Electronic Supplementary Material (ESI) for ChemComm.

A red-shifted photochromic sulfonylurea for the remote control of pancreatic beta cell function

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EXPERIMENTAL PROCEDURES

Chemistry

Solvents for chromatography and reactions were purchased in HPLC grade or distilled over an appropriate drying reagent prior to use. If necessary, solvents were degassed either by freeze-pump-thaw or by bubbling N_2 through the vigorously stirred solution for several min. Unless otherwise stated, all other reagents were used without further purification from commercial sources.

Flash column chromatography was carried out on silica gel 60 (0.040–0.063 mm) purchased from Merck. Reactions and chromatography fractions were monitored by thin layer chromatography (TLC) on Merck silica gel 60 F254 glass plates. The spots were visualized under UV light at $\lambda = 254$ nm.

NMR spectra were recorded in deuterated solvents on a BRUKER Avance III HD 400 (equipped with a CryoProbe™) instrument and calibrated to residual solvent peaks ($^{1}H/^{13}C$ in ppm): DMSO-d_6 (2.50/39.52). Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, br = broad, m = multiplet. Spectra are reported based on appearance, not on theoretical multiplicities derived from structural information.

Low- and high-resolution electrospray (ESI) mass spectra were obtained on a Varian MAT 711 MS instrument operating in either positive or negative ionization modes.

UV/Vis spectra and time-resolved light-dependent **JB558** activity were recorded on a Varian Cary 50 Bio UV-Visible Spectrophotometer using Helma SUPRASIL precision cuvettes (10 mm light path) equipped with an UHP-LED-520 (Prizmatic) connected to an AC/DC switching adapter (Mean Well Enterprises).

LC-MS was performed on an Agilent 1260 Infinity HPLC System, MS-Agilent 1100 Series, Type: 1946D, Model: SL, equipped with a Agilent Zorbax Eclipse Plus C18 (100 x 4.6 mm, particle size 3.5 micron) RP column with a constant flow-rate of 2 mL/min.

Melting points were measured on the apparatus BÜCHI Melting Point B-540 from *BÜCHI Labortechnik AG* or on an EZ-Melt apparatus from *Stanford Research Systems* and are uncorrected.

Infrared spectra (IR) were recorded on a PERKIN ELMER Spectrum BX-59343 instrument as neat materials. For detection a SMITHS DETECTION DuraSam-plIR II Diamond ATR sensor was used. The measured wave numbers are reported in cm^{-1}.

Whole-cell electrophysiology in HEK293T cells

HEK293T cells were incubated at 37 °C (10% CO_2) in Dulbecco´s Modified Eagle Medium (DMEM) + 10% FBS and were split at 80-90% confluency. Detached cells were diluted in growth medium and plated on acid-etched coverslips coated with poly-*L*-lysine in a 24 well plate. 50,000 cells were added to each well in 500 µL standard growth medium along with the DNA (per coverslip: 250 ng Kir6.2, 350 ng SUR1, 50 ng YFP) and JetPRIME transfection reagents according to the manufacturer's instructions (per coverslip: 50 µL JetPRIME buffer, 0.5 µL JetPRIME transfection reagent). The transfection medium was exchanged for normal growth media 4 hr after transfection and electrophysiological experiments were carried out 20-40 hr later. Whole cell patch clamp experiments were performed using a standard electrophysiology setup equipped with a HEKA Patch Clamp EPC10 USB amplifier and PatchMaster software (HEKA Electronik). Micropipettes were generated from “Science Products GB200-F-8P with filament” pipettes using a Narishige PC-10 vertical puller. The patch pipette resistance varied between 3-9 MΩ. The bath solution contained in mM: 3 KCl, 118 NaCl, 25 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES (NaOH to pH 7.4). The pipette solution contained in mM: 90 K-gluconate, 10 NaCl, 10 KCl, 1 $MgCl_2$, 10 EGTA, 60 HEPES (KOH to pH 7.3). Compounds were applied by direct pipette of 1 µL DMSO stock (43.4 mM or 4.34 mM) directly into the recording bath. Voltage clamp measurements were carried out at room temperature with a holding

potential of –60 mV. Compound switching was evaluated at λ = 520 nm using a UH-LED-520 light source and fibre optic placed ~1cm from the sample.

Isolation of rodent islets

C57BL6 mice were maintained in a specific pathogen-free facility under a 12 h light–dark cycle with ad libitum access to water and food. Euthanasia was carried out using a designated schedule-1 method and collagenase solution (1 mg/mL) injected into the bile duct to inflate the pancreas, before isolation of islets using Histopaque gradient separation. Islets were cultured overnight in solution containing Roswell Park Memorial Institute (RPMI) medium supplemented with 10% foetal calf serum and 100 U/mL penicillin & streptomycin 100 µg/mL. All procedures were regulated by the Home Office according to the Animals (Scientific Procedures) Act 1986 of the United Kingdom (PPL 70/7349).

Isolation of human islets

Islets were isolated with necessary local and national ethical permissions, including consent from the next of kin, and cultured as above except for further supplementation with 0.25 mg/mL fungizone. All studies involving human tissue were approved by the National Research Ethics Committee London (Fulham), REC #07/H0711/114.

Calcium and Epac2 imaging

Islets were loaded with Fluo2-AM (10 µM) for 45 min before performing functional multicellular Ca^{2+} imaging (fMCI), as previously described.[1, 2] Briefly, pulsed λ = 491 nm light was delivered using a solid state laser (Cobalt) coupled to a Nipkow spinning disk head (Yokogawa CSU-10) and a Zeiss Axiovert M200 equipped with a 10x/0.3 NA objective (EC Plan-Neofluar, Zeiss). Emitted signals were captured from ʎ = 500-550 nm using a Hammamatsu ImageEM 9100-13 EM-CCD camera. Illumination to induce photoswitching was performed using a λ = 561 ± 5 laser coupled to the microscope with a multimodal fibre optic. Likewise, Epac2 imaging was performed as described,[2, 3] using HEK293T cells transiently transfected with full length Epac2-camps (a kind gift from Prof. Jin Zhang, Johns Hopkins University),[4] and emission filters centred on Cerulean (λ = 470 nm) and Venus (λ = 530 nm) (λ excitation = 440 nm). FRET was calculated as the ratio of Cerulean:Venus fluorescence and signals normalized using R/R_{min}, where R_{min} is the minimum ratio. Finally, Fura-2 (10 µM)-loaded islets were imaged using a monochromator set to alternately excite at 340 nm and 380 nm, with detection at ʎ = 500-550 nm using an Andor Zyla 5.5 sCMOS. Ca^{2+} signals were presented as F340/F380, where F340 and F380 represent fluorescence intensity at 340 nm and 380 nm, respectively. In all cases, HEPES-bicarbonate buffer was used, containing in mM: 120 NaCl, 4.8 KCl, 24 $NaHCO_3$, 0.5 Na_2HPO_4, 5 HEPES, 2.5 $CaCl_2$, 1.2 $MgCl_2$ and 5 D-glucose. Drugs were applied via the perfusion system, as for electrophysiological recordings.

