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Azobenzene photoresponsive elements can be installed on sulfonylureas, yielding optical control over pancreatic beta cell function and insulin release. An obstacle to such photopharmacological approaches remains the use of ultraviolet-blue illumination. Herein, we synthesize and test a novel yellow light-activated sulfonylurea based on a heterocyclic azobenzene bearing a push-pull system.

Type 2 diabetes (T2D) is a modern pandemic currently affecting ~ 6% of the global population. This disease is characterized by diminished insulin secretion from pancreatic beta cells, which together with peripheral resistance to the secreted hormone, leads to defective glucose homeostasis.[1] The resulting elevated glucose concentration drives a variety of complications including heart disease, cancer, retinal degeneration, and nerve and vascular problems.[2]

While current medical treatments work well, they are associated with complications largely due to off-target or persistent actions.[3] Moreover, they are unable to recreate pulsatile insulin release, a more effective signal for glucoregulation.[4] Thus, T2D is ideally suited to photopharmacology, which harnesses the temporal precision of light to spatiotemporally deliver drug activity.[5] We have recently shown that a sulfonylurea possessing an azobenzene photoresponsive element (a.k.a. AzoSulfonylurea) can be used to optically control beta cell function and insulin release *via* its effects on ATP-sensitive potassium (K_{ATP}) channels and Exchange Protein directly Activated by cAMP 2A (Epac2A) signalling.[6]

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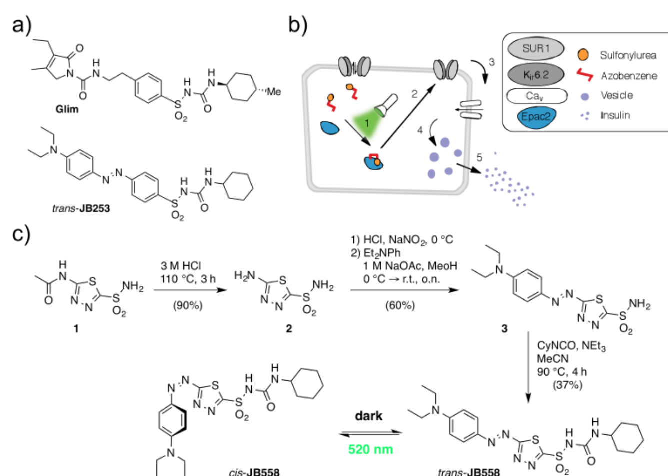
However, a significant barrier to the use of such 'azo-drugs' for T2D treatment is their ultraviolet-blue absorption spectra, increasing phototoxicity and limiting tissue penetration due to photon scattering.[7] By contrast, visible/near infrared wavelengths demonstrate better penetrance in the body. [8]

Spurred on by recent studies of *ortho-* or *para-*substituted azobenzenes,[9-11] we therefore devised a novel approach for the synthesis of wavelength-tuned photopharmaceuticals with red-shifted photochromism. An AzoSulfonylurea based on glimepiride was achieved by installing a heterocyclic aromatic unit, rather than sterically bulky electron-donating halogen or amine moieties (Scheme 1).



Scheme 1 (a) Structures of glimepiride (Glim) and the original blue light-responsive AzoSulfonylurea **JB253** for comparison. (b) The logic of a red-shifted AzoSulfonylurea. Following illumination with green-yellow light (1), the AzoSulfonylurea binds Epac2A, closing K_{ATP} channels (2) and opening voltage-dependent Ca^{2+} channels (Ca_v) (3). This allows optical control of Ca^{2+} influx (4) and insulin secretion (5). (c) Synthesis of the AzoSulfonylurea **JB558** that can be switched from the *trans-* to the *cis-*isomer using green/yellow light.

Starting with the deacetylation of acetazolamide (**1**) in refluxing HCl, heterocycle **2** was obtained that could be further diazotized with *in situ* generated HNO_2. Trapping the resulting diazonium salt with *N, N*-diethylaniline generated sulfonamide azobenzene **3**. Finally, reaction with cyclohexyl isocyanate yielded **JB558** *via* acylation of the sulfonamide, giving unprecedented access to a sulfonylurea containing a heterocyclic azobenzene. While yields were reduced compared to the previously described **JB253** (37% *versus* 97%),[6] this was most likely due to the presence of a less reactive sulfonamide intermediate, as predicted by the lower pK_a value for **3** (7.36, Fig. S1) and **JB558** (2.35, see SI).

