosphere and treated with 1.8 mL of Et₃N (4.5 mmol) in one portion, followed by a solution of [(4-aminobenzyl)-tert-
butoxycarbonylmethylamino]acetic acid tert-butyl ester 3 (2.8 g, 8 mmol) in THF (10 mL), which was added drop-wise over 1 h. The resulting reaction mixture was stirred overnight and after this period, H₂O (50 mL) was added. The mixture was extracted with EtOAc (3 x 50 mL) and the combined organic phases were further extracted with a saturated NaHCO₃ solution (2 x 20 mL). The combined organic phases were dried (Na₂SO₄), filtered and evaporated. Purification by column chromatography (flash silica, EtOAc/petroleum ether 8:2, Rᵣ = 0.9) yielded 0.8 g (0.8 mmol, 55 %) of compound 11 as a pale brown solid. **¹H-NMR (300 MHz, CDCl₃):** δ [ppm] = 1.44 (s, 36H), 1.85–2.02 (m, 8H), 2.65 (t, 3J = 7.3 Hz, 4H), 3.71 (s, 8H), 3.82 (s, 4H), 7.31 (d, 3J = 8.5 Hz, 4H), 7.35 (s, 2H), 7.53 (d, 3J = 8.5 Hz, 4H), 8.08 (br s, 2H); **¹³C-NMR (75 MHz, CDCl₃):** δ = 14.80 (+), 22.48 (−), 28.19 (+), 38.71 (−), 54.98 (−), 56.96 (−), 79.95 (Cquat), 120.18 (+), 129.70 (+), 130.15 (+), 133.54 (Cquat), 133.64 (Cquat), 133.72 (Cquat), 135.52 (Cquat), 139.86 (Cquat), 159.04 (Cquat), 169.59 (Cquat); **FT-IR (ATR):** ν [cm⁻¹] = 3298 (w), 2975 (m), 2924 (w), 1727 (s), 1626 (m), 1599 (m), 1527 (s), 1455 (m), 1410 (m), 1366 (m), 1248 (w), 1216 (w), 1139 (s), 988 (m), 880 (w), 815 (w), 742 (w), **ES-MS (DCM/MeOH + 10 mmol/l NH₄Ac):** m/z (%) = 507.4 (77) [M+2H]^+, 1013.6 (100) [M+H]^+, 1035 (10) [M+Na]^+; **PI LSIMS (MeOH/CH₂Cl₂/NBA):** calcd.: 1012.4689 found: 1012.4817; **MP** = 149–150 °C.

**Synthesis of **{[4-{4-[2-(5-{4-[[(Bis-carboxymethyl-amino)-methyl]-phenylcarbamoyl}-2-methyl-thiophen-3-yl]-cyclopent-1-enyl]-5-methyl-thiophene-2-carbonyl]-amino}-benzyl]-carboxymethyl-amino]-acetic acid hydrochloride (12). **Tetraacid 12.** In a 50 mL round-bottom flask a solution of tetraester 11 (0.2 g, 0.2 mmol) in CH₂Cl₂ (10 mL) was treated with a saturated solution of HCl in Et₂O (4 mL) at 0°C. After the addition of the acid, the cool bath was removed and the mixture was stirred at room temperature for 16 h at which time the solvent was evaporated to dryness to yield 0.1 g (0.12 mmol, 60 %) of compound 12 as a colorless solid. **¹H-NMR (DMSO, 300 MHz):** δ [ppm] = 1.91 (s, 6H), 2.02–2.12 (m, 2H), 2.85 (t, 3J = 7.3 Hz, 4H), 4.02 (s, 8H), 4.33 (s, 4H), 7.47 (d, 3J = 8.5 Hz, 4H), 7.85 (d, 3J = 8.8 Hz, 4H), 8.12 (s, 2H), 10.49
FT-IR (ATR): $v$ [cm$^{-1}$] = 2922 (w), 2854 (w), 1730 (s), 1595 (m), 1518 (s), 1414 (m), 1319 (m), 1246 (m), 1069 (w), 939 (w), 881 (w), 817 (m), 741 (w), 660 (w), 597 (w);

ES-MS (DCM/MeOH + 10 M NH$_4$Ac): m/z (%) = 789.3 (38) [M+H]$^+$, 393.3 (5) [M–2H]$^{2-}$, 787.4 (100) [M–H]$^-$; MP > 200°C.