Cytotoxicity assays

DMSO- or **JB558**-treated islets were incubated with 3 µM of calcein-AM (live) and 2.5 µM of propidium iodide (dead), and absorbance/emission detected at λ = 491/525 nm (calcein) and λ = 561/620 nm (propidium iodide). The area of dead:live cells was calculated as a unitary ratio.

Insulin secretion assays

Batches of six islets were incubated for 1 hr in 0.5 mL of Krebs-HEPES-bicarbonate solution containing in mM: 130 NaCl, 3.6 KCl, 1.5 $CaCl_2$, 0.5 $MgSO_4$, 0.5 NaH_2PO_4, 2 $NaHCO_3$, 10 HEPES, 5 D-glucose and 0.1% (wt/vol) bovine serum albumin, pH 7.4. Treatments were applied as indicated and λ = 560 nm delivered as pulses using a BMG Fluostar Optima platereader. Insulin concentrations in the supernatant were measured using a proprietary Cisbio HTRF assay according to the manufacturer's instructions.

[3H]-Glibenclamide radioassay

HEK293T cells expressing SUR1 were washed twice in assaying buffer, before incubation for 50 min with [3H]-glibenclamide and a concentration series of either glimepiride or **JB558**. Incubation was terminated by rapid filtration through Whatman GF/C filters subsequent to washing. Radioactivity was counted 6 hr after cell and filter lysis in 200 mL Rotiszint EcoPlus using a scintillation counter.

AzoReductase assays

AzoReductase (EC 1.7.1.6) catalyses the reductive cleavage of an azobenzene diazene bond into the corresponding anilines.[5] *Escherichia coli* (BL21 DE3) cells served as a model system as they are known to carry azoreductase (Supplementary Fig. 5).[6]

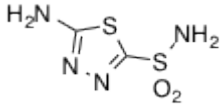
Escherichia coli (BL21 DE3) cells were transformed with pcDNA3.1 (Amp resistance) and grown in LB media with ampicillin to an OD_{600} = 1.0. Methyl red (MR) or **JB558** was added from a 50 mM stock solution in DMSO to a final concentration of 50 μM with or without bacteria. DMSO alone served as control. Solutions were inoculated at 37 °C and 180 rpm and aliquots (100 μL) were taken every 3 min and transferred into Eppendorf tubes. Eppendorf tubes were centrifuged for 1 min (4 °C and 12,000 rpm) and then stored immediately on ice for the duration of the experiment. The supernatant of each Eppendorf tube was transferred into a well of a 96-well plate and absorbance was measured at the respective wavelength with a plate reader. After 27 min, MR was not detectable anymore, assuming azoreductase performed complete diazene cleavage.

Statistics

Multifactorial comparisons were made using one-way ANOVA followed by Bonferonni's post-hoc test. Dose-response curves were fitted using a sigmoid equation (SD attributable to the unexplained variance = 52 cpm). All analyses were conducted using Graphpad Prism (Graphpad Software) and results deemed significant at $P<0.05$.

SYNTHESIS

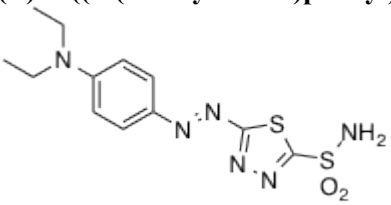
5-Amino-1, 3, 4-thiadiazole-2-sulfonamide (2)



N-(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide (acetazolamide) (3.00 g, 13.5 mmol, 1.0 eq.) was dissolved in 3 M HCl (23.4 mL) and refluxed at 110 °C for 3 h. The mixture was neutralized with 4 M NaOH (17.6 mL) and extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (1 x 200 mL) and concentrated *in vacuo*. 2.18 g (12.1 mmol) of the desired product were obtained in 90% yield as a white fluffy powder.

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 8.08 (br s, 2H), 7.90 (s, 2H).
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 171.7, 157.9.
HRMS (ESI): calc. for $C_2H_5N_4O_2S_2^+$ $[M+H]^+$: 180.9848, found: 180.9849.
R_t (LCMS; MeCN/H_2O/formic acid = 10/90/0.1 → 90/10/0.1 over 7 min) = 0.780 min.
UV/Vis (LCMS): λ_{max} = 278 nm.
IR (ATR): wave number/cm^{-1} = 3396, 3310, 3169, 1684, 1600, 1563, 1492, 1443, 1346, 1176, 1138, 1080, 974, 917 801.
m.p. = 202 °C

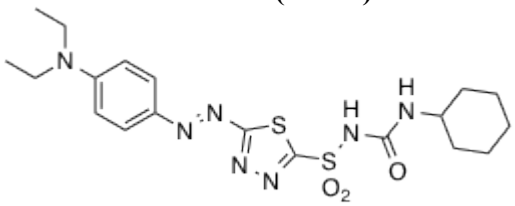
(*E*)-5-((4-(Diethylamino)phenyl)diazenyl)-1, 3, 4-thiadiazole-2-sulfonamide (3)



2 (800 mg, 4.44 mmol, 1.0 eq.) was dissolved in H_2O (32.0 mL) and cHCl (8.00 mL) at 0 °C. A 1.2 M aqueous solution of sodium nitrite (738 mg, 10.7 mmol, 8.91 mL, 2.4 eq.) was added dropwise and the resulting reaction mixture was stirred for 10 min at 0 °C. The prepared yellow solution was added dropwise over 30 min to a solution of *N*, *N*-diethylaniline (748 µL, 4.88 mmol, 1.1 eq.) in MeOH/1 M NaOAc (40.0 mL/10.0 mL) at 0 °C and stirred for 15 min at 0 °C. The reaction was allowed to warm to r.t. and stirred overnight. The reaction mixture was then acidified with 1 M HCl and extracted with EtOAc (3 x 250 mL), washed with brine (1 x 250 mL) and the crude product was filtered through a pore III frit with EtOAc containing a plug of silica (50 mL). 908 mg (2.67 mmol) of **3** were obtained in 60% yield as a deep purple solid.

