On the promise of photopharmacology using photoswitches: a medicinal chemist's perspective

Matthew J. Fuchter,[1,†*]

[1] Department of Chemistry, Imperial College London, Molecular Sciences Research Hub, White City Campus, Wood Lane, London W12 0BZ, United Kingdom.

ABSTRACT

Photopharmacology is a growing area of endeavor that employs photoswitchable ligands to allow for light-dependent pharmacological activity. By coupling light to therapeutic action, improved spatial and temporal selectivity can be achieved and subsequently harnessed for new concepts in therapy. Tremendous progress has already been made, with photopharmacological agents now reported against a wide array of target classes, and light-dependent results demonstrated in a range of live cell and animal models. Several challenges remain however, especially in order for photopharmacology to truly impact the clinical management of disease. This perspective aims to summarize these challenges, particularly with attention to the medicinal chemistry that will be unavoidably required for the further translation of these agents/approaches. By clearly defining challenges for drug hunters, it is hoped that further research into the medicinal chemistry of

photopharmacological agents will be stimulated; ultimately enabling full realization of the huge potential for this exciting field.

Introduction

The coupling of light with therapeutic action has long been of interest to humanity, with ancient civilizations using phototherapeutic approaches against a range of afflictions.[1] While light therapy remains to be a useful clinical option for certain indications – the treatment of neonatal jaundice[2], for example – this perspective is concerned exclusively with the coupling of light action to the therapeutic efficacy of exogenous agents. What added value does a light addressable therapeutic agent offer over a 'conventional' drug? There are many potential answers to this question, which ultimately depend on the agent/approach in question (*vide infra*). One general principle is clear however, which is a result of the spatial selectivity offered by light: If a photoactive drug in a state with low intrinsic (dark) toxicity is administered to a patient, followed by spatially-precise irradiation to convert it into a state with high biological activity in a defined region, then the therapeutic index of the drug can be improved, in principle. Only the irradiated areas will be affected, even if the photoactive drug (in its dark state) is not localized solely in the region of interest. There are several challenges to overcome in the realization of this opportunity however, some of which will be described in this perspective.

The most developed approach that couples light to therapeutic action is photodynamic therapy (PDT, Figure 1A), where a photosensitising dye molecule is administered to patients, followed by

light dosing. Absorption of the light by the dye results in the formation of a dye excited state, which, in turn, results in the formation of a number of reactive intermediates, notable reactive oxygen species (ROS).[3] PDT is an approved therapeutic modality for a number of diseases, from cancer to dermatological and ophthalmic diseases,[4] and related processes are of interest for infectious diseases.[5] Thus, PDT has paved the way for the development of light-based therapeutics. However, the mechanisms of action associated with dye-based photosensitizers are not the only option for such therapeutics and there are other opportunities.

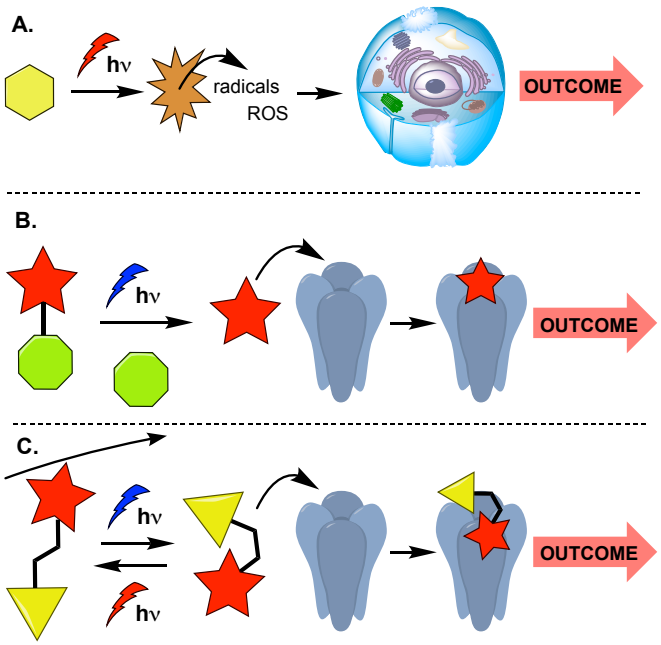


FIGURE 1. Light-dependent pharmacological approaches: (A) Photodynamic therapy (ROS = reactive oxygen species); (B) Irreversible photopharmacology (photodecaging); (C) Reversible photopharmacology with a ligand that is active only in one structural state.

Photopharmacology approaches beyond PDT represent one such opportunity. A photopharmacological agent generally refers to one where light irradiation results in a

photochemical event that changes the molecule's pharmacological activity. 'Photopharmacology' has become a catch-all term for a number of approaches where the photochemistry and pharmacology of biologically active molecules are linked. While other terms, such as optopharmacology and optochemical genetics have also been used, photopharmacology has become the dominant nomenclature and will be used exclusively in this perspective. The photochemistry of photopharmacological agents can be irreversible or reversible, dependent on the molecule in question. The most well-known example of the former is the process of photodecaging (Figure 1B). In such an approach, a photoactive protecting group (a 'cage') is added to a biological ligand such that it masks key pharmacophoric features of the ligand and thereby deactivates its target-based activity. Irradiation results in photochemical cleavage of the group and release of the active biological agent. While a powerful approach in chemical biology, akin to a photoactive prodrug, irreversible photopharmacological agents will not be discussed in this perspective. Interested readers are directed to other comprehensive reviews on the topic.[6]

Reversible photopharmacological agents operate via the incorporation of a molecular photoswitch into their structure (Figure 1C). Such 'photoswitches' are components that can be interconverted into two different structural states, using light as the stimulus to drive at least one of the switching events. By coupling a photoswitch component to a biologically active ligand, light can be used to 'switch' the ligand between two the two states. Assuming the two different structural states have dramatically different binding affinity to the target, one can use light to switch target-based activity 'on' and 'off', in principle. While a variety, and emerging number of new, photoswitches are known,[7] the vast majority of reversible photopharmacology approaches use azo aromatics, such as the prototypical azobenzene (**1**, Figure 2A). For that reason, this perspective will focus exclusively on the prospect of azo arenes for further development in

photopharmacology. There are a number of ways azo arene photoswitches can be incorporated into biological systems for light-dependent investigation. These include approaches for tethering to, or cross-linking with, biomolecules, which again are reviewed elsewhere.[8a] This perspective will focus on non-tethered, freely diffusing photopharmacological agents; arguably the most relevant for translation of photopharmacology into an approach to treat patients in the clinic.

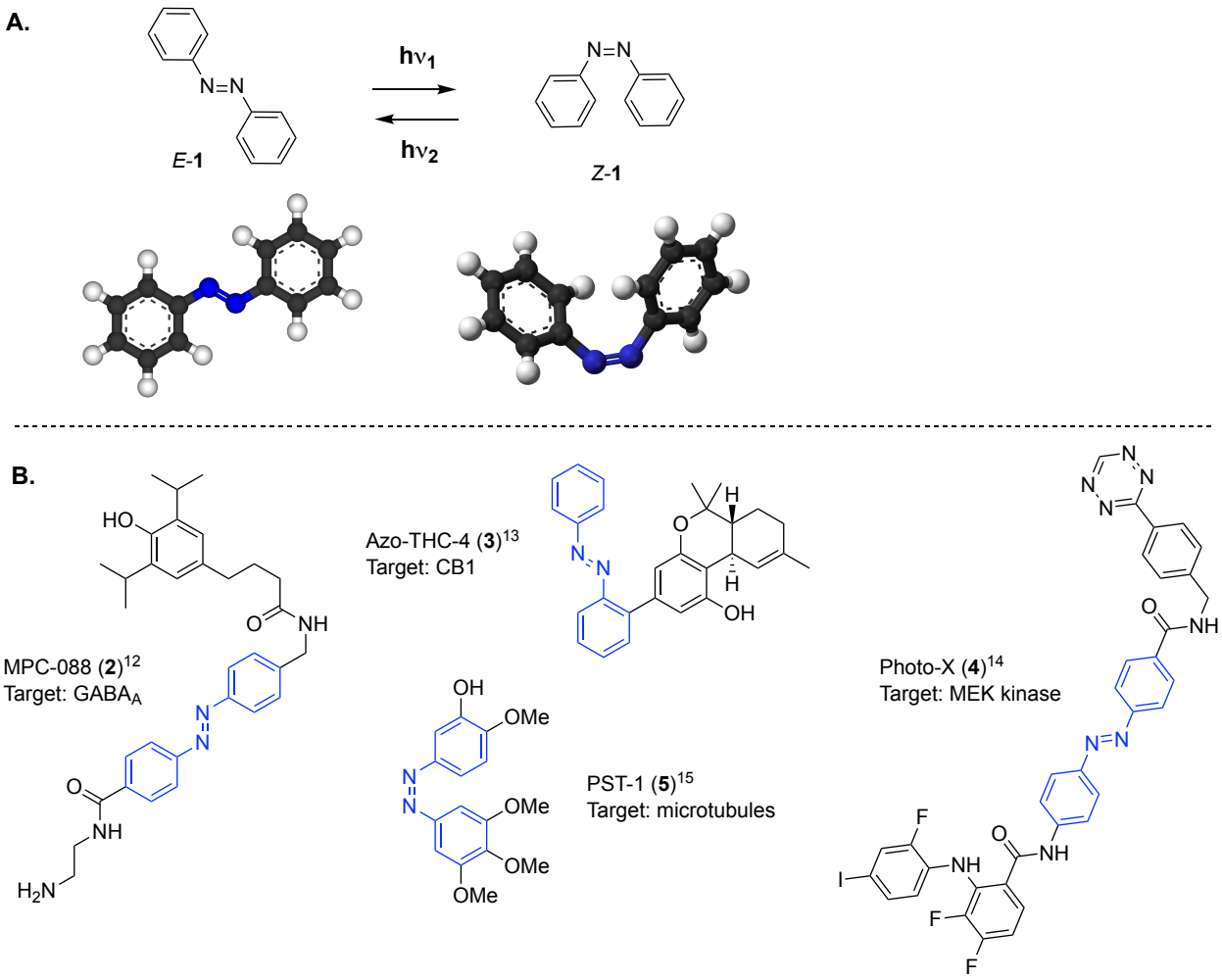


FIGURE 2. (A) Photoisomerization of azobenzene (**1**); (B) Representative azobenzene-derived photopharmacology agents. The azobenzene photoswitch is colored blue.