**Synthesis of the bis(copper) complex 7a.** In a 50 mL round-bottom flask, a suspension of tetraacid 12 (0.14 g, 0.2 mmol) in a mixture of CH$_3$OH and H$_2$O (1:1 v/v, 30 mL) was heated to reflux and treated, at this temperature, with solid NaHCO$_3$ (90 mg, 1 mmol, 6 equiv) in one portion, followed by CuCl$_2$ (60 mg, 0.3 mmol, 2 equiv). After the reaction was heated at reflux for 2 h, the blue precipitate was collected by centrifugation and the blue solid washed with H$_2$O (20 mL), CH$_3$CN (20 mL) and dried yielding compound 7a (0.13 g, 0.15 mmol, 88 %) as a blue solid.

ES-MS (CH$_3$OH + 10 mM NH$_4$OAc): m/z (%) = 911 (100) [M–H]$^+$, 971 (35) [M+CH$_3$COO]$^+$; FT-IR (ATR): $v$ [cm$^{-1}$] = 1595 (s), 1518 (s), 1441 (w), 1412 (w), 1375 (m), 1317 (m), 1249 (m), 1088 (w), 1010 (w), 855 (w), 817 (w), 740 (w), 528 (w); MP > 200 °C; UV (DMSO): $\lambda_{\text{max}}$ = 290 nm (44000).

**Synthesis of 4,4'-(cyclopentene-1,2-diyl)bis(N-(2-(2-methoxyethoxy)ethyl)-5-methylthiophene-2-carboxamide) (8a). Bis(glycol) 8a.** In a 100 mL round-bottom flask, a solution of 1,2-bis(5'-carboxy-2'-methylthien-3'-yl)cyclopentene 2 (0.4 g, 1.1 mmol) in a 1:1 (v/v) mixture of CH$_2$Cl$_2$ and THF (10 mL) was treated with a catalytic amount of DMF (4 drops), followed by a drop-wise addition of SOCl$_2$ (0.3 mL, 0.5 g, 4.52 mmol, 4 equiv). The mixture was stirred at room temperature and the conversion monitored by TLC. After 3 h, the solvent was evaporated in vacuo, the residue was dried under high vacuum and dissolved in THF (20 mL) and treated with pyridine (0.4 mL, 0.35 g, 4.52 mmol, 4 equiv) in one portion, followed by 2-(2-methoxyethoxy)ethylamine (0.4 g, 3.4 mmol, 3 equiv). The resulting red mixture was stirred for 15 h at room temperature at which time the solvent was removed by evaporation. Purification by column chromatography (flash silica, EtOAc/5% CH$_3$OH, $R_f$ = 0.16) afforded 0.3 g (0.6 mmol, 50
% of compound 8a as a colorless oil. $^{1}H$-NMR (300 MHz, CDCl$_3$): $\delta$ [ppm] = 1.89 (s, 6H), 1.99–2.09 (m, 2H), 2.76 (t, $^3J = 7.5$ Hz, 4H), 3.38 (s, 6H), 3.53–3.65 (m, 16H), 6.44 (br s, 1H), 7.22 (s, 2H); $^{13}C$-NMR (75 MHz, CDCl$_3$): $\delta$ [ppm] = 14.70 (+), 22.87 (–), 38.56 (–), 39.54 (–), 59.00 (+), 69.79 (–), 70.13 (–), 71.83 (–), 129.29 (+), 134.45 (C quat), 134.65 (C quat), 136.25 (C quat), 139.86 (C quat), 161.84 (C quat); ES-MS (DCM/CH$_3$OH + 10 mM NH$_4$OAc): m/z (%) = 551.2 (100%) [M+H]$^+$, 568.2 (10 %) [M+NH$_4$]$^+$; FT-IR (ATR): $\nu$ [cm$^{-1}$] = 3345 (m), 2863 (m), 1646 (s), 1559 (w), 1528 (m), 1457 (w), 1427 (w), 1349 (w), 1300 (s), 1246 (m), 1197 (w), 1132 (m), 1088 (s), 1027 (w), 930 (w), 896 (w), 853 (m), 789 (m), 753 (m), 659 (m), 607 (s), 436 (w); PI-EIMS: calcd.: 551.2171, found: 551.2164; UV (DMSO): $\lambda_{\text{max}}$ = 260 nm (32759).

