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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 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) signaling.⁶

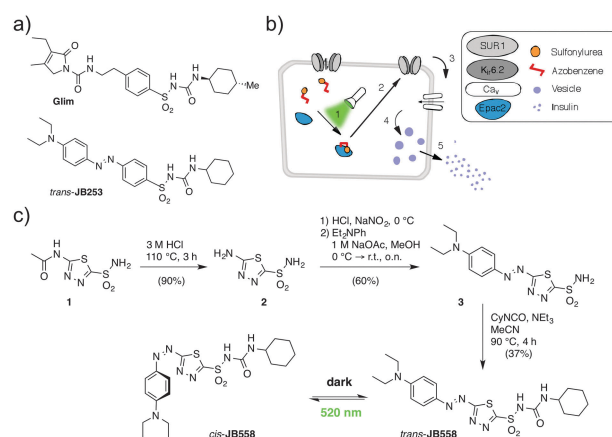
However, a significant barrier to the use of such ‘azo-drugs’ for T2D treatment is their ultraviolet-blue absorption spectra, increasing phototoxicity and limiting tissue penetration due to photon scattering.⁷ By contrast, visible/near infrared wavelengths demonstrate better penetrance in the body.⁸

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

J. Broichhagen,^a J. A. Frank,^a N. R. Johnston,^b R. K. Mitchell,^b K. Šmid,^a
P. Marchetti,^c M. Bugliani,^c G. A. Rutter,^b D. Trauner^{*a} and D. J. Hodson^{*b}

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).

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



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.

^a Department of Chemistry and Center for Integrated Protein Science, LMU Munich, Munich, Germany. E-mail: dirk.trauner@lmu.de

^b Section of Cell Biology and Functional Genomics, Department of Medicine, Imperial College London, London W12 0NN, UK. E-mail: d.hodson@imperial.ac.uk

^c Department of Clinical and Experimental Medicine, Islet Cell Laboratory, University of Pisa, Pisa, Italy

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presence of a less reactive sulfonamide intermediate, as predicted by the lower pK_a value for **3** (7.36, Fig. S1, ESI†) and **JB558** (2.35, see ESI†).

JB558 possessed a red-shifted absorption spectrum ($\lambda_{\text{max}} = 526 \text{ nm}$) in DMSO (Fig. 1a), and could be repeatedly photoconverted to its *cis*-state with green-yellow light ($\lambda = 520 \text{ nm}$) (Fig. 1b). Thermal back relaxation occurred rapidly in the dark and switching kinetics were within the millisecond range ($\tau_{\text{cis}} = 64.9 \pm 1.5 \text{ ms}$; $\tau_{\text{trans}} = 410.8 \pm 12.6 \text{ ms}$), 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, ESI†).

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 $\sim 10\,000$ -fold less affinity than glimepiride ($\text{IC}_{50}(\text{trans-JB558}) = 37.3 \mu\text{M}$; $\text{IC}_{50}(\text{Glim}) = 1.8 \text{ nM}$) (Fig. 2a), it was able to strongly and light-dependently activate an Epac2-camps biosensor containing the sulfonylurea binding domain (Fig. 2b–d).¹²

Electrophysiological recordings of K^+ currents in HEK293T-SUR1-Kir6.2 cells revealed partial K_{ATP} channel blockade by

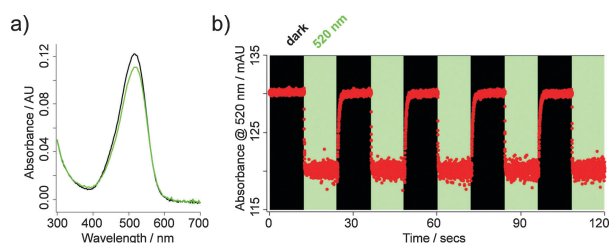


Fig. 1 (a) UV-Vis spectra of **JB558** in DMSO following illumination with $\lambda = 520 \text{ nm}$ (green) or under dark-adapted conditions (black). (b) Robust photoswitching between *cis*- and *trans*-**JB558** induced with $\lambda = 520 \text{ nm}$ and dark, respectively.

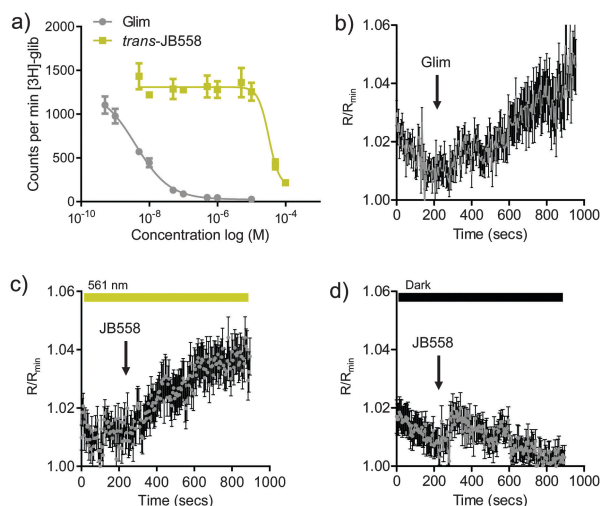


Fig. 2 (a) *trans*-**JB558** and glimepiride (Glim) displace [3H]-glibenclamide from SUR1 ($n = 3$ repeats). (b) Glimepiride decreases FRET (shown here as an increase in R/R_{min}) in HEK293T cells expressing full length Epac2-camps ($n = 32$ cells). (c) As for (b) but *cis*-**JB558** ($\lambda = 561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

trans-**JB558**, presumably due to the momentary stationary state favoring some continued *cis*-isomerization (Fig. S3, ESI†).

