Photoswitchable antagonists for a precise spatiotemporal control of β_2 -adrenoceptors

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Supporting Information

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SUPPLEMENTARY FIGURES

Figure S1. Photochemical characterization of Photoazolol-2 (PZL-2, 50 μM in 0.5% DMSO Buffer). (A) 2D and 3D chemical structures of the photoisomers of **PZL-2**. (B) UV-Vis spectra of the azobenzene under different light conditions. (C) Photostationary state (PSS) quantification by ^1H -NMR. (D) Multiple *cis/trans* isomerization cycles show the stability of the compound over 45 minutes of light application. (E) Half-lifetime estimation of *cis*-**PZL-2** at 25 $^{\circ}\text{C}$; absorbance was measured at 364 nm.

Figure S2. Photochemical characterization of Photoazolol-3 (PZL-3, 50 μ M in 0.5% DMSO Buffer). (A) 2D and 3D chemical structures of the photoisomers of **PZL-3**. (B) UV-Vis spectra of the azobenzene under different light conditions. (C) Photostationary state (PSS) quantification by ^1H -NMR. (D) Multiple *cis/trans* isomerization cycles show the stability of the compound over 45 minutes of light application. (E) Half-lifetime estimation of *cis*-**PZL-3** at 25 $^{\circ}\text{C}$; absorbance was measured at 364 nm.

Figure S3. ¹H NMR studies of the photostationary states (PSS) of Photoazolol-1. A sample containing 0.1 mM **PZL-1** in D₂O was analyzed in dark conditions and after illumination with 380 nm and 550 nm light sources. Grey squares illustrate the NMR signals corresponding to the *trans* isomer and the violet diamonds represent the *cis* signals.

Figure S4. ¹H NMR studies of the photostationary states (PSS) of Photoazolol-2. A sample containing 0.1 mM **PZL-2** in D₂O was analyzed in dark conditions and after illumination with 380 nm and 550 nm light sources. Grey squares illustrate the NMR signals corresponding to the *trans* isomer and the violet diamonds represent the *cis* signals.

Figure S5. ¹H NMR studies of the photostationary states (PSS) of PhotoazoloI-3. A sample containing 0.1 mM **PZL-3** in D₂O was analyzed in dark conditions and after illumination with 380 nm and 550 nm light sources. Grey squares illustrate the NMR signals corresponding to the *trans* isomer and the violet diamonds represent the *cis* signals.

Figure S6. Dose-response curves of **Photoazolol-3** with 3nM Cimaterol in the dark (continuous violet line) and under 380 nm light illumination (discontinuous light violet line). A dose-response of Propranolol (Prop) was tested in the same assay in dark (continuous grey line) and 380 nm light conditions (discontinuous light grey line). Data are shown as the mean $\pm$ SEM of four independent experiments performed in duplicate.

Figure S7. Chemical stability of β_2 -AR agonist cimaterol in aqueous media after light illumination. (A) 0.5 mg/mL solution of cimaterol (1% DMSO in PBS) was continuously illuminated with 405 nm light for different times (0-2h). The different samples were analyzed by HPLC-MS. (B) Cimaterol potency measured over 30 min and after 45 min incubation in the dark or under continuous illumination.

Figure S8. Validation of the effect of Photoazolol-1 and Photoazolol-2 on β_2 -AR.

(A) Dose-response curves of forskolin with constant concentrations of **PLZ-1** and **PLZ-2** (10 nM and 1000 nM respectively) in the dark (continuous lines) or under 380nm light illumination (discontinuous lines). (B) Dose-response curves of **PZL-1** and **PZL-2** with 10 μM forskolin in dark and 380 nm light conditions. Data are shown as the mean $\pm$ SEM of three independent experiments performed in duplicate.

Figure S9. Antagonistic activity of Photoazolol-1. (A) Dose-response curves of cimeterol with different concentrations of **PZL-1** under dark conditions or after 380 nm light illumination. (B) Representation of the pEC_{50} values obtained on the different replicates on panel A. Each data point represents the mean and SEM of four experiments performed in duplicate. Statistical differences from light EC_{50} values are denoted for adjusted p values as follows: * $p < 0.05$ and **** $p < 0.0001$.

Figure S10. Antagonistic activity of Photoazolol-2. (A) Dose-response curves of cimaterol with different concentrations of **PZL-2** under dark conditions or after 380 nm light illumination. (B) Representation of the pEC_{50} values obtained on the different replicates on panel A. Each data point represents the mean and SEM of four experiments performed in duplicate. Statistical differences are denoted for adjusted p values as follows: **** $p < 0.0001$.

Figure S11. Time evolution of receptor activation state in cells treated with Photoazolol-1 and Photoazolol-2 in the dark and under illumination. *In vitro* intracellular cAMP was continuously monitored in the dark for 30 min after a 45 min pre-incubation with the azobenzenes in the dark or under 380 nm light illumination. (A) Cells treated with 10 nM **PZL-1** and 10 nM of the agonist cimaterol. (B) Cells treated with 1 μ M **PZL-2** and 100 nM of the agonist cimaterol. Each data point represents the mean and SEM of three experiments performed in duplicate.

Figure S12. Real-time optical control of β_2 -AR. Time course quantification of intracellular cAMP challenged with the β_2 -AR agonist cimaterol (10 nM and 100 nM, respectively) in the presence of **PZL-1** (A) or **PZL-2** (B). Violet and green boxes correspond to 10 min illumination breaks using 380 nm and 550 nm lights, respectively. **PZL-1** and **PLZ-2** in dark conditions are represented by white dots. Each data point represents the mean and SEM of three to four experiments performed in duplicate.

Figure S13. (A-C) Rigid docking results for carazolol, **PZL-1** in *trans* configuration and **PZL-2** in *trans* configuration, respectively. (D-E) Induced fit results for carazolol, **PZL-1** and **PZL-2**, both in *cis* configuration.

SUPPLEMENTARY TABLES

Table S1. EC₅₀ values of cimaterol Dose-Response curves with different concentrations of Photoazolol-1 under different light conditions (Figure S9).

Conditions	DARK		LIGHT	
	EC ₅₀ (nM)	SEM	EC ₅₀ (nM)	SEM
No PZL-1	3.08	0.80	5.44	1.83
+ 0.1nM	3.74	0.46	5.15	1.93
+ 0.3nM	4.81	0.88	4.40	1.08
+ 1nM	9.49***	1.01	4.57	1.34
+ 3 nM	34.01***	6.21	6.68	1.65
+10 nM	112.68****	16.09	12.97	3.12

Statistical differences between dark and light EC₅₀ values for each concentration were analyzed through an extra sum-of-squares F test for adjusted p values as follows: ***p<0.001 and ****p<0.0001 .

Table S2. EC₅₀ values of cimaterol Dose-Response curves with variable concentrations of Photoazolol-2 under different light conditions (Figure S10).

Conditions	DARK		LIGHT	
	EC ₅₀ (nM)	SEM	EC ₅₀ (nM)	SEM
No PZL-2	5.00	0.88	6.30	0.65
+ 10 nM	4.01*	0.52	6.92	0.86
+30 nM	4.53**	0.94	8.98	2.35
+100 nM	6.67****	1.23	28.95	15.83
+ 300 nM	14.35****	1.12	58.22	23.34
+1000 nM	32.88****	6.94	122.22	32.51

Statistical differences between dark and light EC₅₀ values for each concentration were analyzed through an extra sum-of-squares F test for adjusted p values as follows: *p<0.05, **p<0.01, ****p<0.0001.

NMR SPECTRA, PSS& %EE DETERMINATION

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