Scheme S4. Photochemical ring-closing reactions of compounds 1a, 6a, 7a and 8a.

Photochemical synthesis of the ring-closed isomer 1b. A solution of compound 1a (0.5 mg, 0.0007 mmol) in DMSO (1 mL) was irradiated for 15 min with a 312 nm lamp in an HPLC vial.
yielding a purple solution containing the ring-closed isomer 1b (> 99 %) according to HPLC (NH₄OAc buffer).

Photochemical synthesis of the ring-closed isomer 6b. A solution of compound 6a (0.5 mg, 0.0008 mmol) in DMSO (1 mL) was irradiated for 15 min with a 312 nm lamp in an HPLC vial yielding a purple solution containing the ring-closed isomer 6b (> 99 %) according to HPLC (NH₄OAc buffer).

Photochemical synthesis of the ring-closed isomer 7b. A solution of compound 7a (0.5 mg, 0.0005 mmol) in DMSO (1 mL) was irradiated for 15 min with a 312 nm lamp in an HPLC vial yielding a purple solution of the ring-closed isomer (> 99 %) according to HPLC (NH₄OAc buffer).

Photochemical synthesis of the ring-closed isomer 8b. A solution of compound 8a (3 mg, 0.005 mmol) in CD₂Cl₂ (0.7 mL) was irradiated for 30 min with a 312 nm lamp in a NMR tube light yielding a red solution of a photostationary state containing 91% of the ring-closed isomer according to the ¹H-NMR spectroscopy. The remaining 9% were assigned to the ring-open isomer 8a. ¹H-NMR (300 MHz, CD₂Cl₂): δ [ppm] = 1.87 (quintet, ³J = 7.5 Hz, 2H), 2.00 (s, 6H), 2.78 (t, ³J = 7.4 Hz, 4H), 3.41 (s, 6H), 3.54–3.66 (m, 16H), 6.27 (br s, 2H), 6.65 (s, 2H).
2. Photochromism

2.1 Determination of the photostationary state (PPS) of DTE 1.

Figure S1. Representative HPLC chromatogram for the determination of the PSS of compound 1. The chromatograms of isomer 1a (upper) and the solution of 1b (lower) generated with 312 nm light are shown. The entire peak for 1a disappears and a new one appears at a lower retention time. The small peak appearing at 48 min is attributed to a photo-generated side product. The conversion from the ring-open to the ring-closed isomer is > 99 %.
2.2 Photochemical cycling studies.

a) Bivalent inhibitor 1.

**Figure S2.** Modulated absorbance (abs.) at 294 nm (open squares = 1a) and 545 nm (black circles = 1b) during alternate irradiation of a DMSO (1.08 x 10^{-5} M) solution of 1a with 312 nm light for 34 s, and greater than 420 nm light for 6 min.
b) Bis(sulphonamide) 6.

**Figure S3.** Modulated absorbance (abs.) at 285 nm (open squares = 6a) and 550 nm (black circles = 6b) during alternate irradiation of a DMSO (1.00 x 10^-5 M) solution of 6a with 312 nm light for 26 s, and greater than 420 nm light for 10 min.
c) Bis(IDA) 7.

