

Adrenergic modulation with photochromic ligands

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25 **Abstract:** Adrenoceptors are ubiquitous and mediate important autonomic functions as well as modulating
26 arousal, cognition and pain on a central level. Understanding these physiological processes and their
27 underlying neural circuits requires manipulating adrenergic neurotransmission with high spatio-temporal
28 precision. Here we present a first generation of photochromic ligands (adrenoswitches) obtained via
29 azologization of a class of cyclic amidines related to the known ligand clonidine. Their pharmacology,
30 photochromism, bioavailability and lack of toxicity allow for broad biological applications, as demonstrated by
31 controlling locomotion in zebrafish and pupillary responses in mice.
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34 35 Introduction

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37 Adrenergic neurotransmission plays an essential role over the unconscious regulation of most vital functions
38 in the human body. Heart and respiratory rate, digestion, smooth-muscle contraction, gland secretion,
39 and pupil diameter are modulated by noradrenaline release from sympathetic fibres projecting from the
40 peripheral nervous system (PNS).^[1] The neurotransmitter role is not limited to the PNS, though. Adrenergic
41 neurons firing from the locus coeruleus (LC) towards different areas of the central nervous system mediate
42 alertness, responses to acute stress and danger, pain modulation, arousal, sleep-wake cycles, as well as
43 neuroplasticity and cognitive behaviour.^[2-4] Despite the physiological relevance of adrenergic
44 neurotransmission, tools to precisely modulate its activity and to functionally dissect adrenergic pathways
45 have been scarce. Classical pharmacology, which benefits from high receptor specificity, is inadequate to
46 address cellular processes with high spatio-temporal selectivity. In this regard, light offers a non-invasive and
47 versatile mean to precisely control a variety of biological targets. Photocontrol of adrenergic signalling has
48 been shown with optogenetics^[5-7] and photo-uncaging.^[8] However, while the first technique requires genetic
49 manipulation and can lead to unintended neuroplastic phenomena, the irreversible release of caged ligands
50 displays diffusion-limited resolution and is challenging *in vivo*. Photopharmacology poses an alternative to
51 precisely and reversibly deliver drug activity in space and time by introducing photochromic groups into the
52 structure of bioactive compounds.^[9,10] Successful examples of photoswitchable drugs include ligands of ion
53 channels, receptors and enzymes, peptides, lipids and nucleic acids.^[11-13] However, compounds to photoswitch
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adrenergic neurotransmission are still not available. In this study we aimed at developing adrenergic drug-like molecules that can be reversibly controlled with light.

Results and Discussion

Among the large database of sympatholytic and sympathomimetic agents, we focused on a class of cyclic amidines structurally related to clonidine and other commercialized drugs targeting α -ARs (**Figure 1A**). All adrenergic agents share a common pharmacophoric motif: a primary or secondary aliphatic amine, protonated at physiological pH, and separated by a short linker from a substituted benzene ring.^[14] We postulated that α -adrenergic ligands were suitable candidates for “azologization”^[15,16] as the structural changes required to obtain photo-responsive derivatives could be introduced without altering essential pharmacophoric elements. In addition, the prospect of obtaining dark-inactive adrenergic azologs was supported by the lack of biological activity of *trans*-like epoxydic analogues compared to *cis*-like compounds described in the work of Gentili *et al.*^[17] We thus designed a set of putative photoswitchable adrenergic ligands, named “adrenoswitches”, by replacing the short linker with an azo group while constraining the cyclic amidine moiety to the closest structurally related aromatic derivatives. The *ortho*-dichlorobenzene system, common to clonidine and lofexidine, was maintained to favour both adrenergic activity and photochromism. First, halogen substitution increases the lipophilicity of a molecule, thus improving its absorption and its permeability to the blood brain barrier or the blood ocular barrier.^[18] Moreover, lipophilic substituents at the positions 2 and 6 of the phenyl ring are well tolerated in terms of pharmacological activity by both α_1 - and α_2 -ARs.^[19] Secondly, the photochromism of *ortho*-halogenated azobenzenes offers enhanced thermal stability, longer photoisomerization wavelengths, and higher isomerization ratios.^[20,21]

To identify suitable aromatic heterocycles, in our first analogue (adrenoswitch-1, **Figure 1B**) we substituted the imidazoline ring with an imidazole. As phenylazoimidazoles are known to undergo fast *cis-trans* thermal isomerization (i.e. fast-relaxing photoswitches), in adrenoswitch-2 we sought to reduce the rate of this process with an *N*-methyl imidazole.^[22] In adrenoswitch-3 we introduced a *N,N*-dimethyl imidazolium as a permanent positive charge that might better mimic the electronic properties of the original cyclic amidine in its physiologically protonated form. A similar strategy for the same purpose was using 2-aminothiazole in adrenoswitch-4.

Our library was prepared via a divergent synthetic approach involving a standard azo coupling reaction (**Figure 1B**). Commercially available 2,6-dichloroaniline was converted into the corresponding diazonium salt and reacted under mild basic conditions either with imidazole to provide adrenoswitch-1 or with 2-aminothiazole to afford adrenoswitch-4. Adrenoswitch-2 and adrenoswitch-3 were obtained from adrenoswitch-1 through reactions of mono- or di-*N*-methylation, respectively.

