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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]

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.

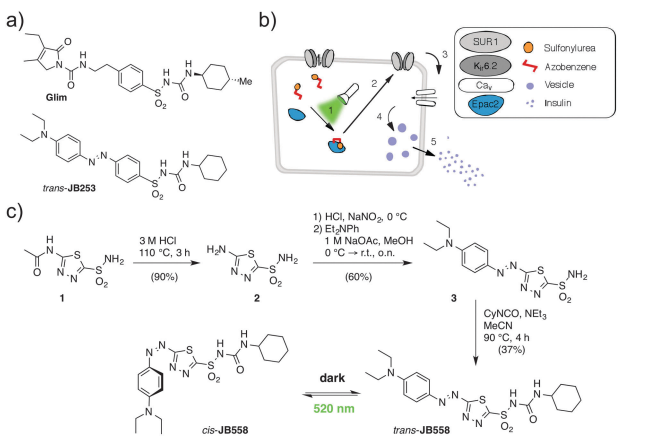
Type 2 diabetes (T2D) is a modern pandemic currently affecting ~6% of the global population. This disease is characterized by diminished insulin secretion from pancreatic beta cells, which together with peripheral resistance to the secreted hormone, leads to defective glucose homeostasis.[1] The resulting 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]

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 (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, 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 ~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 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 *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 ~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).

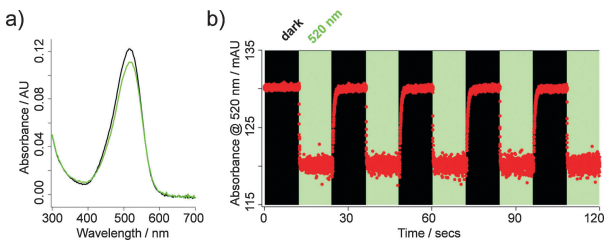

Fig. 1 (a) UV-Vis spectra of **JB558** in DMSO following illumination with λ = 520 nm (green) or under dark-adapted conditions (black). (b) Robust photoswitching between *cis*- and *trans*-**JB558** induced with λ = 520 nm and dark, respectively.

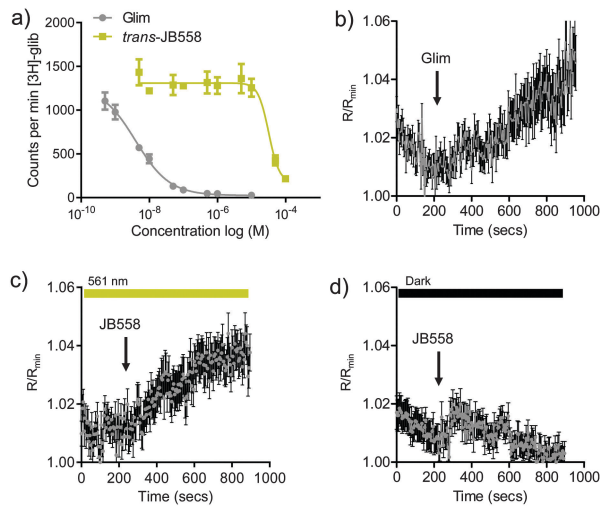

Fig. 2 (a) *trans*-**JB558** and glimepiride (Glim) displace [3H]-glibenclamide from SUR1 (n = 3 repeats). (b) Glimepiride decreases FRET (shown here as an increase in R/R_{min}) in HEK293T cells expressing full length Epac2-camps (n = 32 cells). (c) As for (b) but *cis*-**JB558** (λ = 561 nm) (n = 41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

