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Optical Control of Lysophosphatidic Acid Signaling

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Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.0c02154.

Synthetic procedure for **AzoLPA** including NMR and HRMS characterization data; molecular modeling data for LPA_3 and LPA_5; Ca^{2+} mobilization data control experiments; and a study of **AzoLPA** metabolism (PDF)

The authors declare no competing financial interest.

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Abstract

Lysophosphatidic acid (LPA) is a phospholipid that acts as an extracellular signaling molecule and activates the family of lysophosphatidic acid receptors ($LPA_{1–6}$). These G protein-coupled receptors (GPCRs) are broadly expressed and are particularly important in development as well as in the nervous, cardiovascular, reproductive, gastrointestinal, and pulmonary systems. Here, we report on a photoswitchable analogue of LPA, termed **AzoLPA**, which contains an azobenzene photoswitch embedded in the acyl chain. **AzoLPA** enables optical control of LPA receptor activation, shown through its ability to rapidly control LPA-evoked increases in intracellular Ca^{2+} levels. **AzoLPA** shows greater activation of LPA receptors in its light-induced *cis*-form than its dark-adapted (or 460 nm light-induced) *trans*-form. **AzoLPA** enabled the optical control of neurite retraction through its activation of the LPA_2 receptor.

Lysophosphatidic acid (LPA) is a bioactive lipid that plays key physiological roles in health and disease. LPA targets the lysophospholipid receptors $LPA_{1–6}$, a class of G protein-coupled receptors (GPCRs) that have important roles in the nervous system, immune response, and development.[1–3] Aberrant homeostasis of LPA levels is linked to a number of diseases including cancer,[4] neurological disorders,[5] and cardiovascular diseases.[6] LPA is a potent signaling lipid with K_d values in the low nanomolar range at most LPA receptor subtypes.[7] Additionally, LPA targets a number of intracellular targets, including the nuclear hormone receptor PPARγ,[8] the autotaxin lysophospholipase D,[9] and the ion channel TRPV1.[10] LPA is formed transiently with a high metabolic turnover and complex metabolism since multiple enzymes contribute to its formation and degradation. The study of LPA function is therefore challenging with slow-acting conventional approaches, including pharmacology and genetic manipulations. New methods that facilitate spatiotemporal control over LPA signaling are needed to dissect the many functions of LPA. Optical tools could be particularly useful in this regard. Recently, Schultz and co-workers reported a photocaged version of LPA that allowed for light-induced activation of LPA receptor-dependent effects, including chemotaxis.[11] However, photoactivation of this probe is not reversible, and it has a chemically modified headgroup, which perturbs the amphiphilic character of the molecule and might affect trafficking.[12,13] In recent years, we and others have developed a series of photoswitchable lipids and demonstrated their capacity for the reversible optical control of lipid metabolism and signaling. These photoswitchable lipids have an azobenzene photoswitch incorporated into the hydrophobic tail and mediate optical control of lipid function by reversible, light-induced isomerization between the *trans*- (straight) and *cis*- (bent) isomers. To date, applications of photoswitchable lipids include the modulation of ion channels,[14–17] the fatty acid receptor GPR40,[18] lipid rafts,[19,20] lipid vesicle budding and fission,[21] and protein translocation.[22] Most recently, a photoswitchable version of sphingosine-1-phosphate (S1P), termed **PhotoS1P**, was published.[23] While S1P is a sphingolipid and not a glycerophospholipid like LPA, the GPCRs activated by it are structurally related to the LPA receptors.[24] Motivated by the importance of LPA and the success of our S1P derivatives, we decided to explore the photoswitchable version of LPA.

We now report a new photoswitchable probe, termed **AzoLPA**, which can be used to control endogenous and heterologously expressed LPA receptors and LPA_2 receptor-dependent neurite retraction in NG108.15 neuroblastoma cells. The molecular design of a photoswitchable LPA called for incorporation of the azobenzene N=N double bond near the middle of the lipid tail, which corresponds to the C=C *cis* double bond in the predominant form of LPA, **LPA(18:1)**. The synthesis of **AzoLPA** (Figure 1A) commenced with the phosphorylation of (*S*)-glycidol using di-*tert*-butyl-*N*,*N*-diisopropyl phosphoramidite to yield the phosphorylated glycidol derivative, **1**. Acylation with a mixture of FAAzo-4[14] and the corresponding cesium salt gave the phosphoester, **2**. **AzoLPA** was obtained through depro-tection of **2** with TFA. The photophysical characterization of **AzoLPA** (Figure 1B,C) revealed similar properties to classical azobenzenes and other photoswitchable lipids. The photolipid could be reversibly switched with UV-A (365 nm) and blue light (460 nm) and underwent slow thermal relaxation (Figure 1C).

