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# Emerging Targets in Photopharmacology

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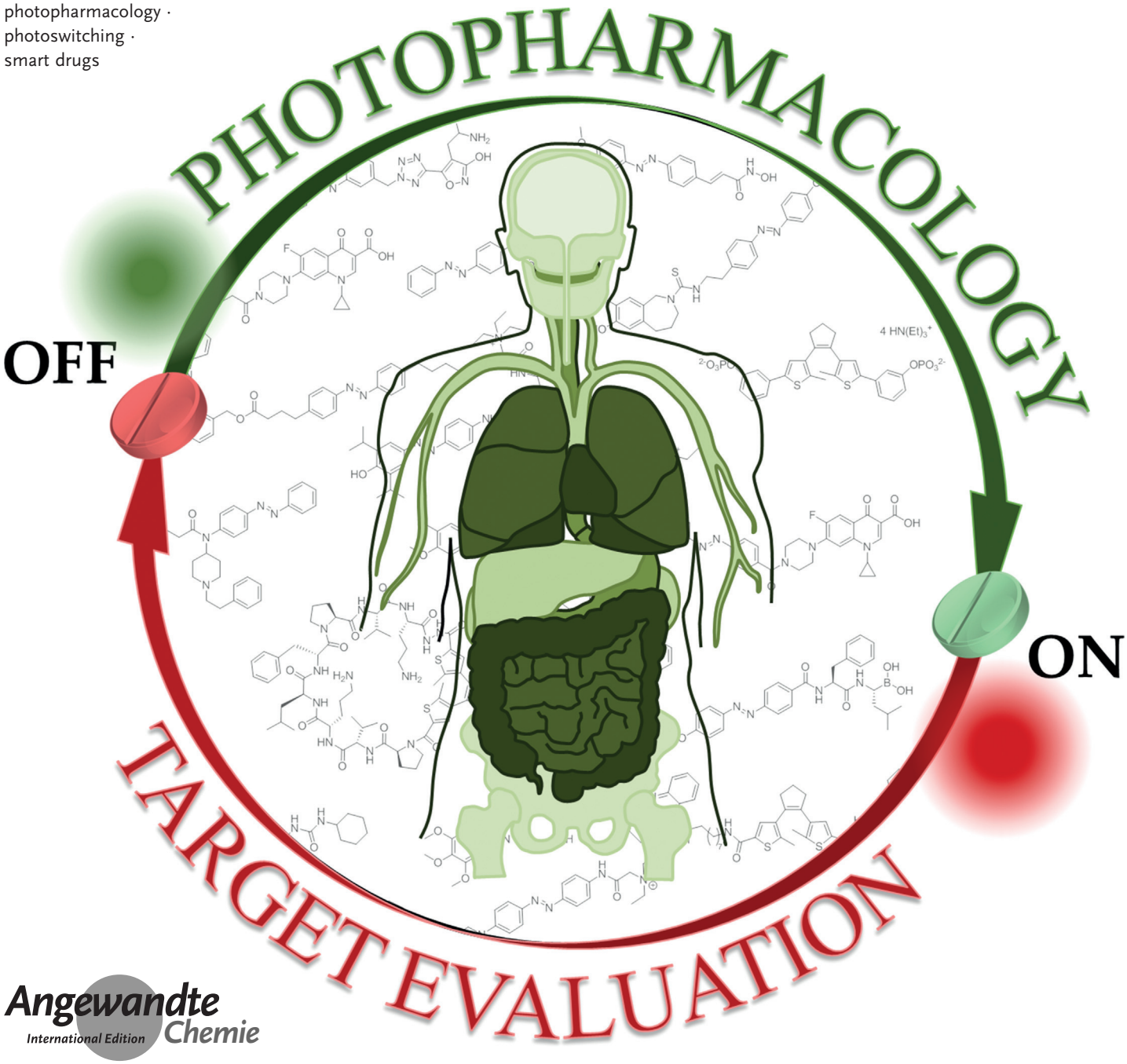
light delivery · light therapy · photopharmacology · photoswitching · smart drugs



*The field of photopharmacology uses molecular photoswitches to establish control over the action of bioactive molecules. It aims to reduce systemic drug toxicity and the emergence of resistance, while achieving unprecedented precision in treatment. By using small molecules, photopharmacology provides a viable alternative to optogenetics. We present here a critical overview of the different pharmacological targets in various organs and a survey of organ systems in the human body that can be addressed in a non-invasive manner. We discuss the prospects for the selective delivery of light to these organs and the specific requirements for light-activatable drugs. We also aim to illustrate the druggability of medicinal targets with recent findings and emphasize where conceptually new approaches have to be explored to provide photopharmacology with future opportunities to bring "smart" molecular design ultimately to the realm of clinical use.*

**From the Contents**



## 1. The Concept of Photopharmacology

The majority of current medical treatments rely on the use of bioactive compounds. These compounds evoke a pharmacological response by interacting with molecular targets in the human body, such as enzymes, receptors, ion channels, and carrier molecules.[1] The selectivity of this interaction is crucial, and the lack thereof leads to the emergence of potentially severe short-, mid-, and long-term side effects in the human body, and also limits increased dose efficacy at the site of action.[2] High levels of selectivity can be attained in several ways: 1) by avoiding cross-interactions upon addressing targets which are not present in humans, for example, of antimicrobial agents;[3] 2) by choosing targets present only in selected organs or overexpressed only in selected diseases, thus, reducing off-target effects, as in some cancer chemotherapies such as immunotherapy,[4] and 3) local administration of the drug, as is the case in ophthalmology.[5] However, in many cases it is not possible to achieve selectivity, because most pharmacological targets are constitutively expressed throughout the body in both healthy and diseased tissues.[4] For example, the epidermal growth factor receptor (EGFR) is present in normal epithelia,[6] besides being overexpressed in head and neck cancer, which thus limits the use of an increased dose of a therapeutic antibody such as cetuximab. Therefore, methods are of special interest that allow remote activation of drugs at the site of action only at a carefully chosen time, irrespective of the target distribution.

Photopharmacology[7,8] (Figure 1a) aims at solving the problem of off-target activity and severe side effects by establishing an external modality for controlling the action of the drug. To achieve this, photopharmacology relies on the design, synthesis, study, and application of drugs whose activity can be regulated with light. The use of such drugs in treatment could prevent systemic and environmental side effects through the selective activation of biological activity/toxicity. The photoactivation can be achieved either extrinsically (from outside the body) or intrinsically (from inside the body or at the site of action) by activated fluorescent compounds (e.g. FRET pairs or quenched probes).

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Photopharmacological agents are bioactive molecules modified with photoswitches, that is, moieties that change their structure upon irradiation with light.[9] Since the pharmacodynamic and pharmacokinetic properties of drugs are directly related to their molecular structure, the photo-induced changes in the structure of photopharmacological agents often allow the use of light to regulate their therapeutic action.

Photopharmacology, although not yet at the stage of clinical development, has the potential to become a privileged way of using light in medicine, since it could lead to the photocontrolled, reversible, selective addressing of targets in the human body by responsive small molecules (drugs), irrespective of the presence of oxygen. The use of light for medical treatment in the photopharmacological approach is inspired by older and more-established methods, including:

1. Photodynamic therapy (PDT, Figure 1b),[10–12] where the light-induced production of singlet oxygen is employed for tissue ablation. Since singlet oxygen is short-lived, its toxicity can be contained in a small volume, thereby leading to spatial selectivity of the therapy.[13] Although PDT is limited to evoking cellular damage, it has found

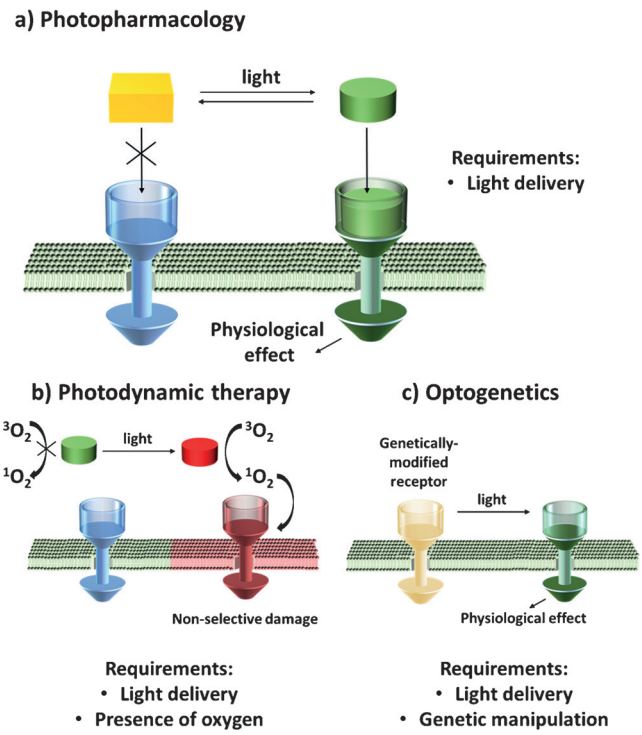


***Figure 1.*** Basic principles and requirements for the light-controlled treatment modalities, illustrated using a cell membrane receptor. a) Photopharmacology uses photoswitchable drugs (here: receptor agonist) that can be reversibly activated with light for interaction with their target receptors or enzymes. b) Photodynamic therapy (PDT) uses dyes that relax from their light-induced excited state by converting available triplet oxygen ($^3O_2$) into highly toxic singlet oxygen ($^1O_2$). c) In optogenetics, genetically engineered, photoresponsive ion channels are used to evoke a specific biological effect with light.

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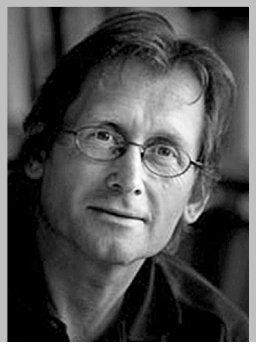


*Ben L. Feringa obtained his PhD in 1978 at the University of Groningen in the Netherlands under the guidance of Prof. Hans Wynberg. After working as a research scientist at Shell, he was appointed full professor at the University of Groningen in 1988 and named the distinguished Jacobus H. van't Hoff Professor of Molecular Sciences in 2004. His research interests include stereochemistry, organic synthesis, asymmetric catalysis, molecular switches and motors, photopharmacology, self-assembly, and nanosystems.*

many applications in clinical therapies and it has inspired the development of medical light-delivery systems. Photopharmacology, which uses light to control distinct drug–target interactions, might provide the possibility for more-selective treatments by taking advantage of the instrumentation and technology developed for PDT.

2. Optogenetics is a valuable photophysiological tool that relies on using light to modulate the activity of genetically engineered ion channels, which are usually derived from photoresponsive rhodopsins (Figure 1c).[14–16] In the future, the use of viral vectors for the editing of human neuron cell genomes will likely become a powerful therapeutic tool, for example in Parkinson's disease. At this point, however, the clinical relevance of optogenetics is limited by the need for challenging genetic manipulation, which is not required in photopharmacology.
3. Other approaches include the use of photoactivated metal complexes,[17] photocaged bioactive compounds,[18] and photoactivated molecules, such as clinically used psoralens.[19]

Currently, photopharmacology is at the stage of defining and evaluating the molecular targets, supported by the results of in vitro studies on receptor binding, enzyme inhibition, and general cellular toxicity. Important breakthroughs have been made in the fields of light-controlled cancer chemotherapy,[20–24] neurology,[25–27] diabetes,[28] and antimicrobial agents,[29] among others. Future milestones on the way to clinically applied photopharmacology will, in our opinion, include in vivo testing and extensive toxicity studies. Also of great importance will be the evaluation of photopharmacology through molecular imaging to study the distribution of the photoactivated drugs and confirm their localized action.[30] Synergistic approaches that rely on molecular imaging and photopharmacology will also contribute to the development of theranostics,[31] in which diagnostics and therapy are combined.

The aim of this Review is to aid the future development of photopharmacology by introducing the concept of "photodruggability" and critically evaluating possible targets for photopharmacological treatment. This discussion will be illustrated with recent examples, mostly from the last two years. For the discussion on the principles behind photopharmacology, the molecular design of photocontrolled drugs, and key requirements for molecular photoswitches, the reader is referred to recent reviews.[7,8]

## 2. Application of Light in Medicine: Opportunities and Challenges

Photopharmacology relies on the delivery of light to targets in the human body. This forms the basis for its selectivity, since light can be delivered with very high spatiotemporal precision. Furthermore, the facile control over the intensity and wavelength of the light could allow dosing of the active drug. On the other hand, the dependence on the delivery of light presents photopharmacology with one of its main challenges: How to deliver photons to the targets in tissues. Although it is well-established that the body has sufficient transparency to high-energy radiation (gamma or X-ray photons), which is used in medical imaging, the lower energy photons from the UV/Vis range are prone to both scattering in tissue and absorption by endogenous chromophores.[32] They also contribute to the photodamage of cells.[33] These processes severely limit the depth of penetration and are responsible for the toxicity of UV light.

