



Cite this: *Chem. Commun.*, 2015, 51, 6018

Received 9th February 2015,
Accepted 21st February 2015

DOI: 10.1039/c5cc01224d

www.rsc.org/chemcomm

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

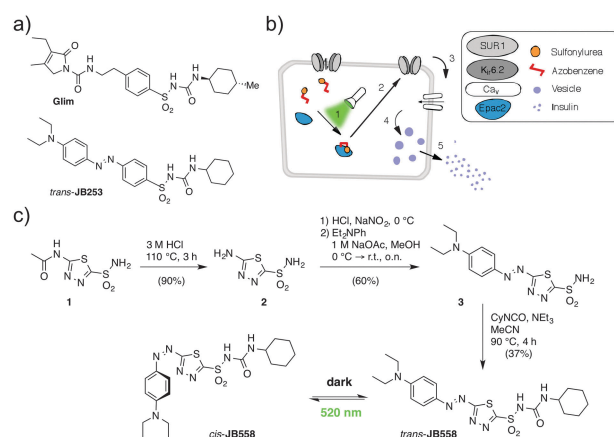
However, a significant barrier to the use of such ‘azo-drugs’ for T2D treatment is their ultraviolet-blue absorption spectra, increasing phototoxicity and limiting tissue penetration due to photon scattering.⁷ By contrast, visible/near infrared wavelengths demonstrate better penetrance in the body.⁸

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

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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_v^{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.

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c5cc01224d



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 ($\lambda_{\max} = 526$ nm) in DMSO (Fig. 1a), and could be repeatedly photoconverted to its *cis*-state with green–yellow light ($\lambda = 520$ nm) (Fig. 1b). Thermal back relaxation occurred rapidly in the dark and switching kinetics were within the millisecond range ($\tau_{cis} = 64.9 \pm 1.5$ ms; $\tau_{trans} = 410.8 \pm 12.6$ 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, 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 glibenclamide ($IC_{50}(\textit{trans}\text{-JB558}) = 37.3$ μM ; $IC_{50}(\text{Glim}) = 1.8$ nM) (Fig. 2a), it was able to strongly and light-dependently activate an Epac2-camps biosensor containing the sulfonyleurea binding domain (Fig. 2b–d).¹²

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

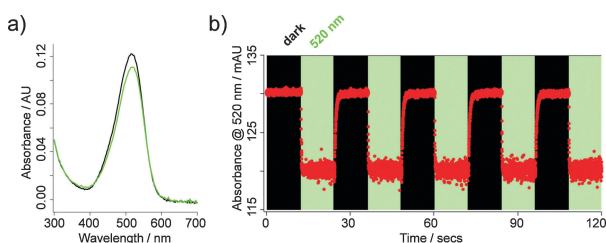


Fig. 1 (a) UV-Vis spectra of **JB558** in DMSO following illumination with $\lambda = 520$ nm (green) or under dark-adapted conditions (black). (b) Robust photoswitching between *cis*- and *trans*-**JB558** induced with $\lambda = 520$ nm and dark, respectively.

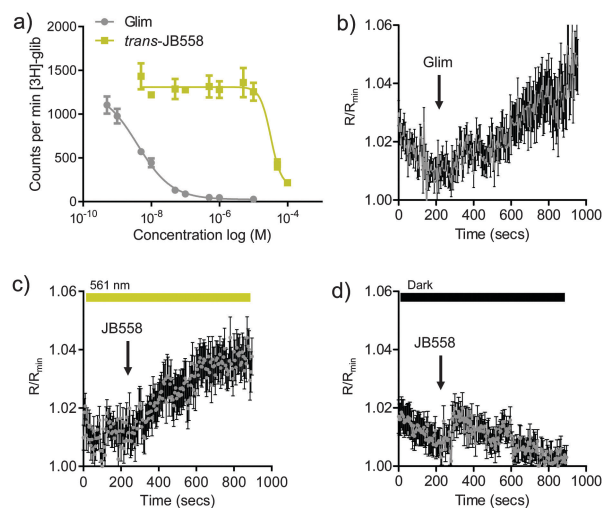


Fig. 2 (a) *trans*-**JB558** and glibenclamide (Glim) displace [3H]-glibenclamide from SUR1 ($n = 3$ repeats). (b) Glibenclamide decreases FRET (shown here as an increase in R/R_{\min}) in HEK293T cells expressing full length Epac2-camps ($n = 32$ cells). (c) As for (b) but *cis*-**JB558** ($\lambda = 561$ 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 sulfonyleurea-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 ($\lambda = 561 \pm 5$ nm) (Fig. 3a and b), but not violet ($\lambda = 405 \pm 5$ nm) light (Fig. 3c). These effects were potentiated using a high concentration of glibenclamide (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).