As these photoswitches have not been described among the reported azoheteroaryls^[23], their photochromic behaviours were then investigated. Slow-relaxing adrenoswitch-2 and -3 were characterized by steady-state UV-Vis absorption spectroscopy (**Figures 1C-F**), while transient UV-Vis absorption spectroscopy was used for fast-relaxing adrenoswitch-1 and -4 (**Figures 1D-E**). Our compounds can be photoisomerized from *trans* to *cis* with (ultra)violet light (365–400 nm) and from *cis* to *trans* with blue or green light (450–500 nm). As intended by design, thermal relaxation rates vary considerably along the series. Measured half-lives spanned from milliseconds (adrenoswitch-4) to seconds (adrenoswitch-1), minutes (adrenoswitch-3), and months (adrenoswitch-2) (**Figures 1E-F**).

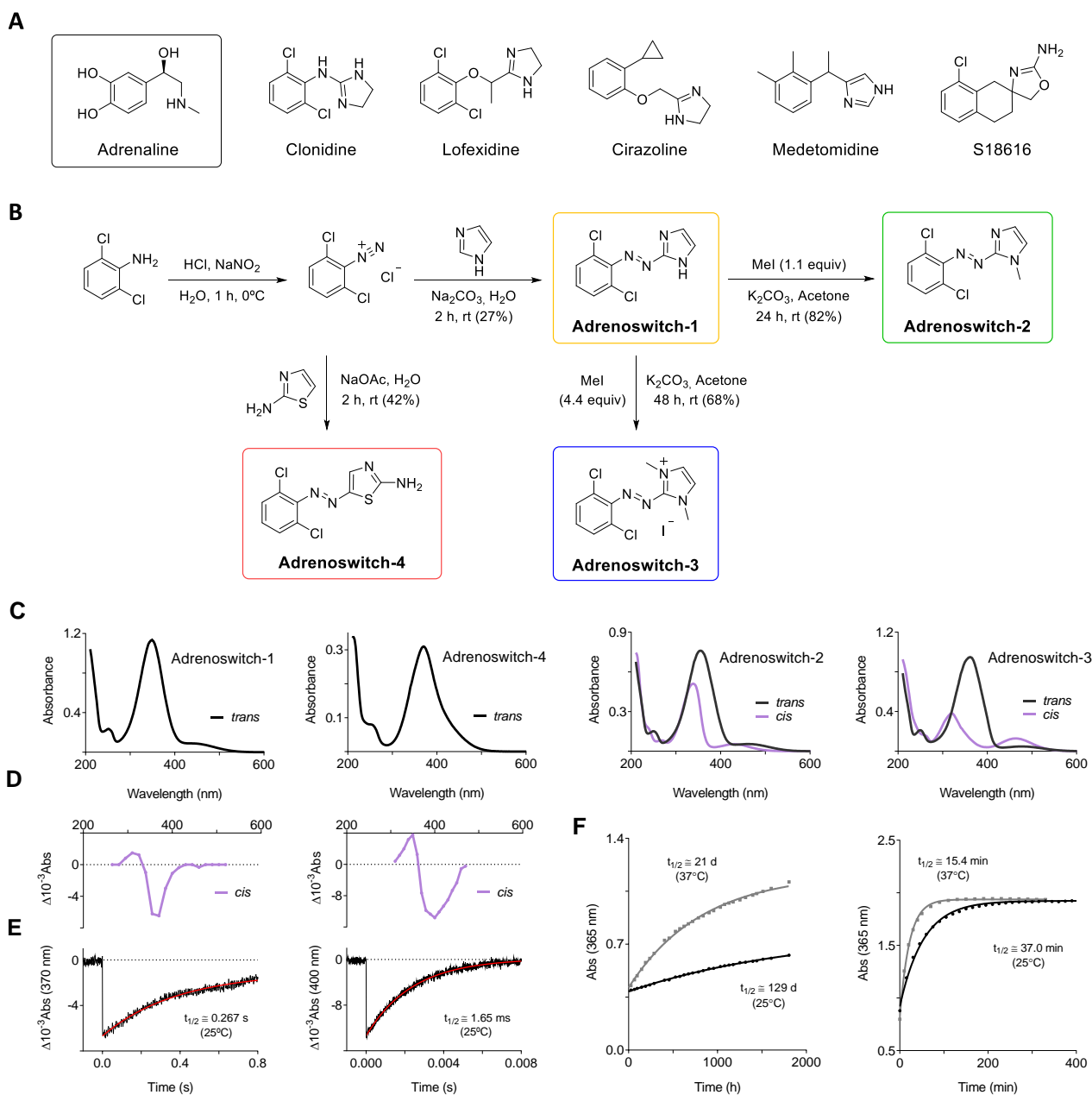


Figure 1. A) Chemical structures of adrenaline and some of the synthetic adrenergic ligands that inspired this work. **B)** Divergent chemical synthesis of adrenoswitches 1-4. **C)** UV-Vis absorption spectra of the *trans* isomers. For slow-relaxing adrenoswitch-2 and -3, UV-Vis absorption spectra of the *cis* isomers are also reported. Spectra were extracted from UPLC chromatograms after elution with a mixture of water and acetonitrile supplemented with trifluoroacetic acid. Spectra of *cis* and *trans* isomers were normalized at their isosbestic points (see SI – Figures S3A-C). **D)** Transient absorption spectra of adrenoswitch-1 and -4 at $t = 0$ upon pulsed excitation at $\lambda_{\text{exc}} = 355 \text{ nm}$ in physiological buffer ($\text{pH} = 7.4, 25^\circ\text{C}$). **E)** Absorption loss and recovery kinetics of adrenoswitch-1 and adrenoswitch-4, measured at $\lambda = 370$ and 400 nm respectively, upon irradiation with a single ns laser pulse ($t = 0$) at $\lambda_{\text{exc}} = 355 \text{ nm}$ in physiological buffer ($\text{pH} = 7.4, 25^\circ\text{C}$). Red lines correspond to monoexponential fitting of the experimental data. **F)** *Cis*-to-*trans* thermal relaxation of adrenoswitch-2 and -3 at 25°C (in black) and 37°C (in gray) under dark conditions. Data were fitted to a monoexponential decay model for *cis* isomers half-lives determination.

With all the information to effectively photoswitch our ligands, we assessed their biological activity as a function of illumination. The affinity of the library towards α_2 -ARs was measured by competitive radioligand binding assay in pre-frontal cortex membranes obtained *post mortem* from human brains. All the adrenoswitches competed in binding to the receptor albeit at weaker affinities than clonidine. Most notably, slow-relaxing adrenoswitch-2 and -3 showed a significant change in binding potency upon UV illumination

(Figure 2A and Figures S4A-B). On the other hand, no difference in binding could be observed for adenoswitch-1 and -4, possibly because these compounds display fast thermal isomerization and the binding assay might reflect mainly the properties of the *trans* isomers. Nonetheless, since in the work of Gentili *et al.* *trans* and *cis*-like epoxy analogues showed similar binding affinities, but produced different biological activities^[17], we decided to further test both slow- and fast-relaxing adenoswitches.