Possible solutions for this challenge can be found in the field of PDT (Figure 1b), which, since its infancy, was presented with the problem of light delivery.[34] A multitude of successful clinical applications of PDT lends credibility to the photopharmacological approach and will inspire its development along two parallel pathways:

1. New developments in the light-delivery systems used in PDT, together with established equipment validated for clinical use, could be modified for application in photopharmacology. Continuing improvements in PDT instrumentation[34] have been made in terms of new light sources (lasers and LEDs), computer-aided delivery systems, endoscopes, fiberoptic devices, and light diffusers. They are aimed at the cost-effective delivery of light with a highly regulated dose and wavelength. For a more in-depth discussion on the delivery of light to tissues, including the newly available light sources, structured illumination, and multiphoton approaches, the reader is referred to recent reviews.[35–37]
2. PDT agents are usually designed to be activated with light with a wavelength of 650–900 nm. Such irradiation in this so-called "near-infrared phototherapeutic window"[34] is known to reach deepest into the tissue, without being limited by absorption by hemoglobin ($\lambda < 650$ nm) and water ($\lambda > 900$ nm).[38] With this requirement in mind, many research groups have recently designed and synthesized molecular photoswitches, mainly based on the azobenzene scaffold, which can be operated in or near the therapeutic window. The design principles and properties of these photoswitches can be found in recent excellent reviews from the groups of Hecht[39] and Woolley.[40] Further developments in this field are eagerly awaited and there is no doubt that they will form the basis of future successful photopharmacological drugs.

## 3. Photodruggability and Classification of Targets for Photopharmacology.

The concept of photodruggability introduced here is related to the druggability,[41] that is, the possibility for a disease-related receptor/enzyme to be targeted by a drug (usually a small molecule) that can bind to it with high affinity and change its activity/properties. Photodruggability encompasses this definition, and further narrows it with the following:

1. The target should be responsive to the light-induced changes in the structure/properties of the photopharmaceutical agent.
2. In cases where photoactivation of the drug in the patient's body is envisaged—to benefit from the spatiotemporal

control—the target must be related to a localized disease, such as a solid tumor or local inflammation.
3. The target should be accessible by light.

With these requirements in mind, we propose here a systematic classification of organs based upon the ease with which light can be delivered to them, inspired by the developments in photodynamic therapy:[11,12,34]

- **Class 1**: easily accessible: skin,[42] eyes (ophthalmoscopy)[43]
- **Class 2**: accessible by endoscopy: GI tract,[44] mouth and throat,[45] sinuses,[46] respiratory system,[47] cervix,[48] biliary tract,[49] bladder,[50] etc.
- **Class 3**: accessible through the skin without incision (lying just below the skin): thyroid, testicles as well as lymph nodes, muscles, and bones lying just under the skin.
- **Class 4**: accessible through minor incision: peritoneum,[51] including pancreas,[52] liver,[53] ovaries, stomach, intestines, kidneys, and spleen; also prostate,[54] most blood vessels,[55] glands, lymph nodes, muscles, and bones.
- **Class 5**: accessible through major incision or intraoperatively: brain[56] and bone marrow.

## 4. Class 1: Superficial Organ Structures

In terms of delivering light, the most easily accessible organs are the skin, eyes, ears, mouth, gastrointestinal tract, and upper and lower airways. We will focus on recent developments in the application of photopharmacology to the eyes and skin. Humans are affected by a multitude of eye diseases that range from macular degeneration, bacterial and viral infections, autoimmune diseases, to color blindness. The control of vision, and ultimately its restoration, is appealing for photopharmacology because the eyes have evolved to interact with light. Conceptually, the desired restoration of light responsiveness can be obtained by the penetration of ambient light through the cornea and iris. Vision is always related to neuronal signaling and, thus, the pharmacology of vision restoration deals with agonists, antagonists, or blockers of membrane channels.

Membrane channels are transmembrane proteins that are important for neuronal communication and the generation/propagation of the action potential.[57] The main classes constitute ionotropic receptors (permeable to ions) and metabotropic receptors (membrane receptors, for example, G-protein-coupled receptors). Membrane channels are responsive to certain types of stimuli, as in the case of ligand-gated ion channels (small molecules, chemical stimuli) and voltage-gated ion channels (transmembrane potential), but mechanical stimuli and temperature responsiveness is also possible. The basic biology and chemistry of membrane channels has been intensively studied.[58–61] More recent fundamental research has been conducted by Bayley and co-workers,[62,63] with additional focus on nanotechnological applications of such channels (e.g. nanopore sequencing).[64–66] Du Bois and co-workers[67–69] have published important studies on the syntheses of neurotoxins and their effects on membrane channels. Certain channel proteins, such as rhodopsin,[70] are naturally photoresponsive, a property which is being widely used in the field of optogenetics (see Figure 1c).[14,71–74]

The concept of artificially gating membrane channels with light was pioneered by the Bayley research group[62–66] (irreversible activation through photocaging) and our group[75–78] (reversible activation through photoswitching), and was further developed by the Trauner research group.[8,79,80] Bayley and co-workers rendered α-hemolysin from *Staphylococcus* photoresponsive through photocaging of a single cysteine residue (Figure 2a). The caged α-hemolysin lost its ability to form pores, but this ability could be recovered by irradiation with UV light. Recently, membrane channels have also been rendered light-responsive through genetic manipulation in an optogenetic approach (see Figure 1c).[14,71–73]

In 2005, our group reported a method to photocontrol a nanovalve derived from a channel protein (Figure 2b–d).[75] Taking inspiration from the well-characterized, mechanosensitive channel of large conductance (MscL) from *Escherichia coli*, we aimed at rendering it photosensitive by modification with a spiropyran photoswitch (Figure 2b,c). By replacing a glycine residue in the M1 helices of the pentameric channel complex by cysteine, which is not naturally present in MscL, we created a site for selective modification inside the protein channel (Figure 2b). A spiropyran-modified MscL derivative was synthesized by reacting this cysteine-derivatized MscL with an iodoacetate-bearing photoswitch (Figure 2c). Upon irradiation with UV light, the spiropyrans switch from a neutral to a charged state, thereby triggering the opening of the MscL pore. Patch-clamp studies and efflux experiments proved that the valve opened when light was applied (Figure 2d). Additionally, a reversible opening and shutting of the MscL channel was observed. This constituted a first step towards the control of channel proteins with light.

A more recent report by the group of Driessen and co-workers[78] focused on the control of protein translocation by the SecYEG complex (Figure 2e,f). Protein translocation in bacteria is mainly controlled by this SecYEG membrane protein channel together with a motor protein SecA. A photoswitchable protein translocation channel was designed by incorporation of an azobenzene into the lateral gate of SecY, which is the main subunit of the SecYEG complex (Figure 2e,f). Translocation assays were performed in vitro using the preprotein proOmpA as a substrate to test the effect of the isomerization of the azobenzene-derivatized SecY. The *trans*-azobenzene/SecY conjugate showed a similar efficiency as the nonconjugated SecY. However, an up to fivefold decrease in translocation was observed upon isomerization to the *cis* isomer. This method is the first to directly control a protein translocation channel with light.

In an effort towards using light-responsive membrane proteins for the restoration of visual responses in rodent models of inherited blindness, Flannery and co-workers aimed at fighting *retinitis pigmentosa* and age-related macular degeneration, which are both diseases that cause blindness through the death of the rod and cone photoreceptors.[81] In their approach, a light-gated ionotropic glutamate receptor (LiGluR) was modified with a maleimide-azobenzene-gluta-

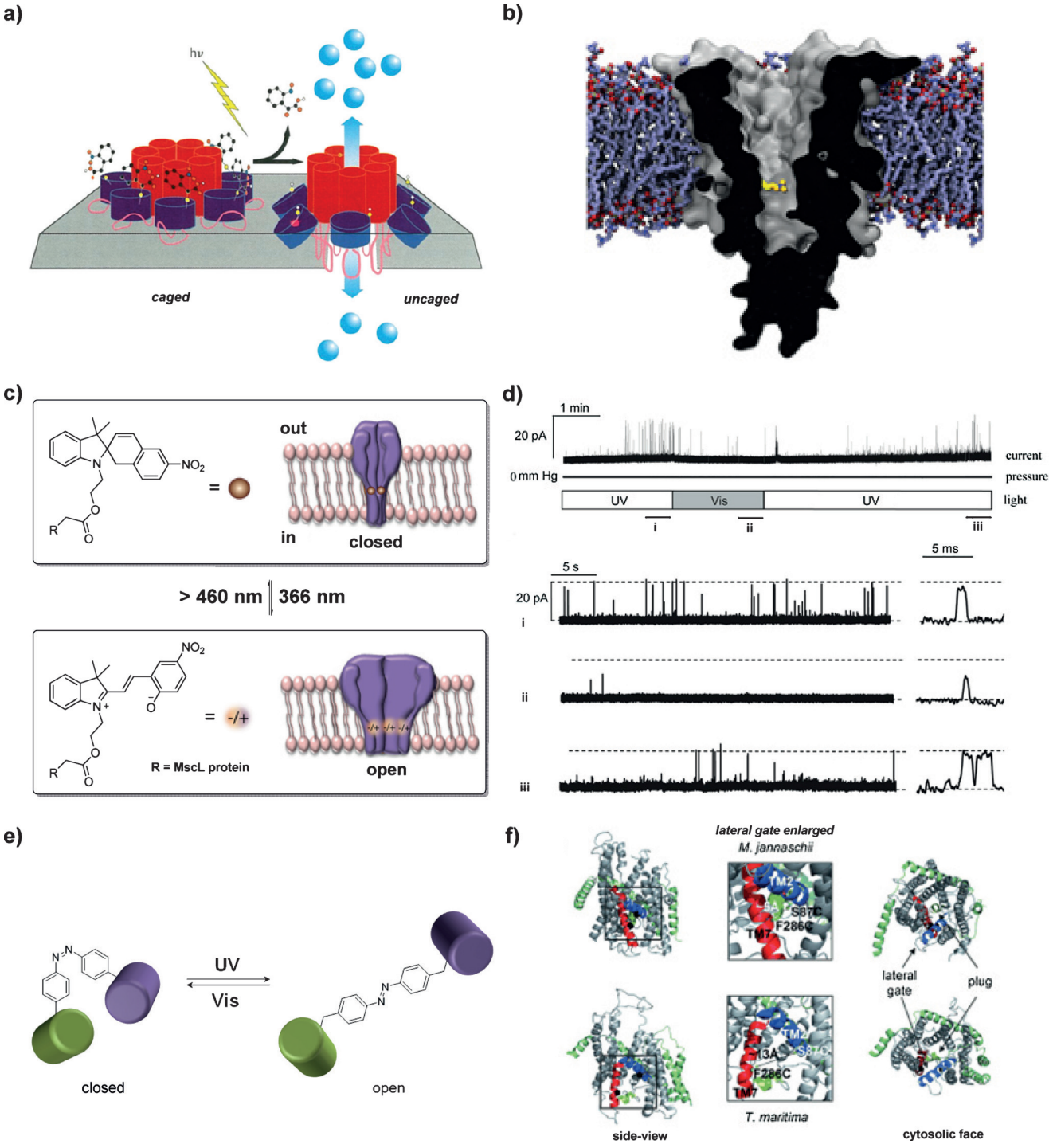



***Figure 2.*** Gating of transmembrane proteins with light: a) Irreversible photocontrol of the staphylococcal α-hemolysin pore-forming complex: Light-mediated uncaging of an engineered cysteine residue to allow pore formation.[63] Reproduced from Ref. [63] with permission, Copyright 1995 Elsevier Inc. b) Reversible photocontrol of the *E. coli* mechanosensitive channel of large conductance (*E. coli* MscL):[75] The structure of the MscL channel is shown as a cross-section (the site of affinity labeling (G22C) with the spiropyran photoswitch is marked in yellow). Reproduced from Ref. [76] with permission, Copyright 2013 American Chemical Society. c) General overview of the light-gating process: Formation of the (zwitterionic) merocyanine isomer leads to localized build-up of charges and, thus, opening of the channel. Reproduced from Ref. [76] with permission, Copyright 2013 American Chemical Society. d) Electrophysiology of the modified MscL: Effect of irradiation on the currents measured. UV light opens the pore (i and iii) and visible light closes the channel (ii). Adapted from Ref. [75] with permission, Copyright 2005 AAAS. e) Reversible gating of the SecYEG protein conducting channel with light[78] by incorporation of an azobenzene photoswitch. f) Structural comparison of SecYEG complexes from *Methanococcus jannaschii* (1RHZ.pdb, lateral gate = closed) and *Thermotoga maritima* (3DIN.pdb, lateral gate = preopen state) in side and cytosolic face views. The lateral gate is enlarged: TM2: blue, TM7: red, and plug domain: yellow, positions of cysteine mutations S87C and F286C of *E. coli*: black spheres. Reproduced from Ref. [78] with permission, Copyright 1999–2016 John Wiley & Sons, Inc.

