Mapping the Azolog Space Enables the Optical Control of New Biological Targets

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Supporting Information

ABSTRACT: Photopharmacology relies on molecules that change their biological activity upon irradiation. Many of these are derived from known drugs by replacing their core with an isoteric azobenzene photoswitch (azologization). The question is how many of the known bioactive ligands could be addressed in such a way. Here, we systematically assess the space of molecules amenable to azologization from databases of bioactive molecules (DrugBank, PDB, CHEMBL) and the Cambridge Structural Database. Shape similarity scoring functions (3DAPfp) and analyses of dihedral angles employed to quantify the structural homology between a bioactive molecule and the cis or trans isomer of its corresponding azolog (‘azoster’) and assess which isomer is likely to be active. Our analysis suggests that a very large number of bioactive ligands (>40 000) is amenable to azologization and that many new biological targets could be addressed with photopharmacology. N-Aryl benzamides, 1,2-diarylethanes, and benzyl phenyl ethers are particularly suited for this approach, while benzylanilines and sulfonamides appear to be less well-matched. On the basis of our analysis, the majority of azosters are expected to be active in their trans form. The broad applicability of our approach is demonstrated with photoswitches that target a nuclear hormone receptor (RAR) and a lipid processing enzyme (LTA4 hydrolase).

RESULTS AND DISCUSSION

Identification of Bioactive Azologs. To confine our search to molecules with known bioactivity, we turned to the databases PDB ligand, DrugBank, and CHEMBL. PDB ligand contains experimentally determined 3D coordinates of molecules in their target-bound conformations, whereas DrugBank and CHEMBL combine 2D data (SMILES strings) with comprehensive drug target information. We also analyzed the Cambridge Structural Database (CSD), the largest source of small molecule single crystal X-ray structures. To identify possible azologable compounds in these databases, we considered all molecules with up to 50 non-hydrogen atoms and selected those containing a pair of aromatic or heteroaromatic ring systems separated by a two-atom linker (e.g., \(-\text{CH}═\text{CH}-\), \(-\text{CH}═\text{CH}_2-\), \(-\text{CH}═\text{NH}-\), \(-\text{CONH}-\), \(-\text{CH}_2═\text{O}-\), \(-\text{CH}_2═\text{S}-\), \(-\text{SO}_2-\text{NH}_2-\), and others). In total, we identified more than 180 000 bioactive molecules that meet these criteria (Table 1), the majority of which were N-aryl benzamides (>60 000), benzyl phenyl ethers (>23 000), biaryl

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sulfonamides (>19,000), and benzyl anilines (>17,000). We also found more than 7000 stilbenes. The 3D coordinates of these molecules were either extracted from the databases (PDB and CSD) or calculated with the 3D structure generator CORINA (Drugbank and ChEMBL).

**Analysis of PDB Ligands and CSD Structures.** We first turned to the azologable PDB ligands because their target-bound conformations are known and can be directly compared to that of the respective cis or trans azologs. We also decided to include the experimental structures from the CSD into this analysis which are usually obtained in much higher resolution. To assess which compounds and linker-types are best suited for azologization, we compared the dihedral angles (ω) defined by the C−X−Y−C linkers (Figure 2). Molecules with ω-values close to 180° correspond to trans-azologs, while ω-values close to 0° match the angles in cis-azologs.

The majority of PDB/CSD structures had dihedral angles close to 180° corresponding to the respective trans-azologs.
Figure 2. Dihedral angle analysis of PDB and CSD compounds with azologable linkers.

Figure 3. Scatter plots showing the 3D-shape similarities of potential azologable compounds toward their cis-azolog (vertical axis) and trans-azolog (horizontal axis), for compounds from PDB (a) and CSD (b). 3D Shape similarities are reported as the city block distance (CBD) in the 3DAPfp, with the lowest value indicating highest similarity. Scatter plots are shown for six highly populated linker groups and rest of the groups merged together (−X−Y−). Each of the scatter plots is marked with the structure of respective linker type. Color scale shows the density of molecules. The compounds further investigated in this study are highlighted on the scatter plots.
For biaryl sulfonamides, benzyl anilines, and benzyl phenyl ethers we observed that a considerable number of representatives had dihedral angle values around 60°. Since this gauche confirmation neither fits the geometry of trans- nor cis-azologs, these compounds are likely less amenable to azologization.

