Supplementary Information

Selective photoswitchable allosteric agonist of a G protein-coupled receptor

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Supplementary Methods

Chemistry

Nuclear magnetic resonance (NMR) spectroscopy

NMR-spectra were acquired with the following spectrometers: Varian INOVA 400 (400 MHz for $^1$H and 101 MHz for $^{13}$C spectroscopy), Bruker Avance III HD 400 with Cryo-head (400 MHz for $^1$H and 101 MHz for $^{13}$C spectroscopy) and Bruker Avance III HD 800 with Cryo-head (800 MHz for $^1$H, 201 MHz for $^{13}$C spectroscopy). Chemical shifts ($\delta$) are reported in parts per million (ppm) relative to tetramethylsilane (TMS). The deuterated solvents CDCl$_3$ and (CD$_3$)$_2$SO were used as internal references. Spin multiplicities are described as follows: s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), six (sixtet), h (heptet), m (multiplet), br (broad) or a combination thereof. Structural analysis was conducted with $^1$H- and $^{13}$C-NMR spectra with the aid of additional 2D spectra (COSY, HMBC, HSQC, NOESY). Spectra analysis was conducted with the software MestReNova v.10.0.1-14719. The atom numbering of the signal assignment does not correspond to IUPAC rules.

Mass spectrometry (MS)

The high-resolution MS spectra were recorded either on Thermo Finnigan MAT 95 (EI: electron ionization) or Thermo Finnigan LTQ FT (ESI: electrospray ionization).

Infrared spectroscopy (IR)

IR spectra were recorded on a PerkinElmer Spectrum BX II FT-IR device equipped with an attenuated total reflection (ATR) measuring unit. For measurements, the neat substances were directly applied as a thin film on the ATR unit. The measured wavenumbers are reported with their relative intensities which were classified as: vs (very strong), s (strong), m (medium), w (weak), vw (very weak), br (broad) or combinations thereof.

UV-Vis spectroscopy (UV-Vis)

UV-Vis spectroscopy was performed on a Varian Cary 60 UV-Visible Spectrometer equipped with an Agilent Technologies PCB 1500 Water Peltier system for temperature control. Samples were
measured using disposable Spectrometer/Photometer Ultra-Micro Cuvettes from BrandTech (10 mm light path, 1 mL sample) and irradiation was performed with a Cairn Research Optoscan Monochromator with Optosource High Intensity Arc Lamp equipped with a 75 W UXL-S50A lamp from USHIO Inc. Japan, set to 15 nm full width at half maximum. Wavelength scans of aBINA (20 µM) were measured after irradiation with different wavelengths of light for 5 min each. Reversible switching was performed by irradiating aBINA (20 µM) with 420 nm or 340 nm for 5 min each. Absorbance was measured at 360 nm over time. Thermal relaxation of aBINA (20 µM) was measured after 5 min irradiation with 420 nm or 340 nm of dark-adapted samples, and subsequently absorption was detected at 360 nm. The monochromator was controlled using a custom MATLAB script. UV-Vis data were analyzed and plotted using Graphpad Prism.

**Photostationary state (PSS)**

Photostationary states were determined by LCMS analysis and performed on an LCMS 1260 Infinity II Agilent Technologies system (Windows 10, OpenLabs CDS Chemstation Software, 6120 Quadrupole LC/MS G7111B quaternary pump, G7129A Infinity II vialsampler, thermostated column compartment, G7117C 1260 diode array detector) with an LC Kinetex column 2.6 µm C18 (50 x 3 mm). Runs were performed at a flow-rate of 1 mL/min with a run-time of 5 min, and a solvent gradient of 0-100% MeCN in water, containing 0.1% formic acid (t<sub>R</sub> (cis) = 3.62 min; t<sub>R</sub> (trans) = 4.31 min). Dark adapted samples (100% trans) were irradiated with the respective wavelength for 10 min under ambient conditions, starting from low to high wavelengths. Samples for LCMS were prepared under red-light conditions in amber vials and immediately subjected to LCMS. The relative ratios of (Z)- and (E)-isomers were determined by detection at the isosbestic points at the respective elution time solvent mixtures (244, 264, 469, 394 nm) and the integration of the UV-absorbances was averaged.