mate tether **1**, by attachment to a genetically engineered cysteine at the active site (Figure 3).[82,83]

Tether **1** was reported by Trauner, Isacoff, and co-workers in 2006[82] and 2007[83] to be a constitutively controllable linker

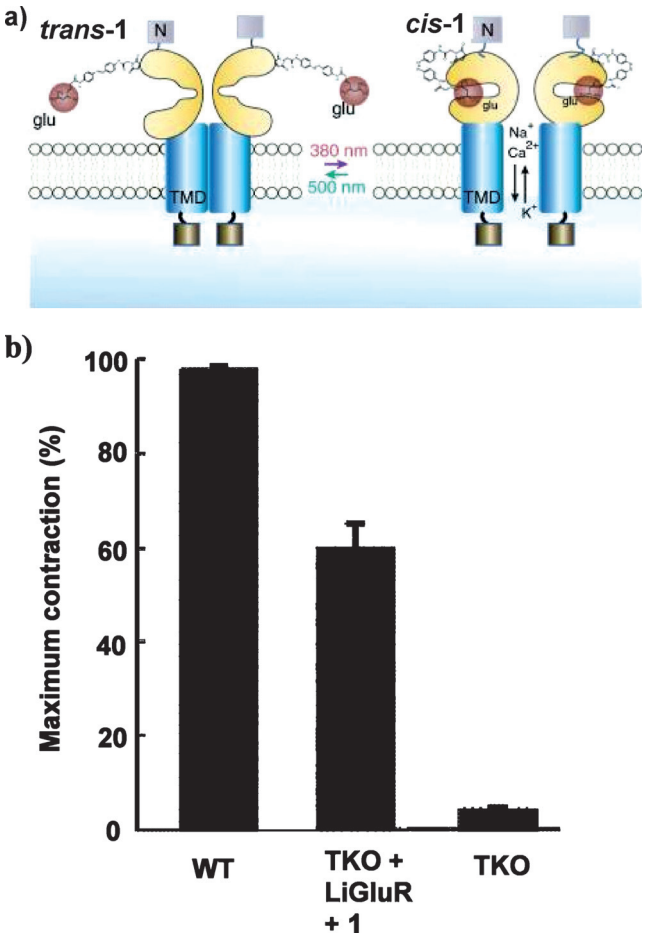
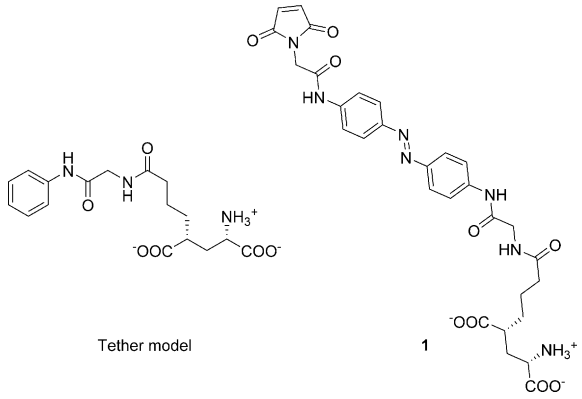


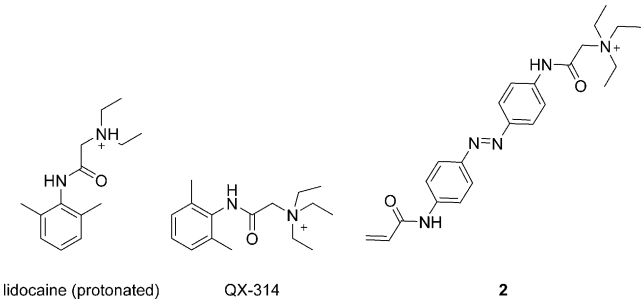

***Figure 3.*** a) Closing and opening of the LiGluR upon *cis–trans* isomerization of the photoswitchable tethered agonist. Opening of the LiGluR allows cations to flow, thereby resulting in depolarization of the membrane upon irradiation with light with a wavelength of 380 nm. b) The puppilary reflex (contraction) was measured on wild-type, triple-knockout (TKO) and triple knockout mice with LiGluR and the photoswitchable agonist. Adapted from Ref. [81] with permission, 2016 American Society of Gene & Cell Therapy.

for the reversible opening and closing of the LiGluR. Adeno-associated viral vectors (AAV)[84] were used to deliver the LiGluR into retinal ganglion cells, thereby restoring the response to the primary visual cortex, the pupillary reflex, and the natural light-avoidance behavior (Figure 3b). Importantly, the delivery of light to the eye is hindered by the impenetrability of the cornea to UV light,[85] so a more-red-shifted analogue of **1** should preferably be utilized. Moreover, red-shifting often coincides with a lower thermal stability of the *cis* isomer, which is beneficial for vision-restoration applications.

A similar approach was taken by Kramer, Trauner, and co-workers, who reported photochemical restoration of visual




responses in blind mice.[26] An acrylamide-azobenzene-quaternary ammonium compound (**2**) was used, which was reported in 2008 to be a $K_v$ channel photoswitch that enables control of neuron excitation.[86,87] The design of compound **2** is




inspired by the positive charge present in lidocaine (in its protonated form). The administration, as in the above-described example, was carried out simply by injection of the photoswitch into the vitreous cavity of the eye, with no surgical intervention required. The injection of **2** led to the restoration of light responses in retinal ganglion cells in mutant mice that lacked rods and cones. It has to be stressed that the reversibility of this system, together with the long-lasting effect of the light-responsiveness, has great potential for the restoration of visual function. However, the drawbacks of this system include the need for high-intensity UV light to trigger the retinal ganglion response, the possible toxicity of the reactive acrylamide moiety, and the inaccuracy of the intravitreal injection, which led to a variable photosensitivity in vivo.[26]

A follow-up study by Kramer and co-workers[88] showed a way to overcome the majority of the drawbacks of the above-mentioned systems by red-shifting the absorption wavelength of the $K_v$ channel photoswitch (Figure 4). The introduction of a strong electron-donating diethylamine group to the system resulted in compound **3**, which can be isomerized with light at a wavelength of 450–550 nm and shows rapid relaxation to the *trans* isomer in the dark.[89] The profound effect of the red-shifted photoswitchable $K_v$ channel blocker **3** is only observed on photoreceptor-degenerated retinas, whereas no effect on healthy retinas was observed in wild-type or triple knockout (TKO) mice. This finding

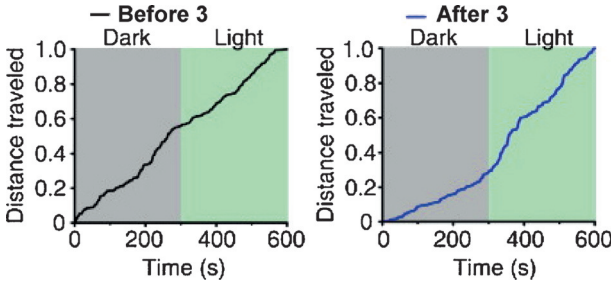
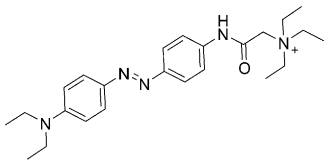


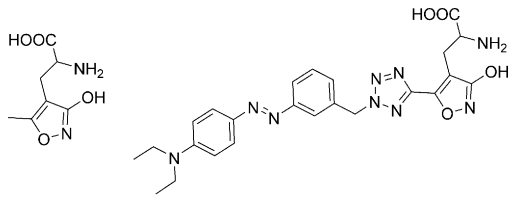

***Figure 4.*** Distance traveled (cumulative) by a rd1 mouse before and after injection of photoswitchable compound **3**. No effect of irradiation was observed before injection, whereas light triggered an increased distance travelled after injection. Adapted from Ref. [88] with permission, Copyright 2014 Elsevier Inc.



**3**

implied that the effect is due to a selective interaction of **3** with regions to which the cell death was constrained.

Recently, another example of the restoration of light sensitivity in blind retinae involved the use of the azobenzene-modified AMPA receptor agonist **4**.[90] This was the first



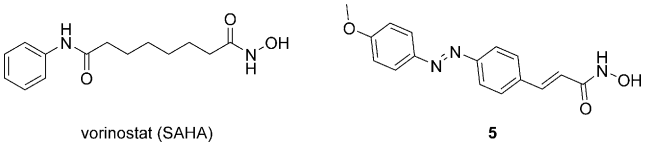
AMPA **4**

example of using a photoswitchable agonist instead of a blocker for the restoration of light sensitivity in blind retinae. The structure of **4** was inspired by the excitatory amino acid AMPA, which has been used for the control of neuronal activity in acute cortical brain slices. However, it is still unknown if AMPA is expressed ubiquitously in damaged retinae.[91–93] This not only has implications for the applicability of this system to vision restoration, but for all attempts to manipulate existent retinae.

In conclusion, a number of publications have appeared in recent years showcasing the potential of the restoration of visual responses in eyes as a target for photopharmacology. The use of phototethered ligands presents a challenge of proving their covalent attachment to the target,[75,77] which furthermore requires genetic engineering, thus making it unsuitable for medical applications at present. However, these groundbreaking studies resulted in the advancement of photopharmacology in the field of ophthalmology. Major challenges include the development of slow-diffusing/degrading small molecules to restore vision for longer periods of time without the need for repeated injections in the vitreous humor. Moreover, an assembly of multiple, switchable small molecules with distinctive wavelengths of irradiation might enable responsiveness to different colors and would bring this field even closer to an applicable system in the fight against blindness.

Skin constitutes another easy-to-reach target for photopharmacological treatment and evaluation strategies as it is the most exposed and easily accessible human organ. We reported the design of photoswitchable histone deacetylase (HDAC) inhibitors,[22] which might pave the way for the application of photopharmacology to skin diseases such as cutaneous T-cell lymphoma and superficial spreading melanoma.

Taking inspiration from the clinically approved drug vorinostat (SAHA, Figure 5a), which is marketed for the treatment of cutaneous T-cell lymphoma, we aimed to render it photoresponsive by the attachment or incorporation of an azobenzene photoswitch (Figure 5b).[22] Differences of up to



vorinostat (SAHA) **5**

39-fold in the activity on HDAC2 were observed upon isomerization of the molecular structure (Figure 5c,d). Moreover, the potency was comparable to the native SAHA, and stable photoswitching was observed together with no reduction by glutathione under physiological conditions. However, as emphasized before, the use of UV light in this example might also cause obstructions for clinical application, because of its toxicity to healthy cells and especially the skin. Therefore, the key next step is the incorporation of more far-red absorbing photoswitches.[39,40,94,95]

## 5. Class 2: Intraluminal and Adjacent Organ Structures

Class 2 organs are accessible by endoscopy and include the sinuses, oropharynx, gastrointestinal tract, respiratory system, bladder, prostate, and cervix. As a result of the easy accessibility of these organs for irradiation with light, localized diseases of the mouth and the respiratory system, as well as different types of cancers (e.g. bladder, cervix, and prostate, as well as gastrointestinal) are privileged targets for photopharmacological treatment and treatment monitoring by optical fluorescence imaging techniques such as molecular-guided endoscopy.[96]

König and co-workers reported the application of photopharmacology for treatment of infectious respiratory diseases, with an important target being tuberculosis.[97] This disease is caused by the pathogenic *Mycobacterium tuberculosis*. Phosphoribosyl isomerase A (*mt*PriA),[98] a branch-point enzyme