Next, we computationally generated cis- and trans-azologs for all PDB/CSD structures identified in our screen using the 3D builder CORINA.²³ We compared the shape similarity of the parent compound to the computed cis- and trans-azologs using the 3D atom pair fingerprint 3DAPfp, which is a 16-dimensional fingerprint counting the number of atom pairs at exponentially increasing distances in a molecule, and which encodes molecular shape.¹⁸ 3D structural homology between parent drug and either azolog isomer was quantified using city block distance (CBD) metric, which is the sum of the absolute differences between the 16 pairs of bit values in the respective 3DAPfp fingerprints, such that a low CBD value indicates a high shape similarity between two molecules. We subsequently analyzed the entire azolog space comparing the CBD values of parent structures with the respective cis/trans azologs. The results from the analysis were scatter plotted by linker type and database (Figure 3) and are individually discussed for the major compound classes below. In addition, we investigated for each class whether the linker engages in hydrogen bonding, which would be partially or fully lost upon azologization. We arbitrarily selected 30 benzyl anilines, N-alkyl benzyl anilines, N-aryl benzamides, and biaryl sulfonamides from the PDB ligands and analyzed the crystal structures for potential target engagement (SI Table 1).

**Azobenzenes.** In addition to the azologable targets analyzed above, PDB and CSD also contained a large number of azobenzenes themselves. Of course, these compounds would be the most straightforward photoswitches to use. In our search for bioactive azobenzenes we found 14 molecules in Drugbank, 45 in PDB, and 1602 in ChEMBL. Similar to the analysis performed above, we analyzed their experimentally determined 3D structures as reported in the PDB and CSD compared the dihedral angles across the C−N=N−C moiety (Figure 2). The majority of the azobenzenes were trans isomers with angles close to 180°, whereas a few examples of cis-azobenzenes existed that had dihedral angles close to 0° (examples: PDB 2H4B, 2M0Z, and 2CLX). None of the diazene units appear to engage in hydrogen bonding, although azobenzenes are protonated at this site under strongly acidic conditions (the pKₐ of methyl orange is 3.4).

**Stilbenes.** Stilbenes provide the most obvious azologization motif since the dihedral angles of trans-stilbenes match those of trans-azobenzenes, while cis-stilbenes would be expected to give cis-active azologs. However, surprisingly few cis-stilbenes have been found in our analysis. Combretastatin A-4 is a notable exception (Figure 3).

**1,2-Diarylethanes.** To the best of our knowledge, 1,2-diarylethanes have not yet been azologed for use in photopharmacology. Their dihedral angle analysis shows that approximately 50% of the PDB ligands bind their biological target with angles closely matching those of trans-azobenzenes. Like stilbenes, they cannot engage in hydrogen bonding at the linker region.

**N-Aryl Benzamides.** The most abundant azologization motifs found are N-aryl benzamides. The dihedral angle and 3D structural homology analyses confirm that these molecules should be prime targets for azologization and are expected to give trans-active compounds. Their linker amide can function as hydrogen bond donor or acceptor. Azologization could therefore lead to a loss of the bioactivity. However, amide linkers are often employed in medicinal chemistry to connect two fragments with an easily formed bond. Indeed, we found that approximately one-third of carboxamide linkers (11/30) do not engage in hydrogen bonding, increasing the chances that the corresponding azolog has comparable potency to the parent compound.

**Benzyl Phenyl Ethers.** Benzyl phenyl ethers have been successfully employed on a few occasions. Our study suggests that this linker class is highly amenable to azologization. The majority of benzyl phenyl ethers possesses dihedral angles around 180°, which primes them as potential trans-azologs. A few benzyl phenyl ethers have gauche angles making them less suitable for azologization. Only 2 of the 30 benzyl phenyl ethers analyzed showed hydrogen bonding to the linker, which is significantly less compared to N-aryl benzamides or benzyl anilines.