**Chemicals**

All chemicals were purchased from *Sigma Aldrich, Fisher Scientific, TCI Europe, Chempur, Alfa Aesar or Acros Organics*. Solvents purchased in technical grade quality and were distilled under reduced pressure and used for purification procedures. Purchased solvents in HPLC- and analytical-grade quality were used without further purification. Oxone® is a mixture of three salts with the chemical formula: 2KHSO₅·KHSO₄·K₂SO₄. Unless otherwise noted, reactions were
performed using dry solvents. Dichloromethane (CH$_2$Cl$_2$) and triethylamine (NEt$_3$) were dried by distillation from CaH$_2$. Tetrahydrofuran (THF) was dried by distillation from sodium and benzophenone. Other dry solvents were purchased from commercial sources (Acros Organics, Fisher Scientific) under inert gas atmosphere and over molecular sieves. All other reagents with a purity of >95% were purchased from commercial sources and used without further purification. For running extra dry reactions with synthetic compounds, stock solutions were prepared in PhMe, the respective amounts transferred into dried glassware and the solvent was removed by stirring under high vacuum (<1 mbar). This procedure was followed by freeze-drying the compound to ensure that H$_2$O was azeotropically removed.

**Chemical Synthesis Methods**

Unless otherwise noted, all reactions were magnetically stirred under inert gas (N$_2$) atmosphere using standard Schlenk techniques. Glassware was evacuated and dried by heating with a heat-gun (set to 550 °C). Drying over Na$_2$SO$_4$ implies stirring with an appropriate amount of anhydrous salt for several minutes followed by filtration through a glass frit and rinsing of the filter cake with additional solvent. Electric heating plates and oil baths were used for reactions at elevated temperature. Stated reaction temperatures refer to the external bath temperature. Cannulas and syringes were used for transfer of reagents or solvents which were flooded with inert gas (3×) before use. Purification by column chromatography was performed under elevated pressure (flash column chromatography) on Geduran® Si60 silica gel (40-63 µm) from Merck KGaA. After flash column chromatography, the concentrated fractions were filtered once through a glass frit. Silica gel F$_{254}$ TLC plates from Merck KGaA were used for monitoring reactions, analyzing fractions of column chromatography and measuring R$_f$ values. To visualize the analytes, TLC plates were irradiated with UV light. Reaction yields refer to spectroscopically pure isolated amounts of compounds.
Chemical Synthesis

2-cyclopentyl-5-nitroisoindolin-1-one (2)

Methyl 2-(bromomethyl)-4-nitrobenzoate (1, 450 mg, 1.64 mmol, 1.0 eq) was dissolved in MeOH (3.28 mL) followed by addition of NEt₃ (0.343 mL, 2.46 mmol, 1.5 eq) and cyclopentylamine (0.162 mL, 1.64 mmol, 1.0 eq). The mixture was stirred in a pressure tube at 100 °C for 4 h. After cooling to rt, the solvent was removed in vacuo. Purification via flash column chromatography (pentane:EtOAc = 4:1) afforded 2-cyclopentyl-5-nitroisoindolin-1-one (2, 251.6 mg, 1.022 mmol, 62%) as a yellow powder.

Rᵣ (pentane:EtOAc = 1:1) = 0.54. (UV)

¹H NMR (400 MHz, CDCl₃) δ (ppm) = 8.38 – 8.31 (m, 2H), 7.98 (d, J = 8.2 Hz, 1H), 4.78 (p, J = 8.0 Hz, 1H), 4.48 (s, 2H), 2.15 – 1.98 (m, 2H), 1.91 – 1.60 (m, 6H).

¹³C NMR (101 MHz, CDCl₃) δ (ppm) = 166.3, 150.0, 142.0, 138.8, 124.7, 124.0, 118.6, 53.3, 46.3, 30.3, 24.2.

HRMS (ESI): calc. for C₁₃H₁₅N₂O₃⁺ [M + H⁺]⁺: 247.1077, found: 247.1077.

