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CHEMPHOTOCHEM Minireviews

Special Issue

On-Command Regulation of Kinase Activity using Photonic Stimuli

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The underlying role that many kinases play in complex cellular pathways as well as disease remains unclear. To better understand the role that kinases play in both health and disease states, the use of light as an external stimulus to modulate kinase activity with high spatiotemporal resolution has gained increasing interest over the years. Herein we highlight the progress made towards the development of light-responsive kinase enzymes and small molecule inhibitors. In these examples, photolabile caging groups and photoswitchable entities have been utilised to modulate either kinase activation or inhibition in a light-controlled manner.

1. Introduction

Biological functions can be impeded/accelerated if a small molecule interacts with the biomolecule of interest. This very simple causality forms the basis for today's rational drug design. The ideal drug should strike the intended biomolecule(s) with perfect selectivity, *i.e.*, not hampering processes other than that/those desired. Unfortunately, ideal drugs do not exist, as the intended mechanism of action is never fully orthogonal to all other bio-relevant processes. This results in undesired side-effects, and is why most drug candidates never make it to the market.

The abovementioned interactions between small molecules and their biomolecular targets depend, of course, on the structure of these species. So, what if one could change dynamically the structure of the molecule using externally controlled stimuli, to convert it to the "active" binding form only at times and locations relevant to the therapy of interest? This would imply an extremely good spatiotemporal precision, and certainly decrease the side-effects. Furthermore, applying this scheme in pre-clinical settings would also provide a valuable research tool in which pharmacodynamical information can be extracted.

This beguiling idea together with the use of light as the external stimulus is today referred to as photopharmacology and is schematically illustrated in Figure 1. Also shown is the 'reverse' variant of this approach, that is, cases where instead the biomolecular targets are equipped with light responsive units. Academic groups world-wide devote their research efforts to this rapidly evolving cross-disciplinary field, and the list of biorelevant systems that have been targeted include lipid membranes,[1] enzymes,[1a,2] nucleic acids,[2a,3] ion channels and various types of receptors.[1–2] The structural changes can be imposed in two principally different fashions: (*i*) Irreversible changes, often by the light-induced cleavage of covalent bonds to separate the "cage" from the "bioactive" (Figure 1A and 1C). This scheme is often referred to as uncaging or photodecaging; (*ii*) Reversible structural changes using molecular photoswitches (photochromic molecules, Figure 1B and 1D). Caging groups come, literally, in many different colours and a selection of these can be found in recent reviews.[4] As for the molecular photoswitches, the azobenzene derivatives are still enjoying clear preference, but alternatives from other photochromic

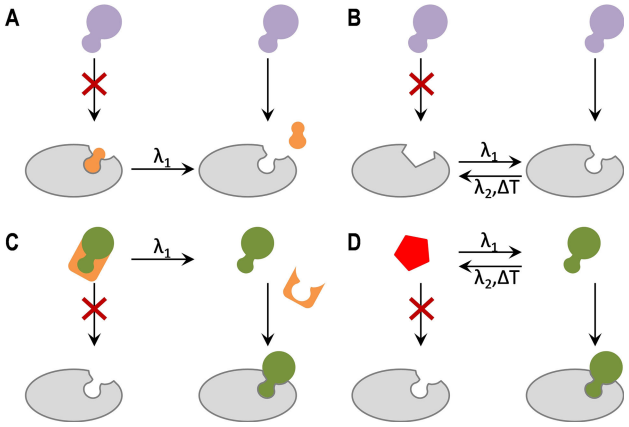

Figure 1. Schematic depiction of photocaging and photoswitchable approaches employed in photopharmacology. (A) Irreversible light-induced activation of enzymatic activity using caging groups; (B) Reversible photocontrol of enzymatic activity (inactive/active) using photoswitchable entities; (C) Irreversible light-induced activation of small molecule inhibitor through the use of a caging group; and (D) Reversible light-induced activation/deactivation of photoswitchable small molecule inhibitor.

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Special Issue
An invited contribution to a Special Issue on Photoresponsive Molecular Switches and Machines

families such as spiropyrans and diarylethenes are also being frequently used in this context.[2,5]

Over the past two decades, the external control of kinase activity using light has been explored. Protein kinases play a fundamental role in a number of cellular functions including cell proliferation, differentiation and apoptosis.[6] It is therefore of no surprise that their inhibition has proven to be a valuable therapeutic approach for the treatment of various diseases.[6] Despite the plethora of kinase inhibitors regularly appearing in the literature, it is evident that the majority of these inhibitors present selectivity issues, significantly impeding their advancement to a clinical setting as a result. Furthermore, the role that many kinases play in a variety of disease states is poorly understood. The relationship between intracellular enzymatic activity and cellular behaviour is typically probed by the use of genetic or siRNA-based approaches, whereby in vitro experiments do not accurately reflect the real-time events or that of the natural cellular environment, while in vivo studies often suffer from poor spatial resolution. As such, the dynamic and optical control of kinase activity in live cells and whole organism settings affords an unprecedented level of spatiotemporal control over biological processes, adding a new layer of experimental opportunity. This minireview serves to highlight the progress made regarding the development of *light-responsive* kinase enzymes as well as small molecule inhibitors in which photocontrolled kinase inhibition has been the main objective.