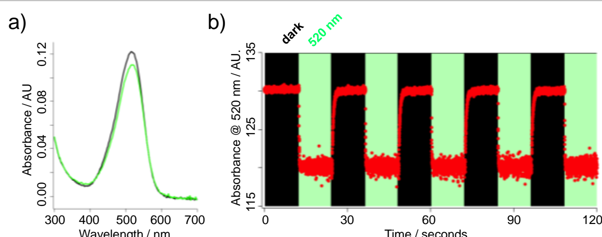


Fig 1 (a) UV-Vis spectra of **JB558** in DMSO following illumination with λ = 520 nm (green) or under dark-adapted conditions (black). (b) Robust photoswitching between *cis*- and *trans*-**JB558** induced with λ = 520 nm and dark, respectively.

JB558 possessed a red-shifted absorption spectra (λ_{max} = 526 nm) in DMSO (Fig. 1a), and could be repeatedly photoconverted to its *cis*-state with green-yellow light (λ = 520 nm) (Fig. 1b). Thermal back relaxation occurred rapidly in the dark and switching kinetics were within the millisecond range (τ_{cis} = 64.9 ± 1.5 ms; τ_{trans} = 410.8 ± 12.6 ms), without obvious decomposition (Fig. 1b). **JB558** was stable in the presence of *Escherichia coli* azoreductase, an enzyme expected to limit oral bioavailability through diazene cleavage in the intestine (Fig. S2).

To determine the binding affinity of **JB558** to the K_{ATP} channel subunit SUR1, as well as Epac2A, [3H]-glibenclamide displacement and FRET assays were performed. While *trans*-**JB558** bound SUR1 with ~10,000-fold less affinity than glimepiride (IC_{50} (*trans*-**JB558**) = 37.3 μM; IC_{50} (Glim) = 1.8 nM) (Fig. 2a), it was able to strongly and light-dependently activate an Epac2A-camps biosensor containing the sulfonylurea binding domain[12] (Fig. 2b-d).

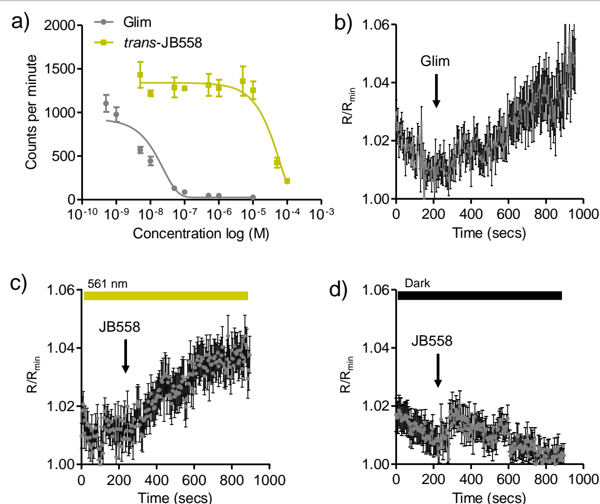


Fig. 2 (a) *trans*-**JB558** and glimepiride (Glim) displace [3H]-glibenclamide from SUR1 (*n* = 3 repeats). (b) Glimepiride decreases FRET (shown here as an increase in R/R_{min}) in HEK293T cells expressing full length Epac2-camps (*n* = 32 cells). (c) As for (b) but *cis*-**JB558** (λ = 561 nm) (*n* = 41 cells). (d) As for (c) but *trans*-**JB558** (dark) (*n* = 37 cells). Values represent mean ± s.e.m.

Electrophysiological recordings of K^+ currents in HEK293T-SUR1-Kir6.2 cells revealed partial K_{ATP} channel blockade by *trans*-**JB558**, presumably due to the momentary stationary state favouring some continued *cis*-isomerisation (Fig. S3).

We next assessed the photoswitching properties of **JB558** in native beta cells where sulfonylurea-mediated K_{ATP} channel-Epac2A signalling is intimately linked to voltage-dependent Ca^{2+} channel (VDCC) activity and insulin exocytosis.[13-16] As expected, **JB558** was able to evoke large increases in intracellular Ca^{2+} concentrations in ~60% of beta cells following exposure to yellow (λ = 561 ± 5 nm)- (Fig. 3a and b), but not violet (λ = 405 ± 5 nm)-light (Fig. 3c). These effects were potentiated using a high concentration of glimepiride (Fig. 3d), and abrogated using diazoxide (Fig. 3e) to force open the K_{ATP} channel pore. Repeated switching of cytosolic Ca^{2+} concentrations could be achieved in the same islet following a brief period of dark exposure to induce *trans*-**JB558** accumulation (Fig. 3f).