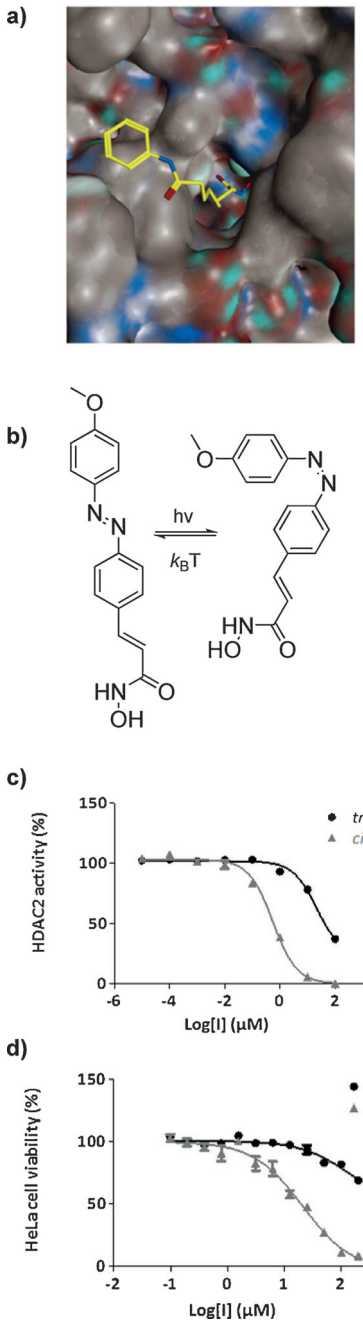
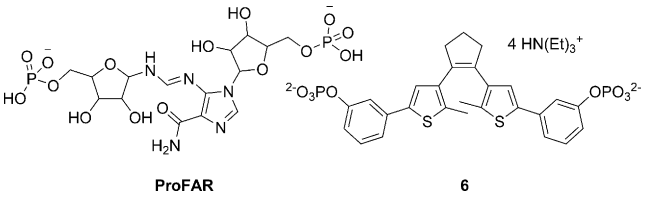


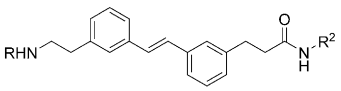

***Figure 5.*** a) The original binding site of the enzyme with complexed SAHA. The aliphatic side chain inserts into the enzymatic channel, with the hydroxamic acid binding to the zinc cation. b) Photoisomerization of photoswitchable SAHA analogue **5**. c) The $IC_{50}$ value for the *trans* and *cis* forms of the inhibitor for inhibition of the HDAC2 enzyme. d) Viability of HeLa cells after 16 h of incubation with various concentrations of each isomeric form of the inhibitor. Adapted from Ref. [22] with permission, 2015 Wiley-VCH.

in the bacterial biosynthesis of tryptophan and histidine, was selected as the target for the photopharmacological approach.[99] The groups of Sterner and König made use of the twofold symmetry of *mt*PriA[100] to develop a set of $C_2$-symmetric inhibitors incorporating a diarylethene[101] photoswitch scaffold and nonswitchable ProFAR, a substrate for *mt*PriA. In vitro tests showed low micromolar inhibition constants ($K_i$; $K_{M,ProFAR} = 8.6\ \mu M$). An up to tenfold difference was observed for **6** between the open and closed forms. The




use of diarylethenes has the advantage of fast photoisomerization and bistability. In this case, the strongly inhibiting isomer is the open form, which can be obtained by irradiation with visible light ($\lambda > 420$ nm). Cyclization is induced with light with a wavelength of 320 nm, which might lower its applicability for direct irradiation. However, bistability is not necessarily a key factor for success in photopharmacology.[8] Importantly, the molecular design uses an adaptive linker between two crucial functional groups, and the diarylethene in this linker modulates the conformational flexibility of the compound. This approach stands in contrast to "azologization".[102]

A different strategy to potentially treat tuberculosis was taken by Gogoll and co-workers.[103] In this case, *M. tuberculosis* ribonucleotide reductase (*mt*RNR) was selected as the target.[104] This enzyme consists of two subunits that form a tetrameric complex. The catalytic activity of the complex requires the interaction of both subunits.[105] Gogoll and coworkers designed a series of short photoswitchable peptidomimetic inhibitors based on a model peptide: a photoswitchable stilbene moiety was incorporated at different positions in the model peptide and different lengths of the peptidomimetic were used, thereby leading to a series of compounds **7**.[103] For longer peptides, the *E* isomer was more active,




**7**

| | | |
|---|---|---|
| Glu-Asp-Asp-Asp-Trp-Asp-Phe | R = H- | $R^2$ = Glu-Asp-Asp-Asp-Trp-Asp-Phe |
| **model peptide** | R = Ac- | $R^2$ = Glu-Asp-Asp-Asp-Trp-Asp-Phe |
| | R = AcTrp- | $R^2$ = Glu-Asp-Asp-Asp-Trp-Asp-Phe |
| | R = Ac- | $R^2$ = Phe |
| | R = Ac- | $R^2$ = Asp-Phe |
| | R = AcGlu | $R^2$ = Asp-Phe |

whereas the opposite was true for shorter peptides. Interestingly, all the compounds were more potent than the parent model peptide, likely because of a hydrophobic interaction between the stilbene and the enzyme pocket.[106] Photoswitching was achieved with light at $\lambda = 300$ nm and relatively long irradiation times (> 1 h). Both factors might be a limitation for clinical use.

Class 2 organs offer ample opportunities for photopharmacological treatment because they offer interesting targets in infectious diseases, inflammation such as Crohn's disease,

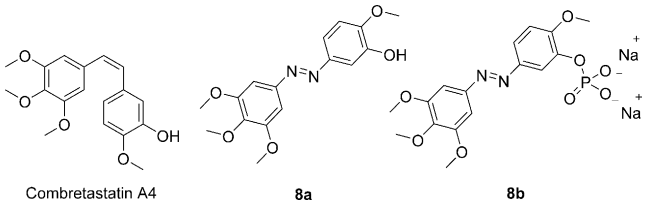
and cancer. Tuberculosis is a severe condition and the initial studies described show the potential of photopharmacology to overcome this disease. Moreover, the photodruggability of Class 2 organs is high because they are accessible by endoscopy, which can easily include multispectral light sources to initiate photoisomerization processes at different wavelengths for switching the photopharmacologic effect "on" and "off".

### 6. Class 3: Organ Structures Lying just Under the Skin

Class 3 organ structures can be accessed by irradiation with light through the skin (without the need for incision). Their location just below the skin allows the use of surface irradiation. However, the penetration depth of the light used to switch the drugs must be chosen appropriately (see above). Class 3 organs include thyroids, testicles, salivary glands, lymph vessels and nodes, muscles, nerves, arteries and veins, as well as bones.

Cancer[107] is a premier target for photopharmacology as it is highly localized and existing therapies are often accompanied by severe systemic side effects, thus posing a tremendous burden to the patient in terms of morbidity and even mortality.[2]

Microtubule dynamics are essential in intracellular transport, motility, and cell proliferation,[108] and has been associated with antiangiogenesis.[109] Combretastatin A4 is a cholchicine-domain microtubule inhibitor that binds to tubulins and thus inhibits their polymerization to form microtubules.[108,110–114] Combretastatin A4 phosphate has shown potency against anaplastic thyroid carcinoma.[115]




Recently, three independent studies reported the development of combretastatin A4 analogues and showcased a powerful example of photopharmacology in anticancer research.[21,116,117] Thorn-Seshold, Trauner, and co-workers performed a series of biological tests on a variety of combretastatin A4 analogues, including water-soluble prodrugs.[21] The most successful compound **8a** showed excellent cytotoxicity in the *cis* form, with an up to 250-fold difference in its potency compared to its *trans* isomer, which shows virtually no biological activity in the dark (Figure 6a). The photoresponse is quick and the *cis* content of the azobenzene mixture proved to directly correlate to the measured cytotoxicity, since only the *cis* form binds to the colchicine domain of tubulin.

Importantly, compound **8b** enabled control over the microtubule assembly/disassembly dynamics in vitro and

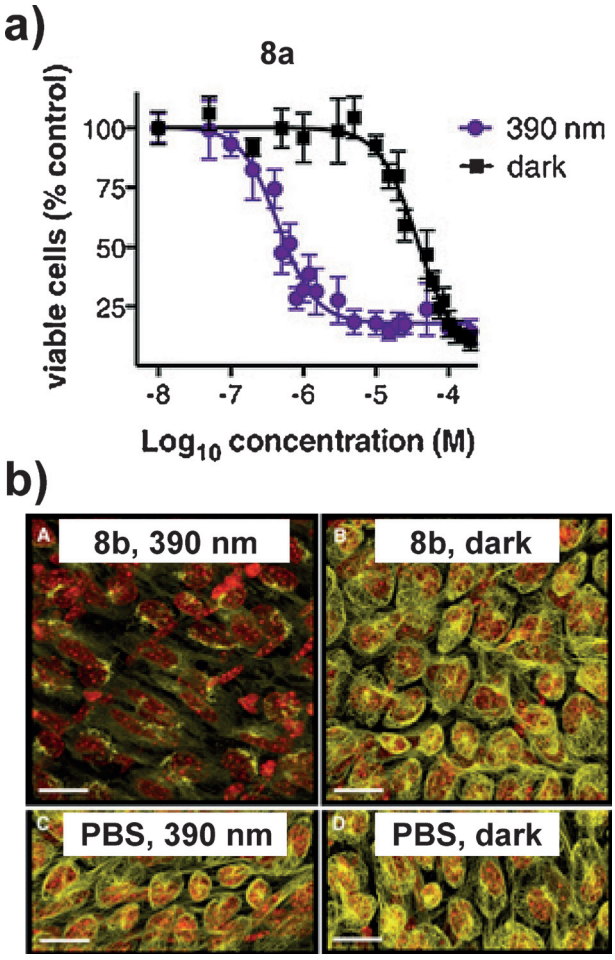



***Figure 6.*** Photoswitchable inhibitors of microtubule polymerization: a) Dose–response curves for the viability of MDA-MB-231 cells in the presence of **8a** either in the dark or under UV light ($\lambda = 390$ nm): **8a** ($EC_{50,dark} = 38$ μM; $EC_{50,390\,nm} = 0.5$ μM). b) In vivo experiment showing the disruption of the microtubule structure with UV light ($\lambda = 390$ nm) in mouse cremaster tissue and 50 μM **8b**: A) **8b**, UV light; B) **8b**, dark; C) buffer, UV light; D) buffer, dark.[21] Adapted from Ref. [21] with permission Copyright 2015 Elsevier Inc.

in vivo with high spatiotemporal precision. It also showed high cytotoxicity in a number of cell lines (Figure 6b). In vivo experiments were performed on *C. elegans* and cremaster muscle tissue of living mice. The light-controlled dynamics of tubulin-polymerization could be observed in real time.[21]

Streu and co-workers reported the same compound **8a** and included data for in vitro and HeLa MTT assays, which showed an increased cytotoxicity of **8a**, similar to the cytotoxicity observed for Combretastatin A4, in the presence of light (Figure 7).[116] Finally, Sheldon et al.[117] found that *cis*-**8a** was easily reduced with glutathione, which can pose a severe problem for biomedical applications. Markedly different thermal stabilities of the *cis* isomers compared to those reported by Borowiak et al.[21] were found. However, the biological results of the cytotoxicity of **8a** in human umbilical vein endothelial cells (HUVEC) and adenocarcinoma epithelial cells (MDA-MB-231) cells match with other reported findings.[21,116]

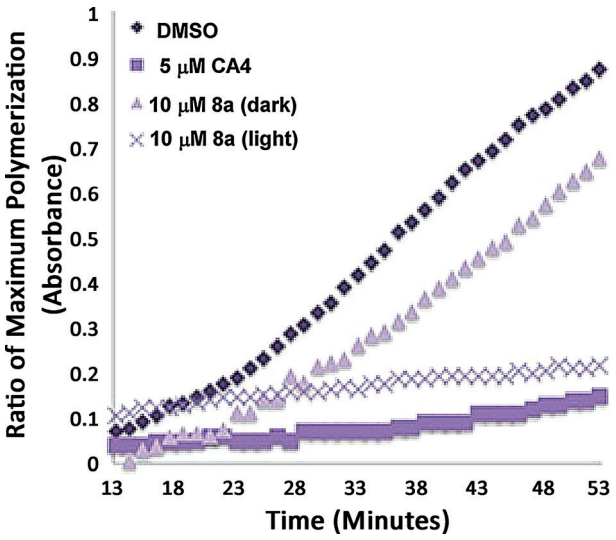



***Figure 7.*** Inhibition of tubulin polymerization of **8a** in vitro (10 μM) either in the dark or under irradiation ($\lambda = 400$ nm).[116] Adapted from Ref. [116] with permission, Copyright 2015 American Chemical Society.