2. Light-Responsive Kinase Enzymes

Photoactivatable enzymes function as valuable tools to probe the role of catalytic activity in a number of cellular pathways with high spatiotemporal resolution. To date, optical control of kinase function has been achieved through the site-specific inclusion of (*i*) photolabile caging groups; and (*ii*) photosensory domains. With the exception of one report by Lawrence and co-workers in 2011,[7] caging moieties have been introduced onto key amino acid residues within the catalytic active site, masking enzymatic activity as a result (Figure 1A). Activation of the caged kinase only occurs when exposed to a suitable wavelength of light. Inspired by the natural photosensory light-oxygen-voltage (LOV) domain present in algae and plants, reversible photocontrol of kinase activity has been achieved by the inclusion of the LOV domain into the kinase of interest.[8] Light induced conformational changes of the LOV domain allosterically regulates the ON/OFF catalytic activity of the kinase (Figure 6). Examples of both photocaged and photoswitchable kinase enzymes are presented below.

2.1. Photocaged Kinase Enzymes

Bayley and co-workers masked the catalytic activity of the cAMP-dependent PKA through the introduction of a photolabile caging group onto either a cysteine (Cys199)[9] or phosphothreonine (Thr-197)[10] residue that reside in the 'activation loop' of the catalytic subunit of PKA. It was postulated that the inclusion of the 2-nitrobenzyl caging group

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Morten Grøtli is a Professor of Medicinal Chemistry at the University of Gothenburg. He studied received his Ph.D. in 1997 after working with Dr. Brian Sproat and Dr. Ramon Eritja at the European Molecular Biology Laboratory in Heidelberg. After a postdoctoral appointment at The Centre for Solid Phase Organic Combinatorial Chemistry, Carlsberg Research Laboratory (Denmark) with Professor Morten Meldal, he began his independent research career at the Biotechnology Centre of Oslo in 2000. In 2002 he moved to the University of Gothenburg as an Associate Professor in Medicinal Chemistry and in 2012 was appointed full Professor. His research interests include the development of kinase inhibitors, photopharmacology, and probes for imaging.

Joakim Andréasson was awarded his PhD from Chalmers University of Technology in 2002. He spent two years as a postdoctoral research fellow with Professor Devens Gust at Arizona State University and then returned to Chalmers starting his independent research group. Currently he is a full Professor. His main research interests are related to the design of molecular photoswitches and their use in molecular information processing and photopharmacology.

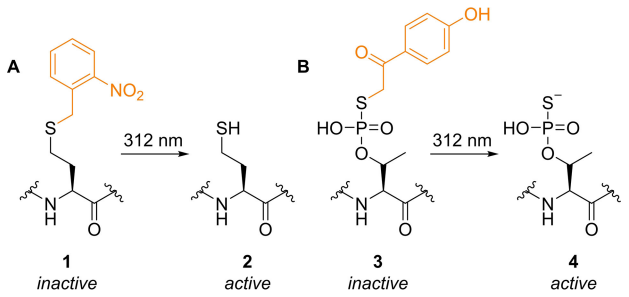

Figure 2. Optical control of PKA activity was realised by the use of photocaged (A) cysteine and (B) phosphothreonine residues present in the activation loop of the catalytic subunit.

(Figure 2A) on the cysteine residue disrupts the native conformation of the activation loop, impeding enzymatic activity as a result. Exposure to UV light (312 nm) saw a 20–30-fold increase in enzymatic activity.[9] The phosphorylation of the threonine residue regulates PKA activity. The 4-hydoxyphenacyl caging group was attached to threonine by the thiophosphorylation of the residue with PKD1 in the presence of ATP(γ)S, followed by the treatment with 4-hydroxyphenacyl bromide. The caged PKA kinase was inactive as the presence of the bulky caging group prevents the kinase from obtaining an active conformation. The catalytic activity of PKA was restored (to 85–90% of the original value) upon irradiation with UV light (312 nm, Figure 2B).[10]

In 2011, Lawrence and co-workers reported on the development of a photoactivatable profluorescent cAMP-dependent kinase enzyme.[7] This was achieved through the use of an active site-directed peptide-based '*caging agent*' for the propinquity labelling of the Cys-343 nonactive site residue in the kinase (Figure 3). The covalently modified kinase exhibited minimal enzymatic activity (<3% residual activity) as well as low fluorescence due to the presence of the appended QSY7 fluorescent quencher. Upon exposure to light, the active site-directed peptide and the appended quencher moiety was cleaved, in turn restoring the catalytic activity of the kinase to 60–80% of the native catalytic activity. The activation of the cAMP-dependent kinase could be further monitored by the 10–20-fold enhancement of the fluorescent signal that arose due to the removal of the quencher and the simultaneous generation of a fluorescent response. The catalytic activity of cAMP-dependent kinases is largely responsible for the disassembly of stress fibres that link the cytoskeleton of the cell with the extracellular matrix. Using the rat embryonic fibroblast cell line REF52, the authors demonstrated that the caged-PKA could

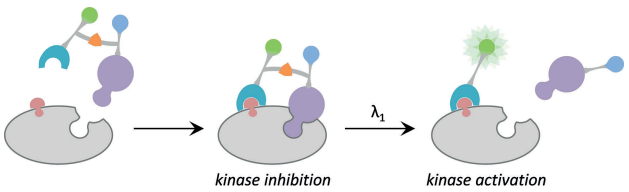

Figure 3. Schematic representation of the active-site directed "caging agent" reported by Lawrence and co-workers.[7]

be used to induce the disassembly of stress fibres in a light-dependent manner.