**N-Benzyl Anilines.** While benzyl anilines have been successfully azologed previously, our analysis suggests that only a few of these compounds have suitable dihedral angles. A surprisingly large proportion of these compounds exhibit...
gauche-like dihedral angles. Approximately one-third of secondary amine linkers (11/30) and tertiary amine linkers (11/30) show hydrogen bonding in the linker region, which is significantly less than in N-aryl benzamides but more than in benzyl phenyl ethers.

**Biaryl Sulfonamides.** Our analysis suggests that biaryl sulfonamides are generally challenging targets for azologization. They almost exclusively exhibit gauche conformations in their bioactive form and do not show a pronounced tendency for structural overlap with either trans- or cis-azologs in our 3D structural homology analysis. Roughly one-third of the biaryl sulfonamide analyzed (11/30) also showed hydrogen bonding in the linker region.

**Analysis of Drugbank and ChEMBL.** Next, we repeated the analysis for Drugbank and ChEMBL using the computationally generated 3D structures (CORINA calculation). With the exception of sulfonamides, all linker types overwhelmingly showed preferred dihedral angles close to 180° (Figure 4).

The scatter plots show similar distributions to that of PDB and CSD compounds, suggesting that the CBD values could indeed be indicative of isomer activity and guide the choice of compounds for azologization (Figure 5). We calibrated this analysis with three known photoswitches, which had previously been developed using an azologization strategy (Figure 6).

These include VU-415374, a positive allosteric modulator of metabotropic glutamate receptor type 4, the ion channel blocker fomocaine, and the microtubule inhibitor combretastatin A-4. For VU-415374 and fomocaine, the 3D structure overlay with the respective trans-azolog showed more overlap and significantly lower CBD scores compared to that of the cis-azolog. For combretastatin A-4 we obtained the reversed result and significantly better overlap between parent drug and cis-azolog. These results matched the experimental results and demonstrate that low CBD scores give good predictions about which isomer of an azolog is active.

**Azologs and Their Biological Targets.** From the analysis above, we selected all molecules from ChEMBL, Drugbank, and PDB having two-atom linkers with experimental or predicted linker dihedral angle falling within the range 0°–20° (cis-azologization targets) or within the range 160°–180° (trans-azologization targets; Table 2).

We analyzed these hits according to biological targets. In total, more than 1200 biological targets in the CHEMBL database could be amenable to azologization. Multiple hits were found for most, which increases the chances to find a useful photopharmaceutical. Strikingly, only a small fraction of these biological targets has been previously addressed by photopharmacology. For example, relatively few enzymes found were put under photocontrol. Kinases present the largest class of biological targets that were identified in our screen (Figure 7). While some efforts in optogenetics have been geared toward the optical control of kinases, only a few studies in photopharmacology have addressed them. Other target classes that could benefit from the spatiotemporal resolution of photopharmacology include transcription factors, GPCRs, ion channels, and transporters.

**Optical Control of RARα Receptor.** To demonstrate the usefulness of our analysis, we synthesized and evaluated a number of hits. To this end, we chose molecules that are easily synthetically accessible and could modulate biological targets that have not been previously addressed with photopharmacology.

![Figure 5](https://example.com/figure5.png) Scatter plots showing the 3D shape similarities of potential azologable compounds toward their cis-azolog (vertical axis) and trans-azolog (horizontal axis), for compounds from PDB (a) and CSD (b). 3D Shape similarities are reported as the city block distance (CBD) in the 3DAPfp, with lowest value indicating highest similarity. Scatter plots are shown for six highly populated linker groups and rest of the groups merged together (~X–Y~). Each of the scatter plots is marked with the structure of respective linker type. Color scale shows the density of molecules. The compounds further investigated in this study are highlighted on the scatter plots.
Nuclear hormone receptors (NHRs) are hormone targeted transcription factors which bind to DNA and regulate various biological processes.24,25 Apart from the modulation of transcription levels on a relatively slow time scale, several NHRs are known to mediate rapid nongenomic effects which are thought to occur through protein−protein interactions.26 Nongenomic functions of NHRs include the regulation of kinases, phosphatase, and ion channels.27 Optical control of NHRs could enable the dissection between genomic and nongenomic mechanisms with the resolution of single cells in complex cellular networks.