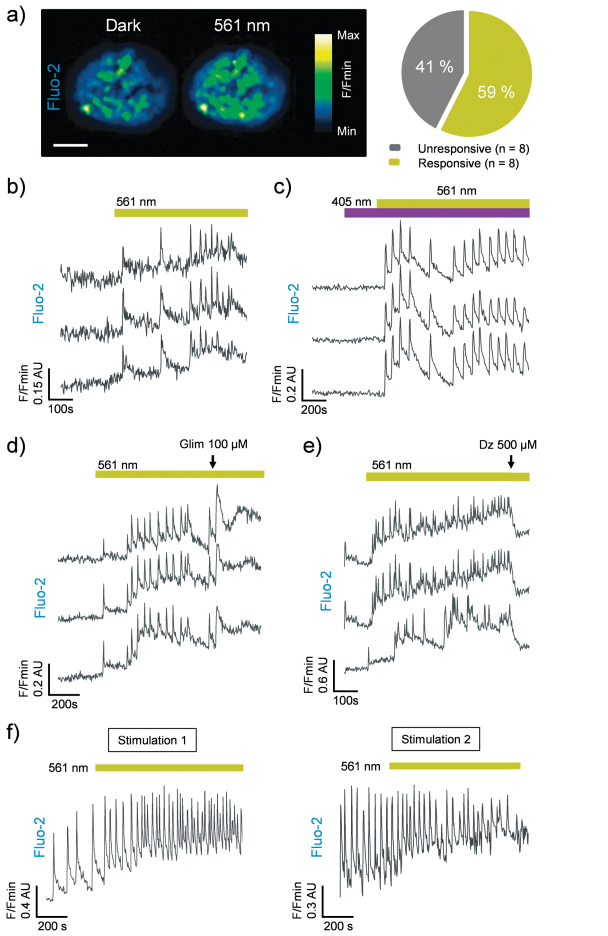

Fig. 3 (a) **JB558** increases intracellular Ca^{2+} concentrations in 59% of beta cells residing within rodent islets of Langerhans following illumination with λ = 561 nm (scale bar = 75 μm) (n = 8 islets). (b) Photoswitching is rapid following exposure to λ = 561 nm. (c) As for (b), but showing the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

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†).

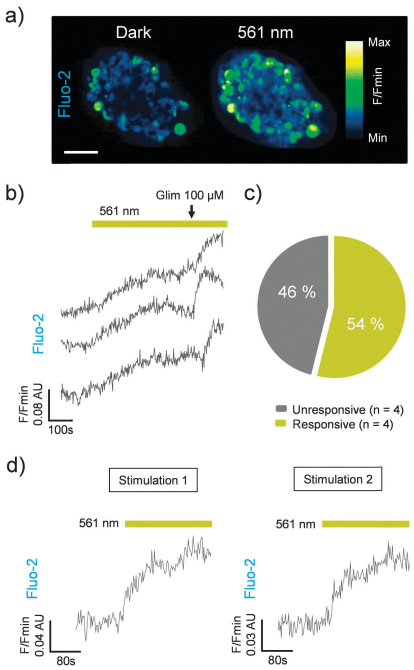

Fig. 4 (a) **JB558** increases intracellular Ca^{2+} concentrations in human beta cells in response to illumination with 561 nm to induce *cis*-formation (scale bar = 50 μm). (b) Photoswitching is rapid following exposure to 561 nm and can be potentiated with glimepiride (Glim). (c) *cis*-**JB588** activates 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.

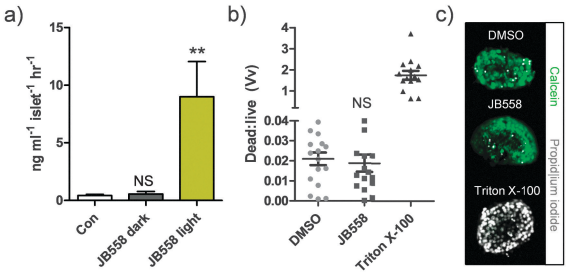

Fig. 5 (a) **JB558**-treated islets respond to illumination with $\lambda = 560$ nm by increasing insulin secretion (**$P < 0.01$ and NS, non-significant *versus* Con; one-way ANOVA). (b) Incubation with **JB558** for 1 h did not adversely affect cell viability *versus* dimethyl sulfoxide (DMSO), as 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 ± 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 (ε_{520nm}(**JB558**) = 1.14×10^5 mol^{-1} 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] enzymes[22] and antibiotics.[23]