We next tested the ability of **AzoLPA** to optically control LPA receptors using Ca^{2+} imaging in human embryonic kidney cells (HEK 293T; Figure 2), which exhibit high endogenous expression of LPA_1 receptor.[25] We employed the red calcium dye X-Rhod-5F,AM with λ_{ex} = 581 nm and λ_{em} = 603 nm, which is orthogonal to the UV-A/blue wavelengths needed to achieve **AzoLPA** photoswitching. When applied in the *trans*-form, **AzoLPA** (25 nM) was inactive and did not yield a significant increase in Ca^{2+} concentration. Upon irradiation with UV-A light, **AzoLPA** could be activated yielding a robust increase in Ca^{2+} concentration (Figure 2B,C), which was also observed after direct addition of preirradiated (365 nm for 1 min) *cis*-**AzoLPA** (25 nM, Figure 2D). Addition of DMSO and subsequent use of UV-A and blue light did not result in Ca^{2+} responses (Figure 2E and Figure S1). A saturating concentration of LPA (250 nM) yielded robust Ca^{2+} responses, which were not modulated through UV-A or blue light (Figure 2E and Figure S1). To demonstrate that the observed responses originated from the optical control of LPA_1 receptor, we applied 10 μM of **Ki16425**,[26] an antagonist for LPA_1 receptor, together with the active photoisomer *cis*-**AzoLPA** (25 nM). **Ki16425** completely inhibited the Ca^{2+} response observed with *cis*-**AzoLPA** demonstrating that these results are indeed based on the optical control of endogenous LPA_1 receptors in HEK293T cells (Figure 2E).

Next, we systematically evaluated the effect of **AzoLPA** on cell lines that do not endogenously express LPA receptors endogenously and were stably transfected with one of the $LPA_{1–5}$ receptors using a Ca^{2+}-mobilization assay.[27] Each untrans-fected cell line employed was confirmed to be nonresponsive to **LPA(18:1)**, *cis*-**AzoLPA**, and *trans*-**AzoLPA** (Figure S2). In agreement with the above Ca^{2+} imaging experiments in HEK 293T cells, *cis*-**AzoLPA** produced an increased response with the LPA_1 receptor compared to *trans*-**AzoLPA** (Figure 3A). The same preference for *cis*-**AzoLPA** was observed by the LPA_2 (Figure 3B) receptor and LPA_4 receptors (Figure 3D). For the LPA_3 receptor, **AzoLPA** (Figure 3C) showed only weak agonism and no significant light-dependent activity. At the LPA_5 receptor, our data suggests potent agonism, but no significant differences were observed between photoisomers (Figure 3E). Thus, **AzoLPA** is an agonist of $LPA_{1–5}$ receptors, with markedly greater potency for $LPA_{1,2,4}$ receptors.

To rationalize the enhanced activity of *cis*-**AzoLPA**, we performed molecular docking studies into a crystal structure of LPA_1 receptor (PDB[29] entry 4Z34[30]) and homology models of $LPA_{2–5}$ receptors. Homology models of $LPA_{2–3}$ receptors were based on the crystal structure of LPA_1 receptor, and homology models of $LPA_{4–5}$ receptors were based on the crystal structure of LPA_6 receptor (PDB[29] entry 5XSZ[31]). Docking results confirm that *cis*-**AzoLPA** resembles the binding pose of bent LPA (18:1) better than *trans*-**AzoLPA** across different receptors (Figure 4 and Figure S3). Docked ligands in the LPA_1 and LPA_4 receptors all showed phosphate headgroup engagement of residues required for LPA recognition (Figure 4A,C: R3.28 in LPA_1 receptor[24] and K2.60 in LPA_4 receptor by analogy to R2.60 in LPA_4[32]). However, *trans*-**AzoLPA** must adopt a nonplanar conformation of the conjugated system (inset of Figure 4A,C), consistent with the poor receptor activation observed compared to both *cis*-**AzoLPA** and **LPA(18:1)** itself. The activity difference between photoisomers at LPA_2 receptor was due to lack of phosphate engagement of R3.28 by *trans*-**AzoLPA** (Figure 4B).