The ability to use light to interconvert azobenzene between two isomeric configurations was discovered in the 1930s.[9] Azobenzene has since become one of the most heavily used photoswitches in a large range of light-addressable applications, and especially in photopharmacology. There is a large change in the end-to-end distance between the *E* and *Z* isomers (Figure 2A), which drives the large structural change of importance to many applications, including photopharmacology. Furthermore, azobenzene is a very efficient photoswitch, exhibiting high extinction coefficients and quantum yields – thereby allowing photoswitching with low-intensity light – and is stable to repeated switching over many cycles. In order to achieve a high degree of switching between the *E* and *Z* isomeric forms, it is important to identify a wavelength where the target isomer absorbs light, but the product isomer does not. Most commonly, UV light is used to switch from *E* to *Z* and visible light to switch from *Z* to *E*, due to the position of the λ_{max} absorptions for the respective azo aromatic isomers. UV light is not without its limitations however, including the potential for (non-specific) phototoxicity and limited tissue penetration. While there are a number of innovations emerging with respect to photoswitch design that allow for red-light switching[10] (i.e. switching using a more penetrant and less toxic wavelength), these new designs are not yet routinely employed in photopharmacology studies. Nonetheless, the need to develop high performance switches that can be addressed with red-light is a recognised photoswitch pharmacology challenege[10b] and so won't be further discussed within this perspective. Finally, it is important to highlight that the *Z* isomer of azo aromatics is metastable and will fully convert back to the *E* isomer over time *via* a thermal (i.e. non-photochemical) mechanism. The rate at which this thermal *Z*-*E* reversion occurs very much depends on the structure of the azo aromatic, being very fast (ps) in some cases and very slow (years) in others.

By incorporating an azobenzene into a variety of ligands, a large amount of tremendously exciting data has been generated; using light to control biological function *in vitro*, in cells and in live animals. While a large number of the known photopharmacological ligands are targeted towards ion channels, an expanding range of biological targets have now been successfully addressed. These also include transporters and pumps, GPCRs, enzymes, and elements of the cytoskeleton. Some representative ligands are included for interest in Figure 2B; but readers are directed to a number of excellent and comprehensive reviews that cover the state of the art with respect to known targets for photopharmacology.[11]

Despite the huge promise for photopharmacology to unpick target-based biology – thereby providing a significant opportunity for target validation – and to potentially deliver novel light-based modalities for clinical usage, there remain a number of challenges in the field. This perspective aims to summarize these challenges, from the perspective of the author, and particularly with attention to the medicinal chemistry that will be unavoidably required for the further translation of these agents into the clinic. By clearly defining the challenges, it is the hope of the author that further research into the medicinal chemistry of photopharmacological agents will be stimulated; ultimately enabling full realization of the huge potential for this exciting field.

The identification of robust hits for photopharmacology projects

To date, the predominate way to identify photopharmacological ligands is to take an established ligand for a desired biological target and then to synthetically modify it, incorporating an

azobenzene (arene) photoswitch. This can be done by identifying appropriate modification sites on the starting ligand that are amenable to functionalization with an azo switch; either through structural biological insight or through simple structure-activity relationship (SAR) analysis. Additionally/alternatively, one can identify a structural feature of the parent ligand that should be amenable to replacement by an isosteric azobenzene; a process termed azologization.[16] The success of these approaches depends on several factors, including the tolerance of the parent ligand to modification/alteration in this fashion and the differential target-based activity of the resultant *E* and *Z* azo arene ligand isomers. When considering differential activity, the *absolute* selectivity between *E* and *Z* isomers is not the only factor at play however; the completeness of isomer photoswitching can also be key. Despite the excellent photochemical performance of azobenzene and its derivatives, the *E*/*Z* isomeric ratio at the optimum photostationary state (PSS) is often lower than desired, for example 80:20. Assuming that light has been used to switch the biological activity of the ligand 'on', this means that only 80% of the active isomer is produced and 20% of the inactive remains (or *visa versa* in the case of switching 'off'). For some targets, given the non-linear nature of biological events and downstream signal amplification, such PSS mixtures are not necessarily a problem and allow dramatic photoswitchable outcomes, even in live animals.[8] However, incomplete switching is a problem for other targets, especially enzymes.[17] Moreover, incomplete switching may 'muddy the water' for interpreting biological data and translating such agents towards the clinic, given the mix of on- and off-target effects for both isomers at different concentrations (*vide infra*).

Clearly, complete photoswitching (ideally in both directions) would give rise to a more precise chemical tool for target validation and potentially to a more attractive photopharmacological agent for further development; for example, allowing reduction of the dose required in order to be able

to switch 'on' activity. It is beyond the scope of this review to discuss how to achieve complete photoswitching through state-of-the-art azo photoswitch design, however, *E* versus *Z* absorption band separation is key. It is the author's opinion that heteroaromatic azo arene photoswitches,[18] which are significantly understudied compared to their azobenzene counterparts, hold much promise in photopharmacology for a number of reasons (*vide infra*). One of those is band separation. For example, the arylazopyrazoles (Figure 3), developed by the author's laboratory, show excellent band separation and therefore near complete photoswitching in both directions.[19] As such, these next generation switches are starting to appear in a number of photopharmacological agents, examples of which are shown in Figure 3.

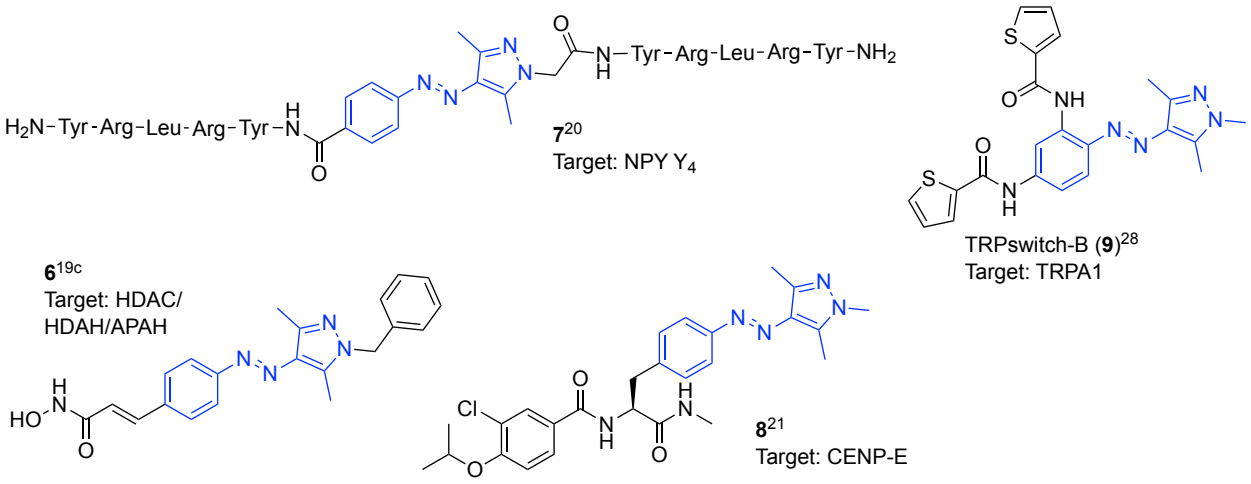


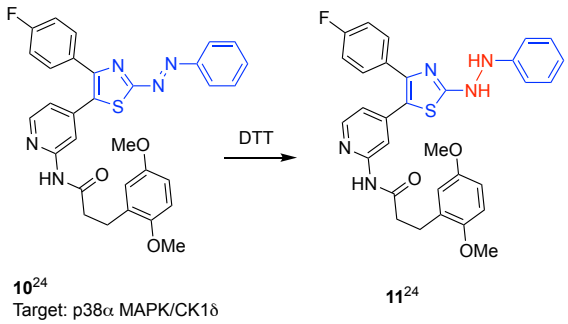
FIGURE 3. Representative photopharmacological agents using arylazopyrazoles. The arylazopyrazole photoswitch is colored blue.

Adapting a known ligand to make it photoswitchable, especially through azologization, is analogous to scaffold hopping in drug discovery.[22] This, obviously, requires a useful ligand for a target of interest as a starting point, and one that is amenable to modification. While this technique

has led to huge advances,[8,11] it is not possible to use azologization for the development of photoswitchable ligands against less-well studied targets (i.e. targets without existing ligands). Furthermore, it is not currently clear whether adapting already known ligands will lead to 'best in class' photopharmacological agents. For example, appending an azobenzene to the periphery of an established ligand may give modest differential activity between *E*/*Z* isomers; but how would that compare to a structurally novel ligand where the azo arene group is incorporated in a more determinant position?