In efforts to realise a general approach to achieve optical control of kinase activation, Chin and co-workers looked at the replacement of a conserved lysine residue in the ATP binding pocket that is critical for the phosphorylation activity of the kinase, with a photocaged lysine derivative (**5a**, Figure 4), using genetic code expansion.[11] The presence of the caging group masked the catalytic activity of the kinase as the lysine residue was no longer available to orientate ATP appropriately in the catalytic domain and thus, ATP could not be accommodated for in the binding site. The caging group was only cleaved off when irradiated with UV light, which concurrently restored the catalytic activity of the kinase enzyme. To date, the authors have used this approach to create photoactivatable versions of the MAP kinase MEK1,[11a] as well as LCK.[11b] The development of such light-activated kinases enables the dissection of signaling transduction pathways as well as the study of the kinetics of the individual steps in these processes.

Deiters and co-workers[12] also employed genetic code expansion to introduce a photocaged lysine group (**5b**, Figure 4) into the MEK1 active site in zebrafish embryos, and successfully demonstrated the effects of in vivo MEK1 activation on embryonic development. The light-induced activation of MEK1 function at predefined time points in zebrafish embryos revealed a time window in which the activity of the MEK/ERK pathway is critical for dorsal/ventral patterning in developing embryos.

2.2. Photoswitchable Kinase Enzymes

Moffat and co-workers described the development of a light-responsive fusion kinase, YF1, by the replacement of the heme-binding Per-Arnt-Sim sensor domain of FixL from *Bradyrhizobium japonicum* with the photoresponsive LOV domain of *Bacillus sabtilis* (YtvA, Figure 5).[13] The parent enzyme, FixL, regulates the gene expression of proteins involved in microaerobic respiration, nitrate respiration and nitrogen fixation. This occurs via a two-step process in which FixL first undergoes autophosphorylation. The phosphate moiety is then transferred to FixJ, the response regulator. The in vitro phosphorylation activity of YF1 was measured using a radioactive assay. In the

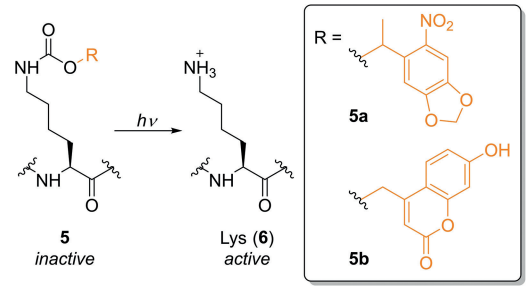

Figure 4. Photocaged lysine residues **5a** and **5b** introduced into the catalytic active site by genetic code expansion.

dark, YF1 exhibited catalytic activity analogous to that of the parent FixL. When exposed to white light, the phosphorylation of FixJ was inhibited by ~1000-fold. The fusion protein YF1 also displayed in vivo kinase activity, in which YF1 was expressed in *E. coli* and its ability to regulate gene expression in a light-dependent manner was determined using a β-galactosidase assay.

In 2016, Dagliyan et al. demonstrated reversible optical control of kinase activity through the inclusion of an allosteric LOV2 photosensory domain into the Src kinase, giving rise to OFF/ON catalytic activity that mimics the inactive/active state of the parent kinase.[14] Irradiation of the LOV2 domain with blue light (455 nm) results in increased flexibility and disorder of its terminal helices. Thereby the inclusion of the LOV2 domain into the Src kinase afforded light-responsive catalytic activity, in which the kinase activity was deactivated due to light-induced conformational changes of the catalytic domain, while upon returning to the dark, catalytic activity was restored (Figure 6). The authors further demonstrated its utility in live cells in which the light-responsive enzyme was expressed in SYF cells that lack Src, Yes and Fyn kinases. As Src is reported to play an important role in cell motility, irradiation of these cells with light resulted in reduced migration rates as well as changes in cell morphodynamics.

Employing a similar approach to that of Dagliyan et al., Anastasiou and co-workers inserted an allosteric photoresponsive LOV2 domain into the pyruvate kinase, PKM2.[15] However, instead of toggling reversibly between distinct inactive/active forms of the kinase, light was used to simply enhance enzymatic activity. Exposure to blue light (460 nm) resulted in light-induced conformational changes of protein structure which afforded a decrease in the Michaelis constant of the enzyme for phosphoenolpyruvate by 30%, in turn correlating to enhanced pyruvate kinase activity. Upon returning to the dark state, the less active conformation of the kinase was restored. The light-responsive PKM2 protein was expressed in HeLa cells and incubated in media containing ^{13}C-labelled glucose. Irradiation with blue light resulted in a significant increase in the labelling of pyruvate from glucose.

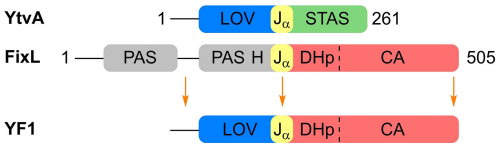

Figure 5. Design of light-responsive fusion protein YF1. Figure adapted with permissions from Reference [13], copyright 2009 Elsevier.