^{1}H NMR (400 MHz, DMSO-d_6): *δ* [ppm] = 8.47 (br s, 2H), 7.86 (d, *J* = 9.2 Hz, 2H), 6.96 (d, *J* = 9.2, 2H), 3.59 (q, *J* = 6.8 Hz, 4H), 1.20 (t, *J* = 7.2 Hz, 6H).
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 183.3, 167.3, 153.8, 142.0, 112.6, 79.2, 44.9, 12.6.
HRMS (ESI): calc. for $C_{12}H_{17}N_6O_2S_2^+$ $[M+H]^+$: 341.0849, found: 341.0846.
UV/Vis (LCMS): λ_{max} = 540 nm.
R_t (LCMS; MeCN/H_2O/formic acid = 10/90/0.1 → 90/10/0.1 over 7 min) = 3.845 min.
IR (ATR): wave number/cm^{-1} = 3303, 3060, 1713, 1602, 1543, 1416, 1357, 1298, 1267, 1215, 1174, 1142, 1068, 909, 829, 788.
m.p. = >275 °C

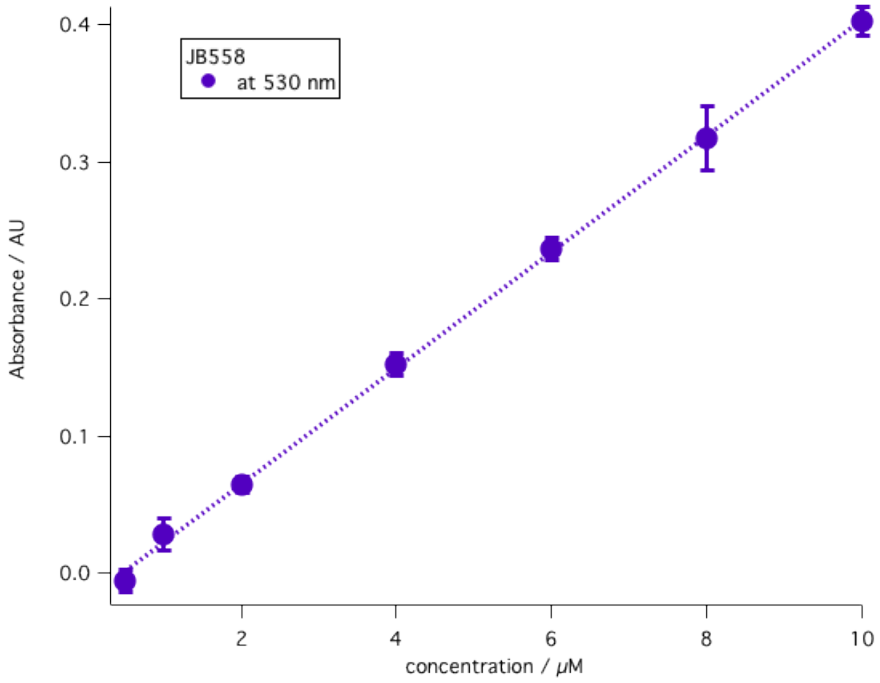
(*E*)-*N*-(Cyclohexylcarbamoyl)-5-((4-(diethylamino)phenyl)-diazenyl)-1, 3, 4-thiadiazole-2-sulfonamide (JB558)



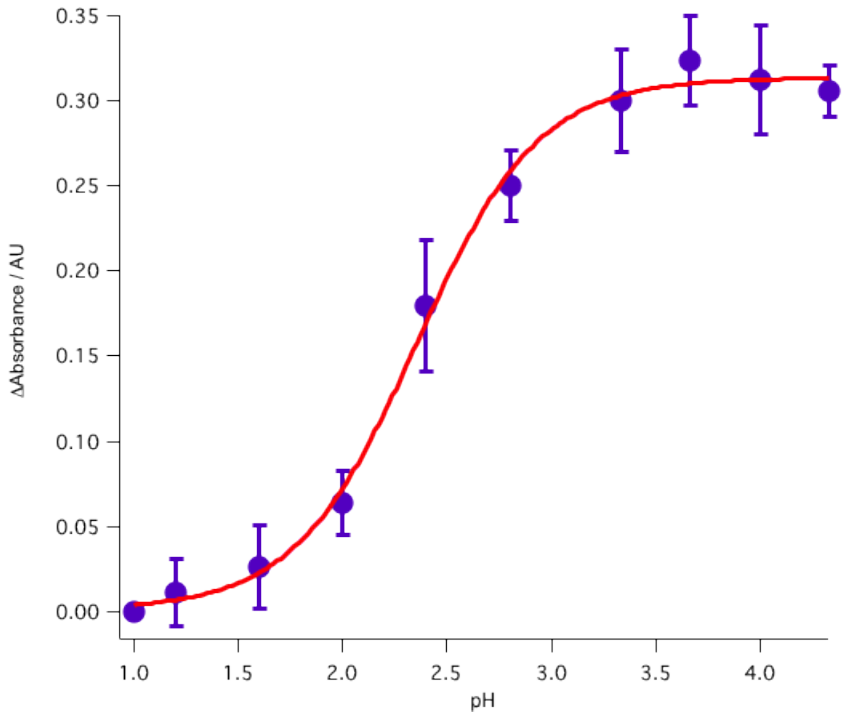
3 (5.1 mg, 15.0 µmol, 1.0 eq.) was dissolved in dry MeCN (4.0 mL) under a N_2-atmosphere. NEt_3 (2.08µL, 15.0 µmol, 1.0 eq.) and cyclohexyl isocyanate (48.0 µL, 376 µmol, 25.0 eq.) were added and the mixture was refluxed for 4 h at 90 °C. The solution was concentrated to 1 mL, filtered through a PTFE filter (CHROMAFIL® Xtra PTFE 0.45) and subjected to RP-HPLC (MeCN/H_2O/formic acid = 5/95/0.1 → 80/20/0.1 over 40 min). 2.6 mg (5.6 µmol) of the desired product was obtained in 37% yield.