IR (Diamond-ATR, neat) υₘₐₓ (cm⁻¹) = 3107 (vw), 3039 (vw), 2961 (vw), 2872 (vw), 1669 (vs), 1625 (vw), 1600 (vw), 1525 (s), 1470 (w), 1454 (m), 1416 (w), 1393 (vs), 1312 (w), 1274 (w), 1240 (w), 1215 (w), 1183 (vw), 1140 (vw), 1117 (vw), 1091 (vw) 1068 (vw), 944 (vw), 918 (vw), 895 (w), 859 (w), 818 (w), 784 (vw), 730 (vs), 673 (w), 665 (m).
5-amino-2-cyclopentylisoindolin-1-one (3)

Nitroaryl (2, 125 mg, 0.508 mmol, 1.0 eq) was dissolved in degassed EtOAc/MeOH (2:1, 21 mL) and Pd/C (10% Pd, 37.8 mg, 35.6 μmol, 0.07 eq) was added. The solution was stirred under H₂ atmosphere (1 atm) for 13 h, filtered through celite and concentrated. Purification via flash column chromatography (hexanes:EtOAc = 1:1) afforded 5-amino-2-cyclopentylisoindolin-1-one (3, 89.4 mg, 0.413 mmol, 81%) as a white foam.

Rₓ (pentane:EtOAc = 1:1) = 0.11. (UV)

¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.61 (d, J = 8.2 Hz, 1H), 6.70 (d, J = 8.2 Hz, 1H), 6.67 (s, 1H), 4.73 (p, J = 8.0 Hz, 1H), 4.24 (s, 2H), 3.98 (br s, 2H), 2.05 – 1.89 (m, 2H), 1.83 – 1.49 (m, 6H).

¹³C NMR (101 MHz, CDCl₃) δ (ppm) = 168.9, 149.7, 143.7, 125.0, 123.9, 114.9, 108.0, 52.4, 45.9, 30.1, 24.2.

HRMS (ESI): calc. for C₁₃H₁₇N₂O⁺ [M + H⁺]: 217.1335, found: 217.1335.

IR (Diamond-ATR, neat) υmax (cm⁻¹) = 3324 (vw), 3210 (vw), 2963 (vw), 1662 (s), 1648 (s), 1633 (m), 1609 (vs), 1495 (w), 1453 (s), 1414 (m), 1373 (w), 1326 (w), 1282 (m), 1238 (w) 1135 (w), 859 (w), 832 (m), 790 (w), 772 (s) 693 (s), 632 (m), 573 (m), 560 (m).
methyl 3'-(2-cyclopentyl-1-oxoisoindolin-5-yl)diazenyl)-[1,1'-biphenyl]-4-carboxylate (6)

Aniline (3, 62.7 mg, 0.290 mmol, 1.0 eq) and oxone® (365 mg, 0.580 mmol, 2.0 eq) were dissolved in CH$_2$Cl$_2$/H$_2$O (1:1, 11.6 mL) and stirred at rt for 14 h 45 min. After diluting the green solution with CH$_2$Cl$_2$ (70 mL), it was washed with H$_2$O (20 mL), 3% aq KHSO$_4$ (20 mL), sat. aq. NaHCO$_3$ (20 mL) and H$_2$O (20 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated. Methyl 3'-amino-[1,1'-biphenyl]-4-carboxylate (5, 65.9 mg, 0.290 mmol, 1.0 eq) was added to the residue and the mixture was dissolved in CH$_2$Cl$_2$/AcOH (1:1, 11.6 mL). The green solution was stirred for 6 h 30 min and gradually turned organe/red. After diluting with CH$_2$Cl$_2$ (70 mL), the organic layer was washed with 1 M aq. HCl (2 x 20 mL), sat. aq. NaHCO$_3$ (20 mL) and sat. aq. NaCl (20 mL) followed by drying over Na$_2$SO$_4$. The solution was concentrated an purified via flash column chromatography (pentane:EtOAc = 4:1) to give methyl 3'-(2-cyclopentyl-1-oxoisoindolin-5-yl)diazenyl)-[1,1'-biphenyl]-4-carboxylate (6, 71.5 mg, 0.163 mmol, 56%) as an orange powder.

Note: Due to the photoswitching properties of the 6 azobenzene moiety, the NMR spectra show a mixture of cis- and trans-isomers. The $^1$H- and $^{13}$C-NMRs are reported for the thermodynamically more stable trans-isomer.

$R_f$ (pentane:EtOAc = 2:1) = 0.59. (visible)

$^1$H NMR (400 MHz, CDCl$_3$) δ (ppm) = 8.24 – 8.12 (m, 3H), 8.06 (d, $J = 8.1$ Hz, 1H), 8.02 – 7.94 (m, 2H), 7.77 (t, $J = 7.2$ Hz, 3H), 7.64 (t, $J = 7.8$ Hz, 1H), 4.81 (p, $J = 8.0$ Hz, 1H), 4.47 (s, 2H), 3.96 (s, 3H), 2.13 – 1.98 (m, 2H), 1.90 – 1.61 (m, 6H).
$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ (ppm) = 167.8, 167.0, 154.6, 153.1, 144.7, 142.1, 141.3, 135.5, 130.4, 129.9, 129.5, 127.3, 124.5, 124.1, 122.9, 122.0, 116.6, 53.0, 52.4, 46.3, 30.3, 24.3.