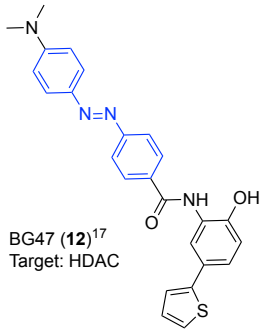
To again draw analogies with current target-based drug discovery, screening may be an alternative option to scaffold hopping. By screening libraries of photoswitchable scaffolds, it may be possible to identify totally new photoswitchable 'hit' ligands for a given target, which can be subsequently optimized and developed. There is an issue with this approach however, that derives from the reliance on azo arenes as photoswitches in photopharmacology. There is now significant knowledge and equally significant debate regarding so-called pan-assay interference compounds (PAINS).[23] PAINS are compounds that, for a variety of reasons, are 'frequent hitters' (and often false positives) against a variety of targets, using a range of assay formats. One chemotype known to have PAINS-type activity is azobenzene.[23b] There are several reasons for this assignment, including the reactivity of azobenzene and the ability for azobenzene to act as a dye. Dye molecules with strong absorbance and/or fluorescence can interfere with the assay readouts of many commonly used target-based assays, giving rise to false positive effects.[23] Reactive molecules, on the other hand, can be false positives due to non-specific (reactive) effects on the target/assay, or by acting as false hits/"bad actors" due to reactivity-derived apparent target affinity; ultimately proving to be non-developable.[23a] A useful case in point was recently reported by Herges and co-workers while attempting to develop new azo photoswitchable kinase inhibitors targeting p38α

MAPK and CK1δ.[24] With significant follow-up, including protein-ligand crystallization, inconsistent kinase activity data between the *E*/*Z* isomers of a 2-azo-thiazole inhibitor (**10**, Figure 4) was found to result from reduction of the azo group (to give a hydrazine, **11**) under the assay conditions. Dithiothreitol (DTT) and/or glutathione (GSH) – common protein stabilization additives for biological assays – were responsible for the reduction of **10** and the resultant hydrazine was more potent than either of the *E* or *Z* isomeric azo forms.



10[24]
Target: p38α MAPK/CK1δ

11[24]



BG47 (**12**)[17]
Target: HDAC

FIGURE 4. Photopharmacological agents reported by Herges and co-workers[24] and Haggarty, Mazitschek and co-workers[17]. Key photoswitch component colored in blue. Hydrazine reduction product highlighted in red.

While these challenges for screening mean that caution must be taken, clearly the issues are not insurmountable. For example, by ensuring that reductive additives are not used, or only used at

low concentrations (or only using less reducible azos, *vide infra*) and by using assay readout technologies less prone to interference it should be possible to robustly assay azo compounds. It is the author's view that the development of robust target-based assays (biochemical and biophysical) that are compatible with diverse azo structures would be highly useful and enabling to photopharmacology. Not only would such assays allow for azo library screening in the hit discovery phase of a photopharmacology project, but they would also allow for robust and quantitative protein-ligand data to be generated. There are many questions in the photopharmacology field surrounding target-ligand interactions that are currently poorly addressed; but could be with appropriate protein targets and assays. For example, it is mostly assumed that ligand photoswitching occurs while the ligand is not bound to its target.[11a] For conformationally dynamic proteins however, it may be possible for ligand photoswitching to occur while bound. This is akin to what is known about azobenzene switching in condensed phases: photoswitching is hindered in rigid and close packed solid-state environments, but possible in more conformationally dynamic systems.[25] Furthermore, by understanding more precisely how protein structure and ligand binding kinetics influence the outcome when using photoswitchable designs, it should be possible to better define design strategies to maximize the potential of certain photopharmacological targets. In this regard, protein conformation again provides a nice example. In the same study by Herges and co-workers,[24] they suggest that "conformational adaption of the protein could also be responsible for an induced-fit type of binding levelling any designed *E*/*Z* affinity differences". This would clearly be an example of protein conformation working against the development of highly *E*/*Z* selective photopharmacological agents. On the other hand, protein conformation could assist in the development of a photopharmacological approach, as has been reported for histone deacetylase (HDAC) targets. In contrast to the photoswitchable hydroxamic

acid HDAC inhibitors developed independently by Feringa, Szymanski and co-workers[26] and Fuchter and co-workers,[19c] Haggarty, Mazitschek and co-workers employed *ortho*-amino or *ortho*-hydroxyl anilide HDAC binders.[17] Such *ortho*-amino/hydroxyl anilide HDAC ligands are well-known from HDAC medicinal chemistry studies to exhibit long HDAC residence times, due to conformational changes in the HDAC protein upon ligand binding.[27] Haggarty, Mazitschek and co-workers hypothesized that combining such long residence times with a rapid isomerising *Z*-active photoswitchable inhibitor would be advantageous. Specifically, such an approach would allow the *Z* isomer ligand to be stabilized and remain bound after light exposure, while the unbound molecules rapidly revert to the ground state. Thus, it would allow one to rely on the quantitative *Z*-*E* thermal conversion (rather than incomplete photoswitching, *vide supra*) to inactivate the inhibitor and to obtain excellent spatial precision in the light-dependent effects. This hypothesis was borne out in the data: a representative compound from their study BG47 (**12**, Figure 4), while exhibiting a *Z*-isomer thermal half-life of 56 μs, had good inhibitory potency in its *Z* isomer (for example IC_{50} against HDAC2 <100 nM), but was inactive in its *E* isomer at the concentration range tested (>100 fold selectivity). By studying the enzyme activity following light exposure, the authors were able to demonstrate that their active inhibitors dissociated following kinetics governed by the dissociation rate constant (k_{off}), rather than the intrinsic photoswitch thermal half-life, with HDAC activity only recovering after many hours. These *in vitro* findings could be demonstrated to also hold in a cellular context, allowing for optical control of gene expression without the need for genome engineering.

As a final comment on screening, assay artefact effects (such as those that arise from PAINS) can be result of the artificial nature of simplified biological assays. One option to avoid such issues would be to conduct phenotypic screening in cells, advanced cellular models, or animals. One such

study was recently undertaken by Chaudhuri, Fuchter and Peterson.[28] A live zebrafish behavior-based screening strategy was used to develop both azobenzene and azopyrazole photoswitchable ligands (for example, **9**, Figure 3) for the transient receptor potential ankyrin 1 (TRPA1) channel. The combination of the resultant 'TRPswitch' compounds with the TRPA1 channel demonstrated a novel step-function photopharmacological system with a unique combination of high conductance, high efficiency, activity against an unmodified vertebrate channel, and capacity for bidirectional optical switching. Phenotypic screening in photopharmacology, just like in conventional drug discovery, does however lead to a range of other challenges, including deconvolution of the target(s)/mechanism of action, complex SAR, etc.[29]

Hit to lead challenges for photopharmacological agents

Another reason for the assignment of azo compounds as PAINS is their propensity to aggregate in biological assays, giving rise to non-specific inhibition.[30] Promiscuous compounds that show such an effect are typically hydrophobic, planar, and rigid; as is the case for azobenzene. Of course, there may be additional molecular structure/functionality within a photopharmacological agent beyond the photoswitch component, which may in turn act to increase the polarity and decrease the overall planarity of the molecule. Alternatively, it may be possible to harness the lipophilic nature of azobenzene for certain applications.[11b] Nonetheless, it is worth questioning whether the intrinsic 'drug like' characteristics of azobenzene could be improved. In this respect, it is worth returning to heteroaromatic azo arene photoswitches.[18] Replacement of a benzene ring for a heteroaromatic ring has long been used as a strategy in medicinal chemistry campaigns to increase

the polarity/solubility of a given series. Heteroaromatic azo photoswitches allow one to take an analogous strategy in photopharmacology, while not necessarily compensating on photoswitch performance (Figure 5A). For example, an arylazopyrazole photoswitch has increased polarity over azobenzene, with improved photoconversion. While photopharmacological agents based on heteroaromatic azos are not as ubiquitous as those based on azobenzenes, they are clearly emerging as a useful class (e. g. Figures 3, 4). Comparative studies on the broader 'drug-like' characteristics of related series of azo photoswitches are so far lacking however. In the future, detailed matched molecular pair analyzes[31] – the determination of how a particular property change is associated with a well-defined structural change – may be worthwhile in order to more robustly compare the physiochemical as well as biological properties of azo compounds. This may give clearer guidelines as to how to define a high-quality azo start point for further medicinal chemistry optimization.

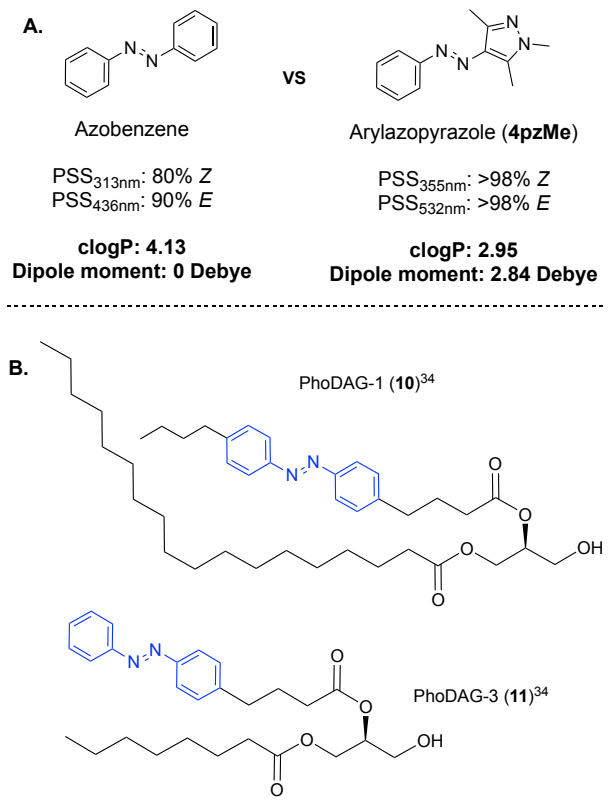


FIGURE 5. (A) Comparison of azobenzene and arylazopyrazole. clogP calculated using Molinspiration; (B) PhoDAGs reported by Trauner and co-workers[34]. Key photoswitch component of PhoDAGs colored in blue.