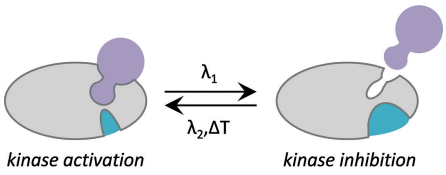

Figure 6. Allosteric control of kinase activity by the inclusion of a LOV2 photosensory domain (blue) into the enzyme. Kinase substrate is depicted in purple.

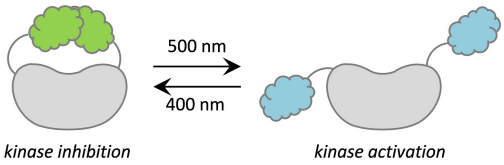

Figure 7. Schematic representation of photoswitchable kinases developed by Lin and co-workers.[16] Figure adapted with premissions from Reference [16], copyright 2017 AAAS.

In 2017, Zhou et al. described the development of photoswitchable kinases by the insertion of two photodissociable dimeric domains into the enzyme.[16] Firstly, the dimeric domain, pdDronpa, was engineered from the photodissociable tetrameric green fluorescent protein, Dronpa145 N. Exposure to 500 nm induced photodissociation as well as a significant decrease in fluorescence. The fluorescent properties and photoassociation was restored when irradiated with 400 nm. The dimeric protein was then introduced into MEK1 at two positions that flanked the active site. In doing so, pdDronpa prevented substrate access, rendering the kinase inactive as a result (Figure 7). Upon exposure to cyan light (500 nm), pdDronpa underwent photodissociation and kinase activity was turned on. This approach was also further extended to other serine/threonine kinases to afford photoswitchable Raf1, MEK2 and CDK5 kinase enzymes. The authors demonstrated how such photoresponsive kinase enzymes could be used for an all-optical method for screening kinase inhibitors, to investigate negative feedback in the Raf-MEK-ERK pathway as well as the effects of in vivo optical control of kinase activity on development and synaptic vesicle transport.

3. Light-Responsive Kinase Inhibitors

The use of light-responsive kinase inhibitors is regarded as a valuable means to probe the underlying molecular events of complex cellular processes. Effective optical control of kinase inhibitors has been achieved by (*i*) the use of photolabile caging groups (Figure 1C); and (*ii*) the inclusion of photochromic moieties into the molecular scaffold of the kinase inhibitor (Figure 1D). The first approach involves the strategic introduction of caging groups onto a region of the bioactive that is essential for binding to the target enzyme, rendering the inhibitor inactive. The inhibitory activity of the bioactive is only restored upon exposure to a certain wavelength of light, in which the caging group is cleaved off and the kinase inhibitor is liberated in its now active form, free to bind to its corresponding target. In the latter approach light is used to toggle reversibly between two forms of the inhibitor, in which one form has been designed to have a higher affinity for the target enzyme than the other.

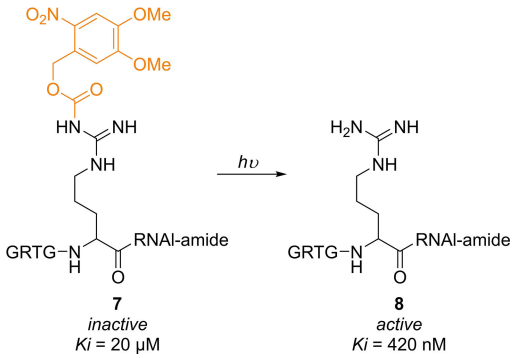

Figure 8. Photolysis of caged inhibitor **7**, affording the protein kinase inhibitor **8** in its active form.

3.1. Photocaged Kinase Inhibitors

While the initial report of a photocaged kinase inhibitor appeared in the literature in 1998,[17] it was not until 2012,[18] when the next photocaged inhibitor was published. Despite the utility of employing light to trigger the activation of kinase inhibitors at pre-determined time points in a biological setting, only a handful of photocaged kinase inhibitors have been documented in the literature.

Lawrence and co-workers reported the first caged kinase inhibitor **7** (Figure 8), for the photocontrolled inhibition of the cAMP-dependent kinase, PKA.[17] The 6-nitroveratryloxycarbonyl (NVOC) caging group was introduced onto the arginine moiety of the peptide-based kinase inhibitor **8**, as it serves as an important substrate recognition element for PKA. The caged-inhibitor **7** functioned as a poor inhibitor of PKA ($K_i = 20$ μM). Irradiation of the caged inhibitor with a Xe-arc lamp (> 300 nm) resulted in the generation of the active kinase inhibitor ($K_i =$ 420 nM). The utility of this caged inhibitor was further demonstrated in a rat embryo fibroblast cell line (REFs), which when treated with a PKA activator (CPT-cAMP) underwent pronounced morphological changes. These morphological changes in response to the CPT-cAMP stimulus were not observed only in the cells that were treated with the caged inhibitor **7** and subsequently irradiated with light.