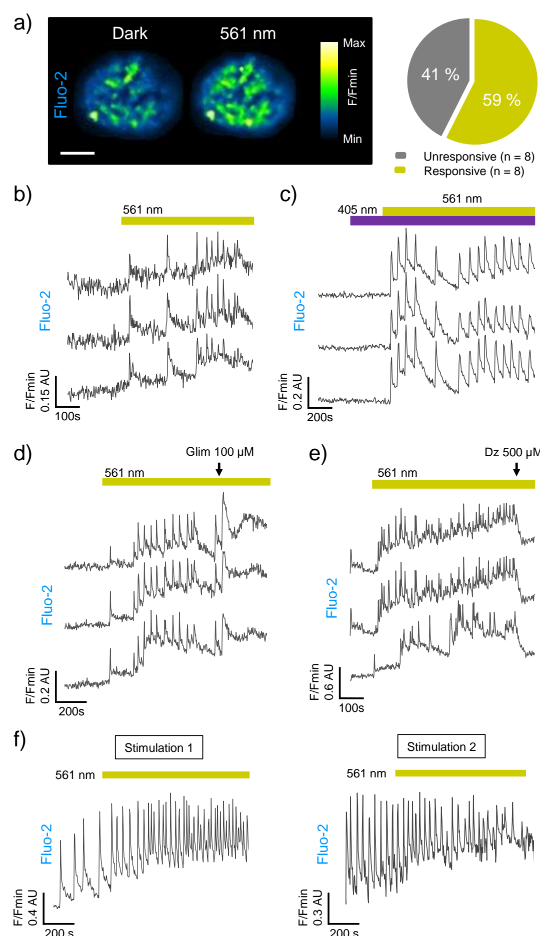


Fig. 3 (a) **JB558** increases intracellular Ca^{2+} concentrations in 59% of beta cells residing within rodent islets of Langerhans following illumination with

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λ = 561 nm (scale bar = 75 μm) (n = 8 islets). (b) Photoswitching is rapid following exposure to λ = 561 nm. (c) As for (b), but showing the absence of photoswitching with λ = 405 nm. (d) A high concentration (100 μM) of glimepiride (Glim) augments **JB558**-stimulated Ca^{2+} rises. (e) Diazoxide (Dz) reverses *cis*-**JB558**-induced Ca^{2+} fluxes. (f) Reversible manipulation of Ca^{2+} transients can be achieved in the same islet following thermal back relaxation of **JB558** in the dark (5 min between stimulation 1 and 2). Traces represent n = 6-10 recordings from 3 animals. Islets were maintained in 5 mM D-glucose throughout.

Similar to the results observed in rodent tissue, *cis*-**JB588** was able to confer light-sensitivity on Ca^{2+}-spiking activity in human pancreatic islets (Fig. 4a-c), and this effect could be reversed following 5 min relaxation in the dark (Fig. 4d).

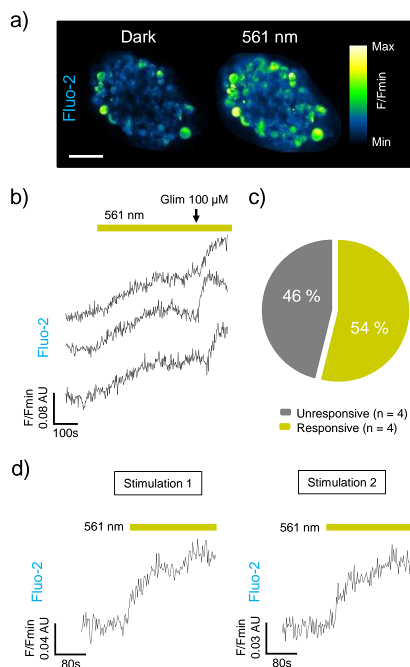

Fig. 4 (a) **JB558** increases intracellular Ca^{2+} concentrations in human beta cells in response to illumination with 561 nm to induce *cis*-formation (scale bar = 50 μm). (b) Photoswitching is rapid following exposure to 561 nm and can be potentiated with glimepiride (Glim). (c) *cis*-**JB588** activates 54% of beta cells (n = 4 islets). (d) Reversible manipulation of Ca^{2+} rises following thermal back relaxation of **JB558** in the dark (5 min between stimulation 1 and 2). Traces represent n = 3-9 recordings from a single donor. Islets were maintained in 5 mM D-glucose throughout.