With respect to ligand quality, there are now many efficiency metrics in medicinal chemistry that are used to judge the quality of a hit and how such quality changes in the process of hit-lead-candidate optimization.[32] Perhaps the simplest incarnation of these metrics is Ligand Efficiency (LE), which was first proposed as a method for comparing molecules according to their average target binding energy *per atom*. Effectively, such an analysis allows one to consider how much of a ligand's molecular framework is involved in target binding and, as such, to prioritize low molecular weight 'efficient' ligands at all stages of optimization. This, in turn, allows for the

development of higher quality candidate molecules, which contain very little superfluous molecular structure/weight. The author is not aware of any study in photopharmacology where the ligand efficiency of a photopharmacological series has been studied or discussed explicitly. Such considerations, however, have clearly proved useful to practitioners of medicinal chemistry and will likely be equally useful in the translation of photopharmacological agents towards the clinic. Intuitively, it is the author's belief that heteroaromatic azo arene photoswitches may again prove advantageous in the development of ligand efficient photopharmacological agents. This is due to the fact that heteroatoms already within the central photoswitch component have the capacity to form directional polar contacts in the binding site of the target. Therefore, it should be possible to use heteroaromatic rings within the azo switch motif, not just to tune photoswitching performance, but also to increase binding energy through the formation of new protein-ligand contacts. This should ensure the development ligand efficient photopharmacological agents.

Medicinal chemistry optimization is ultimately a multiparameter exercise.[33] Practitioners aim to improve target potency, selectivity, ADME/PK, toxicology, etc., within a given medicinal chemistry campaign. This is no small feat! Trade-offs inevitably have to be made in order to identify a molecule with a sufficient *balance* of properties for a given target candidate profile. The need to balance multiple properties/activities will analogously be the case for ligand optimization in photopharmacology, but with the additional (and significant!) caveats that: 1) the photoswitching properties of the ligand cannot be negatively compromised during optimizsation; and 2) the 'standard' medicinal chemistry metrics - target potency, selectivity, ADME/PK, toxicology, etc – will not just differ between analogues of a series, but also between *E* and *Z* isomers *of the same molecule*. Point 1 may be simple to address if the azo aromatic is peripherally attached and electronically decoupled to the core pharmacophore. In such a case, optimization of

the pharmacophoric part of the ligand should not overly impact photoswitching performance (PSS, λ_{max}, photoswitching quantum yield, thermal half-life of *Z* isomer, etc). If, however, the azo arene forms a central component of the ligand – as advocated above – then photochemical performance and properties will shift during ligand optimization. This issue will mean that there are yet further properties to balance during ligand optimization for a photopharmacological agent over a conventional ligand. Point 2 is perhaps even more challenging to address. The medicinal chemist will not just need to balance the physiochemical and pharmacological properties of a single molecule, but both *E* and *Z* isomers too. While current photopharmacological endeavors are mostly concerned with the selectivity of the *E* and *Z* isomer of a given agent against the target/biological endpoint of interest, the wider change in properties/activity between the two isomers may provide additional challenges, and potentially opportunities, during ligand optimization. A representative example is permeability. Current photopharmacology studies do not routinely investigate how *E* and *Z* isomers differ in their cellular permeability. A study by Schultz, Trauner and co-workers previously reported the development of photoswitchable diacylglycerols (PhoDAGs, Figure 5B) as a means to enable the optical control of protein kinase C.[34] As part of this study, to rationalize the analogue-dependent effects observed, they studied the membrane localization and uptake of their PhoDAGs. Their results were interpreted to mean that PhoDAG-1 (**13**) remains trapped on the plasma membrane, whereas PhoDAG-3 (**14**) accumulates at inner membranes. While this study was specific to molecules expected to localize in membranes and not strictly measuring permeability though the membrane(s), changes in cellularly permeability are to be expected for photopharmacological agents; not just between analogues of a similar series, but also between *E* and *Z* isomers of the same molecule. For cellular and *in vivo* experiments, differential cellular permeability of *E* and *Z* isomers may mask on-target effects, especially if permeability assays have

not been employed. Conversely, different permeability or cell localization between *E*/*Z* isomers may be an opportunity to enhance low differential target selectivity. Regardless, it is clear that measurement of a broader subset of differential physiochemical and pharmacological characteristics between isomers and analogues, including permeability, will be key to robust progression of photopharmacological agents in the future.

Biological stability and toxicity of azo arenes

The growth of the dye industry in the 19th century led to a prevalence of azo aromatic compounds. Especially before the modern-day dogmas associated with expert medicinal chemistry, such azo dyes were investigated as pharmacological agents in a variety of contexts, from neurological diseases to cancer. The most famous output from these endeavors was the pioneer antibiotic Prontosil (**15**, Figure 6A), one of the earliest antimicrobial drugs.[35] The origins of **15** are thought to stem from Nobel laurate Paul Ehrlich and his interest in using dye molecules to stain bacteria.[36] Further collaboration between chemists at Bayer (and its parent company, IG Farben) and the German pathologist Gerhard Domagk led to the discovery of antibiotic **15**; winning Gerhard Domagk the 1939 Nobel Prize in Physiology or Medicine.[37] In terms of mechanism of action, **15** functions as a prodrug: the azo functionality is reduced *in vivo* to produce the active sulphanilamide (**16**), which ultimately kills bacteria through folic acid deficiency (Figure 6A).

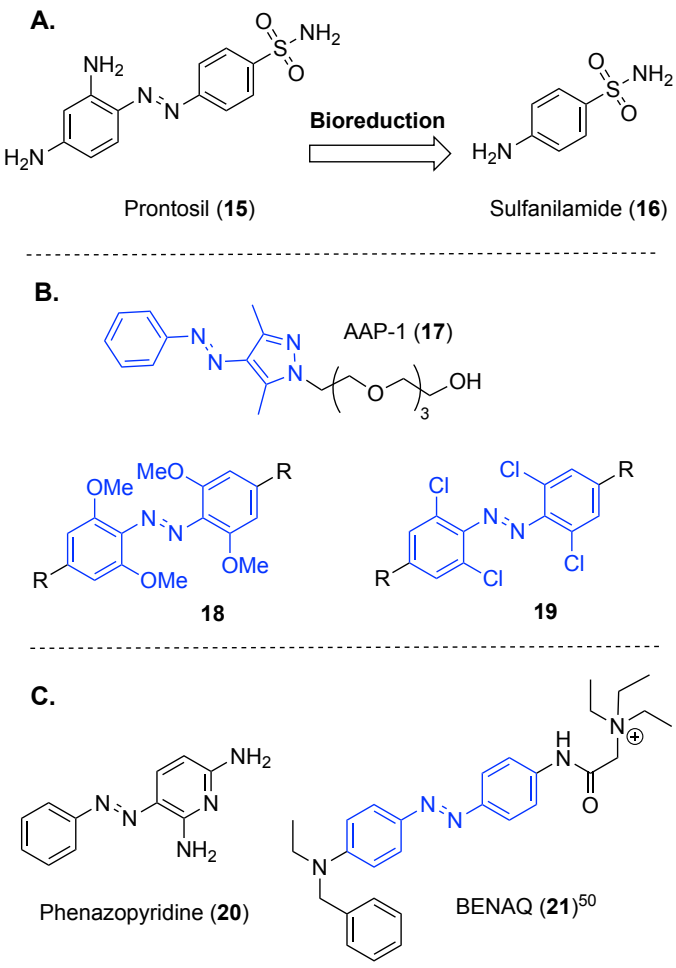


FIGURE 6. (A) The azo antibiotic drug Prontosil; (B) Azo arenes studied for GSH reduction; (C) Azo arenes studied for PK. Key photoswitch components colored in blue.

One thing that is clear from this historical precedent, is the potential for metabolic cleavage of the azo bond *in vivo*. Such metabolism is clearly an issue for photopharmacological studies using azo photoswitches: potential light-dependent activity is of no use if the photoswitching functionality has low biological stability *in vivo*. The rate and type of metabolism for azo aromatics is not constant however, and significantly depends on chemical structure.[38] Therefore, it should

certainty be possible to use medicinal chemistry to improve the *in vivo* stability of azo-based photopharmacological agents.

The metabolic transformation that has perhaps received most attention in the photopharmacology literature is chemical reduction by the biological reductant, GSH (*vide supra*). This is a prevalent means for the biological reduction of azos[38] and is relatively easy to assay. The ability to reduce the -N=N- bond using GSH is partly determined by redox potential: electron-poor azos are more readily reduced than equivalent electron-rich molecules. This trend therefore provides one key guideline to the design of azo aromatics that should be stable to GSH: the introduction of substituents that increase the electron-rich nature of the azobenzene. Interestingly, replacement of one of the benzene rings with a heteroaromatic ring can be equally effective to adjust redox potential. For example, Bart Jan Ravoo and co-workers reported that arylazopyrazoles are remarkably stable to GSH; most likely a result of the electron-rich pyrazole ring. The arylazopyrazole series they reported (a representative example being AAP-1, **17**, Figure 6B) showed excellent stability against 10mM of the reduced form of GSH for at least 22 h. In comparison, some azobenzenes have been reported to be reduced relatively rapidly under the same conditions (for example **18**, $t_{½}$ = 1 h).[39] Other factors affect the rate of reaction with GSH however. Woolley and co-workers have pioneered the development of tetra-*ortho*-substituted azobenzenes as a means to improve several properties of azobenzenes, including improved *Z*-isomer lifetime and the capacity for red-light switching.[10b] Surprisingly however, they found their tetra-*ortho*-chloro-substituted azobenzene (**19**, Figure 6B) to be far more resistant to GSH than the equivalent tetra-*ortho*-methoxy-substituted analogue (**18**).[40] This is counterintuitive to what would be expected given the comparatively electron-poor nature of the tetra-*ortho*-chloro-substituted analogue. Woolley and co-workers attribute this outcome to a change in reduction mechanism.