To demonstrate that **AzoLPA** can be used for the optical control of other LPA receptor-dependent physiological path-ways, we next turned to neurite outgrowth assays. Neurite branch outgrowth and retraction are critical for the regulation of neural networks,[5] and **LPA(18:1)** induces pronounced ROCK-pathway-dependent neurite retraction.[33,34] We used the neuronal cell line NG108.15 to study light-dependence of neurite retraction and cell-rounding with different concentrations of *trans*-**AzoLPA** or *cis*-**AzoLPA**. NG108.15 cells primarily express LPA_2 receptors (Figure S4). In accordance with the pharmacological data shown in Figure 3B, we observed that *cis*-**AzoLPA** was significantly more potent than *trans*-**AzoLPA** (Figure 5B,C). At physiologically relevant concentrations (100 nM), *cis*-**AzoLPA** is as potent as **LPA(18:1)** itself. The cell viability is not compromised by *cis*-**AzoLPA** (Figure S5).

In summary, we have disclosed the design, development, and application of a photoswitchable analogue of lysophosphatidic acid, **AzoLPA**. This photolipid was synthesized by incorporation of an azobenzene photoswitch into the lipid tail. Our approach preserves the integrity of the lipid headgroup and only perturbs the lipid tail while enabling light-dependent modulation of lipid function. We show that **AzoLPA** provides precise optical control of LPA receptor function using dynamic live cell Ca^{2+} release experiments. We further demonstrate the capacity of this tool to control neurite branching with light. Optical control of neurite branching in development could allow for the study of nervous system development with opportunities for spatiotemporal control. Beyond applications in neuroscience, **AzoLPA** could allow for the optical control of LPA-dependent physiological pathways in the study of development, reproduction, and vascular biology. This new tool might further be applicable to the optical control of intracellular LPA targets, including TRPV1, ATX, or PPARγ.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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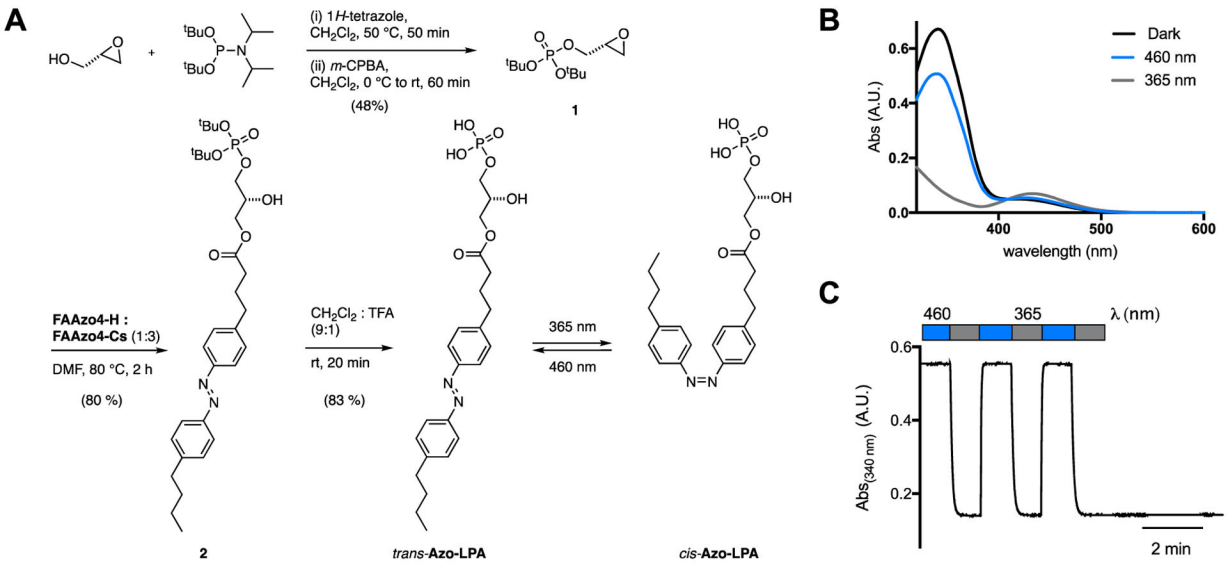

Figure 1.