Our computational screen for azologization motifs led to the identification of several hits for a number of nuclear hormone receptors, including retinoic X receptor, retinoic acid receptor, and others.

Table 2. Azologable Compounds

| database      | no. of azologable cmpds | no. of cmpds. as per top five linker types |
|---------------|-------------------------|----------------------------------------|
| PDB Ligand    | 949                     | 577, 37, 5, 117, 70                     |
| DrugBank      | 281                     | 91, 54, 32, 40, 16                      |
| ChEMBL        | 40,719                  | 16,097, 4,746, 3,915, 7,785, 1,186      |

Figure 6. (a) Structures of previously reported azologable compounds. The two atom linkers which were replaced by the diazo group are highlighted in red. (b) 3D-overlays of parent azologable compounds (gray) with corresponding trans-azologs (yellow). (c) 3D-overlays of parent azologable compounds (gray) with corresponding cis-azologs (yellow).

Figure 7. Heatmaps showing (a) number of unique ChEMBL compounds and (b) number of unique ChEMBL targets as a function of linker type and protein target family. Only azologable compounds within the correct predicted dihedral angle windows having IC_{50} and EC_{50} values of <10 μM (~41 K compounds) were considered for this analysis.
thyroid hormone receptor, peroxisome proliferator-activated receptor, liver X receptor, and estrogen receptor. We decided to synthesize the azolog of one of these compounds targeting retinoic acid receptor α (RARα). The potent RARα agonist Am80 is commercially available and widely used in biological research. At the same time our screen suggests that Am80 is an ideal target for azologization (3DAPfp CBD: 14; 3DAPfp cis: 147; Figure 8 A). Interestingly, the azolog of Am80 was already synthesized in 1989 and evaluated in SAR studies (CHEMBL 13150). However, its potential for optical control has never been evaluated, and the compound was exclusively tested in its nonirradiated form. We resynthesized the azolog of Am80, termed Azo80 (Figure 8B), using Baeyer-Mills conditions with subsequent ester hydrolysis (Figure 8C).

Figure 8. Computational prediction, design, and synthesis of Azo80. (A) 3D overlays of parent azologable compounds (gray) with corresponding cis- and trans- azologs (yellow) and 3DAPfp scores of 3D shape similarity comparison. (B) Design of Azo80 based on the azologization of the N-aryl benzamides Am80. (C) Chemical synthesis of Azo80.

Figure 9. Photophysical and biological evaluation of Azo80. (A) The UV−vis spectrum of Azo80 in the dark-adapted (black, trans), 365 nm adapted (gray, cis), and 460 nm adapted (blue, trans) photostationary states. (B) Reversible cycling between isomers with alternating illumination at 365/460 nm. (C) Schematic depiction of RARα activation with a small molecule photoswitch leading to corepressor/coactivator exchange and transcription of target genes (here: luciferase−luc). (D) Dose responses of Am80, cis-Azo80, and trans-Azo80 in a luminescent reporter cell line after 22 h. Samples were run in duplicates and in two independent experiments. Error bars represent mean ± SD (E) Control and rescue (reversibility) experiments. Samples were run in triplicates. Error bars represent SEM *** p < 0.001, n.s., not significant, student’s t-test.
Figure 10. Synthesis and isomerization of LTA₄₇-Photoswitch. (A) 3D-overlays of parent azologable compounds (gray) with corresponding cis- and trans- azologs (yellow) and 3DAPfp scores of 3D shape similarity comparison. (B) Design of LTA₄₇-Photoswitch based on the azologization of a benzyl phenyl ethers. (C) Chemical synthesis of LTA₄₇-Photoswitch.