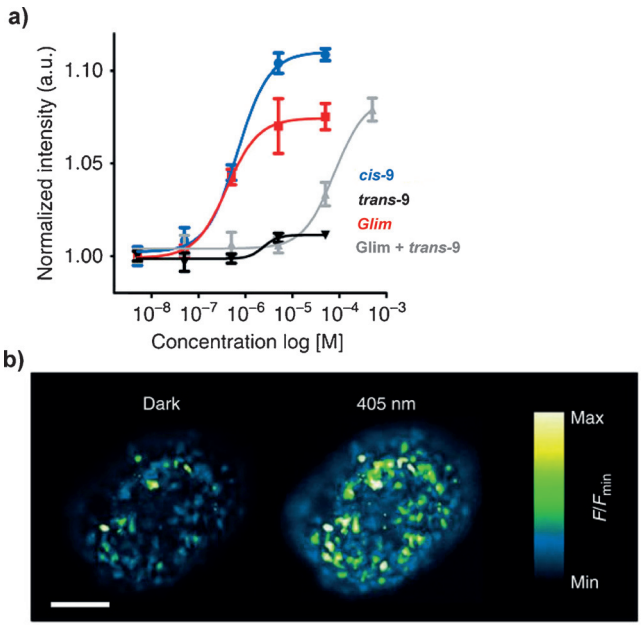
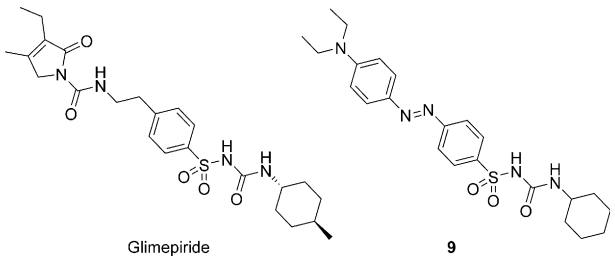



***Figure 8.*** a) Concentration–response curves for *cis*-**9**, *trans*-**9**, and Glimepride for the stimulation of $Ca^{2+}$ ions. b) Islets treated with **9** and residing in β cells display a large increase in cytosolic $Ca^{2+}$ following isomerization upon irradiation with light with a wavelength of 405 nm. Adapted from Ref. [28] with permission, 2014 Macmillan Publishers Limited.

## 7. Class 4: Deeper Lying Organ Structures

Organs such as the pancreas and bile ducts can be reached by intraluminal endoscopy. Upon incision, almost all the internal organs can be accessed, such as the liver, pancreas, spleen, small and large bowel, bladder, kidneys, and adrenal glands. Increasing interest in the pancreas has been observed in recent years, because of its role in diabetes. The need to control insulin levels in a precise temporal manner makes diabetes an excellent target for photopharmacology.

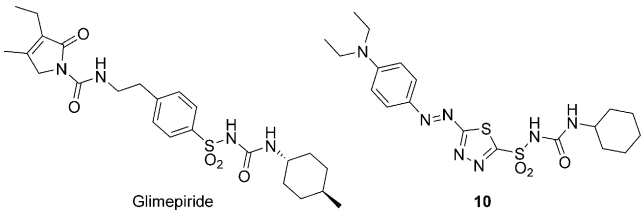
In 2014, Trauner and co-workers published a study that brought photopharmacology into diabetes research.[28] By applying photoswitchable sulfonylurea derivative **9**, it was




shown that both insulin release and pancreatic beta cell function could be controlled with UV light (Figure 8). The light-responsive sulfonylurea **9** was derived from Glimepiride,[118] which is a known stimulator of pancreatic beta cells to release insulin and thus lower the blood-sugar level. It is known that sulfonylurea derivatives boost insulin release to restore glucose levels by action on ATP-sensitive $K^+$ channels. However, the major drawback of the use of both insulin and sulfonylurea derivatives is the increased risk of hypoglycemia, that is, prolonged periods of dangerously low blood-sugar levels, which is a result of long-lasting, excessive secretion of insulin.

The greatest advantage of the concept presented is the possibly reduced risk of developing hypoglycemia and cardiovascular diseases, by means of increased control of insulin secretion towards its peak demand for a short period of time, thereby averting excessive insulin concentrations. However, limitations of the presented design include the use of short-wavelength ($\lambda = 400$ nm) light, the lack of thermal stability and low potency (17.6 μM, compared with 8.3 nM for Glimepiride), together with a small difference in the activity between both the *trans* and *cis* isomers. A bistable switching with longer wavelength light, combined with larger differences in potency between the isomers and higher overall potency, would drive this elegant proof-of-concept closer towards clinical application.

A more recent example by Hodson, Trauner, and co-workers addressed some of these challenges.[119] Incorporation of a heterocyclic azobenzene in the target molecule led to compound **10** with a red-shifted absorption spectrum ($\lambda_{max} = 500$ nm). However, a drop in potency of five orders of magnitude was observed compared to Glimepiride, which



shows the limitation of this molecular design. Despite this drawback, even with small amounts of *cis* isomer (no PSS data were included) obtained upon irradiation, elevated concentrations of $Ca^{2+}$ were observed, thus proving the ability to control pancreatic β cells with light in a reversible manner.

A recent report by the Trauner group was based on an incretin switch that enabled insulin secretion to be controlled with light (Figure 9).[120] As in the earlier examples, pancreatic β cells were treated, in this case with a glucagen-like peptide-1 derivative **11**, based on Lira, which allowed spatial control over $Ca^{2+}$ levels. Remarkably, the *trans* isomer enhanced calcium influx, whereas cAMP generation was induced by the *cis* isomer (Figure 9). This showcases the possibility to switch between two distinctive pathways (calcium influx and cAMP generation) upon isomerization with light.

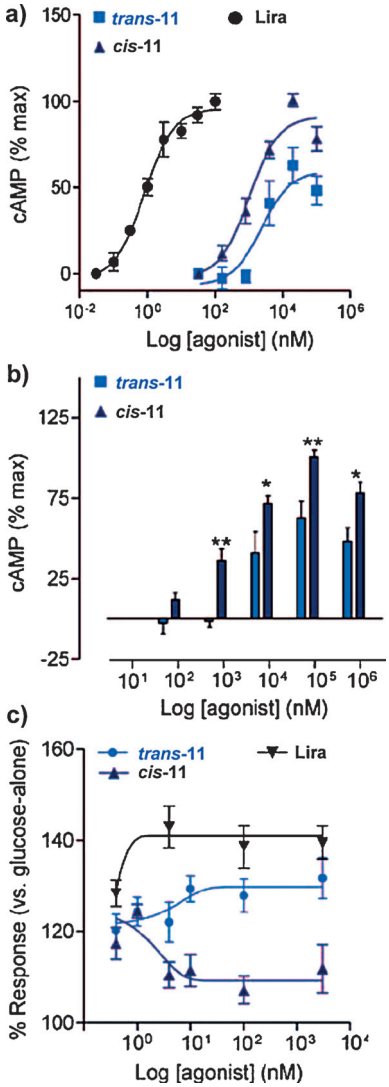
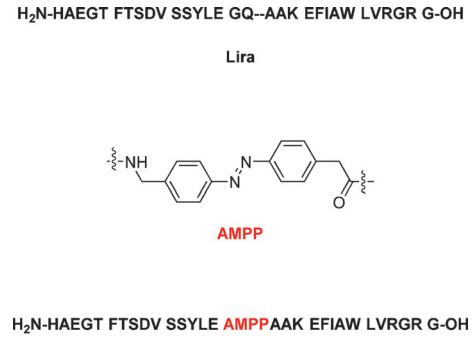



***Figure 9.*** a) cAMP responses upon photoswitching showing the higher activity of the *cis* isomer. b) cAMP responses in pancreatic β cells, showing increased cytosolic $Ca^{2+}$ levels in *trans*-**11** (blue light) versus *cis*-**11** (UV light). c) $Ca^{2+}$ signaling, showing a difference between the *cis* and *trans* isomers of LirAzo at concentrations greater than 101 nM. Adapted from Ref. [120] with permission, Copyright 1999–2016 John Wiley & Sons, Inc.



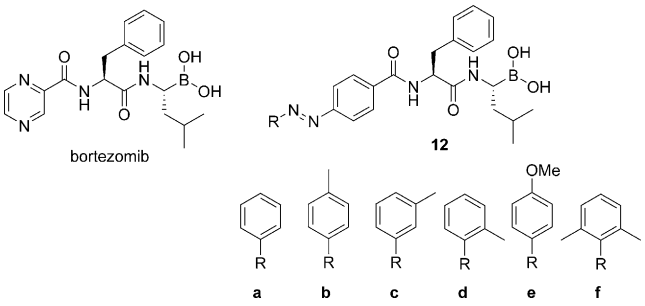

However, as in earlier reports, the limitation of this system for clinical applications is the need for UV light for the *trans* to *cis* isomerization. This drawback is effectively counteracted by the bistability of this system, which might allow the use of a harmless pre-irradiation which allows the function of the bioactive molecule to be controlled before uptake.

In conclusion, impressive efforts have been made towards the light-controlled release of insulin. However, it has to be stressed that the poor accessibility of the pancreatic β cells in vivo should stimulate research towards the applicability of different wavelengths of light or advanced delivery tools. Despite these drawbacks, it could be highly advantageous for diabetes research to gain spatiotemporal control over the function of β cells.

## 8. Class 5: Organ Structures Impermeable to Light

Class 5 organ structures, which include bone marrow and the brain, are the most difficult to irradiate, because of the opacity of bone tissue.

Bone marrow is vital for hematopoiesis, and diseases affecting it are often very severe. Multiple myeloma is the proliferation and accumulation of malignant clonal plasma cells in the bone marrow.[121] Treatment is difficult, and the drugs show adverse side effects on healthy tissue,[122] thus rendering local activation by light beneficial. The chemotherapeutic bortezomib[123,124] (a proteasome inhibitor) has proven successful against multiple myeloma and also mantle cell lymphoma. The light-responsive variants **12a—f**, reported by our group, which constitute the first reported examples of photoswitchable anticancer drugs,[20] were tested in RAJI cell lysate and for cell toxicity in HeLa cells. The

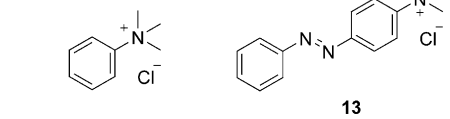


photoswitchable compounds were found to show two- to threefold differences in activity, with different selectivities towards the different active sites of the proteasome. The different inhibitors also showed activity in MTT cytotoxicity assays on HeLa cells. However, the use of UV light, especially for delivery to bone marrow, is a drawback towards clinical applications. Moreover, a larger difference in activity between the two isomers would be beneficial.

The brain is by far the most complex organ in the human body, with considerable unknown areas of function such as memory. Membrane channels lie at the heart of the physiological function of the brain. Targeting such channels, however, is difficult. Delivering light to the brain is always connected with an invasive surgical intervention. Recently, the delivery of light to the brain has received increased attention through the advent of optogenetics.[71–74] In these studies, ways were established to get light into the brain of a model organism (e.g. mice).

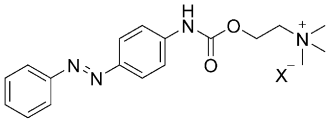
The curing of neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's disease is an important challenge in healthcare. Acetylcholinesterase (AChE) hydrolyzes acetylcholine (ACh), a neurotransmitter that stimulates nicotinic and muscarinic acetylcholine receptors.[125] AChE inhibition has also been associated with myasthenia gravis and glaucoma.[125] Erlanger and co-workers pioneered the concept of photopharmacology around 1969,[126–129] after reporting the control of AChE activity with light by using azobenzene-based inhibitors. Inspired by the known AChE inhibitor phenyltrimethylammonium,[130] compounds **13** and **14** were developed. Both photoisomers of **13** and **14** inhibited




Phenyltrimethylammonium Ion



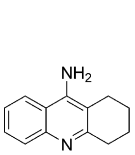

**13**



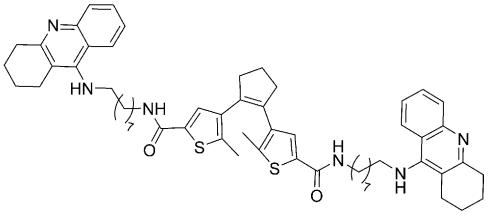

**14** X = Cl or I

AChE, with only a minor difference in activity (the *trans* isomers were more potent). Photocontrol of **14** (X = I) using sunlight was shown, together with the modulation of the membrane potential of electric organ cells of the electric eel (**14,** X = Cl). These initial proof-of-concept studies (although medically not particularly relevant) have had a tremendous influence on the field and its later focus (e.g. photocontrol of membrane channels[8,63,75,131] and enzymatic activity).[132] It is worth mentioning that Erlanger and co-workers not only pioneered soluble bioactive photoswitches, but also tethered photoswitches. They used azobenzenes as photoswitchable tether molecules to obtain a photoswitchable nicotinic acetylcholine receptor as early as 1980.[133]

The drug tacrine[134,135] is an acetylcholinesterase (AChE) inhibitor used for the treatment of Alzheimer's disease (AD).[136] However, it shows dose-dependent hepatotoxicity. Decker, König, and co-workers designed a diarylethene-based photoswitchable AChE inhibitor based on tacrine.[137] Compound **15** bears two tacrine moieties at the end of flexible linkers. It enables the photocontrol of β-amyloid aggregation associated with AD,[138,139] a noncholinergic activity where the peripheral anionic site (PAS) is potentially involved.[139,140]



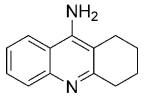
tacrine (THA)



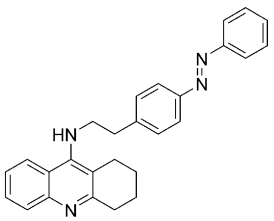
**15**

The inhibitor itself shows nanomolar inhibition of AChE (Figure 10a). Only a minor difference in inhibitory activity between the ring-opened and ring-closed forms was observed. However, the modes of inhibition (observable by different Hill slopes) seem markedly different (Figure 10a). Importantly, the ring-opened form of **15** is a strong inhibitor of β-amyloid aggregation,[138,139] whereas the ring-closed form shows much lower levels of inhibition.