Synthesis and photophysical properties of **AzoLPA**. (A) Chemical synthesis of **AzoLPA**. (B) UV–vis spectra of **AzoLPA** in the dark-adapted (black, *trans*), 365 nm adapted (gray, *cis*), and 460 nm adapted (blue, *trans*) photostationary states (50 μM, DMSO). (C) Reversible cycling between photoisomers with alternating illumination at 365/460 nm.

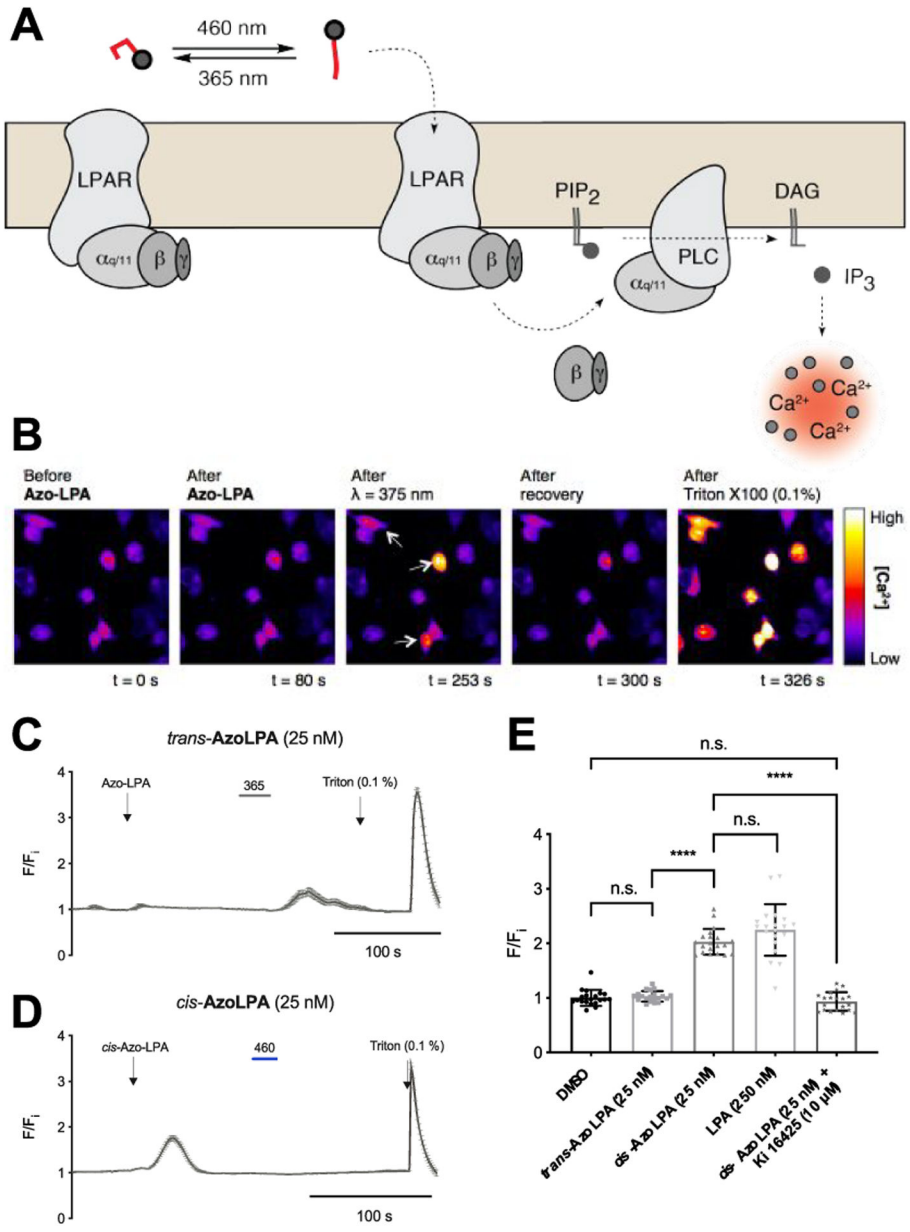

Figure 2.