Figure 11. Photophysical evaluation and LTA₄₇-hydrolase peptidase assay with LTA₄₇-H-Photoswitch. (A, B) Enzymatic reactions catalyzed by LTA₄₇-hydrolase. (C) The UV−vis spectrum of LTA₄₇-H-Photoswitch in the dark-adapted (black, trans), 365 nm adapted (gray, cis), and 460 nm adapted (blue, trans) photostationary states. (D) Reversible cycling between isomers with alternating illumination at 365/460 nm. (E) Schematic depiction of l-alanine 4-nitroanilide cleavage by LTA₄₇ (PDB: 2VJ8). (F) LTA₄₇ peptidase assay with LTA₄₇ (1.1 μg) and L-alanine 4-nitroanilide (1 mM) in the presence and absence of cis-LTA₄₇-H-Photoswitch at different concentrations. Samples were irradiated with 460 nm light after 4 min to yield trans-LTA₄₇-H-Photoswitch. The slope of 4-nitroaniline absorption (λ = 410 nm) was plotted. (G, H) Representative traces of 4-nitroaniline absorption (λ = 410 nm) before and after application of 460 nm light. Samples were run in triplicates. Error bars represent SEM ** p < 0.01, n.s., not significant, student’s t-test.
The photophysical properties of Azo80 are similar to those of a classical nonsubstituted azobenzene. Azo80 can be efficiently switched between cis/trans using 365/460 nm light and is bistable (Figure 9A,B). To test Azo80 for the ability to photocontrol RARα, we used a reporter gene assay in which the activation of RARα induces transcription of luciferase (Figure 9C). Upon addition of luciferase substrate after 24 h incubation, a luminescent signal proportional to luciferase transcription and RARα activation was quantified. We were pleased to find that the EC50 of cis-Azo80 (1.6 × 10^-8 M) is significantly lower than that of trans-Azo80 (6.0 × 10^-9 M) (Figure 9D). In a subsequent control experiment (Figure 9E), we demonstrated that light does not mediate or alter transcription levels in the absence of Azo80, while reversible optical control is achieved with Azo80. To demonstrate reversibility, Azo80 was added to cells as 365 nm-adapted cis-Azo80 and after 5 min the cells were illuminated with 460 nm light for 2 min to reactivate Azo80. Similar levels of transcription were observed in this rescue experiment compared to the experiment with dark-adapted trans-Azo80.

Optical Control of Leuktriene-A4 Hydrolase. Lipid photopharmacology is a rapidly growing field, and photo-switchable lipids have enabled the control of a wide range of biological pathways. In this context, the optical modulation of lipid metabolic networks could provide important insights. Our computational screen identified a number of small molecules that target various lipid metabolizing enzymes, including fatty acid amide hydrolases, lipoxygenases, phospholipases, leukotriene hydrolases, lipid kinases, and phosphorylases. Many of these enzymes could be interesting targets for photopharmacology. We decided to synthesize and test a photoswitchable inhibitor of the enzyme Leukotriene A4 hydrolase (LTA4H). LTA4H is a dual enzyme that functions both as a hydrolase and as aminopeptidase (Figure 11). It catalyzes the conversion of LTA4 to LTB4 and the degradation of chemoattractant tripeptide molecules.

Multiple inhibitors for LTA4H have been in development as the degradation of chemoattractant tripeptide molecules. Upon addition of luciferase substrate after 24 h incubation, a luminescent signal proportional to luciferase transcription and RARα activation was quantified. We were pleased to find that the EC50 of cis-Azo80 (1.6 × 10^-8 M) is significantly lower than that of trans-Azo80 (6.0 × 10^-9 M) (Figure 9D). In a subsequent control experiment (Figure 9E), we demonstrated that light does not mediate or alter transcription levels in the absence of Azo80, while reversible optical control is achieved with Azo80. To demonstrate reversibility, Azo80 was added to cells as 365 nm-adapted cis-Azo80 and after 5 min the cells were illuminated with 460 nm light for 2 min to reactivate Azo80. Similar levels of transcription were observed in this rescue experiment compared to the experiment with dark-adapted trans-Azo80. The trans-activity of Azo80 is coherent with the computational prediction, which suggested better 3D homology of Am80 with its trans-azolog.