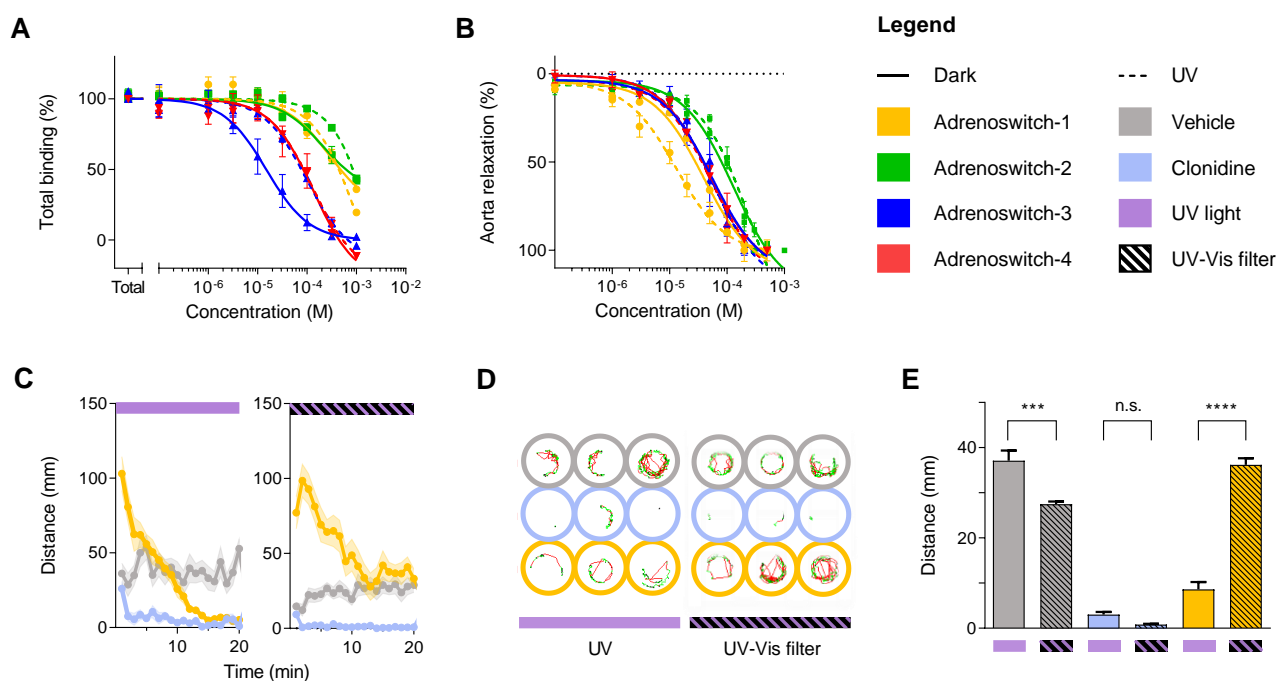


Figure 2. **A)** Competition binding curves of dark-relaxed (*trans*, solid lines) and UV-illuminated (*cis*, dashed lines) adrenergic photoswitches to α_2 -ARs in human brain pre-frontal cortex tissue. Binding is expressed as percentage of radioligand - [³H]RX821002 - bound to the receptor in the presence of the competitor. Data are means \pm SEM of two independent experiments run in duplicates or triplicates. **B)** Dose-response curves comparing the vasodilatory potencies of dark-adapted (*trans*, solid lines) and *cis*-enriched (dashed lines) adenoswitches administered in the dark or under UV illumination to rat aortic rings where the endothelium had been preserved. Vessels were pre-contracted with 10^{-6} M phenylephrine after treatment with 10^{-3} M *N*⁶-methyl-L-arginine acetate (L-NMMA), a nitric oxide synthase inhibitor. Relaxation is expressed as percentages of the reference contraction induced by PE. Data are means \pm SEM (adenoswitch-1, $n=4$ (per condition); adenoswitch-2, (*trans*) $n=6$ (*cis*) $n=5$; adenoswitch-3, (*trans*) $n=3$ (*cis*) $n=4$; adenoswitch-4, $n=2$ (per condition)). **C-D)** *Danio rerio* 7 days post-fertilisation larvae swimming distances and trajectories (movements with velocities over $6 \text{ mm}\cdot\text{s}^{-1}$) after treatment with vehicle (grey wells), $50 \mu\text{M}$ clonidine (light-blue wells) and $50 \mu\text{M}$ adenoswitch-1 (yellow wells). Conditions were simultaneously analysed under 365 nm UV illumination (left panels, *cis*-enriched adenoswitch-1) and under a UV-Vis filter (right panels, *trans* adenoswitch-1) that only transmits infrared light for movement recording. **E)** Quantification of the swimming distances shown in **C)** at $t=20$ minutes. Data are means \pm SEM ($n=12$ per treatment). Statistical differences between UV- and non-UV-exposed larvae were determined by two-way ANOVA with Tukey's multiple comparison test (n.s., not significant; ***, p -value <0.01 ; ****, p -value <0.001).

Since the adrenergic system is a major regulator of the vascular tone, we characterized the adrenergic effects of our compounds by measuring their vascular activity in *ex vivo* rat aortic rings.^[24–26] Besides being generally recognized as a potent central hypotensive agent^[27], clonidine is also known to induce vasodilation by acting directly on vascular adrenoceptors.^[25] This peripheral response involves stimulation of endothelial α_2 -ARs as well as partial agonism towards α_1 -ARs of the vascular smooth muscle, and results in relaxation of endothelium-intact strips previously contracted with phenylephrine (PE).^[26,28–30] When testing if our analogues displayed a similar behaviour, we observed that exposure to UV light alone could induce relaxation of the vascular smooth muscle, substantially abolishing the contraction evoked by PE.^[31] These intrinsic photoresponses are due to nitric oxide (NO) release either from endogenous or exogenous donors or from storage vesicles^[32,33]. To minimize unintended photorelaxation, we pre-incubated the vessels in