Optical control of endogenous LPA receptors in HEK293T cells. (A) Schematic depiction of the optical control of LPA receptor-induced Ca^{2+} release. (B) Representative images of Ca^{2+} response before and after addition of **AzoLPA** (25 nM), irradiation with 375 nm light, and addition of Triton X100. Ca^{2+} responses after treatment with *cis*-**AzoLPA** (C), *trans*-**AzoLPA** (D), irradiation with light, and after treatment with Triton X100. (E) Quantification of Ca^{2+} responses as F_{max} normalized to Triton X100. Parts C–E include data from at least 20 cells from two independent experiments. Error bars represent mean ± SEM; **** $p <$ 0.0001, n.s., not significant, Student's *t*-test.

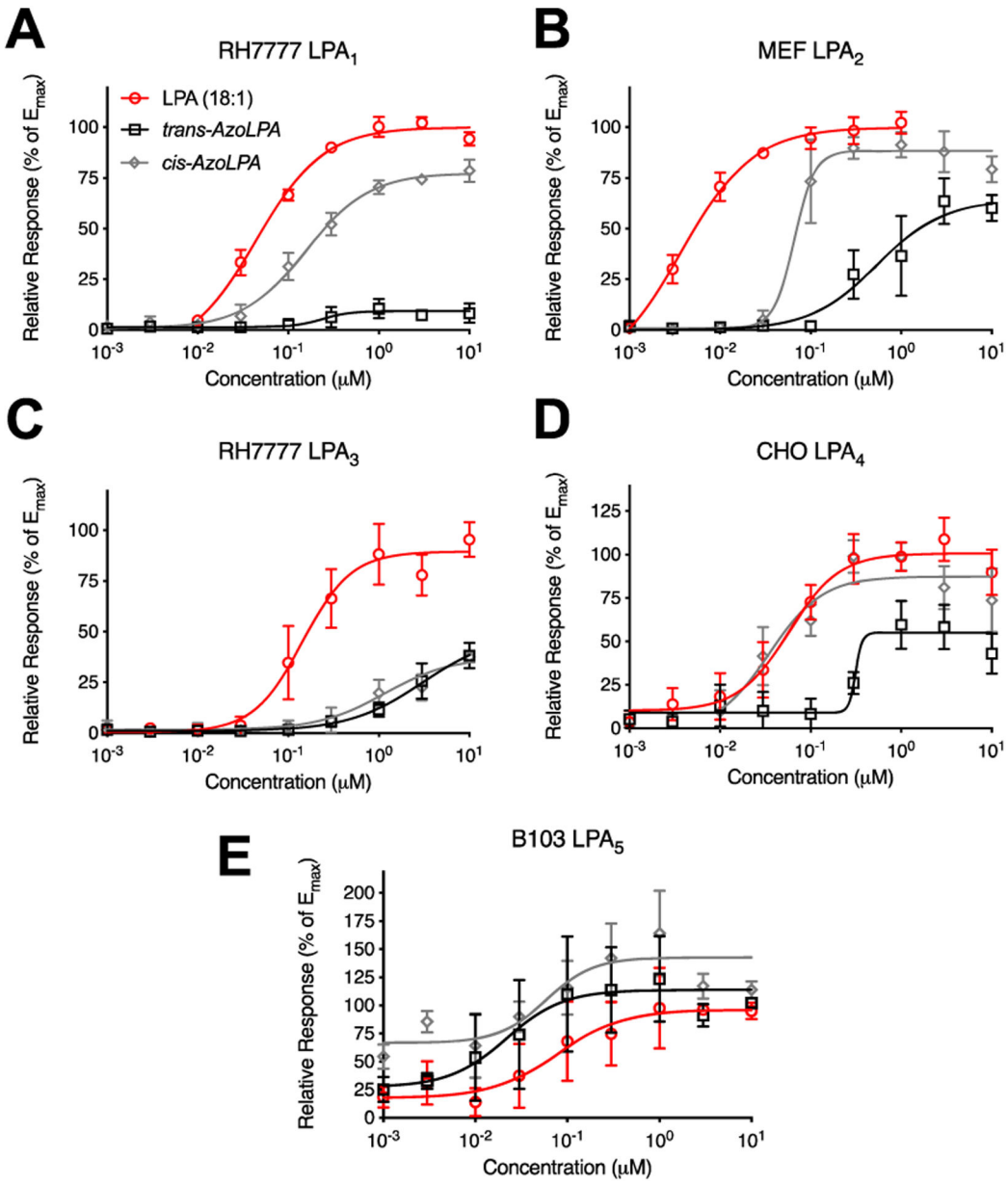

Figure 3.

Optical control of $LPA_{1–5}$ receptor-mediated Ca^{2+} release. Fura2-AM calcium imaging in cells stably transfected with $LPA_{1–5}$ receptors. Dose response of LPA (18:1), *trans*-**AzoLPA** and *cis*-**AzoLPA** in RH7777 LPA_1 receptor (A), MEF LPA_2 receptor (B), RH7777 LPA_3 receptor (C), CHO LPA_4 receptor (D), and B103 LPA_5 receptor (E) cells. A minimum of two independent experiments that included triplicate samples were performed. Data points were normalized to maximal LPA response for each receptor. Error bars represent mean ± SD.

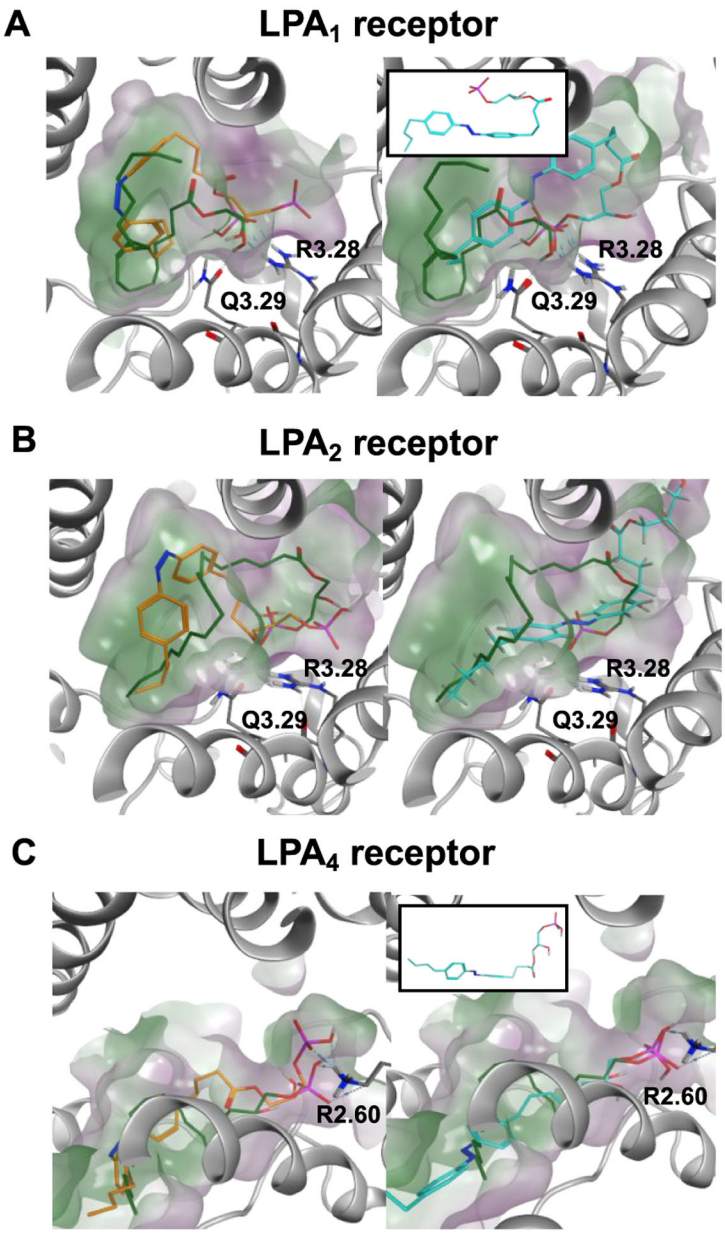

Figure 4.