^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] = 7.86 (d, J = 9.2 Hz, 2H), 6.97 (d, J = 9.6 Hz, 2H), 6.68 (m, 1H), 3.60 (q, J = 7.2 Hz, 4H), 3.54–3.47 (m, 1H), 1.75–1.60 (m, 4H), 1.55–1.45 (m, 2H), 1.25–1.14 (m, 10H).
^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] = 184.2, 164.4 (formic acid), 163.1, 158.2, 154.0, 150.6, 142.1, 112.8, 48.6, 45.0, 32.2, 24.3, 12.6.
HRMS (ESI): calc. for $C_{19}H_{28}N_7O_3S_2^+$ $[M+H]^+$: 466.1690, found: 466.1690.
UV/Vis (LCMS): λ_{max} = 545 nm.
R_t (LCMS; MeCN/H_2O/formic acid = 10/90/0.1 → 90/10/0.1 over 7 min) = 4.691 min.
IR (ATR): wave number/cm^{-1} = 2933, 1682, 1603, 1530, 1450, 1323, 1305, 1265, 1208, 1139, 1072, 1009, 907, 826, 788, 696, 655.
m.p. = 171 °C

Extinction coefficient JB558 (Ringer solution): $\varepsilon_{530\ nm} = 114{,}000\ mol^{-1}\ cm^{-1}$.



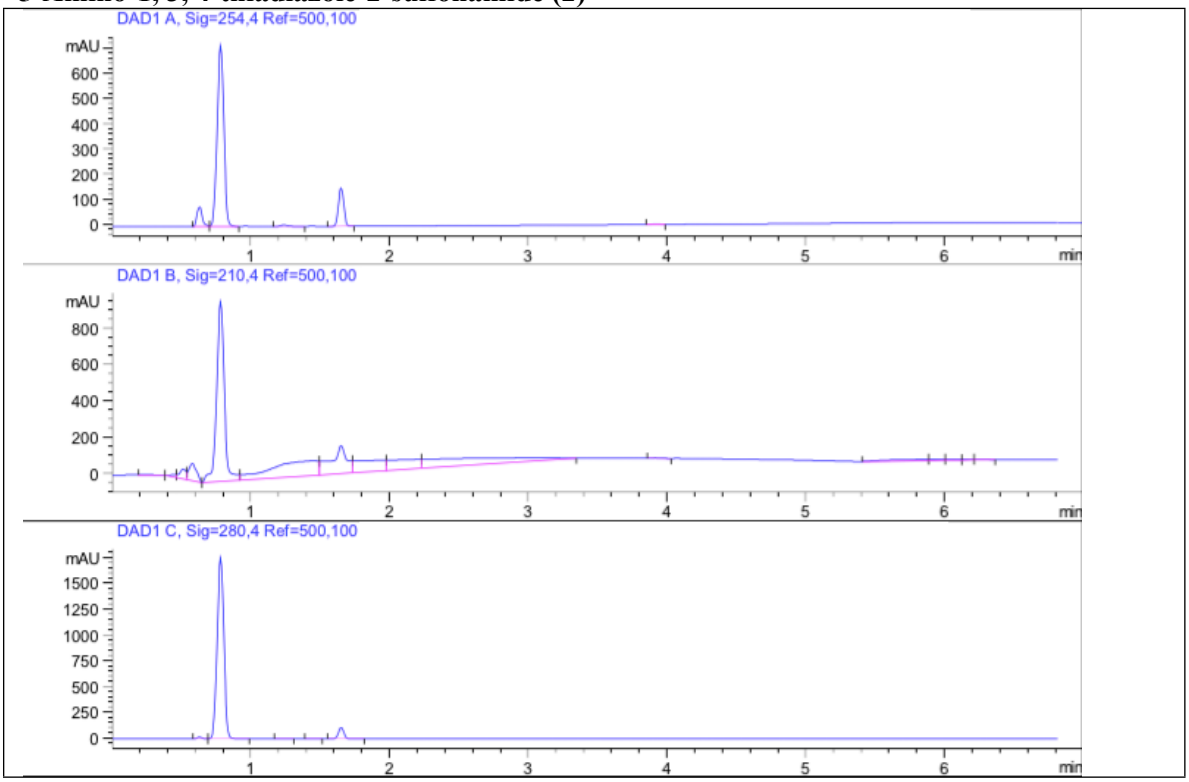
***pK*$_a$ JB558** (aqueous buffer/DMSO = 4/1) = 2.36 ± 0.05; measured according to Martinez *et al.*.[7]