1 a suitable nitric oxide synthase (NOS) inhibitor, namely *N*^G-methyl-L-arginine acetate (L-NMMA) which is
2 reported to be unaffected by light^[34]. Remarkably, all adenoswitches displayed high vasodilatory efficacies
3 (maximal evoked response E_{max} , **Figure 2B**) with adenoswitch-1 coming through as the most potent
4 compound and the only one displaying a light-dependent behaviour in this assay (3x increase in potency upon
5 UV illumination, **Figure S8A**). Although this assay was useful to identify and rank the adrenergic efficacy and
6 potency of adenoswitches, it had important shortcomings. Intrinsic photoresponses forced us to use a NOS
7 inhibitor, thus partially blocking endothelium-dependent relaxation responses to α_2 -AR agonists^[26,35].
8 Moreover, the model proved complex to clarify adenoswitches vascular pharmacodynamics as the observed
9 antihypertensive responses might involve both α_1 and α_2 -ARs and do not exclude further contributions from
10 other non-adrenergic targets, most notably imidazoline receptors.^[36–38]

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12 **We next characterized the activity of adenoswitches *in vivo*.** As proof-of-concept applications, we used the
13 most promising compound of the series, adenoswitch-1, to remotely manipulate locomotion in zebrafish and
14 pupillary responses in mice.

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16 Zebrafish (*Danio rerio*) is a well-established animal model for both neurobehavioural^[39] and toxicological
17 studies^[40], and the transparency of their larvae makes them convenient for photopharmacological
18 applications.^[41,42] Besides, the expression and function of zebrafish ARs have been studied^[43] and the effect of
19 dexmedetomidine, a central-acting and structurally-related α_2 -AR agonist, has been described by Ruuskanen
20 *et al.*^[44] In analogy to the sedative effect reported for dexmedetomidine, clonidine (50 μ M) caused locomotor
21 inhibition when compared to untreated animals (**Figure 2C-D**). Clonidine effect was not significantly altered
22 by UV light, although in vehicle-treated animals we observed an increase of the swimming distances as a result
23 of their photomotor response to illumination.^[39] In contrast, larvae treated with adenoswitch-1 (50 μ M)
24 responded to UV light by progressively reducing their locomotion to levels comparable to clonidine-treated
25 animals under UV (**Figures 2D-E** and **Figures S9-S10**). Therefore, adenoswitch-1 displayed a clonidine-like
26 behaviour upon UV-activation, while its *trans* isomer was unable to evoke sedation. Although this response is
27 unequivocally light-induced, thermal relaxation of the compound was not enough to revert the behavioral
28 effect (**Figure 1E** and **Figure S10**). Since other non-adrenergic receptors might evoke locomotion responses,
29 we further studied adenoswitch-1 in another *in vivo* model.