Recently, another tacrine-based inhibitor **16** was reported with a photoswitchable azobenzene unit.[27] Compound **16** is active in its *cis* state and shows AChE inhibition in enzymatic assays and in mouse trachea (tracheal tensometry assay).[141]

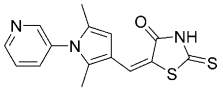


tacrine (THA)



**16**

TrpA1[142,143] is expressed in sensory neurons and its pharmacology promises treatments in nociception, spinal trauma therapy, and chronic inflammation (Figure 11). Peterson and co-workers[144] have taken a rather nonclassical approach to photopharmacology: Instead of a rational photoswitch design based on defined structure–activity studies and structural data,[7,8] the basis for this work was a behavioral screen of 10000 compounds to test for the photoresponsiveness of zebrafish embryos (*Danio rerio*). A photoactive rhodanine-based compound **17** was identified from this screen. The activation of TrpA1 channels was achieved in HEK293T cells expressing hTrpA1



**17**

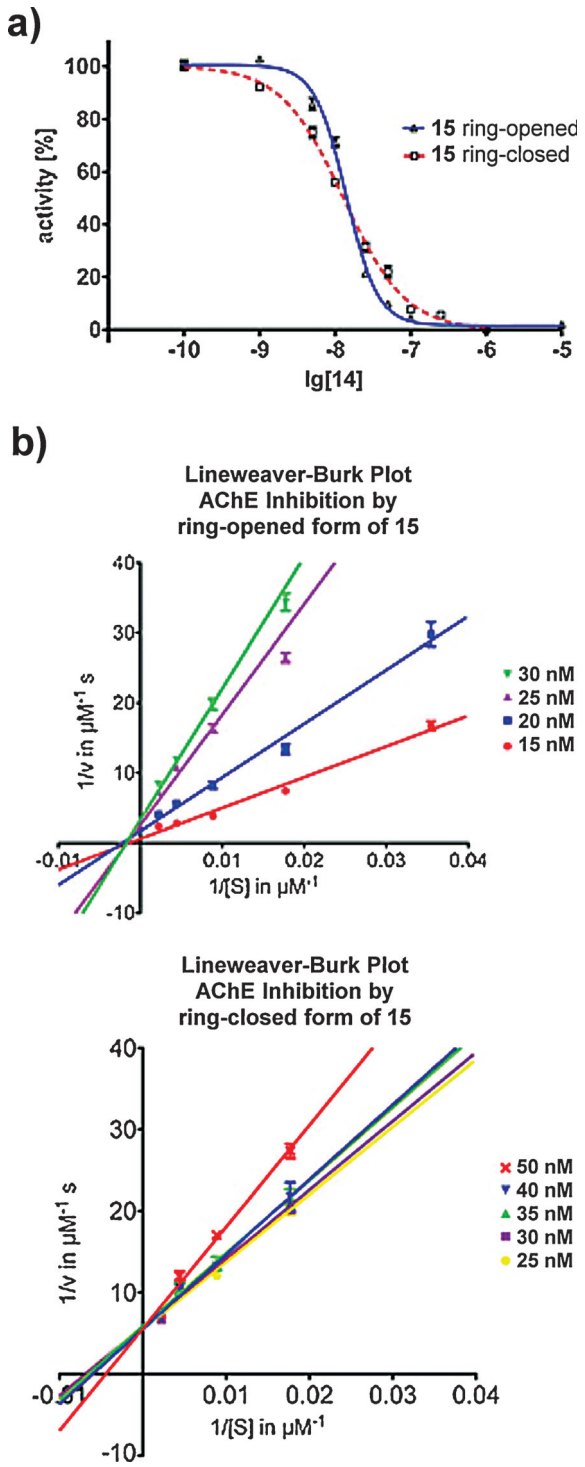



***Figure 10.*** Photoswitching the biological activity of AChE inhibitors: a) Dose–response curve for **15** on electric eel AChE for both photoisomers (ring-opened, blue solid line; ring-closed, red dashed line). Different Hill slopes are clearly visible (ring opened: $n_H = 2.1$; ring closed: $n_H = 1.0$), whereas the $IC_{50}$ value remains about the same. b) The ring-opened form acts as a noncompetitive inhibitor and the ring-closed form as a competitive inhibitor of AChE, as is apparent in Lineweaver–Burk plots.[137] Adapted from Ref. [137] with permission, Copyright 2014 American Chemical Society.

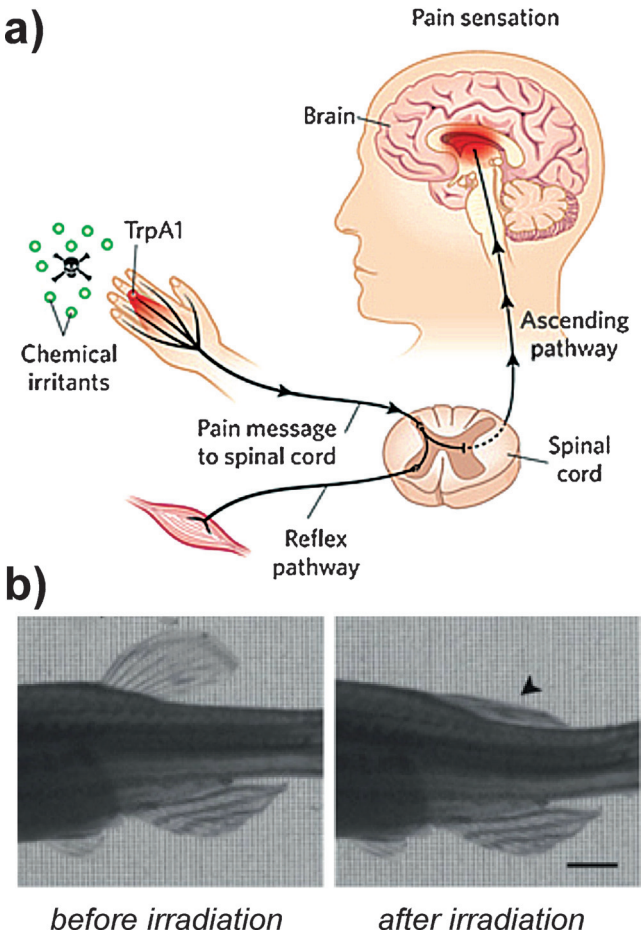



***Figure 11.*** Photocontrolled activation of TrpA1: a) Schematic representation of TrpA1 activation through noxious stimuli followed by signal transduction to the spinal cord (motor reflexes) and brain (pain perception).[145] Reproduced from Ref. [145] with permission, 2013 Macmillan Publishers Limited. b) Effect of the irradiation of spinalized zebrafish (treated with **17**) on motor excitation: Photostimulation of the dorsal fin (scale bar = 2.5 mm).[144] Adapted from Ref. [144] with permission, 2013 Macmillan Publishers Limited.

(the human variant) and in mouse wild-type sensory neurons of dorsal root ganglia (DRG).

Illumination of animals with a severed spinal cord leads to muscle contractions in paralyzed body parts (Figure 11 b). Zebrafish treated with **17** were stimulated for dorsal fin contraction by laser irradiation at a wavelength of 405 nm. Localized stimulation of body parts resulted in their respective movements, results that were explained by activation of spinal reflex arcs.

Despite the potential promiscuity of the rhodanine substructure, the experimental results reported are interesting and showcase that phenotype- or behavior-based chemical screening could also work for photopharmacological applications.

Finally, it has to be emphasized that the research groups of Driessen, Feringa, König, Trauner, Isacoff, and Kramer have recently conducted seminal work on the photopharmacology of membrane channels and receptors.[8,75,78,131] Giving a com-

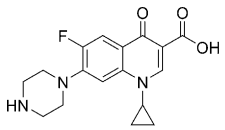
prehensive overview is beyond the aim of this Review and the reader is referred to recent reviews on the topic.[8,76,80,131]

In conclusion, despite the inaccessibility of the brain to simple irradiation with light, photopharmacology of the brain has undergone tremendous progress. This is probably due to the spectacular results obtained with optogenetics,[73] and these approaches might be translated to photopharmacology with small responsive molecules. The case of optogenetics shows elegantly that different fields can influence each other in a beneficial way. Optogenetics has provided methods for irradiating the brain, whereas photopharmacology has provided tools to render neurons photoresponsive, without the need to rely on genetic engineering.

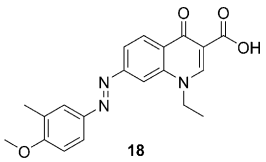
## 9. Other Clinical Applications

This class consists of unclassifiable targets in terms of organs or organ structures. It comprises methods to fight pain,[146] infections, inflammations, and other neoplastic, metabolic, and neurological disorders. Infections are frequently treated by systemic application of antibiotics. However, the antibiotics build-up in the body and affect not only the pathogen of interest but almost all other bacteria present in the body (such as the microbiome in the gastrointestinal and respiratory tract). Moreover, the release of antibiotics in the environment by large-scale use of antibiotics in society (i.e. food industry, hospitals)[147,148] is a significant threat to humanity because of the rapid build-up of multiresistant bacteria. Therefore, activation at the site of infection and subsequent deactivation after effective use is even more important than spatial activation.

Our research group focused on the synthesis and biological evaluation of photoswitchable antibiotics.[29] Ciprofloxacin is an intensively used antibiotic for the treatment of bacterial infections ranging from urinary tract to the meninges.[149] Its antibacterial activity stems from binding to DNA gyrase and blockage of DNA replication.[150]




ciprofloxacin




18

Replacement of the piperazine moiety in ciprofloxacin by an azobenzene photoswitch led to the selective turning "on" and "off" of the activity of compound **18** upon irradiation with UV light (Figure 12a). The compound showed activity against both *Escherichia coli* and *Micrococcus luteus*. Upon irradiation with light of $\lambda = 365$ nm, the activity of **18** was increased up to eightfold (*M. luteus*). Here, in contrast to the applications described above, the use of UV light is not a problem, since the irradiation can be performed outside the body before administration. Thermal deactivation (Figure 12b) prevents the accumulation of active antibiotics in the environment. However, to obtain better spatial control in the human body for the local treatment of infections and to prevent in vivo build-up of resistance, a red-light-switchable antibiotic would be highly desirable.

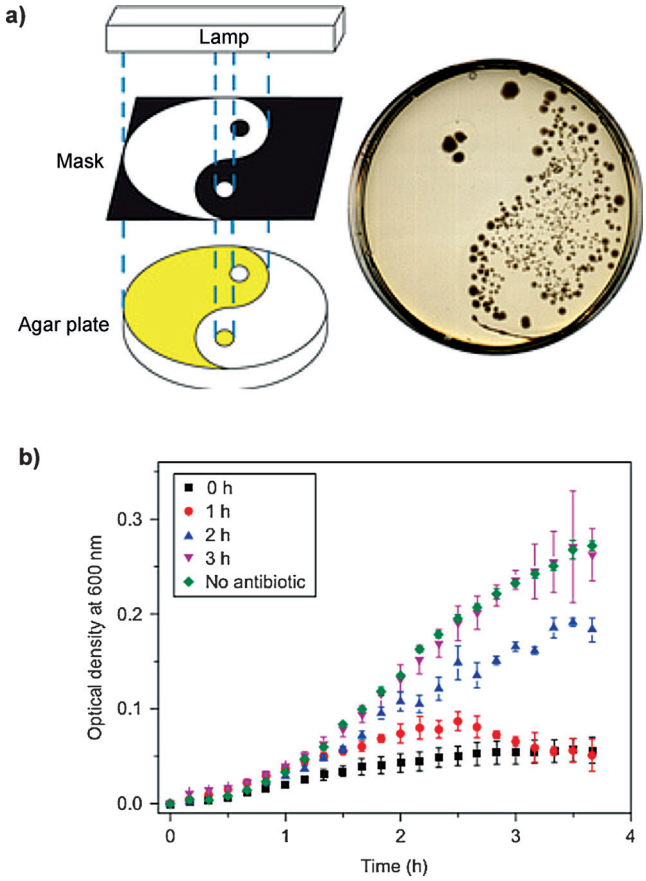



***Figure 12.*** Optical control of antibacterial activity. a) Bacterial patterning showing the light-activated cytotoxicity to *E. coli*. Only the covered areas, not exposed to light, show bacterial growth. b) Autoinactivation of photoswitchable antibiotic **18.** Thermal *cis–trans* relaxation turns off the antibacterial activity after 2–3 h. Reproduced from Ref. [29] with permission 2013, Macmillan Publishers Limited.