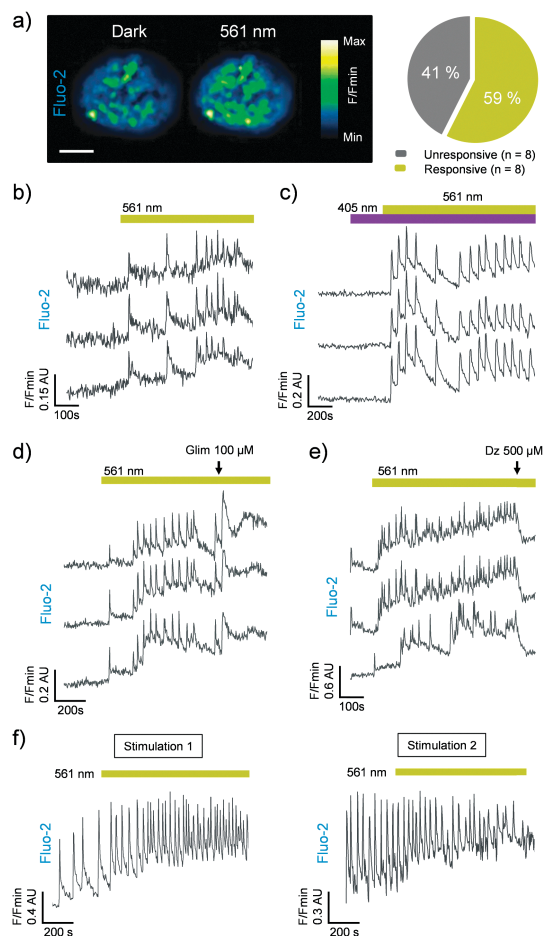


Fig. 3 (a) **JB558** increases intracellular Ca^{2+} concentrations in 59% of beta cells residing within rodent islets of Langerhans following illumination with $\lambda = 561$ nm (scale bar = 75 μm) ($n = 8$ islets). (b) Photoswitching is rapid following exposure to $\lambda = 561$ nm. (c) As for (b),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

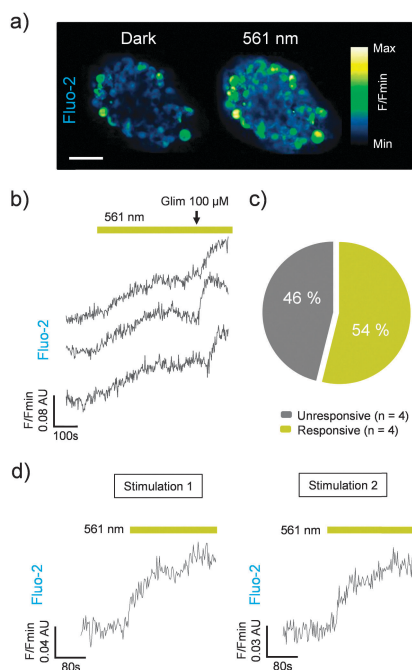


Fig. 4 (a) **JB558** increases intracellular Ca^{2+} concentrations in human beta cells in response to illumination with 561 nm to induce *cis*-formation (scale bar = 50 μm). (b) Photoswitching is rapid following exposure to 561 nm and can be potentiated with glibenclamide (Glim). (c) *cis*-**JB558**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

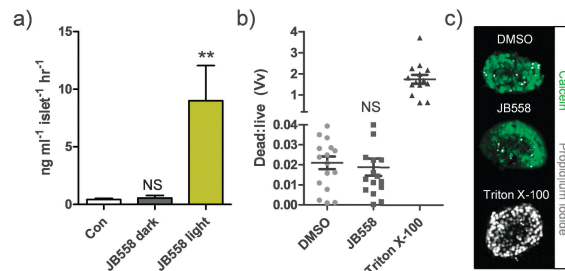


Fig. 5 (a) **JB558**-treated islets respond to illumination with $\lambda = 560$ nm by increasing insulin secretion (** $P < 0.01$ and NS, non-significant versus Con; one-way ANOVA). (b) Incubation with **JB558** for 1 h did not adversely affect cell viability versus dimethyl sulfoxide (DMSO), as assessed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

J.B. was supported by a European Foundation for the Study of Diabetes (EFSD) Albert Renold Young Scientist Fellowship and a Studienstiftung des deutschen Volkes PhD studentship. N.R.J. was supported by a Diabetes UK RW and JM Collins Studentship (12/0004601). J.A.F. was supported by a Collaborative Research Centre Grant (SFB1032). G.A.R. was supported by Wellcome Trust Senior Investigator (WT098424AIA), MRC Programme (MR/J0003042/1), Diabetes UK Project Grant (11/0004210) and Royal Society Wolfson Research Merit Awards. D.T. was supported by an



Advanced Grant from the European Research Commission (268795). D.J.H. was supported by a Diabetes UK R.D. Lawrence Research Fellowship (12/0004431). The work leading to this publication has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement no. 155005 (IMIDIA), resources of which are composed of a financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution (G.A.R.).

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