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31 Among the available animal models for assessing autonomic responses, we sought one in which adrenoceptors
32 played a major physiological role and in which the use of photoswitchable drugs may have actual
33 investigational and therapeutic implications. Modulating pupil responses fulfilled both criteria and it seemed
34 particularly attractive as the target tissue is naturally accessible to light. In addition, pupillometry allows for a
35 selective evaluation of α -adrenergic activity and it excludes the involvement of imidazoline receptors.^[45–47] As
36 pupils adapt their diameter to changes in light intensity (pupillary light reflex, PLR), we resorted to genetically
37 engineered blind mice (*Opn4xRd10*) for a first assessment of the compound activity. *Opn4xRd10* mice do not
38 express melanopsin and their rods and cones degenerate two months after their birth, thus becoming
39 physiologically insensitive to any luminous stimuli. We tested if adenoswitch-1 enabled photoregulation of
40 pupil diameter after topical administration (1 mM, 0.02% w/v) to isoflurane-anesthetized animals. The
41 compound exerted mydriasis only under UV illumination and the effect fully reversed in the dark, when
42 adenoswitch-1 rapidly relaxes to the *trans* configuration (**Figures 3A-B-C** and **Supplementary Movie S1**).
43 These pupillary responses were reproducible in at least two cycles of alternating UV light and darkness, and
44 were neither elicited by application of the vehicle nor by exposure to UV light alone. The maximum
45 photoresponses were consistently observed after approximately 20 min from its administration (**Figure 3C**),
46 in agreement with the pharmacodynamics of adrenergic ligands with similar structure and lipophilicity.^[48] As
47 mydriasis is mediated by postsynaptic α_1 -adrenoceptors of the iris dilator muscle, the results of **Figures 3A-B-**
48 **C** unambiguously demonstrate that adenoswitch-1 modulates endogenous ARs with light. Taking into account
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its molecular structure and the pharmacological characterization, we consider that the compound is likely to act as a partial agonist towards α_1 -ARs. Nonetheless, a thorough characterization of adenoswitches' pharmacological profiles would be needed to evaluate their specificity and selectivity over adrenergic and non-adrenergic receptors.