Ordinarily, it is thought that the thiol functionality in GSH attacks one of the azo nitrogen atoms, followed by protonation of the second azo nitrogen atom to give a sulfenyl hydrazine derivative.[41] Instead, Woolley and co-workers hypothesized that the enhanced rate of reduction for **18** is due to a higher effective pKa of the azo group. This results in protonation to give an azonium species – something they have later exploited for infrared switching[10b,42] – which is, in turn, more reactive towards reaction by GSH. Whether this trade off between intrinsic azo redox potential and azo pKa has wider implications for azo GSH reduction remains to be seen. The author notes that the arylazopyrazoles (e.g. **17**) appear to show high GSH stability (*vide supra*), despite increased azo pKa through mesomeric stabilization of the resultant azonium.[43]

GSH-based reduction is not the only relevant metabolic reaction for azo compounds *in vivo* however. It is also well known that azo aromatics can be reduced by enzymatic reductants. For example, bacteria are known to contain reductase enzymes that reduce azo aromatics. Intestinal bacteria are likely to be the major source of azoreductase for many azo aromatics when dosed orally to humans.[38] Additionally, NADPH-dependent reductase enzymes are present in liver microsomes and so are likely to be another major source of azo reductive metabolism.[38] While there is a reasonably large body of data surrounding the enzymatic metabolism of azo dyes,[38] enzymatic reductive assays are not routinely used for the investigation of photopharmacological agents.[44] Therefore, it remains unclear whether some of the emerging strategies to 'protect' the -N=N- bond from reduction by GSH (*vide supra*) will hold true for enzymatic reduction.

Finally, there are other metabolic reactions to consider beyond azo reduction; particularly oxidative metabolism. Azo dyes have indeed been shown to undergo significant oxidative metabolism *in vivo*.[38] Such reactions primarily occur on the azobenzene aromatic ring(s) – ring

hydroxylation, dealkylation of alkyl amine substituents, etc – and leave the azo linkage intact. Therefore, while oxidative metabolism will not result in removal of the key azo functional group required for photoswitching, it will affect the clearance of the compound *in vivo*. This may, in turn, limit the usefulness of some photopharmacological agents, through mismatched pharmacokinetics for a given application (*vide infra*).

Aside from metabolism, there are wider concerns over the toxicity of azo compounds. Interestingly, using an azo prodrug (**15**) proved to be a safer way to deliver the highly insoluble sulfanilamide antibiotic: an ethylene glycol formulation called "Elixir Sulfanilamide" resulted in the death of more than 100 Americans and led to the Federal Food, Drug, and Cosmetic Act (FFDCA) in 1938.[45] Nonetheless, toxicity associated with azo aromatics has been long known,[38] especially the ability of azobenzenes to act as carcinogens and mutagens. The precise predominant toxicity concern appears to correlate to the biological stability of the azo bond.[38] Previous studies in animals using azo dyes have suggested that carcinogenicity appears to require an intact azo linkage; which ultimately results in the formation of adducts with DNA. The liver has been particularly eported to be the major site of tumorigenesis for most azo dyes. In contrast, the mutagenic activity of azo dyes has been assigned to occur following azo bond reduction. Full reduction of an azobenzene would give two aniline molecules. Anilines are well known toxophores (following, for example, further metabolic oxidation) and are avoided in medicinal chemistry campaigns.[46] Despite these concerns, just as for metabolism, the precise (off-target) toxicology of an azo compound will depend on its chemical structure and it should be possible to use medicinal chemistry to improve/remove any serious liabilities. It is also perhaps worth distinguishing between the potential to cause cancer (as judged in an animal model) and actual cancer incidence in humans when using therapeutically relevant doses. For example, phenazopyridine (**20**, Figure

6C) is an azo drug used for its local analgesic effects on the urinary tract. While it has been shown to be able to cause liver and colon cancer in animal models, it has not been conclusively demonstrated to cause cancer in humans[47] despite many years of usage. One thing is clear however: short- and long-term toxicity must be avoided if light-based therapeutics can really be used to improve therapeutic index over non-light based approaches (*vide supra*).

It would seem that in order to facilitate the translation of azo-based photopharmacological agents, far more work is needed to determine metabolic and toxicological liabilities for such agents; particularly as a function of molecular structure. While further usage of established *in vitro* assays (microsomal preparations, Ames test, etc) should enable this endeavor, further *in vivo* characterization is also drastically needed. Currently, *in vivo* pharmacokinetics (PK) and animal tolerability/toxicology (particularly in higher organisms, as opposed to zebrafish) are very rarely measured or reported for new azo photopharmacological agents;[44] although recent reports are emerging that include this data for other photoswitch classes.[48] The most translationally developed application for azo photopharmacological agents is in the area of vision restoration.[49] While such studies have included assessment of *in vivo* PK and toxicology, these measurements were performed using a specific delivery methodology relevant to the particular application; namely, intravitreal injection – injection directly into the space in the back of the eye called the vitreous cavity. Recent results for vision restoration photopharmacological agents seem promising. For example, following intravitreal injection to rabbits, BENAQ (**21**, Figure 6C) exhibited a half-life in the retina of 24 days, was undetectable in plasma (due to negligible penetration through the blood-retina barrier), and caused no pathological changes to the eye.[50] Whether other applications, particularly those requiring systemic delivery, will be able to achieve equally positive results *in vivo* will require further assessment.

Balancing kinetics

As stated in the introduction, the *Z* isomer of an azo aromatic is only metastable and will undergo thermal *Z*-*E* reversion at a rate that depends on the structure of the photoswitch. There is significant discussion at photopharmacology meetings over the optimum rate for thermal reversion; however, as has already been highlighted by others,[8a] the required balance between photochemical and thermal chemistry for a given photoswitch will depend on the precise biological system to be investigated. In cases where the *Z*-isomer has a significantly long *Z*-*E* isomerization half-life (> days), the photoswitch will be bistable under the time window of most experiments. This may be advantageous for a range of target classes and will allow for complete optical control of activation and deactivation using different wavelengths of light. Alternatively, in cases where the *Z*-isomer undergoes rapid *Z*-*E* isomerization (< seconds), automatic deactivation of the photoswitch will occur (assuming the *Z* isomer is the active species) following removal of the light source. Fast automatic deactivation may be useful, for example, for neural systems with millisecond response times,[8a] or was highlighted to be useful for the photoswitchable HDAC inhibitors described above. The kinetics of *Z*-*E* thermal isomerization is not the only temporal aspect of concern however, especially once photopharmacological agents are used *in vivo*. The pharmacokinetics of the agent, and how this differs between *E* and *Z* isomers, will also be critical to the success of a given photopharmacological approach. Overall, appropriate balance between a range of kinetic processes will be needed to realize the full potential of photopharmacological agents as therapeutics. Thermal *Z*-*E* isomerization kinetics will need to be balanced against target binding kinetics, as well as absorption, distribution and clearance kinetics *in vivo*.

Given the paucity of quantitative PK data for azo photopharmacological agents (*vide supra*), appropriate case studies are lacking for further examination of this challenge. Nonetheless, a very nice conceptual idea was put forward by Feringa, Szymanski, Velema and co-workers with respect to photoswitchable antibiotics,[51] and this will be used to provide a useful framework for discussion. The quinolone class of antibiotics are used to treat a variety of bacterial infections including those from both gram-positive and gram-negative organisms.[52] The targets for these antibiotics are the homologous type II topoisomerase enzymes, DNA gyrase and topoisomerase IV. Feringa, Szymanski, Velema and co-workers prepared a series of photoswitchable quinolones in order to develop photopharmacological antibiotic agents. When quinolone-sensitive *Escherichia coli* were treated with the representative compound **22** (Figure 7A), a clear difference in the inhibitory effect was observed for the non-irradiated (MIC $\geq$ 64 μg/mL) and irradiated (MIC = 16 μg/mL) samples. This data demonstrated that **22** is active when it is photoswitched to its *Z* form and inactive (or at least significantly less active) in the *E* isomer. The half-life for *Z*-**22** to thermally revert to the inactive *E* isomer was shown to be 2 h. It was hypothesized that thermal auto-inactivation over time would be a useful attribute for an antibiotic. This is because a substantial percentage of antibiotics consumed in healthcare and agriculture are excreted unmetabolized, leading to their build-up in the environment. Such environmental exposure is thought to be a major contributor to the development of bacterial resistance. By developing antibiotics that auto-inactivate, environmental exposure following secretion should only occur for the inactive isomer, preventing the development of microbial resistance, in theory. Taking the 2 h *Z*-*E* half-life into account, Feringa, Szymanski, Velema and co-workers demonstrated auto-inactivation over time in bacterial liquid culture.[51a]

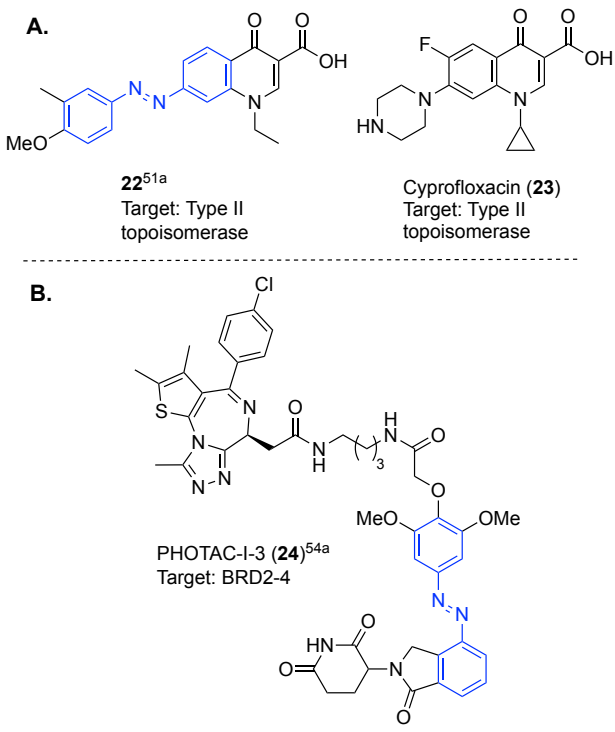


FIGURE 7. (A) Photoswitchable antibiotic reported by Feringa, Szymanski, Velema and co-workers[51a]; (B) Photoswitchable PROTAC reported by Trauner and co-workers[54a]. Key photoswitch components colored in blue.