The large number of potential molecular targets (>1200) that should be addressable with photopharmacology suggests that this approach to optical control is very versatile. It should be noted, however, that methods other than azologization further increase the reach of photopharmacology. Many photoswitches were designed through extension of the core with azobenzenes instead of replacement ("azo-extension" approach). An n-alkyl chain has been replaced with an azobenzene in the optical control of glycerophospho- and sphingolipids. In addition, photoswitches have been successfully installed in the backbone or side chain of biopolymers, such as nucleic acids and peptides. While "azologization" is the most straightforward design strategy, all existing strategies complement each other to provide an exceptionally versatile photopharmacological toolbox.

Experimental Section

Processing of Database and Azologs Generation. DrugBank (version 5, https://www.drugbank.ca/), PDB ligands (http://ligand-expo.rcsb.org/l-d-download.html), and ChEMBL (version 22, https://www.ebi.ac.uk/chembl/) databases were downloaded in SDF format from the respective database Web site in year 2017. CSD was copied from a licensed CD to Dr. Jürg Hauser, University of Bern. Molecules were processed using an in-house developed Java program utilizing the JChem chemistry library from ChemAxon Pvt. Ltd. (https://www.chemaxon.com/). Counter ions were removed, valence errors were checked, and molecules were ionized at pH 7.4. Molecules containing ≥26 and ≤50 heavy atoms, ≤4 stereocenters, 1 Ar-(two atom linker)-Ar, and no Ar-(diazo linker)-Ar were retained in the database. "Ar" stands for aromatic carbon. The two atoms in linker are acylclic atoms.
and may or may not be substituted. For each database, duplicate molecules were removed based on unique smiles comparisons. The resulting molecules in these processed databases are considered as potential azologable compounds. Afterward for each of the azologable molecules, Ar-(two atom linker)-Ar was replaced by Ar-(diazo linker)-Ar, and corresponding trans- and cis-azologs were generated. It should be noted that, whenever the difference in number of atoms between parent azologable molecules and corresponding azologs were more than six atoms, the corresponding molecules were not considered in the study. This was because some of the two atom linkers in parent molecules were substituted by large groups.

**Similarity Calculation.** For each drug and its two isomeric azolog, we generated the lowest energy 3D conformer using the CORINA program available from Molecular Networks Pvt. Ltd. For the parent azologable compounds in PDB and CSD databases experimental 3D coordinates were used. To compare molecules, we used an in-house developed 3D atom pair fingerprint as a measure of overall shape similarity.36 For each parent drug and its corresponding cis and trans azolog 3D atom pair fingerprints were computed, and similarities between them were quantified using city block distance metric.

**Chemical Synthesis.** All reagents and solvents were purchased from commercial sources (Sigma-Aldrich, TCI Europe N.V., Strem Chemicals, etc.) and were used without further purification. Solvents were obtained from Fisher Scientific. Reactions were monitored by TLC on precoated, Merck Silica gel 60 F 254 glass backed plates, and the chromatograms were first visualized by UV irradiation at λ = 254 nm. Flash silica gel chromatography was performed using silica gel (SiO2, particle size 40–63 μm) purchased from SiliCycle. NMR spectra were measured on a BRUKER Avance III HD 400 (equipped with a CryoProbe). Multiplicities in the following experimental procedures are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to the residual proton in the NMR solvent (CDCl3 = 7.26; MeOD: δ = 3.31). Carbon chemical shifts are expressed in ppm (δ scale) and are referenced to the carbon resonance of the NMR solvent ([CDCl3; δ = 77.16; MeOD: δ = 49.00]). NOTE: Due to the trans/cis isomerization of some compounds containing an azobenzene functionality, more signals were observed in the 1H and 13C NMR measurement.1H NMR (400 MHz, CDCl3) δ 8.32–8.04 (m, 3H), 7.89 (d, J = 1.8 Hz, 1H), 7.86 (d, J = 7.7 Hz, 2H), 7.63 (dd, J = 8.5, 1.9 Hz, 1H), 7.40 (d, J = 8.5 Hz, 1H), 1.69 (s, 4H), 1.33 (s, 6H), 1.28 (s, 6H). 13C NMR (100 MHz, CDCl3) δ 155.5, 150.7, 149.5, 146.2, 131.0, 127.6, 123.6, 123.4, 122.4, 118.5, 35.0, 34.9, 34.8, 34.6, 31.8, 31.7. HRMS: m/z calc. for C18H22N3O4 (M + H)+: 335.1765, found: 335.1758.