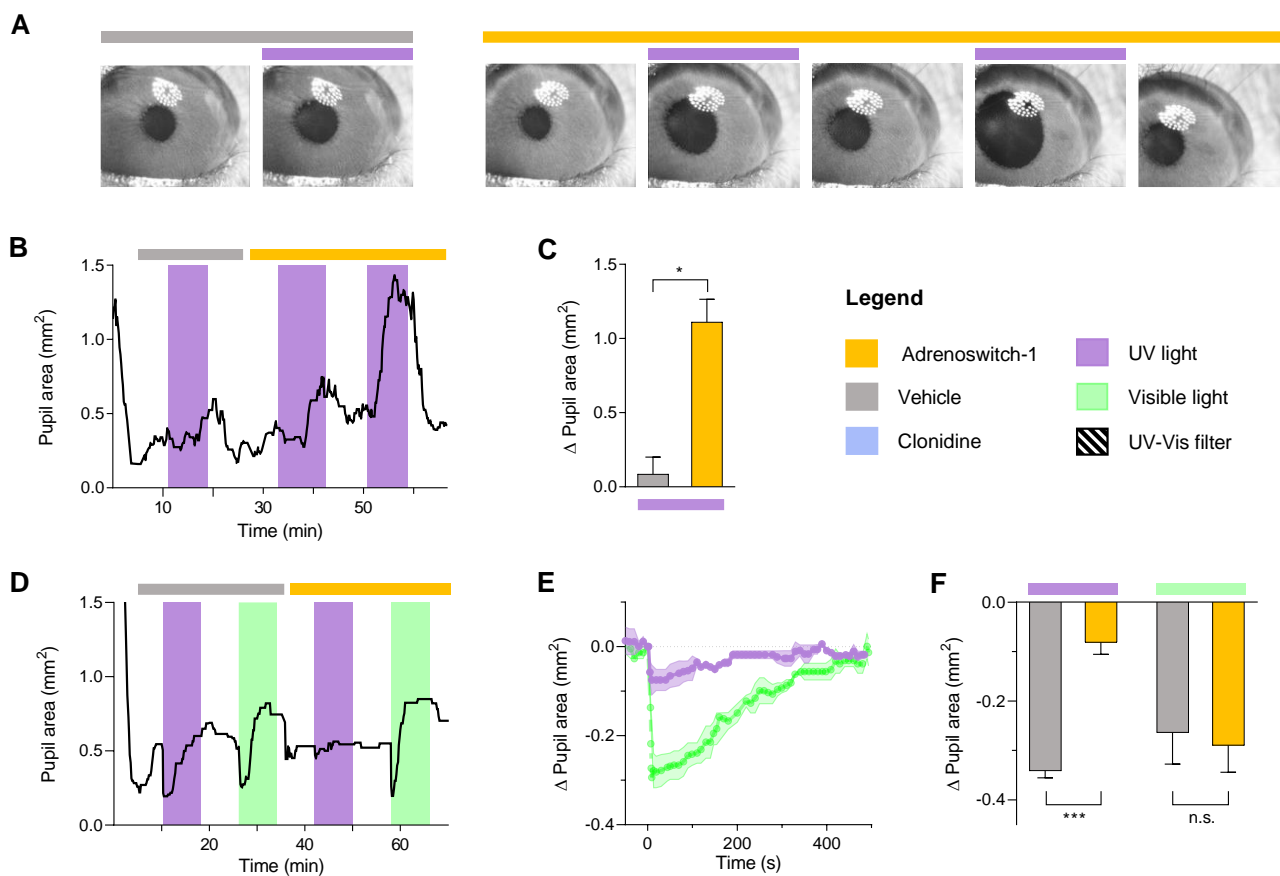


Figure 3. A-B) Pupillary responses in an isoflurane-anesthetized *Opn4xRd10* blind mouse first treated with vehicle and then administered 1 mM adenoswitch-1 in its right eye. Adenoswitch-1 exerted mydriasis only upon UV illumination. Neither the vehicle nor UV light alone elicited any pupillary responses in the animals. **A complete movie of the experiment (100x speed) is available as SI (Supplementary Movie S1).** **C)** Change in area of vehicle vs. adenoswitch-1 treated pupils of *Opn4xRd10* blind mice at t=20 minutes from administration of the drug under UV illumination. Data are means \pm SEM ($n=4$ per condition). Statistical differences were determined by Student's *t* test for unpaired observations. **D)** Pupillary responses in an isoflurane-anesthetized C57BL/6J wildtype mouse first treated with vehicle and then administered 1 mM adenoswitch-1 in its right eye. The animal was exposed to 5-min cycles of UV, darkness and green light. UV and green light elicited a pupillary light reflex (PLR). Adenoswitch-1 abolished the PLR only under concomitant UV illumination, while the response was maintained under visible light. **E)** Comparison of UV and green light evoked PLR in adenoswitch-1-treated animals. Data are means \pm SEM ($n=2$ per condition). **F)** PLR quantification in anesthetized wildtype mice. Pupil contraction evoked by exposure to UV and green light was compared between vehicle- and adenoswitch-1-treated animals. Data are mean \pm SEM ($n=2$ per condition). Statistical differences were determined by Student's *t* test for unpaired observations.

Finally, we tested if adenoswitch-1 could be used in wildtype animals to selectively decouple pupil tone from environmental illumination. As shown in **Figures 3D-F**, vehicle-treated C57BL/6J mice respond to violet and green light exposure with similar rapid PLRs. After topical administration of adenoswitch-1, the miosis caused by UV light was blocked whereas the response to green light was maintained (**Figures 3D-E-F**). As the UV luminous stimulus is triggering a miotic reflex while simultaneously activating a photo-regulated mydriatic agent (adenoswitch-1), the two effects appear to cancel each other out. These pupillary responses were reproducible in all the cycles of alternating UV, darkness and green light and persisted after several washings of the cornea.

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In contrast to anticholinergic mydriatics, the pharmacological action of adenoswitch-1 can be selectively controlled using specific wavelengths, can be fully reversed on demand, and does not alter the pupil basal tone. These unique properties might allow deeper insights into the role of PLR in vision adaptation dynamics. Moreover, as retinal responses are closely related to both area and intensity of the visual stimulus, adenoswitches might become unique tools to investigate photoresponse kinetics and their mechanisms in retinal ganglion cells.^[49]

On-demand regulation of pupil diameter might also have ophthalmic applications. For example, slow-relaxing pre-illuminated adenoswitches could induce a mydriatic response that spontaneously reverses upon progressive exposure of the eye to natural light. Procedures like examination of the retina or other structures of the eye's fundus require fully dilated pupils. Although mydriasis can be readily achieved with topic cholinergic antagonists, post-exam pupils' dilation lasts hours and causes photophobia, increased intra-ocular pressure and blurry vision. As these side effects are highly impairing, patients would benefit from drugs whose mydriatic effect can be rapidly reverted.

Conclusion

In summary, we have rationally designed novel arylazoheteroarene photoswitches and characterised their adrenergic action *in vitro* and *in vivo*, including the control of locomotor activity in zebrafish larvae and pupil responses in mice. **These results enable multiple applications that were previously inaccessible for adrenergic neurotransmission and open the way to achieve optimized photopharmacological properties based on this scaffold.** On-demand modulation at specific locations might allow to single out adrenergic projections from the LC and to dissect their function in circuits and behaviour with high resolution.^[50,51] Moreover, adenoswitches can enable photocontrol over a variety of physiological functions, like pupillary responses, and help unravel their physiology *in vivo*.