HRMS (EI): calc. for C$_{27}$H$_{25}$N$_3$O$_3$+ [M]$^+$: 439.1890, found: 439.1890.

IR (Diamond-ATR, neat) $\nu_{\text{max}}$ (cm$^{-1}$) = 2921 (vw), 1718 (m), 1671 (vs), 1606 (w), 1440 (w), 1399 (w), 1267 (m), 1195 (m), 1109 (m), 1016 (w), 893 (vw), 858 (w), 798 (w), 778 (w), 769 (s), 701 (m), 686 (m).
3'-((2-cyclopentyl-1-oxoisooindolin-5-yl)diazenyl)-[1,1'-biphenyl]-4-carboxylic acid (aBINA)

Methyl ester (6, 20.0 mg, 45.5 µmol, 1.0 eq) was dissolved in THF/H₂O (10:1, 1.25 mL) and LiOH-H₂O (9.5 mg, 0.23 mmol, 5.0 eq) was added. After stirring the reaction at rt for 6 h, it was diluted with EtOAc (35 mL) and washed with 1 M aq. HCl (10 mL) as well as sat. aq. NaCl (10 mL). The organic layer was dried over Na₂SO₄ and concentrated. Purification via flash column chromatography (CH₂Cl₂:AcOH = 99:1) afforded 3'-((2-cyclopentyl-1-oxoisooindolin-5-yl)diazenyl)-[1,1'-biphenyl]-4-carboxylic acid (aBINA, 13.2 mg, 31.0 µmol, 68%) as an orange powder.

Note: Due to the photoswitching properties of the aBINA azobenzene moiety, the NMR spectra show a mixture of cis- and trans-isomers. The ¹H- and ¹³C-NMRs are reported for the thermodynamically more stable trans-isomer.

Rᵣ (pentane:EtOAc:AcOH = 9:1:1%) = 0.29. (visible)

¹H NMR (800 MHz, (CD₃)SO) δ (ppm) 8.25 (t, J = 1.9 Hz, 1H), 8.10 (d, J = 0.7 Hz, 1H), 8.07 – 8.03 (m, 3H), 8.00 – 7.95 (m, 2H), 7.87 (dd, J = 8.1, 6.1 Hz, 3H), 7.75 (t, J = 7.8 Hz, 1H), 4.68 – 4.57 (m, 3H), 1.94 – 1.88 (m, 2H), 1.80 – 1.75 (m, 2H), 1.75 – 1.69 (m, 2H), 1.67 – 1.61 (m, 2H).

¹³C NMR (201 MHz, (CD₃)SO) δ (ppm) = 168.2, 166.7, 154.2, 152.9, 143.6, 142.4, 141.3, 135.5, 130.8, 130.5, 127.1, 124.1, 123.7, 122.4, 121.7, 117.6, 52.9, 46.6, 30.2, 24.3.

According to the 2D-NMR spectra, one carbon signal was not observed observed due to line broadening.
HRMS (ESI): calc. for $C_{26}H_{22}N_3O_3^-$ [M – H$^-$]: 424.1667, found: 424.1669.

IR (Diamond-ATR, neat) $\nu_{\text{max}}$ (cm$^{-1}$) = 3556 (vw), 2922 (w), 2855 (vw), 1671 (vs), 1606 (m), 1455 (w), 1428 (m), 1407 (m), 1354 (w), 1291 (m), 1246 (w), 1205 (w), 1182 (w), 1152 (w), 1120 (w), 1017 (w), 937 (w), 917 (w), 883 (w), 856 (m), 803 (m), 771 (s), 723 (w), 702 (m), 690 (s), 675 (w).
All constructs were cloned into mammalian expression vectors. For the mGluR mediated-GIRK activation assay, HEK293T cells were seeded onto 18 mm coverslips and transiently transfected overnight with Lipofectamine 2000 and the following constructs: A wildtype mGluR (0.7 µg), GIRK1(F137S) (0.7 µg), and tdTomato (0.1 µg).