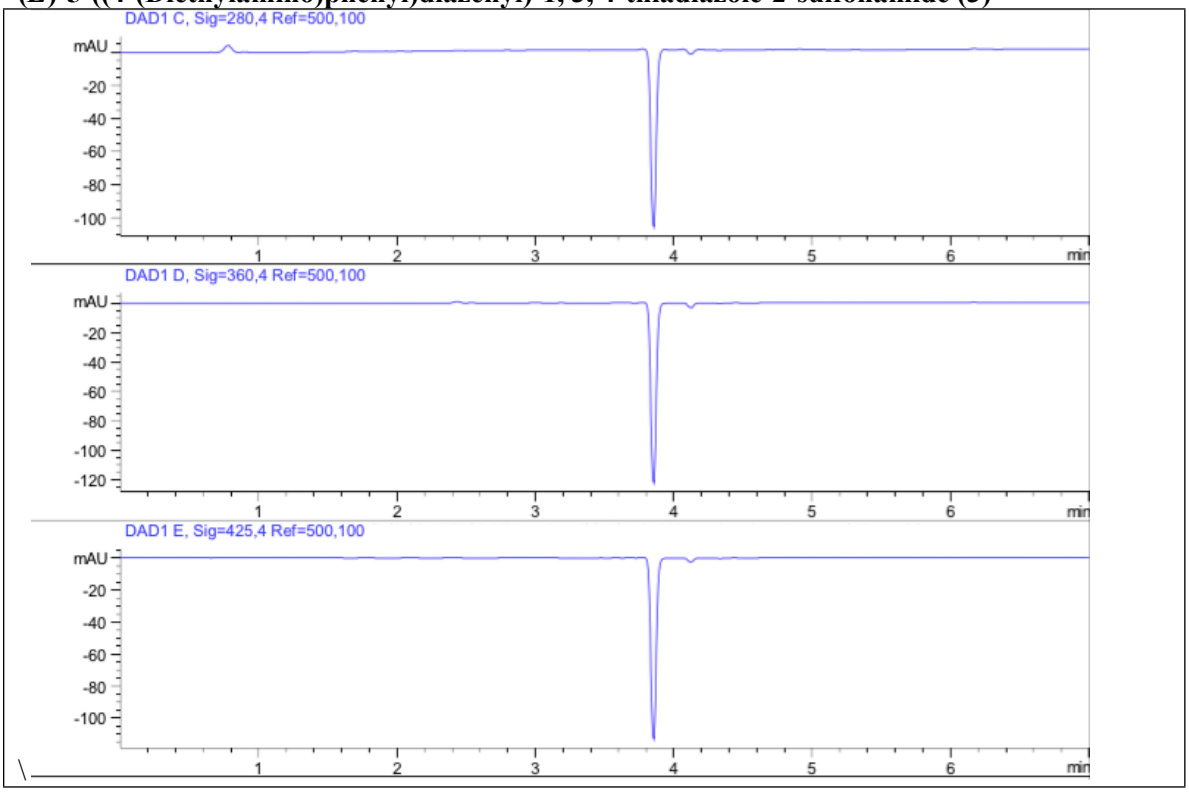
LIQUID CHROMATOGRAPHY TRACES

Note that the reference is set to λ_{ref} = 500 nm, resulting in negative absorption peaks in the chromatogram for red-shifted compounds **3** and **JB558**.

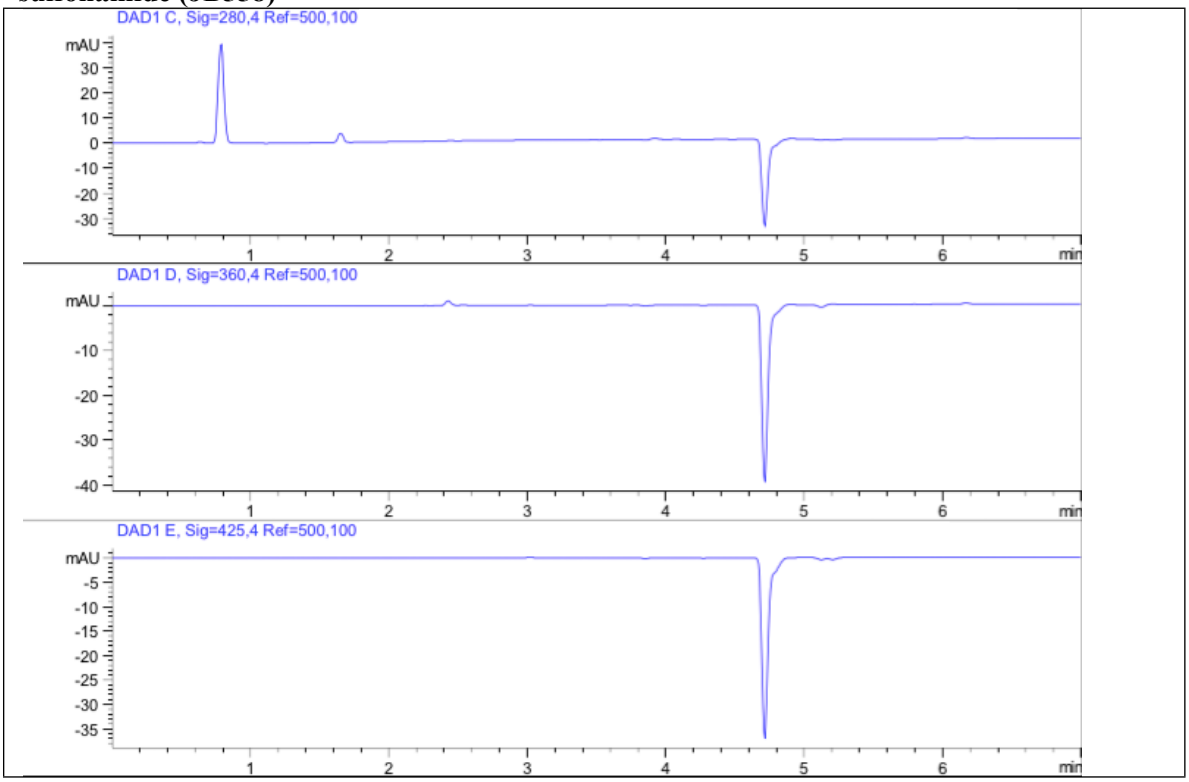
5-Amino-1, 3, 4-thiadiazole-2-sulfonamide (2)



(*E*)-5-((4-(Diethylamino)phenyl)diazenyl)-1, 3, 4-thiadiazole-2-sulfonamide (3)