A more recent approach[151] relied on modifying the widely used antibiotic Gramicidin S by incorporation of a diarylethene photoswitch. Upon irradiation with UV light, which results in electrocyclization of the diarylethene units, a dramatic decrease was observed in the antimicrobial activity of **19** (16 times) on *Staphylococcus aureus*. The decrease in activity was attributed to an overall decrease in the amphiphilicity of this membranolytic molecule. Upon closure of the diarylethene unit, the cyclic ring structure of the Gramicidin S analogue became sterically confined and therefore less able to bind to and adapt itself to the amphiphilic interface of the bacterial membrane target (Figure 13).[*]

Compound **19** can be activated using visible light ($\lambda =$ 30 nm), with important implications for deeper-tissue penetration. However, to take advantage of the reversibility of the

[*] Please note: Changes have been made to Paragraphs 4 and 5 of Section 9 of this manuscript since its publication in *Angewandte Chemie* Early View. The Editor.

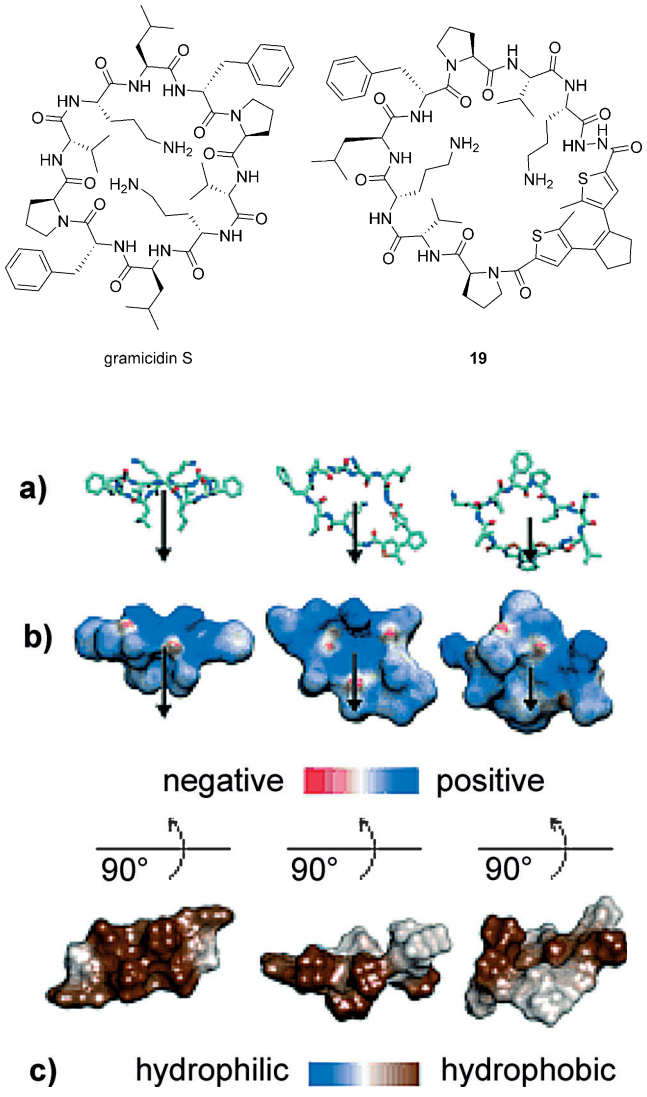
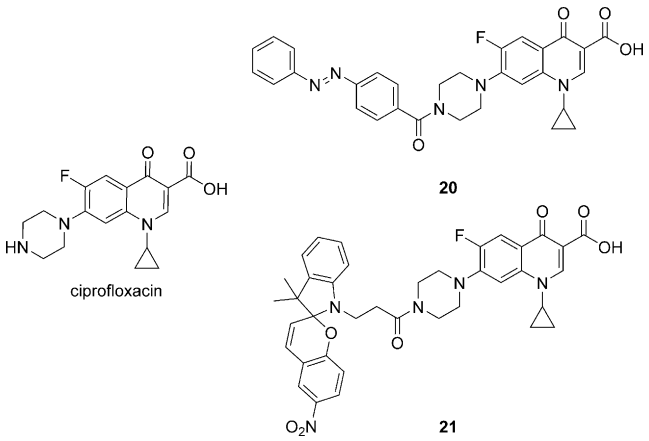



***Figure 13.*** MD simulations of Gramidicin S (left) and its photoswitchable analogues **19** in the "open" (center) and "closed" (right) forms. a) Side views of the hydrophobic moment vectors, and the relative length of the vectors (arrows). b) The solvent-accessible surfaces of the molecules, showing a significant difference between the open and closed form. c) Kyte–Doolittle hydrophobicities, showing a large change in the hydrophobicity upon photocyclization. Adapted from Ref. [151] with permission. Copyright 1999–2016 John Wiley & Sons.

switching process and to be able to inactivate the drug outside its site of action, a prolonged (> 20 min) irradiation with highly toxic light with a wavelength of 256 nm was needed, thus presenting a possible drawback of the current design.

As an extension of earlier reports from our group, we developed a method to incorporate photoswitches into bioactive molecules in a single step.[152] By using this approach, an existing drug has been derivatized with either an azobenzene or a spiropyran. Starting from ciprofloxacin, the piperazine group was modified by coupling with a photoswitch in a single step. A difference in activity was observed with **21** on *Escherichia coli* and with **20** on *Micrococcus luteus*. Moreover, a 50-fold increase in activity was observed for **20** compared to the original ciprofloxacin. Despite the small

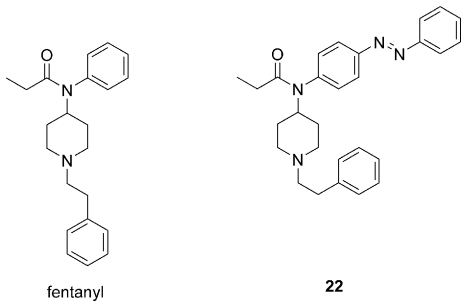



change in activity upon isomerization and the need for UV light, this approach showcased a simple strategy to render bioactive molecules photoswitchable.

Pain perception is a central topic in medicine.[153,154] Photopharmacology in anesthetics brings the benefit of high spatiotemporal control over light delivery to ensure highly localized action. Anesthetics are often nonselective and have long residence time, often triggering addictive behavior.[155] Photopharmacology could prevent such side effects as, even when used in high doses, the compound would be activated only locally.

Fentanyl is a synthetic opioid widely used in transdermal patches.[156,157] It is a potent agonist of the μ-opioid receptor (MOR)[158] and belongs to the family of G-protein-coupled receptors (GPCRs), which are distributed mainly in the digestive tract, the brain, and the spinal cord.[159–161]

The groups of Gorostiza and Trauner pioneered in 2014 the general concept of rendering GPCRs photoswitchable.[159,162] Compound **22**, which is based on fentanyl, was developed. *trans*-**22** shows MOR channel blocking activity in




electrophysiological measurements on HEK293t cells transfected with human MOR and GIRK1&2 (Figure 14). GIRK1&2 are potassium channels that respond to MORs and are required for electrophysiological read-out.[163] Irradiation at a wavelength of 420–480 nm thus leads to MOR-mediated $K^+$ influx through the GIRK channels. The *cis*-**22** form was inactive, which would limit its applicability because it can only be selectively turned "off" by direct irradiation.

This concept was then extended to two other photoswitchable anesthetics.[164] In contrast to GPCRs, this class of

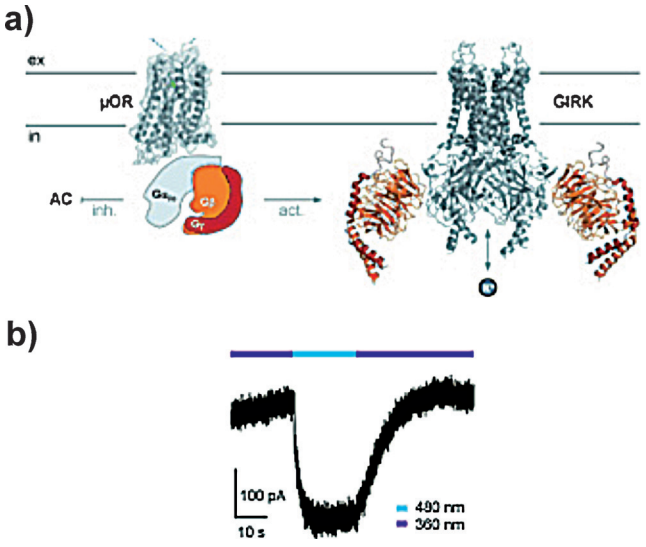
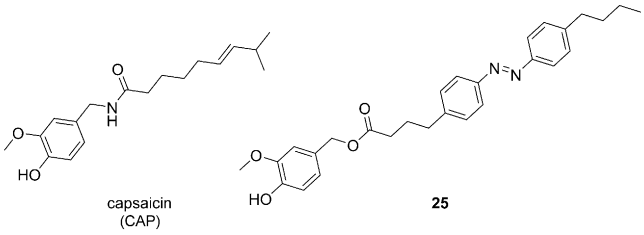



***Figure 14.*** Photoswitchable MOR agonist: a) Principle of the experimental system: Activation of MORs leads to indirect GIRK1&2 activation, thereby resulting in a measurable $K^+$ influx. b) **22** (25 mM) triggers inward $K^+$ currents through MOR activation when switched to its *trans* isoform at $\lambda = 480$ nm.[159] Adapted from Ref. [159] with permission, Copyright 1999–2016 John Wiley & Sons, Inc.



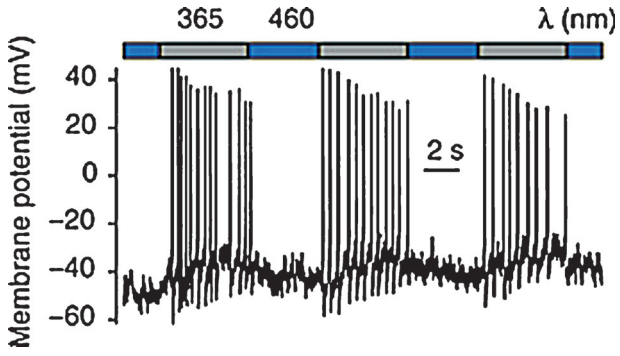
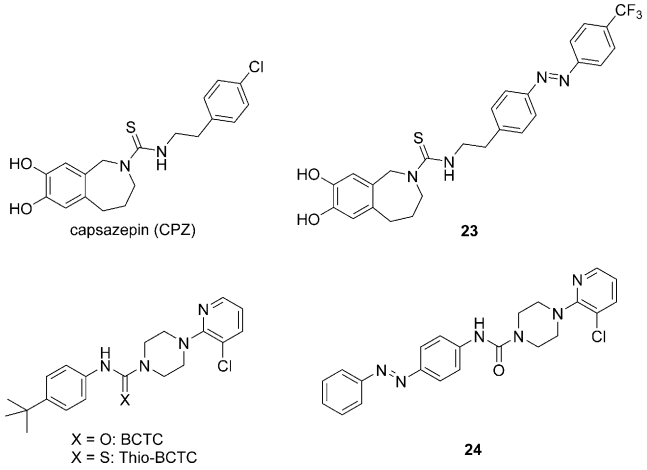




***Figure 15.*** Photoswitching the electrophysiological activity of wt-DRG neurons: Application of **25** (200 nM) to wt-DRG neurons elicited action potentials when the membrane was clamped at 0 pA.[173] Adapted from Ref. [173] with permission, 2015 Macmillan Publishers Limited.

drugs acts on the vanilloid receptor 1 (TrpV1). This channel receptor reacts to a multitude of noxious signals[165,166] and allows (upon activation) the passage of $Ca^{2+}$ ions, as well as other cations ($Na^+$ or $K^+$).[167] An often used agonist of TrpV1 is vanilloid capsaicin (CAP).[168] Two potent antagonists of TrpV1 action are capsazepin (CPZ)[169,170] and thio-BCTC.[171,172] Their photoswitchable derivatives **23** and **24** were synthesized and tested. Compound **23** shows modality-

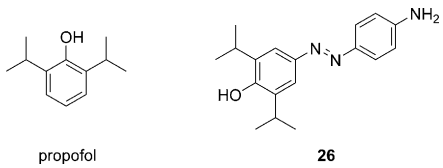



selective antagonism in TrpV1-transfected HEK cells: *cis*-**23** inhibits CAP-induced TrpV1 currents, while *trans*-**23** inhibits TrpV1 upon voltage gating. The BCTC-based drug **24** is an antagonist for voltage-activated TrpV1 currents, but shows no modality selectivity.