In efforts to achieve spatiotemporal inhibition of Rho kinase, Morckel et al.[18] developed a photocaged Rho kinase inhibitor

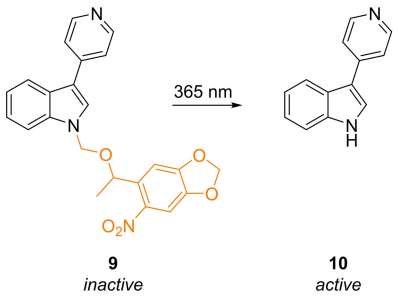

Figure 9. Photoactivation of Rho kinase inhibitor **10** upon irradiation of caged derivative **9** with UV light (365 nm).

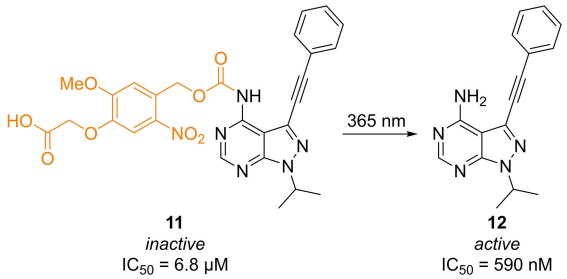

Figure 10. Photolysis of caged inhibitor **11**, affording the RET inhibitor **12** in its active form.

by the installation of the 6-nitropiperonyloxymethyl (NPOM) caging group onto a small-molecule Rho inhibitor. While no IC_{50} or K_i values are reported, the inhibitory activity of the caged Rho inhibitor **9** was shown to be light-dependent (Figure 9). When caged (compound **9**), inhibition of Rho activity was not observed. However, upon exposure to UV light (365 nm) and the subsequent release of the Rho inhibitor in its active form (compound **10**), the catalytic activity of Rho was inhibited by 91.2%. To further demonstrate the efficacy of the caged inhibitor, the authors employed **9** as a probe to regulate Rho activity in zebrafish embryos, in which Rho activity and its effect on gut epithelial morphology was controlled in a light-dependent manner.

In 2015, Bliman et al.[19] described the development of a caged RET kinase inhibitor (**11**). Masking of the inhibitory properties of the RET inhibitor **12** (previously reported by the Grøtli group)[20] was achieved by the inclusion of a NVOC-derived photolabile group onto the exocyclic amino moiety that partakes in key interactions within the hinge region of the ATP-binding site of the RET kinase (Figure 10). In a cell free assay, **11** modestly inhibited RET with an IC_{50} value of 6.8 μM. Photolysis of **11** with 365 nm afforded the active RET inhibitor **12**, in which a 12-fold increase in RET inhibition was observed ($IC_{50} = 590$ nM). Furthermore, the authors demonstrated the utility of the photocaged inhibitor **11** as a tool to probe the effects of spatiotemporal RET inhibition on motoneuron development in zebrafish embryos.

Peifer and co-workers have also contributed to this field with the development of photocaged derivatives of the FDA approved kinase inhibitors imatinib[21] and vermurafenib[22] (Figure 11). The inclusion of the 4,5-dimethyoxy-2-nitrobenzyl (DMNB) caging group at the amide moiety of the benzanilide group rendered the cage imatinib derivative **13** a poor inhibitor of PDGF–R ($IC_{50} = 5.8$ μM). The decaging reaction proceeded upon exposure to UV light (365 nm) to release the known inhibitor, imatinib, in its active form, with an IC_{50} value of 0.089 μM.[21]

The photocaged vermurafenib derivative **14** was realised through the introduction of the same DMNB photolabile group onto the central azaindole-NH moiety of vermurafenib, known to partake in important interactions with the hinge region of the ATP-binding site of the BRAF kinase.[22] A binding affinity of 440 nM was observed for the caged compound **14** for BRAF, which is 43-fold weaker than that of vermurafenib itself ($K_d =$

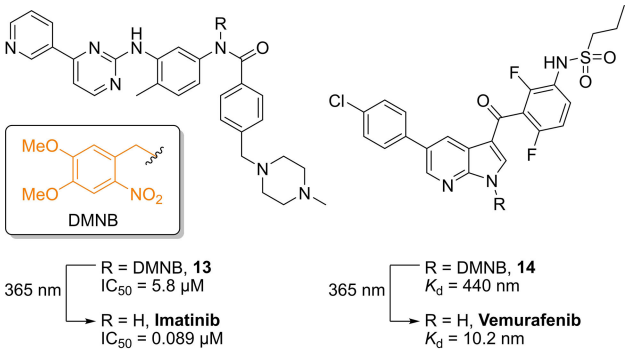

Figure 11. Photoactivation of known kinase inhibitors, imatinib and vemurafenib, upon irradiation with UV light (365 nm).

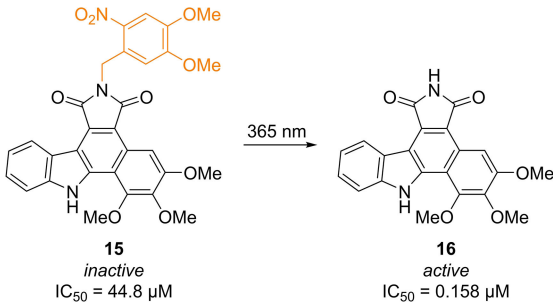

Figure 12. Photolysis of caged inhibitor **15**, affording the VEGFR-2 inhibitor **16** in its active form.

10.2 nM). To demonstrate the utility of compound **14**, the melanoma cell line SkMel13 was treated with **14**, in which no antiproliferative activity was observed. Only when the treated cells were exposed to UV light (365 nm) was the antiproliferative activity of vermurafenib restored (GI_{50} = 1.5 μM).