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Notes and references

1 G. A. Rutter, *Mol. Aspects Med.*, 2001, **22**, 247–284.
2 J. M. Forbes and M. E. Cooper, *Physiol. Rev.*, 2013, **93**, 137–188.
3 S. Bolen, L. Feldman, J. Vassy, L. Wilson, H. C. Yeh, S. Marinopoulos, C. Wiley, E. Selvin, R. Wilson, E. B. Bass and F. L. Brancati, *Ann. Intern. Med.*, 2007, **147**, 386–399.
4 S. Seino, T. Shibasaki and K. Minami, *J. Clin. Invest.*, 2011, **121**, 2118–2125.
5 W. A. Velema, W. Szymanski and B. L. Feringa, *J. Am. Chem. Soc.*, 2014, **136**, 2178–2191.
6 J. Broichhagen, M. Schönberger, S. C. Cork, J. A. Frank, P. Marchetti, M. Bugliani, A. M. J. Shapiro, S. Trapp, G. A. Rutter, D. J. Hodson and D. Trauner, *Nat. Commun.*, 2014, **5**, 5116.
7 J. V. Frangioni, *Curr. Opin. Chem. Biol.*, 2003, **7**, 626–634.
8 A. Bakker, B. Smith, P. Ainslie and K. Smith, *Applied Aspects of Ultrasonography in Humans*, InTech, Near-Infrared Spectroscopy, 2012.
9 M. A. Kienzler, A. Reiner, E. Trautman, S. Yoo, D. Trauner and E. Y. Isacoff, *J. Am. Chem. Soc.*, 2013, **135**, 17683–17686.
10 A. Rullo, A. Reiner, A. Reiter, D. Trauner, E. Y. Isacoff and G. A. Woolley, *Chem. Commun.*, 2014, **50**, 14613–14615.
11 A. A. Beharry, O. Sadovski and G. A. Woolley, *J. Am. Chem. Soc.*, 2011, **133**, 19684–19687.
12 K. J. Herbst, C. Coltharp, L. M. Amzel and J. Zhang, *Chem. Biol.*, 2011, **18**, 243–251.
13 C. L. Zhang, M. Katoh, T. Shibasaki, K. Minami, Y. Sunaga, H. Takahashi, N. Yokoi, M. Iwasaki, T. Miki and S. Seino, *Science*, 2009, **325**, 607–610.
14 G. A. Rutter and D. J. Hodson, *Cell. Mol. Life Sci.*, 2014, **72**, 453–467.
15 M. Almahariq, F. C. Mei and X. Cheng, *Trends Endocrinol. Metab.*, 2014, **25**, 60–71.
16 L. Aguilar-Bryan, J. P. t. Clement, G. Gonzalez, K. Kunjilwar, A. Babenko and J. Bryan, *Physiol. Rev.*, 1998, **78**, 227–245.
17 C. A. Leech, O. G. Chepurny and G. G. Holz, *Vitam. Horm.*, 2010, **84**, 279–302.
18 T. Tsalkova, F. C. Mei, S. Li, O. G. Chepurny, C. A. Leech, T. Liu, G. G. Holz, V. L. Woods, Jr. and X. Cheng, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 18613–18618.
19 M. Stein, S. J. Middendorp, V. Carta, E. Pejo, D. E. Raines, S. A. Forman, E. Sigel and D. Trauner, *Angew. Chem., Int. Ed. Engl.*, 2012, **51**, 10500–10504.
20 M. Volgraf, P. Gorostiza, R. Numano, R. H. Kramer, E. Y. Isacoff and D. Trauner, *Nat. Chem. Biol.*, 2006, **2**, 47–52.
21 I. Tochitsky, M. R. Banghart, A. Mourot, J. Z. Yao, B. Gaub, R. H. Kramer and D. Trauner, *Nat. Chem.*, 2012, **4**, 105–111.
22 J. Broichhagen, I. Jurastow, K. Iwan, W. Kummer and D. Trauner, *Angew. Chem., Int. Ed. Engl.*, 2014, **53**, 7657–7660.
23 W. A. Velema, J. P. van der Berg, M. J. Hansen, W. Szymanski, A. J. Driessen and B. L. Feringa, *Nat. Chem.*, 2013, **5**, 924–928.