To complete the picture, a photoswitchable agonist of TrpV1 was developed. Compound **25**, an "azologue" of CAP, activates pain sensation by making use of photoswitchable fatty acids (Figure 15).[173] *cis*-**25** activates TrpV1 signaling. Some background activity, however, was also observed with the *trans* isomer. Reversible photocontrolled activation of TrpV1 receptors was achieved in HEK293T cells (expressing TrpV1-YVP) and C-fiber nociceptors.

Compound **25** was selective for TrpV1-expressing neurons in wild-type mouse dorsal root ganglion neurons. Moreover, inflammatory-related sensitization of TrpV1 (TrpV1-mediated hyperalgesia) was also observed for **25**.

A third target for pain perception and anesthetics are $GABA_A$ receptors. These receptors have inhibitory effects on the postsynaptic neuron, eliciting a chloride influx and leading to its hyperpolarization.[174] The anesthetic propofol[175] is postulated to act as an allosteric potentiator of $GABA_A$ receptors, among others. It is widely used as a rapid intravenous anesthetic. A propofol-based photoswitchable drug **26** was reported.[176] *trans*-**26** showed potentiating effects

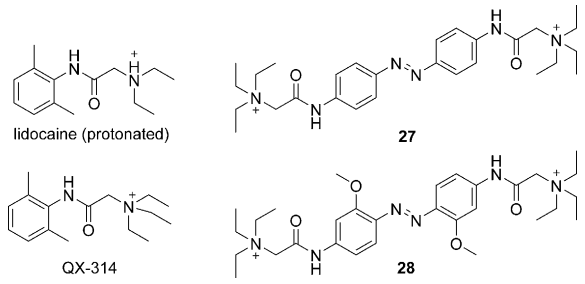



on $\alpha_1\beta_2\gamma_2$-$GABA_A$ receptors expressed both in *Xenopus* oocytes and HEK cells, whereas *cis*-**26** was inactive.[176] Compound **26** shows a fast photoresponse, and the *cis* isomer has a short thermal half-life (seconds in aqueous buffer). The anesthetic effect of **26** was demonstrated in

in vivo experiments with albino *Xenopus laevis* tadpoles, which showed a loss of righting reflexes.[177]

Voltage-gated sodium and potassium channels respond to changes in the polarization across the lipid bilayer and are crucial for triggering and relaying action potentials.[61] They can be inhibited by channel blockers based on the quaternary ammonium structural motif (such as protonated lidocaine,[155] and QX-314).[178] As quaternary ammonium based drugs




cannot cross the lipid bilayer, they have to enter the cell by import channels (e.g. TrpV1)[178] and then block channels intracellularly. Such import channels get activated by noxious stimuli (see above), and prolonged activation results in them opening up for larger ions.[61,155,179,180] Based on this mechanism of action, a photoswitchable pain-selective anesthetic was developed by Trauner, Kramer, and co-workers.[155] *trans*-**27** blocks $K_v$, $Na_v$, and voltage-gated $Ca^{2+}$ channels in several cell lines, when applied through a patch-clamp electrode in the neuron (Figure 16). *cis*-**27** is inactive; thus, *trans-cis* isomerization results in unblocking of the pore and regenerates the neuronal excitability. HEK293 cells coexpressing the Shaker $K^+$ channel and TrpV1 underwent CAP-induced uptake of **27**. Such uptake could be prevented by TrpV1 blockers (here: BCTC). This photoinhibition of uptake could be used to establish anesthetic action in vivo in mice: The nocifensive blinking response (Frey hair test)[181] of a mouse model was significantly reduced when **27** and CAP were coapplied to the cornea (Figure 16). Photoswitching to the inactive *cis*-**27** resulted in rescue/regain of the blinking response.

UV light is required to achieve photoswitching of **27**, which is a disadvantage for photopharmacological applications. Thus, a red-shifted variant **28**[182] with slightly lower activity was developed. The presence of the methoxy groups in the 2,2′-positions enables it to be switched with blue light of $\lambda = 420$ nm (less harmful, but still not medically relevant). Shaker-IR ($K_v$) can be reversibly blocked in HEC293 cells and $Na_v$ in mouse neuroblastoma cell (NG108-15 cells). The same is true for $Na_v$ and $K_v$ channels in brain slices of mouse cortical pyramidal neurons. It should be noted that *trans*-**28** is the active form in all these cases.

In conclusion, remarkable examples of reversible control of nociception by light have been reported in recent years. Photoswitchable anesthetics and analgesics offer an exciting opportunity for photopharmacology, with many interesting possible applications.

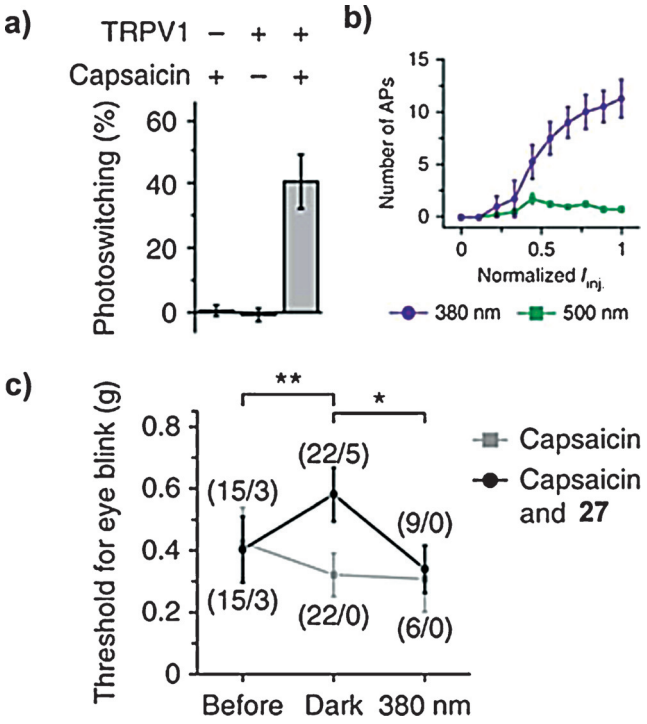



***Figure 16.*** Channel blockers based on a quaternary ammonium structure: a) Requirement of TrpV1 activation to allow photoswitching of $K^+$ currents through blocking of Shaker $K^+$ channels in cells with or without TrpV1 upon application of **25** (1 mM) and capsaicin (1 μM). b) Difference in action potential elicitation by incremental injections under irradiation at either $\lambda = 380$ or 500 nm in dissociated rat hippocampal neurons with 100 μM **27**: Quantification of the number of triggered action potentials. c) Results of the Frey hair test on the nocifensive blinking response of rats when pressure is applied to the cornea in the presence and absence of **27**. The eye was treated with capsaicin (10 μM) and **27** (20 mM) or capsaicin alone. The difference in the normalized blink threshold was about fivefold, an effect that could be reversed by irradiation with UV light ($\lambda = 380$ nm). The number of rats and the number of rats that did not respond to the maximum force applied (1 g) are indicated in parentheses.[155] Adapted from Ref. [155] with permission, 2012 Macmillan Publishers Limited.

## 10. Conclusion and Outlook

Photopharmacology has undergone rapid development in the past decade. Researchers from fields as diverse as chemistry, medicine, pharmacy, and molecular biology are realizing the clinical potential of this approach, and new and exciting applications are being reported. Here, we have given a critical overview of the different pharmacological targets (i.e. diseases/organs) in the human body, the prospects for delivering light to these organs, their specific requirements for the molecular design of a photoactivatable drug, and whether and how these challenges can be addressed. We believe that, at this early stage of development of the field, a perspective with a focus on medical applications is highly warranted to inspire efforts toward medically relevant targets. This Review is also meant as a starting point and stimulus for chemists to enter this exciting field, with opportunities to bring "smart" molecular design ultimately to the realm of clinical use. In our

opinion, the following future developments will be vital for a successful development of this rapidly expanding field:

1. Target evaluation: the photopharmacological approach should always take into consideration localized diseases with targets where the delivery of light is feasible. Reversible light activation is, therefore, not just a feature, but should bring additional value to a drug (i.e. reduce the build-up of environmental resistance, reduced side effects, increased control over activity, precise targeting, etc.).
2. Optical properties: a major goal for photopharmacology is to move the absorption bands of photoswitchable drugs into the optical window between $\lambda = 650$ nm and 900 nm (see below). The use of red-shifted azobenzene derivatives up to $\lambda = 450$ nm (n-$\pi^*$ transition) is simply not enough to bring the responsive drugs to the clinic.
3. Photoswitch stability and toxicity: the development of new photocontrolled drugs must be matched by continuous efforts in studying their cellular stability and toxicity. For azobenzenes, in particular, there is already a large body of literature[7] that describes the influence of structural features on their stability under reducing conditions, together with the toxicity of photoswitches and the products of their degradation. Similar extensive studies for other photoswitches are needed to establish the feasibility of their application in photopharmacology.
4. Light delivery: currently, an external delivery of light is envisioned in photopharmacological treatments. However, one should not exclude the possibility of using internal, exogenous light sources, such as luminescent compounds. This possibility would provide multiple benefits. Firstly, the problems of the penetration of light through the skin and tissue could be avoided, since light would be delivered directly at the side of action. Secondly, an additional level of selectivity could be attained if the luminescent source was specifically targeted to the disease. Finally, in this way, photopharmacology could be used in a theranostic approach, bringing together molecular imaging (diagnostics) and targeted drug activation (therapy). Alternatively, photochemical upconversion methods might offer attractive alternatives to apply near-IR irradiation that can penetrate deeper. For such purposes, one could envision combining photopharmacology with optical imaging, where luminescent compounds are used for the localization of the disease. Another possibility is to incorporate positron emission tomography (PET) radioactive tracers, by using Cerenkov photons for activation[183] of the photoresponsive drugs. Clearly, much research is needed to establish if the photon flux in these methods is efficient enough for photoswitching. However, initial research on the use of light emitted by luciferase for the photoswitching of spiropyran-based MRI contrast agents[184] supports the feasibility of using internal, exogenous light delivery systems for local drug activation.
5. Cross-activity: it is important to realize that the two isomers of the photoswitchable drugs are often not simply "on" and "off" forms, but they can have different biological activities towards completely different targets.[120,137] This selectivity towards different functions might under certain circumstances be desirable, but often can be problematic. Thus, researchers should bear this aspect in mind when transitioning to in vivo experiments.
6. Phenotypic screening: so far, photopharmacology has only made use of rational drug design, starting from a known drug or bioactive compound and based on structure–activity relationship studies. In contrast, phenotypic screening for photoswitchable drugs—as was outlined for the case of compound **17** (Figure 11)[144]—may give new insights into targets and methods in photopharmacology.
7. Synthetic methods and mechanistic studies: photopharmacology is dependent on the availability and synthetic accessibility of photoswitches. Thus, new developments of robust, rapidly synthesized chromophores are in high demand.
8. Rescue protocols: Thorn-Seshold and co-workers[21] have pioneered approaches where a certain area of the "on" irradiation pulses is spatially confined by an outer ring of "off"-switching pulses. It proved successful (no drug activity observed after switching "off") and might, thus, be used to protect certain tissues/areas. Requirements for such applications are, however, fast-responding photoswitches that switch on the millisecond scale with visible light at low doses.

The aim of this critical Review is to provide the field with an overview toward clinical possibilities in light-guided therapy. We hope that this Review will serve as an inspiration for realistic choices of molecular targets and will contribute to focused efforts in applying photopharmacology. This requires the combined expertise of several disciplines, but the remarkable progress made in the past few years alone, as discussed here, illustrates the potential to control medicinally relevant biological functions with light by using a photopharmacological approach. It is a long and winding road from the molecular design of bioactive compounds with intrinsic photoswitches to clinical application, but the ultimate reward will be innovative approaches to interfere with complex biological pathways and address life-threatening diseases.

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