**Electrophysiology**

HEK293T cells were sparsely seeded and maintained in DMEM (Invitrogen) with 10% fetal bovine serum on poly-L-lysine-coated coverslips at 37°C and 5% CO2. For electrophysiology, HEK293T cells were voltage clamped in whole-cell configuration 16-24 hours after transfection. The extracellular solution contained 120 mM KCl, 25 mM NaCl, 10 mM HEPES, 2 mM CaCl2, and 1 mM, MgCl2, pH 7.4. Glass pipettes with a resistance of 3-7 MΩ were filled with intracellular solution containing 120 mM Gluconic acid δ-lactone, 15 mM CsCl, 10 mM BAPTA, 10 mM HEPES, 1 mM CaCl2, 3 MgCl2, 3 mM MgATP, pH 7.2. Cells were voltage clamped to -60 or -80 mV using an Axopatch 200A (Molecular Devices) amplifier. Compounds were applied using a gravity-driven perfusion system and illumination was applied to the entire field of view using a Polychrome V monochromator (TILL Photonics) through a 20x objective (0.5 mW/mm2 at 340 nm or 4 mW/mm2 at 420 nm) or using a DG4 (Sutter) through a 20x objective. pClamp software was used for both data acquisition and control of illumination. The selection criteria for electrophysiological experiments are that a cell (i) expresses the fluorescent protein transfection marker, and (ii) responds to glutamate, indicating the presence of mGluR and GIRK. We do not exclude individual cells unless a recording is of poor quality (e.g., unstable baseline).

Primary cortical neurons were maintained in neurobasal medium supplemented with B27 and glutamax at 37°C and 5% CO2. The neurons were current clamped in whole-cell configuration. The extracellular solution contained 135 mM NaCl, 5.4 mM KCl, 10 mM HEPES, 10 mM glucose, 2 mM CaCl2, and 1 mM MgCl2, pH 7.4. The intracellular solution contained 125 mM K-Gluconate, 15 mM KCl, 5 mM EGTA, 10 mM HEPES, 0.5 mM CaCl2, 2 mM MgATP, 0.3 mM NaGTP, and 10 mM creatine phosphate, pH 7.2. aBINA was applied using a gravity-driven perfusion system and illumination was applied to the entire field of view using a DG4 (Sutter) through a 20x objective. pClamp software was used for both data acquisition and control of illumination.
**Structural Modeling**

A homology model of the transmembrane domain of mGluR2 was generated using Modeller 9 (University of California San Francisco, San Francisco). The model was based on mGluR5 because of the high relative degree of homology between mGluR2 and mGluR5. The amino acid sequence of mGluR2 was aligned to mGluR5 in BLAST and the TM segments were then structurally aligned with the crystal structure of mGluR5 bound to the negative allosteric modulator mavoglurant (pdb: 4O09). To dock the BINA or aBINA in the mGluR2 model, the ligands were first prepared in ChemDraw (PerkinElmer) and Chimera (developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco and supported by NIGMS P41-GM103311). They were then docked in the mGluR2 model using Autodock Vina4. The following allosteric binding site residues in mGluR2 were allowed to rotate during the docking procedure: V613, C616, F623, R635, F643, M728, S731, L732, N735, I739, T769, I772, W773, F776, F780, M794, S797, V798, and S801. All molecular representations were prepared using Chimera.
NMR Spectra

2-cyclopentyl-5-nitroisoindolin-1-one (2)
5-amino-2-cyclopentylisoindolin-1-one (3)

$^1$H-NMR (400 MHz, CDCl$_3$)