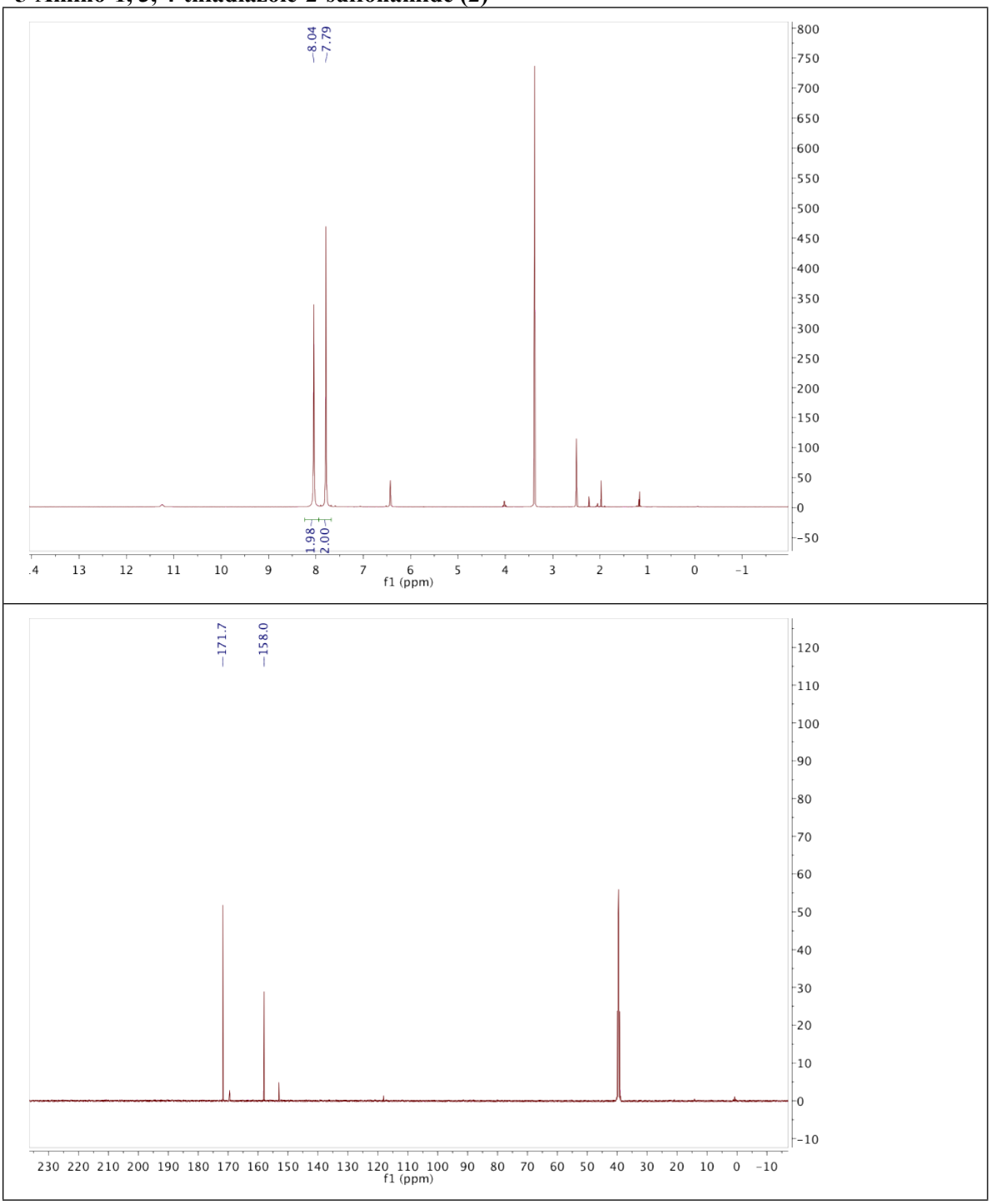


(*E*)-*N*-(Cyclohexylcarbamoyl)-5-((4-(diethylamino)phenyl)-diazenyl)-1, 3, 4-thiadiazole-2-sulfonamide (JB558)