To link photocontrol of Ca^{2+} levels with insulin secretion, batches of rodent islets were incubated with **JB558** and exposed to either dark (no illumination) or light (λ = 560 ± 10 nm). **JB558**–treated islets kept under dark conditions were no different to controls (5 mM glucose-alone) (Fig. 5a), suggesting that the observed stationary state K_{ATP} channel block was insufficient to elicit exocytosis. By contrast, irradiation dramatically stimulated insulin release (Fig. 5a). Finally, cytotoxicity assays demonstrated that **JB558** did not adversely affect cell viability, as assessed using the vital stain calcein and the necrosis indicator propidium iodide (Fig. 5b and c).

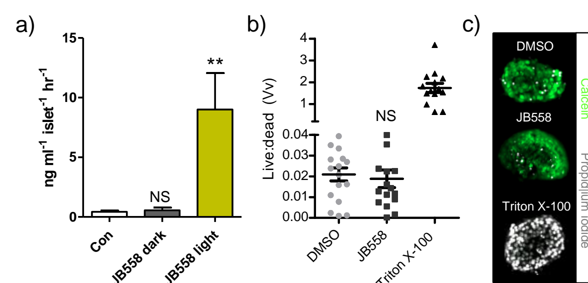

Fig. 5 (a) **JB558**-treated islets respond to illumination with λ = 560 nm by increasing insulin secretion (**P<0.01 and NS, non-significant *versus* Con; one-way ANOVA). (b) Incubation with **JB558** for 1 hr did not adversely alter cell viability *versus* dimethyl sulfoxide (DMSO), as assessed by the ratio of calcein (live):propidium iodide (dead) fluorescence (positive control; Triton X-100) (NS, non-significant *versus* Con; one-way ANOVA). (c) Representative images of islets stained with calcein and propidium iodide. In all cases, n = 36 islets per treatment group from 6 animals. Values represent mean ± s.e.m.

The data presented here outline a synthetic route for the production of AzoSulfonylureas with red-shifted photochromism. Consistent with its sulfonylurea backbone, **JB558** was able to bind SUR1 and activate Epac2A. Formation of *cis*-**JB558** occurred with green-yellow light (λ = 520–561 nm), and thermal back relaxation in the dark yielded *trans*-**JB558**. While photoconversion between *cis*- and *trans*- forms was rapid in solution, it was slower in the tissue setting, taking minutes for reisomerisation. An effect of Fluo-2 excitation on the isomer equilibrium cannot be excluded, although illumination at λ = 491 nm *per se* was unable to evoke Ca^{2+} rises in either Fluo-2- or Fura2 (λ = 340 nm/380 nm)-loaded islets (Fig. S4).

A more plausible explanation is the inactivation of beta cell Epac2A-signalling, which may lag behind that of **JB558** due to persistent mobilisation of intracellular Ca^{2+}.[1, 17] Such tissue effects may be desirable for the development of photopharmaceuticals, since pulsed illumination would reduce phototoxicity, while sustaining compound activity to match long-lasting (dozens of minutes) insulin peaks.[4] Indeed, **JB558** displayed almost 3-fold more potency than its blue-light activated predecessor **JB253**,[6] most likely due to slower back-relaxation during the light pulses used in the secretion assays.

Neither were we able to detect photoswitching of K^+ currents in HEK293T cells overexpressing K_{ATP} channels, free from orthogonal wavelengths (Fig. S5). This was likely because HEK293T cells do not express sufficient Epac2A to allow **JB558** to properly toggle K_{ATP} activity,[13, 15, 18] and/or the inability to deliver sufficient illumination using the non-coherent source on our patch-clamp setup ($\varepsilon_{520\ nm}$ (**JB558**) = 1.14 x 10^5 mol^{-1} cm^{-1}; see SI).

Nonetheless, we clearly show that **JB558** light-dependently binds Epac2A, allowing optical control of cell function and insulin secretion with λ = 560 nm in the most physiologically-relevant testbed, *viz* the islets of Langerhans. Thus, **JB558** represents a blueprint for red-shifted AzoSulfonylureas based upon heterocyclic azobenzenes. Further studies are now warranted to improve isomerisation kinetics in tissue to improve the use of **JB558** as a research tool for rapid K_{ATP} channel manipulation. Importantly, similar synthetic approaches may also be applicable to other

clinically-relevant azobenzene-possessing compounds where steric hindrance may affect molecule motion *e.g.* neuromodulators,[19] neurotransmitters,[20, 21] enzymes[22] and antibiotics. [23]

Notes and references

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

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