$^{13}$C-NMR (101 MHz, CDCl$_3$)
methyl 3\'-(2-cyclopentyl-1-oxoisooindolin-5-yl)diazenyl)-[1,1\'-biphenyl]-4-carboxylate (6)
3'-(2-cyclopentyl-1-oxoisooindolin-5-yl)diazeny1-[1,1'-biphenyl]-4-carboxylic acid (aBINA)
Figure S1. Comparison of photoswitchable allosteric modulators and photoswitchable allosteric agonists. GPCRs are turned on and off with precise timing in response to the release and removal/degradation of endogenous ligands from the extracellular space. The dysregulation of endogenous ligands can alter the frequency and/or magnitude of receptor activation. When converted to the active isomeric configuration (blue), photoswitchable allosteric modulators (PAMs and NAMs) can control the magnitude but not the frequency of receptor activation in response to endogenous ligand. In contrast, photoswitchable allosteric agonists control both the magnitude and frequency of activation, and thus are better suited for restoring physiological receptor signaling.
**Figure S2. Extended photophysical characterization of aBINA.** Absorbance spectra of aBINA (20 µM) under different wavelengths of light and maximum absorption at 335 nm in (A, B) 10% DMSO and (C, D) 100% DMSO. The photo-stationary state (PSS) of aBINA (20 µM) at different wavelengths of light according to LCMS in (E) 10% DMSO or (F) 100% DMSO. (G, H) The trans and cis configurations of aBINA are stable over several hours. (I) Consistent with the photophysical properties of cis-azobenzene, the cis-aBINA relaxes to the trans configuration over time (4.6 ± 0.6 days, n = 4).
Figure S3. Schematic of the mGluR2-dependent GIRK activation assay. HEK293T cells were co-transfected with an mGluR and GIRK1(F137S). Agonist-induced receptor activation results in the release of Gβγ from endogenous heterotrimeric G_{i/o}. Gβγ subsequently binds GIRK channels, resulting in channel opening and increased inward-current assessed by whole-cell patch clamp recordings.
Figure S4. The photo-effect of aBINA in the GIRK assay requires mGluR2. (A, B) aBINA has no effect on inward-current in the absence of mGluR2 in GIRK-expressing HEK293T cells. (C) Summary of the effect of aBINA relative to BaCl2, a GIRK channel blocker, on GIRK-expressing HEK293T cells. (D, E) aBINA has no effect on a mutant of mGluR2 wherein the allosteric binding site is crippled (N735D). Glutamate can activate this receptor because the glutamate binding site is intact. (F) Summary of the effect of aBINA on mGluR2(N735D).
Figure S5. mGlur2 activation in response to aBINA can be reversed with 360 nm light. (A) mGlur2 activation in response to the *trans* configuration of aBINA under 420 nm light in the GIRK assay can be reversed by switching to *cis* configuration of aBINA under 360 nm light. (B) Summary of the effect of aBINA on mGlur2 under 420 nm light and 360 nm light.
Figure S6. Comparison of aBINA and its parent compound BINA. (A) Representative trace of mGluR2 activation in response to BINA in the GIRK assay. (B) Dose-response curve of BINA-induced mGluR2 activation in the GIRK assay. n = 3-4 cells per concentration. (C) Comparison of the potency (EC50) and hill slope ($n_H$) of aBINA under 420 nm light, aBINA under 340 nm light, and BINA in the GIRK assay. The hill slope for aBINA and BINA are supra-linear compared to glutamate in this assay\(^1\), suggesting positive cooperativity in the binding to and/or activation of the mGluR2 dimer by BINA and aBINA.
Figure S7. The *cis* configuration of aBINA has no effect on mGluR2. To determine whether the *cis* configuration of aBINA is an agonist of mGluR2, the experimentally derived dose-response curve of aBINA under 340 nm light (grey solid line) was compared to a simulated curve (grey dotted line) wherein (i) a small fraction of aBINA is in the active trans configuration, as determined by LCMS (Fig. S2), and (ii) *cis*-aBINA is assumed to be completely inactive. The experimental and simulated 340 nm curves are nearly identical, indicating that *cis*-aBINA has no effect on mGluR2 over the concentration range tested.
Figure S8. The activation of mGluR2 with the aBINA can be terminated with washout. Unlike the parent compound BINA, the effect of aBINA on mGluR2 can be terminated by washout. Shown are the kinetics of photoswitching mGluR2 with aBINA with 420 nm light and 340 nm light, as well as deactivation in response to washing out the trans configuration of aBINA under 420 nm light from solution.
Figure S9. aBINA potentiates glutamate-induced activation of mGluR2. (A) mGluR2 is unaffected by a low-dose of glutamate (100 nM) in the GIRK assay. (B) Summary of the effect of low-dose glutamate on mGluR2. Low-dose glutamate is potentiated by the approximate EC25 concentration (C) and the EC70 concentration (D) of aBINA under 420 nm light. (E) Summary of the potentiation of low-dose glutamate by 100 nM aBINA under 420 nm light. n = 3 cells for the 30 nM aBINA condition and n = 4 cells for 100 nM aBINA condition.
Figure S10. aBINA is selective for mGluR2. Examples traces of the effect of aBINA in the GIRK assay on (A) mGluR3, (B) mGluR1, (C) mGluR4, and (D) mGluR7.
References

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