While this data provides proof of the concept for thermal auto-inactivation of an antibiotic, things become far more complicated when considering the translation of the approach into the clinic. The *in vivo* PK of compound **22** was not measured; however, for the sake of argument, let's assume the PK of **22** is similar to the approved quinolone antibiotic Ciprofloxacin (**23**, Figure 7A) [Note, given the metabolism described above, this is probably not a valid assumption]. Following oral dosing, **23** reaches a maximal serum concentration after 1 to 2 hours and the serum elimination half-life is approximately 4 hours.[53] This would mean that the optimum timing for the start of light dosing for an equivalent photopharmacological quinolone (e.g. **22**) would be between 1-2 hours,

during the phase of maximum systemic exposure. The optimum *duration* of light irradiation – ignoring for the time being light intensity, penetration, etc – is difficult to predict, however. While in static culture, light irradiation would produce the active *Z* isomer with a subsequent half-life of 2 hours, *in vivo*, the active isomer would diffuse away from the site of action over time and be subsequently cleared. Therefore, following cessation of irradiation, the active *Z* isomer at the site of action would be replaced by the more systemically prevalent *E* isomer over time, as a function of the PK. Continuous irradiation would potentially solve this problem: ensuring that the *E* isomer is continually converted to the active *Z* isomer at the site of action. This comes with its own set of considerations however, including the safety of long doses of UV-light (for a conventional azobenzene switch) and the kinetics of antibacterial action. Therefore, one would need to carefully balance the active drug concentration at the site of interest (from drug dose, PK, and PSS) and duration of action (from target binding kinetics, antibacterial effect, PK and light dose duration) in order to have the required antibiotic effect. Such considerations may not be trivial, especially considering that the *E* and *Z* isomers may well exhibit different PK! For example, the *Z* isomer of azobenzene has been reported to be more rapidly reduced by GSH than the *E* isomer.[24] Irrespective of action at the target site, assuming an elimination half-life of 4 hours (and no significant difference in PK between *E* and *Z* isomers), the measured *Z*-*E* thermal isomerization half-life (2 h) should indeed result in the majority of (non-metabolized) compound **22** being excreted in the inactive state. Whether or not this outcome can truly prevent the build-up of environmental bacterial resistance will depend on many factors including the differential selectivity between *E* and *Z* isomers (i.e. whether the 'inactive' still engages the target at higher concentration), prior resistance against related antibiotics, the (bio)stability of the azo antibiotic in the environment and the precise resistance mechanisms (target-based versus compound-based) that may develop.

Ultimately, photopharmacology is generating a lot of creative and elegant ideas for how the spatial and temporal selectivity that comes with a photoaddressable drug could be harnessed for new concepts in therapy. Balancing the photochemical and thermal processes at the site of target action are, however, further complicated once a project progresses to *in vivo* experiments in animals: as would be required for translation into novel medicines. As new photopharmacological approaches are defined, these will bring further challenges due to the interplay of multiple kinetic processes. A good example is the recent innovation of photoswitchable proteolysis targeting chimeras (PROTACs, for example **24**, Figure 7B).[54] PROTACs are bifunctional small molecules that divert the endogenous protein regulating machinery – the ubiquitin proteasome system (UPS) – to direct specific degradation of a selected target protein.[55] Importantly, the role of a PROTAC reagent is catalytic, simply facilitating the interaction between a E3 ligase complex and target protein, and the pharmacodynamic (PD) action of the agent depends on a number of factors, including the turnover of the biological target. As such, PROTAC reagents have shown very interesting PK-PD effects.[56] How light addressability fits into this strategy, especially with respect to the development of photoswitchable PROTAC *drugs* (as opposed to chemical tools), remains to be rigorously determined.

The challenge of balancing the multiple kinetic processes involved in drug action is intrinsic to the work of a medicinal chemist. Thus, the additional challenges presented by photopharmacology are certainly not insurmountable; but they will require further rigorous *in vivo* work, in order to translate novel photopharmacological concepts and agents into the clinic. Such *in vivo* work will, in turn, allow the definition of clearer criteria (required target selectivity, PK parameters, light dosing, etc) for medicinal chemists who are focused on photopharmacology projects.

Conclusions and perspectives

Photopharmacology endows therapeutics with light addressability. This, in turn, allows for improved spatial and temporal selectivity in drug action, which can be harnessed in the generation of new concepts for therapy. These include using light to improve therapeutic index, the ability to temporally modulate target action with enhanced precision, and the potential for light activated but auto-inactivated drugs (such as antibiotics). Tremendous progress has already been made, with photopharmacological agents now reported against a wide array of target classes - ion channels, transporters and pumps, GPCRs, enzymes, mitotic targets. Light-dependent activity has been demonstrated in a range of live cell and animal (particularly zebrafish) models, and translationally promising results have been reported in the area of vision restoration. The aim of this article was to provide, from the author's point of view, a perspective of some of the challenges that will need to be overcome in order to fully realize the potential of photopharmacology in the future. Focus was placed on the medicinal chemistry challenges associated with the development of photopharmacological agents, rather than technical/logistical challenges associated with light delivery. Light (especially at short wavelengths) does not penetrate tissue efficiently, due to absorption by biomolecules, which include heme and flavoproteins. While such limited penetration of light through tissue has long been used as a criticism of light-based therapeutics, advances in engineering are addressing this challenge, including wireless subdermal electronics and multimodal optical fibres.[8b] In such approaches, through implantation or fibre optics, the irradiation source is physically placed adjacent to the site to be irradiated. This negates the need for deep light penetration, at least when addressing a well-defined target irradiation site. Thus, advanced photopharmacological agents could be plausibly coupled with advanced and biocompatible light sources in future applications.

Many of the challenges stated in this perspective are 'business as usual' for a medicinal chemist: the need to identify robust hits, progress the highest quality scaffolds, address ADMET liabilities, improve PK, etc. The fact that the agents in question are light addressable however, brings a whole new 'flavor' to proceedings; including the need to address all the challenges above while retaining optimal photochemical and thermochemical properties of the agent in question. The focus of the discussion has been on freely diffusing reversible photopharmacological agents, but it is likely that several of the challenges stated will equally applicable to other approaches: irreversible photopharmacology, tethered ligands, etc. Therefore further translational work, particularly from medicinal chemists, is not only important to the progression of photopharmacology, but is likely to be an exciting and fruitful endeavor.

As a concluding point, it is interesting to question whether the approval of an optimized photopharmacological agent for clinical use would encounter any regulatory challenges, given that the *E* and *Z* isomers would likely exhibit wholly different pharmacology - different on/off target effects, PK, toxicology, etc. This situation is further compounded by the fact that, whether due to incomplete photoswitching or *Z*-*E* thermal isomerization, mixtures of these isomers may be present within a patient at a given time. Perhaps the closest analogy to this situation would be the regulatory guidelines associated with racemates, especially to enantiomers of a drug that interconvert. The EMA guidelines[57] define two scenarios in this context: "One of the following two situations may occur: 1) Rapid interconversion "*in vivo*". If the interconversion rate "*in vivo*" is appreciably higher than the apparent distribution and elimination rates of the enantiomers, only the racemate should be studied as the active substance. 2) No or slow inversion "*in vivo*", allowing for separate enantiomer effects on [the] fate in the organism." It therefore seems logical that in the case of an azo photopharmacological agent with a very long *Z*-*E* thermal half-life, separation and

single isomer characterization could be performed and (if favorable) should satisfy regulators. Conversely, in the case of an azo photopharmacological agent with a very short *Z*-*E* thermal half-life, it should be possible to experimentally demonstrate that the interconversion rate is appreciably higher than the apparent distribution and elimination rates of the isomer(s). There is arguably a gray area in the middle for azos with an intermediate half-life (hours-days): as is often observed for many azo photoswitches! Further regulatory requirements that may be relevant for photopharmacological agents could include measurements of photostability.[58]

From ancient civilizations to the present day, the coupling of light with therapeutic action continues to drive innovation and invention in the treatment of human disease. By addressing challenges important to the translation of photopharmacological agents, it should be possible to realize the huge potential of photopharmacology in the clinic. In the opinion of the author, the future of photopharmacology is bright!

AUTHOR INFORMATION

Corresponding Author

*M.J.F. e-mail: m.fuchter@imperial.ac.uk

Present Address

†CSIRO Manufacturing, Bag 10, Clayton South, Victoria 3169, Australia

ACKNOWLEDGEMENTS

M.J.F. would like to thank the EPSRC for an Established Career Fellowship (EP/R00188X/1) and the Commonwealth Scientific and Industrial Research Organization (CSIRO) Research Office and CSIRO Manufacturing for the provision of support through a Distinguished Visiting Researcher position.

ABBREVIATIONS

PDT, photodynamic therapy; GPCR, G-protein coupled receptor; SAR, structure-activity relationship; PSS, photostationary state; DTT, dithiothreitol; GSH, glutathione; LE, ligand efficiency; PK, pharmacokinetics; PD, pharmacodynamics.