Acknowledgements:

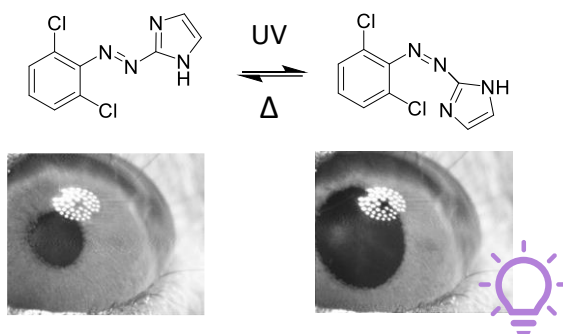
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- [1] A. W. Tank, D. L. Wong, *Compr. Physiol.* **2015**, *5*, 1–15.
 - [2] E. Samuels, E. Szabadi, *Curr. Neuropharmacol.* **2008**, *6*, 254–285.
 - [3] M. Llorca-Torrallba, G. Borges, F. Neto, J. A. Mico, E. Berrocoso, *Neuroscience* **2016**, *338*, 93–113.
 - [4] S. J. Sara, *Nat. Rev. Neurosci.* **2009**, *10*, 211–223.
 - [5] J. M. Kim, J. Hwa, P. Garriga, P. J. Reeves, U. L. RajBhandary, H. G. Khorana, *Biochemistry* **2005**, *44*, 2284–2292.
 - [6] P. Makowka, T. Bruegmann, V. Dusend, D. Malan, T. Beiert, M. Hesse, B. K. Fleischmann, P. Sasse, *Nat. Commun.* **2019**, *10*, 1281.
 - [7] E. R. Siuda, J. G. McCall, R. Al-Hasani, G. Shin, S. Il Park, M. J. Schmidt, S. L. Anderson, W. J. Planer, J. A. Rogers, M. R. Bruchas, *Nat. Commun.* **2015**, *6*, 8480.
 - [8] S. Muralidharan, G. M. Maher, W. A. Boyle, J. M. Nerbonne, *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 5199–5203.
 - [9] W. A. Velema, W. Szymanski, B. L. Feringa, *J. Am. Chem. Soc.* **2014**, *136*, 2178–2191.
 - [10] M. J. Fuchter, *J. Med. Chem.* **2020**, DOI 10.1021/acs.jmedchem.0c00629.
 - [11] K. Hüll, J. Morstein, D. Trauner, *Chem. Rev.* **2018**, *118*, 10710–10747.
 - [12] M. M. Lerch, M. J. Hansen, G. M. van Dam, W. Szymanski, B. L. Feringa, *Angew. Chemie - Int. Ed.* **2016**, *55*, 10978–10999.
 - [13] P. Paoletti, G. C. R. Ellis-Davies, A. Mourot, *Nat. Rev. Neurosci.* **2019**, *20*, 514–532.
 - [14] R. K. Griffith, in *Foye's Princ. Med. Chem.* (Eds.: T.L. Lemke, D.A. Williams), Lippincott Williams & Wilkins, **2013**, pp. 348–356.
 - [15] S. Pittolo, X. Gómez-Santacana, K. Eckelt, X. Rovira, J. Dalton, C. Goudet, J. P. Pin, A. Llobet, J. Giraldo, A. Llebaria, P. Gorostiza, *Nat. Chem. Biol.* **2014**, *10*, 813–815.
 - [16] M. Schoenberger, A. Damijonaitis, Z. Zhang, D. Nagel, D. Trauner, *ACS Chem. Neurosci.* **2014**, *5*, 514–518.
 - [17] F. Gentili, F. Ghelfi, M. Giannella, A. Piergentili, M. Pignini, W. Quaglia, C. Vesprini, P. A. Crassous, H. Paris, A. Carrieri, *J. Med. Chem.* **2004**, *47*, 6160–6173.
 - [18] G. Thomas, in *Med. Chem. An Introd.*, Wiley, **2008**, pp. 83–84.
 - [19] A. J. Nichols, R. R. Ruffolo, in *Alpha-Adrenoceptors Mol. Biol. Biochem. Pharmacol.*, **1991**, pp. 75–114.
 - [20] D. Bléger, J. Schwarz, A. M. Brouwer, S. Hecht, *J. Am. Chem. Soc.* **2012**, *134*, 20597–20600.
 - [21] J. Calbo, A. R. Thawani, R. S. L. Gibson, A. J. P. White, M. J. Fuchter, *Beilstein J. Org. Chem.* **2019**, *15*, 2753–2764.
 - [22] J. Otsuki, K. Suwa, K. K. Sarker, C. Sinha, *J. Phys. Chem. A* **2007**, *111*, 1403–1409.
 - [23] S. Crespi, N. A. Simeth, B. König, *Nat. Rev. Chem.* **2019**, *3*, 133–146.
 - [24] B. Civantos Calzada, A. Aleixandre De Artiñano, *Pharmacol. Res.* **2001**, *44*, 195–208.
 - [25] S. Guimarães, D. Moura, *Pharmacol. Rev.* **2001**, *53*, 319–356.
 - [26] P. M. Vanhoutte, *J. Cardiovasc. Pharmacol.* **2001**, *38*, 796–808.
 - [27] L. Isaac, *J. Cardiovasc. Pharmacol.* **1980**, *2*, 5–20.
 - [28] E. G. Silva, T. Feres, L. M. Vianna, P. Okuyama, T. B. Paiva, *J. Pharmacol. Exp. Ther.* **1996**, *277*, 872–876.
 - [29] S. Iwanaga, O. Shibata, A. Tsuda, S. Hashimoto, T. Makita, S. Cho, K. Sumikawa, *Res Commun Mol*

Pathol Pharmacol. **1998**, *102*, 137–47.