**LTA4H-Photoswitch.** A solution of 1-(2-chloroethyl)-pyrrolidine-HCl (51.4 mg, 0.30 mmol, 1.2 equiv), 4-phenylazophenol (50.0 mg, 0.25 mmol, 1.0 equiv), and K2CO3 (104 mg, 0.76 mmol, 3.0 equiv) in DMF (3 mL) was stirred at 85 °C for 16 h. The solution was cooled, water was added, and extracted with EtOAc. The combined organic phase was dried over Na2SO4 and concentrated in vacuo. Purification by flash column chromatography with CH2Cl2 → 10% MeOH in CH2Cl2 yielded LTA4H-Photoswitch (38.6 mg, 0.13 mmol, 52%) as an orange liquid.1H NMR (400 MHz, CDCl3): δ 7.93–7.81 (m, 4H), 7.55–7.39 (m, 3H), 7.11–7.04 (m, 2H), 4.19 (t, J = 5.6 Hz, 2H), 2.94 (t, J = 5.6 Hz, 2H), 2.68 (t, J = 6.6 Hz, 4H), 1.86–1.78 (m, 4H). 13C NMR (100 MHz, MeOD): δ 162.8, 154.0, 148.3, 131.6, 130.2, 125.8, 125.3, 115.9, 67.9, 55.5, 24.2. HRMS: m/z calc. for C18H22N3O4 (M + H)+: 296.1757, found: 296.1757.

**RRα Reporter Gene Assay.** A cell-based human RARα (NR1B1) driven luciferase reporter assay from INDIGO Bioscience (State College, PA) was adapted and used for the biological evaluation of Azo80. In brief, a 10 mM stock solution of Am80 or Azo80 was diluted with the provided cell screening medium to a final concentration of 2.5 μM. A 4-fold dilution series was prepared using this initial concentration and cell screening medium. The dilutions were added to reporter cells in white-bottom 96-well plates. For trans-Azo80, dilutions were added in the dark and cells were incubated for 22 h in the dark. For cis-Azo80, dilutions were irradiated at 365 nm for 3 min, and cells were incubated for 22 h in the presence of a 370 nm LED Cell Disco with light pulses for 75 ms/15 s. To minimize variations in the dilutions, the same dilutions were used for both experiments before and after irradiation. For the rescue experiment, Azo80 was added to cells as 365 nm adapted cis-Azo80 and cells were illuminated after 5 min with 460 nm light for 2 min to reactivate Azo80. After 22 h medium was ejected, and the supplied luciferase detection reagents were added and quantified using a BMG Labtech FLUOSstar Omega plate reader.

**LTA4H-Peptidase Assay.** Recombinant LTA4H was purchased from Cayman Chemicals and stored at −80 °C. LTA4H (1.1 μg) was incubated with L-alanine-p-nitroanilide (1 mM), in 50 mM HEPES (pH = 7.5), 100 mM KCl, 1 mg/mL BSA, 1% DMSO in the presence and absence of LTA4H-Photoswitch. LTA4H-Photoswitch was illuminated with 365
nm light for 3 min before addition of L-alanine-p-nitroanilide, and the reaction was initiated with LTA4H added last. The absorption at 410 nm was recorded for 4 min. After 4 min a 460 nm LED was used to illuminate the sample in the cuvette, and the absorption was recorded for 4 min under constant illumination.

**Safety Statement.** No unexpected or unusually high safety hazards were encountered in this line of research.

**ASSOCIATED CONTENT**

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscentsci.8b00881.

Analysis of linker–protein contacts, analysis of correlation between similarity scatter plot and dihedral angle, compound characterization by NMR and HRMS (PDF)

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**Notes**

The authors declare no competing financial interest.

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