BIOGRAPHY

Matthew J. Fuchter is a Professor of Chemistry, an ESPRC Established Career Fellow, and co-Director of the Centre for Drug Discovery Science at Imperial College. From a PhD concerning PDT agents, to his recent work on heteroaromatic azo arene photoswitches, he has long been interested in the coupling of light to therapeutic action. Beyond photopharmacology, his research concerns the development of chemistry-led approaches to interrogate function in chemistry, materials and medicine. His work has resulted in a number of awards, including the Royal Society of Chemistry's Harrison-Meldola Memorial Prize (2014), the Imperial College President's Medal for Excellence in Innovation and Entrepreneurship (2017), the Tetrahedron Young Investigator Award for Bioorganic and Medicinal Chemistry (2018), and a Blavatnik Award for Young Scientists in the United Kingdom (2020).

REFERENCES

1. Ackroyd, R.; Kelty, C.; Brown, N.; Reed, M. The history of photodetection and photodynamic therapy. *Photochem. Photobiol.* **2001**, *74*, 656-669.

2. Broichhagen, J.; Trauner, D. Bilirubin in a new light. *Angew. Chem. Int. Ed.* **2013**, *52*, 13868-13870.

3. a) Redmond, R. W.; Kochevar, I. E. Spatially-resolved cellular responses to singlet oxygen. *Photochem. Photobiol.* **2006**, *82*, 1178-1186. b) Blázquez-Castro, A.; Breitenbach, T.; Ogilby, P. R. Singlet oxygen and ROS in a new light: low-dose subcellular photodynamic treatment enhances proliferation at the single cell level. *Photochem. Photobiol. Sci*. **2014**, *13*, 1235-1240.

4. Huang, Z. A review of progress in clinical photodynamic therapy. *Technol. Cancer Res. Treat*. **2005**, *4*, 283-293.

5. Wainwright, M. Photodynamic antimicrobial chemotherapy (PACT). *J. Antimicrob. Chemother.* **1998**, *42*, 13-28.

6. For example: a) Klań, P.; Solomek, T.; Bochet, C.G.; Blanc, A.; Givens, R.; Rubina, M.; Popik, V.; Kostikov, A.; Wirz, J. Photoremovable protecting groups in chemistry and biology: reaction mechanisms and efficacy. *Chem. Rev*. **2013**, *113*, 119-191; b) Vorobev, A. Y.; Moskalensky, A. E. Long-wavelength photoremovable protecting groups: on the way to *in vivo* application. *Comput. Struct. Biotechnol. J*. **2020**, *18*, 27-34.

7. Harris, J. D.; Moran, M. J.; Aprahamian, I. New molecular switch architectures. *Proc. Natl. Acad. Sci. U.S.A.* **2018**, *115*, 9414-9422.

8. a) Hüll, K.; Morstein, J.; Trauner, D. *In vivo* photopharmacology. *Chem. Rev*. **2018**, *118*, 10710-10747. b) Morstein, J.; Trauner, D. New players in phototherapy: photopharmacology and bio-integrated optoelectronics. *Curr. Opin. Chem. Biol.* **2019**, *50*, 145-151.

9. Hartley, G. The *cis*-form of azobenzene. *Nature*, **1937**, *140*, 281-281.

10. For select examples of red-light switching see: a) Hammerich, M.; Schutt, C.; Stahler, C.; Lentes, P.; Rohricht, F.; Hoppner, R.; Herges, R. Heterodiazocines: synthesis and photochromic properties, *trans* to *cis* switching within the bio-optical window *J. Am. Chem. Soc*. **2016**, *138*, 13111-13114. b) Dong, M.; Babalhavaeji, A.; Samanta, S.; Beharry, A. A.; Woolley, G. A. Red-shifting azobenzene photoswitches for *in vivo* use. *Acc. Chem. Res*. **2015**, *48*, 2662-2670. c) Konrad, D. B.; Savasci, G.; Allmendinger, L.; Trauner, D.; Ochsenfeld, C.; Ali, A. M. Computational design and synthesis of a deeply red-shifted and bistable azobenzene. *J. Am. Chem. Soc*. **2020**, *142*, 6538–6547.

11. a) Velema, W. A.; Szymanski, W.; Feringa, B. L. Photopharmacology: beyond proof of principle. *J. Am. Chem. Soc.* **2014**, *136*, 2178-2191. b) Broichhagen, J.; Frank, J. A.; Trauner, D. A roadmap to success in photopharmacology. *Acc. Chem. Res.* **2015**, *48*, 1947-1960. c) Lerch, M. M.; Hansen, M. J.; van Dam, G. M.; Szymanski, W.; Feringa, B. L. Emerging targets in photopharmacology. *Angew. Chem. Int. Ed.* **2016**, *55*, 10978-10999. d) Reeßing, F.; Szymanski, W. *Curr. Med. Chem*. **2017**, *42*, 4905-4950. e) Albert, L.; Vázquez, O. Photoswitchable peptides for spatiotemporal control of biological functions. *Chem. Commun.* **2019**, *55*, 10192-10213.

12. Yue, L.; Pawlowski, M.; Dellal, S. S.; Xie, A.; Feng, F.; Otis, T. S.; Bruzik, K. S.; Qian, H.; Pepperberg, D. R. Robust photoregulation of $GABA_A$ receptors by allosteric modulation with a propofol analogue. *Nat. Commun*. **2012**, *3*, 1095.

13. Westphal, M. V.; Schafroth, M. A.; Sarott, R. C.; Imhof, M. A.; Bold, C. P.; Leippe, P.; Dhopeshwarkar, A.; Grandner, J. M.; Katritch, V.; Mackie, K.; Trauner, D.; Carreira, E. M.; Frank, J. A. Synthesis of photoswitchable Δ9-tetrahydrocannabinol derivatives enables optical control of cannabinoid receptor 1 signaling. *J. Am. Chem. Soc*. **2017**, *139*, 18206−18212.

14. Tsai, Y.-H.; Essig, S.; James, J. R.; Lang, K.; Chin, J. W. Selective, rapid and optically switchable regulation of protein function in live mammalian cells. *Nat. Chem*. **2015**, *7*, 554−561.

15. Borowiak, M.; Nahaboo, W.; Reynders, M.; Nekolla, K.; Jalinot, P.; Hasserodt, J.; Rehberg, M.; Delattre, M.; Zahler, S.; Vollmar, A.; Trauner, D.; Thorn-Seshold, O. Photoswitchable inhibitors of microtubule dynamics optically control mitosis and cell death. *Cell*, **2015**, *162*, 403−411.

16. Morstein, J.; Awale, M.; Reymond, J. -L.; Trauner, D. Mapping the azolog space enables the optical control of new biological targets. *ACS Cent. Sci*. **2019**, *5*, 607-618.

17. Reis, S. A.; Ghosh, B.; Hendricks, J. A.; Szantai-Kis, D. M.; Törk, L.; Ross, K. N.; Lamb, J.; Read-Button, W.; Zheng, B.; Wang, H.; Salthouse, C.; Haggarty, S. J.; Mazitschek, R. Light controlled modulation of gene expression by chemical optoepigenetic probes. *Nat. Chem. Biol*. **2016**, *12*, 317-323.

18. Crespi, S.; Simeth, N. A.; König, B. Heteroaryl azo dyes as molecular photoswitches. *Nat. Rev. Chem*. **2019**, *3*, 133-146.

19. a) Weston, C. E.; Richardson, R. D.; Haycock, P. R.; White, A. J. P.; Fuchter, M. J. Arylazopyrazoles: azoheteroarene photoswitches offering quantitative isomerization and long thermal half-lives. *J. Am. Chem. Soc*. **2014**, *136*, 11878-11881. b) Calbo, J.; Weston, C. E.; White,

A. J. P.; Rzepa, H.; Contreras-García, J.; Fuchter, M. J. Tuning azoheteroarene photoswitch performance through heteroaryl design. *J. Am. Chem. Soc*. **2017**, *139*, 1261-1274. c) Weston, C. E.; Kraemer, A.; Colin, F.; Yildiz, O.; Baud, M. G. J.; Meyer-Almes, F. –J.; Fuchter, M. J. Towards photopharmacological antimicrobial chemotherapy using photoswitchable amidohydrolase inhibitors. *ACS Infect. Dis*. **2017**, *3*, 152-161. d) Calbo, J.; Thawani, A. R.; Gibson, R. S. L.; White, A. J. P. Fuchter, M. J. A combinatorial approach to improving the performance of azoarene photoswitches. *Beilstein J. Org. Chem*. **2019**, *15*, 2753-2764.

20. Lachmann, D.; Konieczny, A.; Keller, M.; König, B. Photochromic peptidic NPY Y4 receptor ligands. *Org. Biomol. Chem.* **2019**, *17*, 2467-2478.

21. Mafy, N. N.; Matsuo, K.; Hiruma, S.; Uehara, R.; Tamaoki, N. Photoswitchable CENP-E inhibitor enabling the dynamic control of chromosome movement and mitotic progression. *J. Am. Chem. Soc*. **2020**, *142*, 1763-1767.

22. Sun, H.; Tawa, G.; Wallqvist, A. Classification of scaffold hopping approaches. *Drug Discov. Today.* **2012**, *17*, 310–324.

23. a) Baell, J. B.; Nissink, J. W. M. Seven Year Itch: Pan-assay interference compounds (PAINS) in 2017—utility and limitations. *ACS Chem Biol*. **2018**, *13*, 36-44. b) Baell, J. B.; Holloway, G. A. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. *J. Med. Chem*. **2010**, *53*, 2719-2740.

24. Schehr, M.; Ianes, C.; Weisner, J.; Heintze, L.; Müller, M. P.; Pichlo, C.; Charl, J.; Brunstein, E.; Ewert, J.; Lehr, M.; Baumann, U.; Rauh, D.; Knippschild, U.; Peifer, C.; Herges, R. 2-Azo-2-diazocine-thiazols and 2-azo-imidazoles as photoswitchable kinase inhibitors: limitations and

pitfalls of the photoswitchable inhibitor approach. *Photochem. Photobiol. Sci.* **2019**, *18*, 1398-1407.