Peifer and co-workers have also described the development of the photocaged inhibitor **15** for the receptor tyrosine kinase, VEGFR-2 (Figure 12).[23] The inhibitor properties of the known VEGFR-2 inhibitor **16**[24] (IC_{50} = 0.158 μM), was masked by the inclusion of the DMNB caging group onto the NH moiety of the maleimide hinge binding motif (IC_{50} = 44.8 μM). The antiproliferative activity of **15** was evaluated on VEGFR-2 dependent PC-3 cells, in which a GI_{50} value of 34.6 μM was observed prior to UV irradiation. However, upon exposure to 365 nm, the DMNB caging group was removed and the antiproliferative properties of **16** restored, in which a GI_{50} value of 0.2 μM was obtained.

In contrast to the development of caged kinase inhibitors, in which the decaging reaction results in the release of the inhibitor in its active form, Lawrence and co-workers describe an alternate approach whereby light is used to trigger the *deactivation* of a peptide-derived inhibitor.[25] A known bivalent peptide-derived Src inhibitor was modified to contain a photolabile moiety that serves to position the peptide appropriately for kinase inhibition. Exposure to light splits the bivalent inhibitor, resulting in the release of the inhibitor from its binding site as well as the restoration of the catalytic activity of the kinase (Figure 13).

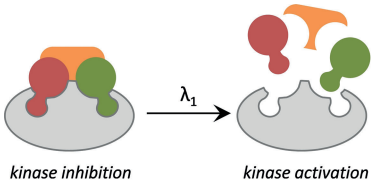

Figure 13. Schematic representation of photodeactivatable Src kinase inhibitor described by Lawrence and co-workers.[25]

3.2. Photoswitchable Kinase Inhibitors

In 2014, Yi et al. developed photoswitchable inhibitory peptides of cyclic-AMP dependent kinase (PKA) and MLCK (Figure 14).[26] Known inhibitory peptide sequences of PKA and MLCK were incorporated into the natural photosensory LOV2 domain. In the absence of light, the inhibitor sits close to the LOV2 domain and is unable to bind to its corresponding target due to steric bulk. However, when exposed to blue light (460 nm), the LOV2 domain undergoes significant conformational changes and orientates the inhibitor appropriately for binding to the kinase. For cells expressing the photoswitchable PKA inhibitor, PKA phosphorylation was reduced by ~50% when exposed to blue light. Furthermore, the PKA inhibitory peptide exhibited light-dependent inhibition of CREB phosphorylation in live cells. The inhibitory properties of the photoswitchable MLCK inhibitor was also shown to be light-dependent, in which a ~70% decrease in MLCK activity was observed when exposed to light (460 nm). When COS-7 cells were treated with the light-responsive MLCK inhibitor, blue light could be used to induce changes in cell morphodynamics.

The first example of a photoswitchable small molecule kinase inhibitor was described by Ferreira et al. in 2015.[27] The development of the photoswitchable RET kinase inhibitor **17** was achieved by exchanging the alkyne moiety of the known RET inhibitor **12**[20] with a photochromic azobenzene moiety (Figure 15). Docking studies suggested that the *E*-form of **17** could bind to the ATP-binding site of the RET kinase in an analogous fashion to that of **12**, while the geometry of the *Z*-form (**17-*Z***) would not be accommodated for in the RET active site, and thereby serve as a poor inhibitor of RET. Photoisomerisation of the *E*→*Z* form was achieved by irradiation with

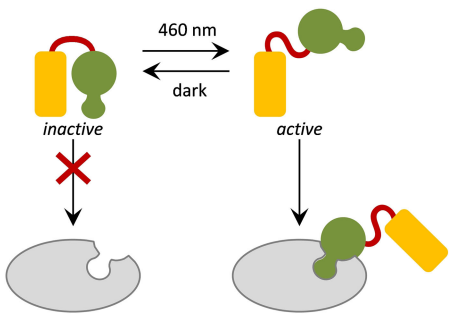

Figure 14. Schematic representation of photoswitchable inhibitory peptide reported by Hahn and co-workers.[26]

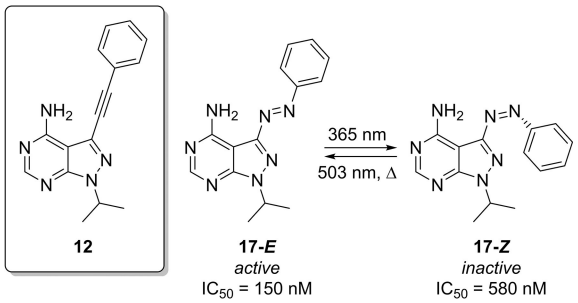

Figure 15. Photoswitchable RET inhibitor **17** derived from known RET inhibitor **12**.

365 nm light, in which a photostationary distribution of 87% of **17-*Z*** was obtained. The reverse reaction could also be achieved either thermally or upon exposure to visible light (503 nm). Furthermore, the reversible photoisomerization cycle could be repeated 10 times without exhibiting any signs of photo-fatigue. Cell free studies in a RET biochemical assay showed a 3.8-fold difference in inhibitory properties between the two photo-isomers, with IC_{50} values of 150 nM and 580 nM for the *E*-form and the photo-enriched *Z*-form, respectively. The inhibitory effects of the photoswitchable RET inhibitor **17** was also demonstrated in a whole cell functional assay, in which IC_{50} values for the active *E*-form and the inactive *Z*-form of 3.8 μM and 12 μM, respectively, were obtained.