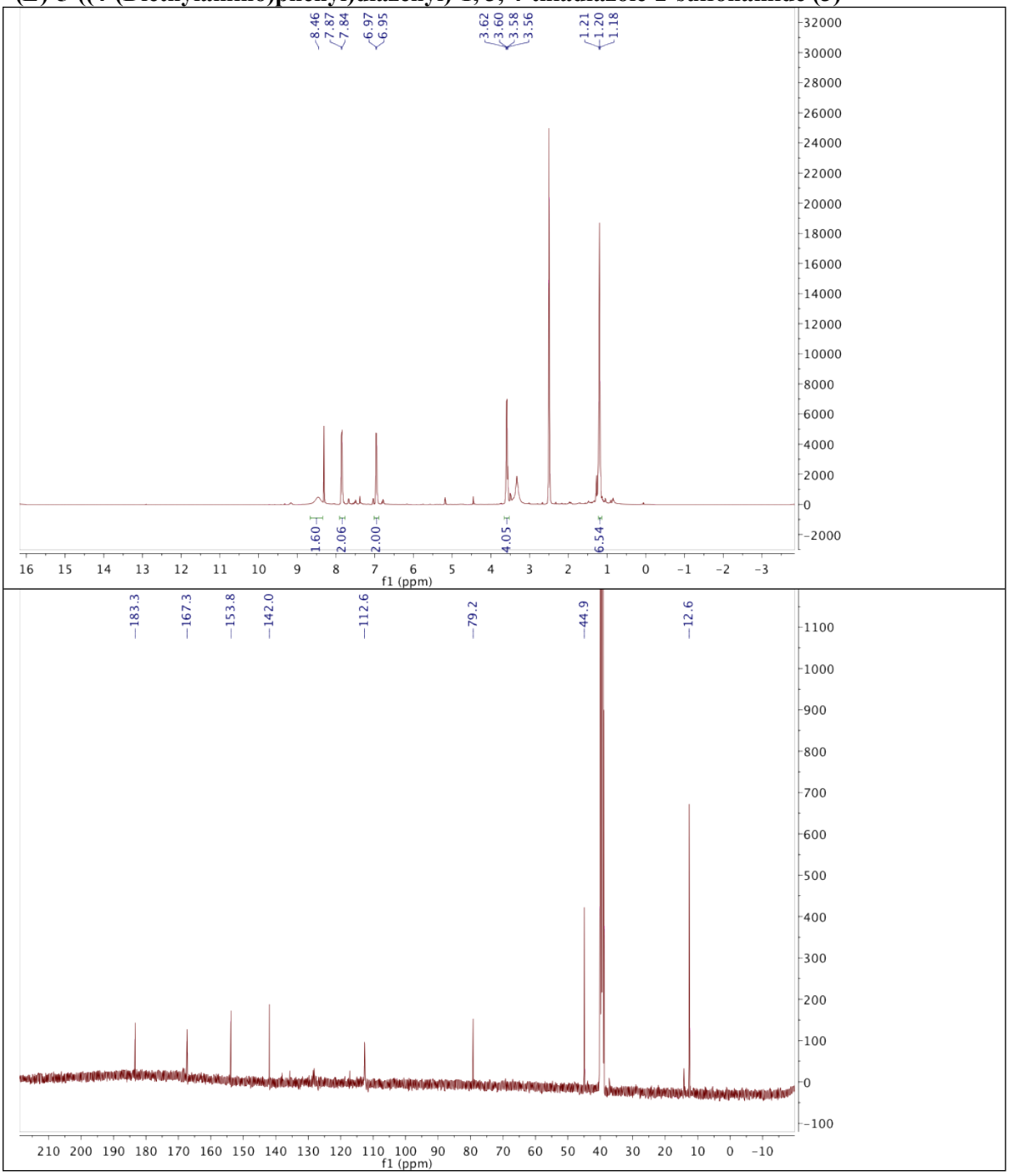


SPECTRAL DATA

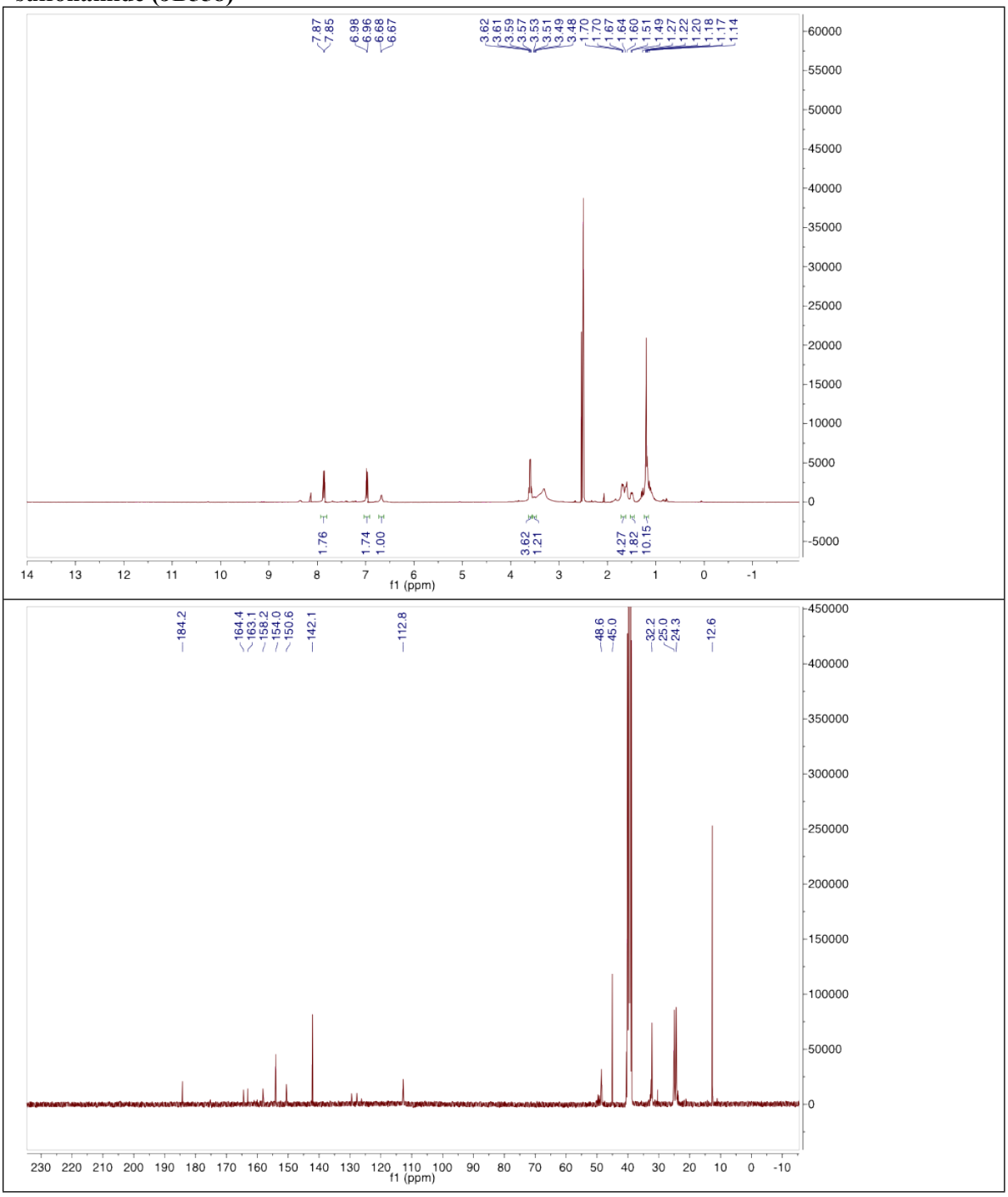
5-Amino-1, 3, 4-thiadiazole-2-sulfonamide (2)



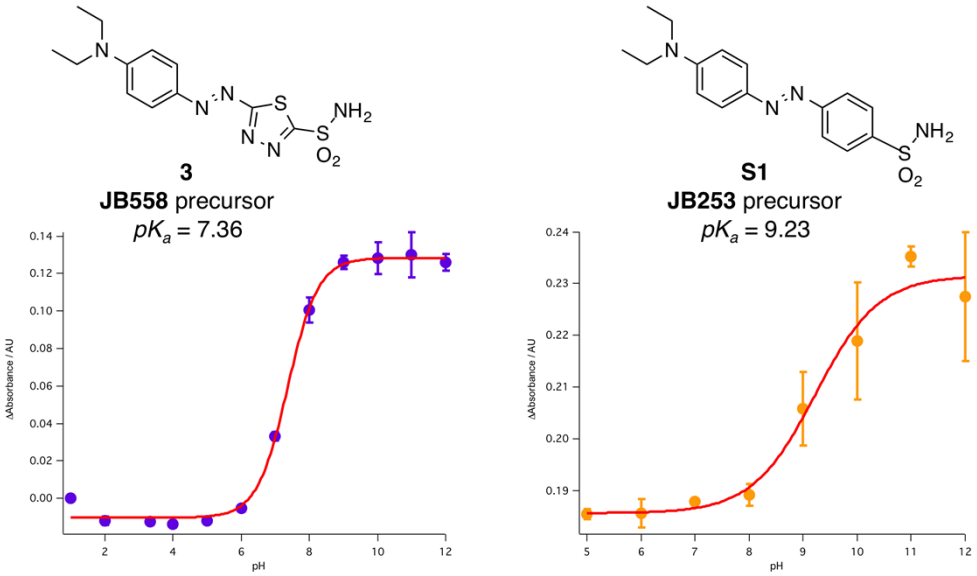
(*E*)-5-((4-(Diethylamino)phenyl)diazenyl)-1, 3, 4-thiadiazole-2-sulfonamide (3)



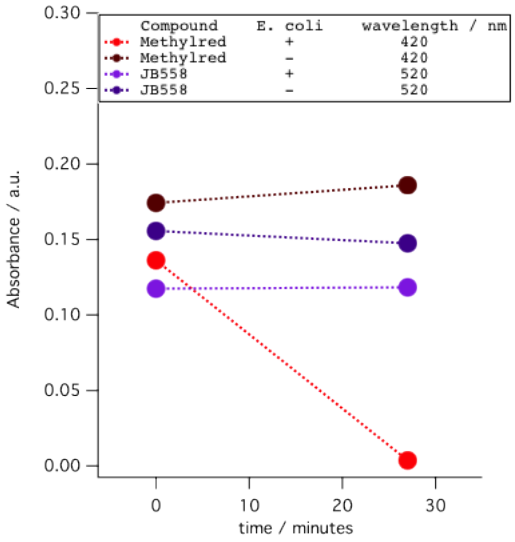
(*E*)-*N*-(Cyclohexylcarbamoyl)-5-((4-(diethylamino)phenyl)-diazenyl)-1, 3, 4-thiadiazole-2-sulfonamide (JB558)