25. Gonzalez, A.; Kengmana, E. S.; Fonseca, M. V.; Han, G. G. D. Solid-state photoswitching molecules: structural design for isomerization in condensed phase. *Mater. Today Adv*. **2020**, *6*, 100058.

26. Szymanski, W.; Ourailidou, M. E.; Velema, W. A.; Dekker, F. J.; Feringa, B. L. Light-controlled histone deacetylase (HDAC) inhibitors: towards photopharmacological chemotherapy. *Chem. Eur. J*. **2015**, *21*, 16517-16524.

27. Bradner, J. E.; West, N.; Grachan, M. L.; Greenberg, E. F.; Haggarty, S. J.; Warnow, T.; Mazitschek, R. Chemical phylogenetics of histone deacetylases. *Nat. Chem. Biol.* **2010**, *6*, 238-243.

28. Pui-Ying Lam, Aditya R. Thawani, Enrique Balderas, Andrew J.P. White, Dipayan Chaudhuri, Matthew J. Fuchter and Randall T. Peterson, unpublished results.

29. Moffat, J. G.; Vincent, F.; Lee, J. A.; Eder, J.; Prunotto, M. Opportunities and challenges in phenotypic drug discovery: an industry perspective. *Nat. Rev. Drug Dis*. **2017**, *16*, 531-543.

30. McGovern, S. L.; Caselli, E.; Grigorieff, N.; Shoichet, B. K. A Common mechanism underlying promiscuous inhibitors from virtual and high-throughput screening. *J. Med. Chem*. **2002**, *45*, 1712-1722.

31. Griffen, E.; Leach, A. G.; Robb, G. R.; Warner, D. J. Matched molecular pairs as a medicinal chemistry tool. *J. Med. Chem*. **2011**, *54*, 7739-7750.

32. Hopkins, A. L.; Keserü, G. M.; Leeson, P. D.; Rees, D. C.; Reynolds, C. H. The role of ligand efficiency metrics in drug discovery. *Nat. Rev. Drug Dis.* **2014**, *13*, 105-121.

33. For a representative discussion, see Gleeson, M. P.; Hersey, A.; Montanari, D.; Overington, J. Probing the links between *in vitro* potency, ADMET and physicochemical parameters. *Nat. Rev. Drug Dis.* **2011**, *10*, 197-208.

34. Frank, J.; Yushchenko, D.; Hodson, D.; Lipstein, N.; Nagpal, J.; Rutter, G. A.; Rhee, J. -S.; Gottschalk, A.; Brose, N.; Schultz, C.; Trauner, D. Photoswitchable diacylglycerols enable optical control of protein kinase C. *Nat. Chem. Biol*. **2016**, *12*, 755-762.

35. Lesch, J. E. The First Miracle Drugs: How the Sulfa Drugs Transformed Medicine. Oxford University Press USA: New York, 2006.

36. Science History Institute Biography. https://www.sciencehistory.org/historical-profile/paul-ehrlich (accessed Apr 8, 2020).

37. Nobel Prize Biography. https://www.nobelprize.org/prizes/medicine/1939/domagk/biographical/ (accessed Apr 8, 2020).

38. Levine, W. G. Metabolism of azo dyes: implication for detoxication and activation. *Drug Metab. Rev.* **1991**, *23*, 253-309.

39. Stricker, L.; Böckmann, M.; Kirse, T. M.; Doltsinis, N. L.; Ravoo, B. J. Arylazopyrazole photoswitches in aqueous solution: substituent effects, photophysical properties, and host–guest chemistry. *Chem. Eur. J.* **2018**, *24*, 8639-8647.

40. Samanta, S.; Beharry, A. A.; Sadovski, O.; McCormick, T. M.; Babalhavaeji, A.; Tropepe, V.; Woolley, G. A. Photoswitching azo compounds in vivo with red light. *J. Am. Chem. Soc*. **2013**, *135*, 9777-9784.

41. Kosower, E. M.; Kanety-Londner, H. Glutathione. 13. Mechanism of thiol oxidation by diazenedicarboxylic acid derivatives. *J. Am. Chem. Soc*. **1976**, *98*, 3001-3007.

42. Dong, M.; Babalhavaeji, A.; Collins, C. V.; Jarrah, K.; Sadovski, O.; Dai, Q.; Woolley, G. A. Near-infrared photoswitching of azobenzenes under physiological conditions. *J. Am. Chem. Soc*. **2017**, *139*, 13483-13486.

43. Gibson, R. S. L.; Calbo, J.; Fuchter, M. J. Chemical Z-E isomer switching of arylazopyrazoles using acid. *ChemPhotoChem*, **2019**, *3*, 372-377.

44. For an example, see Mehta, Z. B.; Johnston, N. R.; Nguyen-Tu, M.; Broichhagen, J.; Schultz, P.; Larner, D. P.; Leclerc, I.; Trauner, D.; Rutter, G. A.; Hodson. D. J. Remote control of glucose homeostasis *in vivo* using photopharmacology. *Sci. Rep*. **2017**, *7*, 291.

45. The Atlantic. https://www.theatlantic.com/technology/archive/2018/01/the-accidental-poison-that-founded-the-modern-fda/550574/ (accessed Apr 8, 2020).

46. Limban, C.; Nuţă, D. C.; Chiriţă C.; Negreș, S.; Arsene, A. L.; Goumenou, M.; Karakitsios, S. P.; Tsatsakis, A. M.; Sarigiannise, D. A. The use of structural alerts to avoid the toxicity of pharmaceuticals. *Toxicol Rep*. **2018**, *5*, 943-953.

47. "Phenazopyridine Hydrochloride". Report on Carcinogens, Fourteenth Edition (2016). National Toxicology Program.

https://ntp.niehs.nih.gov/ntp/roc/content/profiles/phenazopyridinehydrochloride.pdf (accessed Apr 8, 2020).

48. Babii, B.; Afonin, S.; Schober, T.; Garmanchuk, L. V.; Ostapchenko, L. I.; Yurchenko, V.; Zozulya, S.; Tarasov, O.; Pishel, I.; Ulrich, A. S.; Komarov, I. V. Peptide drugs for photopharmacology: how much of a safety advantage can be gained by photocontrol? *Future Drug. Discov.* **2020**, *2*, FDD28.

49. Tochitsky, I.; Kienzler, M. A.; Isacoff, E.; Kramer, R. H. Restoring vision to the blind with chemical photoswitches. *Chem. Rev*. **2018**, *118*, 10748-10773.

50. Tochitsky, I.; Trautman, J.; Gallerani, N.; Malis, J. G.; Kramer, R. H. Restoring visual function to the blind retina with a potent, safe and long-lasting photoswitch. *Sci. Rep*. **2017**, *7*, 45487.

51. a) Velema, W. A.; van der Berg, J. P.; Hansen, M. J.; Szymanski, W.; Driessen, A. J. M.; Feringa, B. L. Optical control of antibacterial activity. *Nat. Chem.* **2013**, *5*, 924-928. b) Velema, W. A.; Hansen, M. J.; Lerch, M. M.; Driessen, A. J. M.; Szymanski, W.; Feringa, B. L. Ciprofloxacin–photoswitch conjugates: a facile strategy for photopharmacology. *Bioconjugate Chem*. **2015**, *26*, 2592-2597. c) Wegener, M.; Hansen, M. J.; Driessen, A. J. M.; Szymanski, W.; Feringa, B. L. Photocontrol of antibacterial activity: shifting from uv to red light activation. *J. Am. Chem. Soc.* **2017**, *139*, 17979-17986.

52. Bardell-Cox, O. A.; White, A. J. P.; Aragón, L.; Fuchter, M. J. Synthetic studies on the reverse antibiotic natural products, the nybomycins. *Med. Chem. Commun*. **2019**, *10*, 1438-1444 references therein.

53. FDA summary. https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/019537s087,020780s044lbl.pdf (accessed Apr 8, 2020).

54. a) Reynders, M.; Matsuura, B. S.; Bérouti, M.; Simoneschi, D.; Marzio, A.; Pagano, M.; Trauner, D. PHOTACs enable optical control of protein degradation, *Sci. Adv.* **2020**, *6*, eaay5064. b) Pfaff, P.; Samarasinghe, K. T. G.; Crews, C. M.; Carreira, E. M. Reversible spatiotemporal control of induced protein degradation by bistable photoPROTACs. *ACS Cent. Sci*. **2019**, *5*, 1682-1690. c) Jin, Y. -H.; Lu, M. -C.; Wang, Y.; Shan, W. -X.; Wang, X. -Y.; You, Q. -D. Azo-PROTAC: novel light-controlled small-molecule tool for protein knockdown. *J. Med. Chem*. **2020**, *63*, 4644–4654.

55. Pettersson, M.; Crews, C. M. PROteolysis TArgeting Chimeras (PROTACs) — past, present and future. *Drug Discov. Today: Technol.* **2019**, *31*, 15-27.

56. Mares, A.; Miah, A. H.; Smith, I. E. D.; Rackham, M.; Thawani, A. R.; Cryan, J.; Haile, P. A.; Votta, B. J.; Beal, A. M.; Capriotti, C.; Reilly, M. A.; Fisher, D. T.; Zinn, N.; Bantscheff, M.; MacDonald, T. T.; Vossenkamper, A.; Dace, P.; Churcher, I.; Benowitz, A. B.; Watt, G.; Denyer, J.; Scott-Stevens, P.; Harling, J. D. Extended pharmacodynamic responses observed upon PROTAC-mediated degradation of RIPK2. *Commun. Biol.* **2020**, *3*, 140.

57. EMA Guidelines. https://www.ema.europa.eu/en/documents/scientific-guideline/investigation-chiral-active-substances_en.pdf (accessed Apr 8, 2020).

58. EMA Guidelines. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-1-b-photostability-testing-new-active-substances-medicinal-products-step-5_en.pdf (accessed Apr 8, 2020).

TABLE OF CONTENTS GRAPHIC