Recognising the structural similarities between the known bisindolylmaleimide (BIM) family of ATP kinase inhibitors (**18**) and the photochromic diarylmaleimide moiety, Branda and co-workers describe the development of a photochromic PKC inhibitor in which the photochromic diarylmaleimide moiety has been incorporated into the pharmacophore of a BIM-derived kinase inhibitor (Figure 16).[28] The *s-trans-s-trans* conformation of the open form (**19-*i***) can be readily accommodated for in the ATP-binding site of PKC, thus serving as the active form. However, in order to photoisomerise to the inactive closed form (**19-*C***), **19-*i*** must reorientate to adopt a *s-cis-s-cis* conformation (**19-*O***). Unfortunately, the photoinduced ring-closing reaction of diarylmaleimide derivatives is highly dependent on the polarity of the solvent system, and cannot occur in highly polar environments.[29] This is largely due to the presence of a twisted intramolecular charge transfer state that is populated upon photoexcitation and surpasses that of the ring-closing reaction.[29] As the photocyclisation reaction is suppressed in aqueous medium, reversible photoisomerisation between the open and closed form of **19** had to be conducted in organic solvent. Nevertheless, the authors were able to demonstrate that the in vitro activation of **19** could be readily achieved when an isolated sample of **19-*C*** was irradiated with 490 nm to afford the active open form, **19-*i***, which inhibited PKC with an IC_{50} of 580 nM.

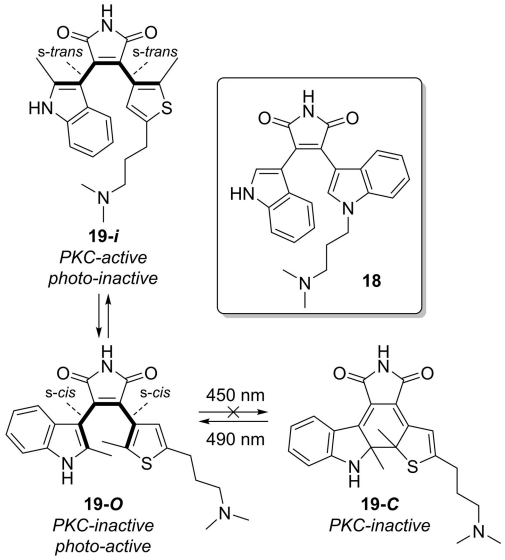

Figure 16. Photochromic PKC inhibitor **19** derived from the known PKC inhibitor **18**.

In efforts to achieve both isoform selective and optically controlled MEK inhibition, Chin and co-workers employed a bioorthogonal ligand tethering (BOLT) approach, in which the MEK1/2 inhibitor **20** is covalently attached to a modified amino acid residue on the protein surface of MEK1 through a photoswitchable linker (compound **22**, Figure 17).[30] When in the *trans*-form, the allosteric inhibitor should be able to bind to the kinase, thus preventing phosphorylation, while in the *cis*-form, the inhibitor is no longer positioned appropriately to bind to MEK1, restoring the catalytic activity as a result. Reversible optical control of MEK1 catalytic activity in live HEK293ET cells was observed, in which 360 and 440 nm was used to toggle between the *cis*/*trans*-isomers.

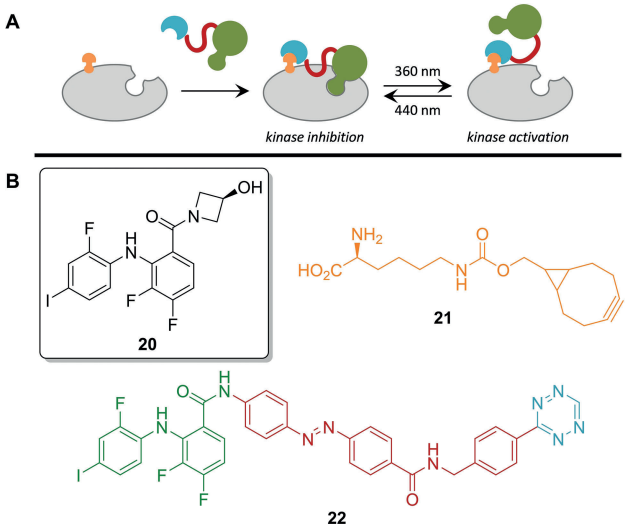

Figure 17. Optical control of MEK1 activity using a photoswitchable BOLT approach. (A) The MEK1/2 inhibitor (green) is conjugated to a reactive group (blue) via a photoswitchable linker (red). Following incubation with the ligand (compound **22**), the target kinase is inhibited. Upon exposure to 360 nm, the linker isomerises from the *trans*- to the *cis*-form, resulting in the activation of the kinase; (B). MEK1/2 inhibitor **22**. Unnatural amino acid **21** introduced onto the protein surface of MEK1. The inhibitor is conjugated to the reactive group via an azobenzene linker **22**. Figure (A) adapted with permissions from Reference [30], copyright 2015 Nature Publishing Group.