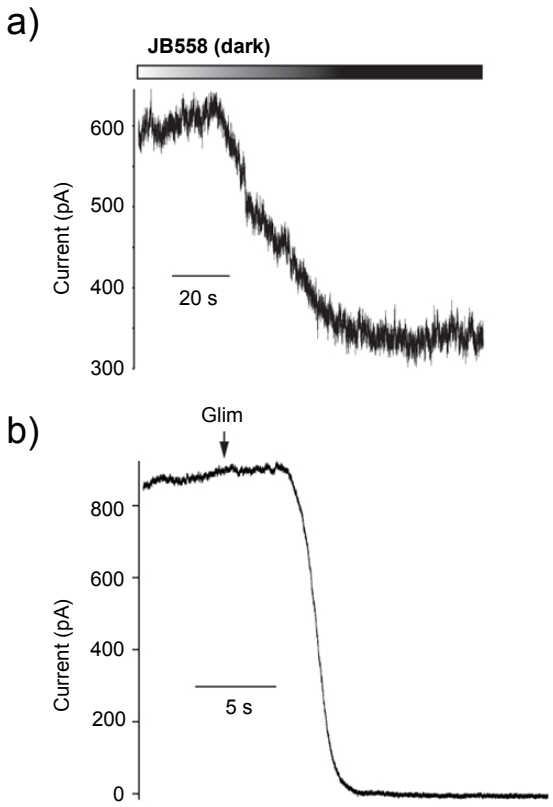
SUPPLEMENTARY FIGURES



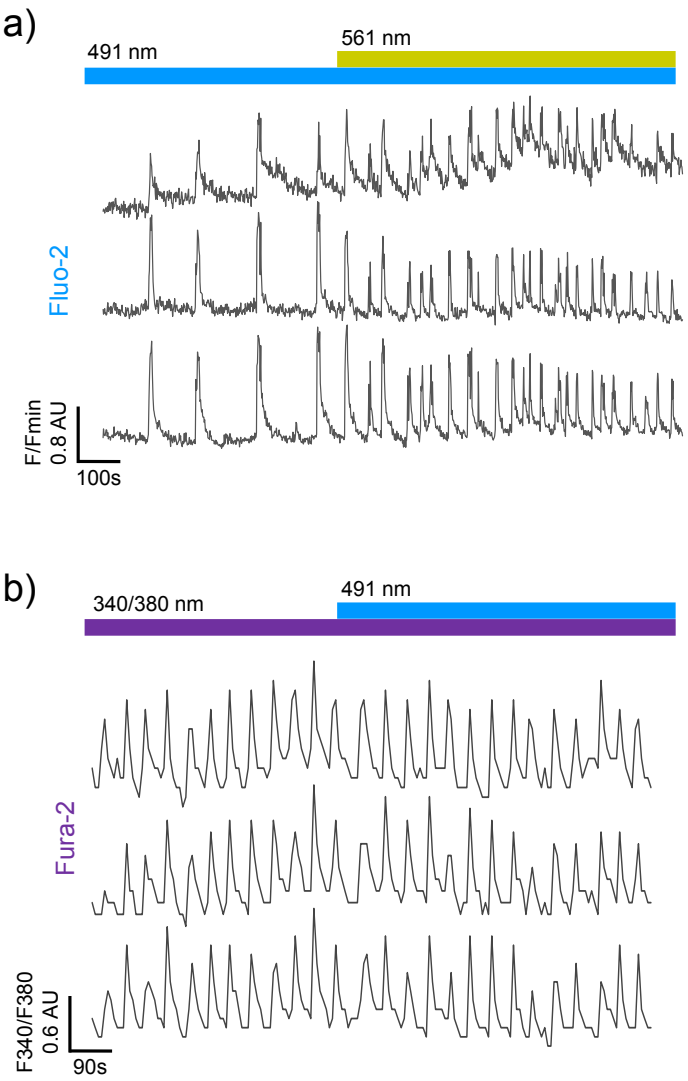
Supplementary Fig. 1: pK_a 3 (aqueous buffer/DMSO = 4/1) = 7.36 ± 0.06 and **pK_a S1** (aqueous buffer/DMSO = 4/1) = 9.23 ± 0.22; measured according to Martinez *et al.*.[7]



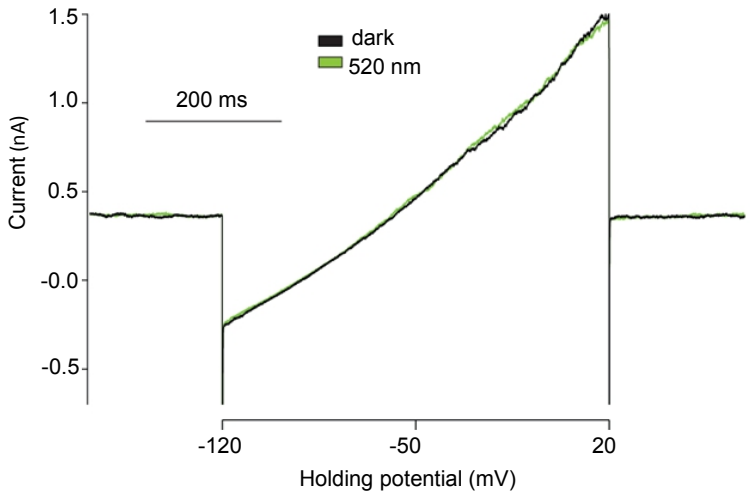
Supplementary Fig. 2: AzoReductase from *Escherichia coli* (BL21 DE3) is able to cleave methylred (MR) but not **JB558**.



Supplementary Fig. 3 (a) Representative whole cell voltage clamp recording in a HEK293T cell showing that 20 μM **JB558** causes partial block (~ 280 pA) of K_{ATP} channel activity in its dark-adapted state (holding potential = –60 mV). (b) As for (a) but following application of 100 μM glimepiride (Glim), which produces complete K_{ATP} channel block (~ 850 pA).



Supplementary Fig. 4: a) **JB558**-treated islets respond to excitation with λ = 561 nm, but not λ = 491 nm, with alterations to Ca^{2+}-spiking frequency and amplitude (representative trace from *n* = 3 recordings). b) Fura-2-loaded (λ = 340/340 nm) islets incubated with **JB558** do not respond to illumination with λ = 491 with changes in $[Ca^{2+}]_i$.



Supplementary Fig. 5: Voltage ramp experiments (from –120 to +20 mV over 1 s) in HEK293T cells show no light-dependent block of K_{ATP} channel activity in the presence of dark-adapted (black) or illuminated (green) 20 μM **JB558**.

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