- [30] E. F. Castillo, C. S. Ortíz, R. M. López, A. Ruíz, J. M. Vélez, C. Castillo, *Fundam. Clin. Pharmacol.* **2006**, *20*, 339–349.
- [31] R. F. Furchgott, S. J. Ehrreich, E. Greenblatt, *J. Gen. Physiol.* **1961**, *44*, 499–519.
- [32] K. L. Andrews, J. J. McGuire, C. R. Triggle, *Br. J. Pharmacol.* **2003**, *138*, 932–940.
- [33] F. W. Flitney, I. L. Megson, *J. Physiol.* **2003**, *550*, 819–828.
- [34] J. A. Bauer, H. Fung, *Pharmacol. Lett.* **1993**, *54*, 1–4.
- [35] J. C. Molin, L. M. Bendhack, *Vascul. Pharmacol.* **2004**, *42*, 1–6.
- [36] S. Regunathan, C. Youngson, H. Wang, D. J. Reis, *Ann. New York Acad. Sci.* **1995**, *12*, 580–90.
- [37] G. J. Molderings, M. Göthert, *Gen. Pharmacol.* **1999**, *32*, 17–22.
- [38] M. F. Chen, J. T. Tsai, L. J. Chen, T. P. Wu, J. J. Yang, L. Te Yin, Y. L. Yang, T. A. Chiang, H. L. Lu, M. C. Wu, *Biomed Res. Int.* **2014**, *2014*, 1–7.
- [39] D. Kokel, J. Bryan, C. Laggner, R. White, C. Y. J, R. Mateus, D. Healey, S. Kim, A. A. Werdich, J. Stephen, C. A. Macrae, B. Shoichet, R. T. Peterson, *Nat Chem Biol* **2010**, *6*, 231–237.
- [40] A. L. Rubinstein, *Expert Opin. Drug Metab. Toxicol.* **2006**, *2*, 231–240.
- [41] C. Matera, A. M. J. Gomila, N. Camarero, M. Libergoli, C. Soler, P. Gorostiza, *J. Am. Chem. Soc.* **2018**, *140*, 15764–15773.
- [42] K. Rustler, A. Gomila, G. Maleeva, P. Gorostiza, P. Bregestovski, B. König, *Chem. – A Eur. J.* **2020**, *26*, in press.
- [43] J. O. Ruuskanen, J. Laurila, H. Xhaard, V. V. Rantanen, K. Vuoriluoto, S. Wurster, A. Marjamäki, M. Vainio, M. S. Johnson, M. Scheinin, *Br. J. Pharmacol.* **2005**, *144*, 165–177.
- [44] J. O. Ruuskanen, N. Peitsaro, J. V. M. Kaslin, P. Panula, M. Scheinin, *J. Neurochem.* **2005**, *94*, 1559–1569.
- [45] J. Raczak-Gutknecht, T. Frackowiak, A. Nasal, R. Kaliszan, *Pharmacol. Reports* **2013**, *65*, 305–312.
- [46] Y. Yu, M. C. Koss, *Auton. Neurosci. Basic Clin.* **2005**, *117*, 17–24.
- [47] H. Ishikawa, D. D. Miller, N. Patil, *Naunyn. Schmiedebergs. Arch. Pharmacol.* **1996**, *354*, 765–772.
- [48] H. C. A. Innemee, A. de Jonge, J. C. A. van Meel, P. B. M. W. M. Timmermans, P. A. van Zwieten, *NaunynSchmiedebergs Arch. Pharmacol.* **1981**, *316*, 294–298.
- [49] K. Y. Wong, F. A. Dunn, D. M. Berson, *Neuron* **2005**, *48*, 1001–1010.
- [50] S. Pittolo, H. Lee, A. Lladó, S. Tosi, M. Bosch, L. Bardia, X. Gómez-Santacana, A. Llebaria, E. Soriano, J. Colombelli, K. E. Poskanzer, G. Perea, P. Gorostiza, *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 13680–13689.
- [51] G. Cabré, A. Garrido-Charles, M. Moreno, M. Bosch, M. Porta-de-la-Riva, M. Krieg, M. Gascón-Moya, N. Camarero, R. Gelabert, J. M. Lluch, F. Busqué, J. Hernando, P. Gorostiza, R. Alibés, *Nat. Commun.* **2019**, *10*, 907.

Adrenoswitches



Adrenoswitches, a class of novel arylazoheteroarene photoswitches, were developed to achieve on-demand modulation of adrenergic neurotransmission by means of illumination at specific wavelengths. Their versatility to photocontrol biological systems is demonstrated *in vivo* employing two different animal models, zebrafish and mice.

Keywords: Adrenergic – Azo compounds – Biological activity – Neurotransmitters – Photochromism