**Figure S4.** Modulated absorbance (abs.) at 285 nm (open squares = 7a) and 545 nm (black circles = 7b) during alternate irradiation of a DMSO (1.0 x 10^{-5} M) solution of 7a with 312 nm light for 40 s, and greater than 420 nm light for 5 min.
d) bis(glycol) 8.

Figure S5. Modulated absorbance (abs.) at 280 nm (open squares = 8a) and 545 nm (black circles = 8b) during alternate irradiation of a DMSO (5.52 \times 10^{-5} \text{ M}) solution of 8a with 312 nm light for 85 s, and greater than 420 nm light for 16 min.
3. Enzyme activity assay (EC 4.2.1.1).

3.1 Materials and methods

<table>
<thead>
<tr>
<th>stock solutions</th>
<th>code</th>
</tr>
</thead>
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<tr>
<td>Tris sulfate buffer 20 mM, pH 8.3 at 0°C</td>
<td>A</td>
</tr>
<tr>
<td>([Phenol red] = 5.6 x 10^{-4} M)</td>
<td></td>
</tr>
<tr>
<td>distilled water</td>
<td>B</td>
</tr>
<tr>
<td>CO₂ water (2.46 x 10^{-3} M)(^\text{[a]})</td>
<td>C</td>
</tr>
<tr>
<td>enzyme solution (3.33 x 10^{-6} M)(^\text{[b]})</td>
<td>D</td>
</tr>
</tbody>
</table>

\(^\text{[a]}\) Prepared by bubbling CO₂ from a gas bottle through 200 mL distilled water at 0°C for 10 min. The final concentration was determined by precipitation with an aqueous 0.1 M Ba(OH)\(_2\) solution and back-titration with 1 N HCl solution.\(^\text{[b]}\) Diluted from a 3.33 x 10^{-5} M stock solution.

<table>
<thead>
<tr>
<th>blank samples(^\text{[a]})</th>
<th>test samples(^\text{[a]})</th>
</tr>
</thead>
<tbody>
<tr>
<td>solution vol (mL)</td>
<td>solution vol (mL)</td>
</tr>
<tr>
<td>A 3.0</td>
<td>A 3.0</td>
</tr>
<tr>
<td>B 0.05</td>
<td>D 0.05</td>
</tr>
<tr>
<td>C 1.0</td>
<td>B 1.0</td>
</tr>
</tbody>
</table>

\(^\text{[a]}\) Stirred at 100 rpm.

The enzyme activity was determined using the following equation:

$$\frac{t_{\text{blank}} - t_{\text{test}}}{t_{\text{test}}}$$

The binding affinity was determined using the Cheng-Prusoff equation:

$$K_i = \frac{IC_{50}}{l = \left(\frac{C_{\text{substrate}}}{K_m}\right)}$$
3.2 Results.

a) Bis(sulphonamide) 6.

![Figure S6. Inhibition measurements of bis(sulphonamide) 6 compared with sulphanilamide 4.]

<table>
<thead>
<tr>
<th>inhibitor</th>
<th>IC_{50} (μM)</th>
<th>K (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ring-open</td>
<td>ring-closed</td>
<td>ring-open</td>
</tr>
<tr>
<td>4</td>
<td>0.46 ± 0.01</td>
<td>0.29 ± 0.007</td>
</tr>
<tr>
<td>6</td>
<td>0.53 ± 0.007</td>
<td>0.57 ± 0.01</td>
</tr>
</tbody>
</table>

b) Bis(IDA) 7.

![Figure S7. Inhibition measurements of bis(IDA) 7 compared with sulphanilamide 4.]

<table>
<thead>
<tr>
<th>inhibitor</th>
<th>IC_{50} (μM)</th>
<th>K (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ring-open</td>
<td>ring-closed</td>
<td>ring-open</td>
</tr>
<tr>
<td>4</td>
<td>0.46 ± 0.01</td>
<td>0.29 ± 0.007</td>
</tr>
<tr>
<td>7</td>
<td>1.55 ± 0.8</td>
<td>1.46 ± 0.15</td>
</tr>
</tbody>
</table>

References.