Molecular docking of **AzoLPA**. Computationally predicted poses for **LPA(18:1)** (green), *trans*-**AzoLPA** (cyan), and *cis*-**AzoLPA** (orange) docked into LPA_1 receptor (A), LPA_2 receptor (B), and LPA_4 receptor (C). Pocket surfaces are highlighted in green (hydrophobic) and violet (hydrophilic). The numerals represent key residues involved in target engagement according to the Ballesteros– Weinstein system.[28]

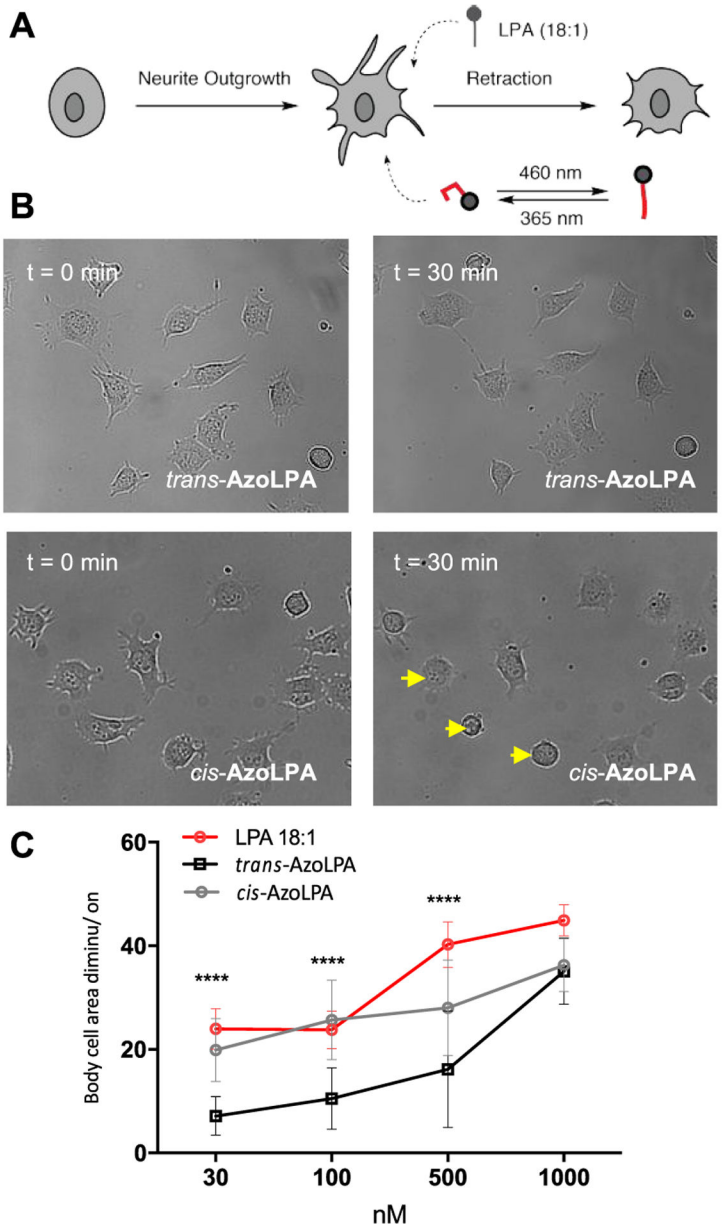

Figure 5.

Optical control of neurite branch retraction in NG108.15 cells. (A) Scheme of LPA or **AzoLPA**-induced neurite retraction. (B) Representative images of NG108.15 cells after addition of *trans*-**AzoLPA** or *cis*-**AzoLPA** after 0 and 30 min. (C) Quantification of body cell rounding at different concentrations of LPA (18:1), *trans*-**AzoLPA**, and *cis*-**AzoLPA** after 30 min of treatment. Samples were run at least in three independent experiments. *p*-values for *trans*-**AzoLPA** vs *cis*-**AzoLPA**. **** $p < 0.0001$, Mann–Whitney test. Error bars represent mean ± SD.