We next assessed the photoswitching properties of **JB558** in native beta cells where sulfonylurea-mediated K_{ATP} channel-Epac2A signaling 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 $\sim 60\%$ of beta cells following exposure to yellow ($\lambda = 561 \pm 5 \text{ nm}$) (Fig. 3a and b), but not violet ($\lambda = 405 \pm 5 \text{ nm}$) 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).

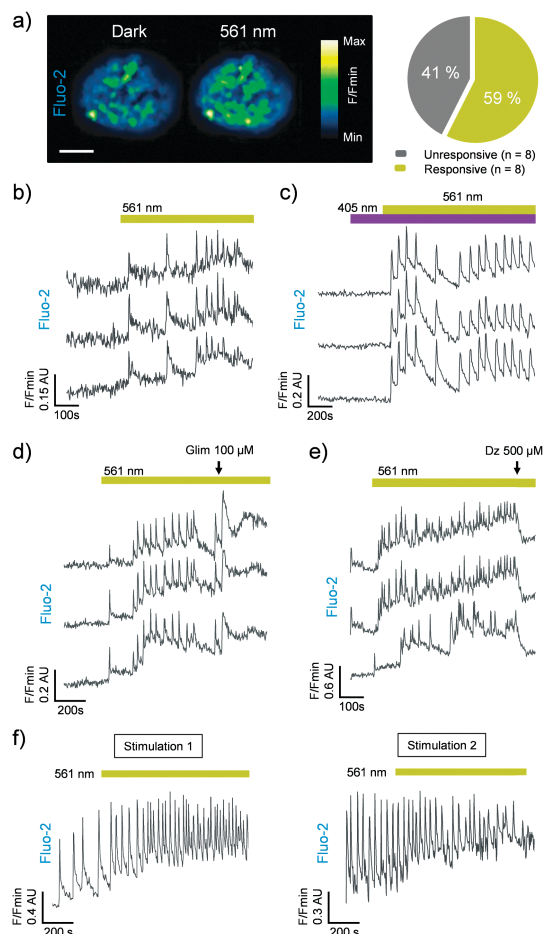


Fig. 3 (a) **JB558** increases intracellular Ca^{2+} concentrations in 59% of beta cells residing within rodent islets of Langerhans following illumination with $\lambda = 561 \text{ nm}$ (scale bar = $75 \mu\text{m}$) ($n = 8$ islets). (b) Photoswitching is rapid following exposure to $\lambda = 561 \text{ nm}$. (c) As for (b), but showing the absence of photoswitching with $\lambda = 405 \text{ nm}$. (d) A high concentration 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*-**JB558** 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).

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 ($\lambda = 560 \pm 10$ 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).

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 ($\lambda = 520$ – 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 reisomerization. An effect of Fluo-2 excitation on the isomer equilibrium cannot be excluded, although illumination at $\lambda = 491$ nm *per se* was unable to evoke Ca^{2+} rises in either Fluo-2- or Fura2 ($\lambda = 340$ nm/380 nm)-loaded islets (Fig. S4, ESI†).

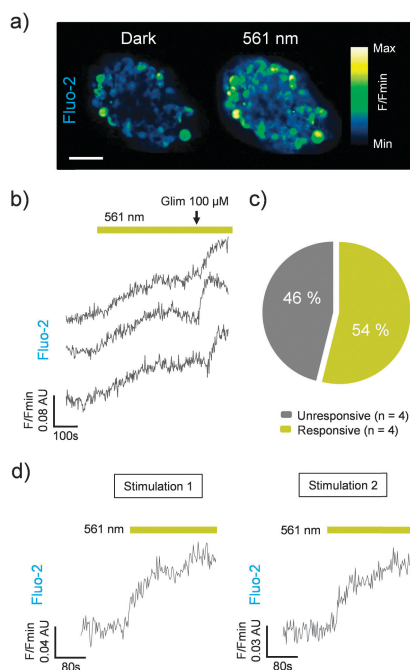


Fig. 4 (a) **JB558** increases intracellular Ca^{2+} concentrations in human beta cells in response to illumination with 561 nm to induce *cis*-formation (scale bar = 50 μm). (b) Photoswitching is rapid following exposure to 561 nm and can be potentiated with glimepiride (Glim). (c) *cis*-**JB558** activates 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.

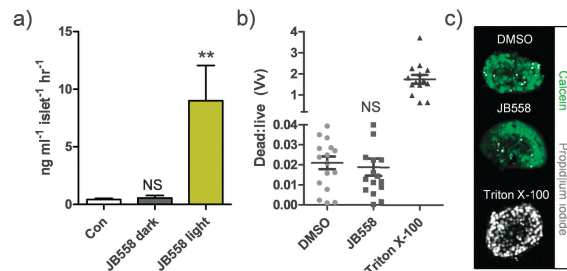


Fig. 5 (a) **JB558**-treated islets respond to illumination with $\lambda = 560$ nm by increasing insulin secretion (** $P < 0.01$ and NS, non-significant versus Con; one-way ANOVA). (b) Incubation with **JB558** for 1 h did not adversely affect cell viability versus dimethyl sulfoxide (DMSO), as assessed by the ratio of propidium iodide (dead):calcein (live) 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 \pm s.e.m.

A more plausible explanation is the inactivation of beta cell Epac2A-signaling, which may lag behind that of **JB558** due to persistent mobilization 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, ESI†). 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 ($\epsilon_{520\text{nm}}(\text{JB558}) = 1.14 \times 10^5 \text{ mol}^{-1} \text{ cm}^{-1}$; see ESI†).

Nonetheless, we clearly show that **JB558** light-dependently binds Epac2A, allowing optical control of cell function and insulin secretion with $\lambda = 560$ 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 isomerization 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} enzymes²² and antibiotics.²³

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