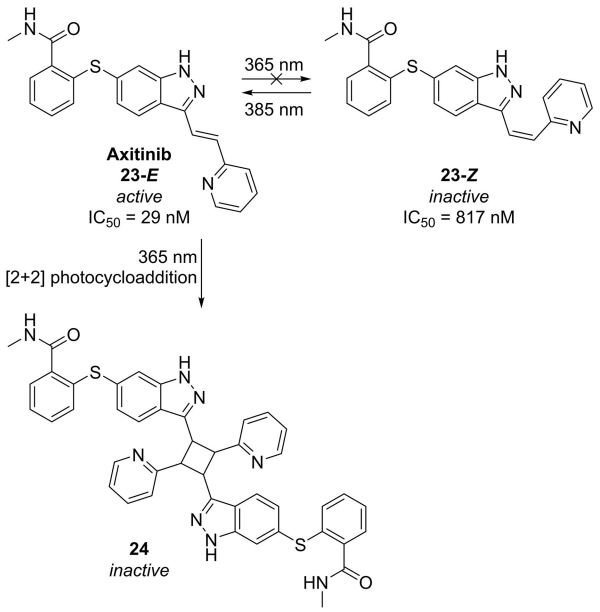

Figure 18. Photoinduced isomerisation of known VEGFR2 inhibitor Axitinib (**23-*Z***→**23-*E***) upon exposure to 385 nm, as well as the [2+2] photocycloaddition of **23-*E*** to **24** when irradiated with light of wavelength 365 nm.

Most recently, Schmidt et al. investigated the photoinduced isomerisation of the known VEGFR2 kinase inhibitor, Axitinib (**23-*E***), which consists of the photoresponsive stilbene moiety that should allow for reversible *E*/*Z*-isomerisation (Figure 18).[31] As molecular modelling studies showed that in the *Z*-isomer (**23-*Z***) is unable to participate in critical interactions with the hinge region of the active site and therefore should function as a poor inhibitor of VEGFR2, it was envisioned that photoinduced isomerisation could be employed to regulate the inhibitory activity of Axitinib 'OFF' and 'ON'. While reversible switching was readily demonstrated in DMSO, unfortunately *E*→*Z* photoisomerisation in aqueous solution was not realised as a competing irreversible [2+2]-photocycloaddition reaction to afford the inactive Axitinib dimer **24** was observed. The authors demonstrated optical control of Axitinib in a VEGFR2 kinase assay. In the absence of light, **23-*Z*** exhibited an IC_{50} value of 817 nM. Upon exposure to 385 nm light, **23-*Z*** was photoisomerised to the corresponding active form, **23-*E***, in which an IC_{50} value of 29 nM was obtained. When these experiments were repeated in a HUVEC proliferation assay, **23-*Z*** exhibited an IC_{50} value of 26 μM. However, after the light-induced *Z*→*E* isomerisation (385 nm), the inhibitory activity was found to increase by only a factor of 2.6 (**23-*E*** IC_{50} = 10 μM), suggesting that the *Z*→*E* photoisomerisation was not as efficient in a cellular context.

4. Summary and Outlook

Light serves as a valuable external stimulus to probe biorelevant mechanisms as it is non-invasive, can be delivered with high spatiotemporal resolution and is readily controlled by simply adjusting the wavelength and intensity. To date, much effort has been devoted to the development of valuable light-responsive tools to study complex cellular pathways. The role of many kinases in signal transduction pathways and various diseases is poorly understood. In this minireview, we highlight the progress made towards the development of light-responsive kinase enzymes and small molecule inhibitors to probe the role of kinases in both health and disease states.

Photolabile caging moieties have been strategically introduced onto (*i*) kinase enzymes to mask their catalytic activity; and (*ii*) small molecule kinase inhibitors to prevent them from binding to their corresponding target. Catalytic activity or the ability to bind to the target is only restored when exposed to light. To date, all examples employ the use of UV light to initiate the decaging reaction, therefore impeding their utility in more advanced animal model systems. The toxicity of UV light towards biological samples is well established, in which extensive exposure may result in mutations and induce cell apoptosis.[32] Furthermore, UV light exhibits poor tissue penetration in which depths of only a few millimetres can be reached.[33] To the best of our knowledge, photocaged kinase enzymes and inhibitors that employ longer wavelengths have yet to be reported. As such, we should turn our focus to employing caging groups that utilise longer wavelengths, as well as the use of two photon methods.

Unfortunately, the use of caging groups does not allow for reversible optical control over kinase activation/inhibition. The activation of either the kinase itself or the small molecule inhibitor can only occur at a single predefined time point. The use of photoswitchable entities enables one to obtain dynamic and reversible control over both kinase activation and inhibition, however, other critical parameters have to be considered when photoswitches are employed. These include:

(a) The ratio of the two photoisomers at the photostationary state. The OFF/ON changes in catalytic activity directly correlate to the percentage of the sample that undergoes photoisomerisation to the respective isomeric form. Ideally, a quantitative conversion from one form to the other occurs when exposed to light. However, this is often not the case due to spectral overlap of the two forms.
(b) The thermal stability of the photo-enriched isomer following irradiation. Preferably, the photo-enriched species is stable in the dark until photoisomerisation to the respective isomer occurs.
(c) The photostability of the photoswitch should be good enough to allow for repeated toggling between the two forms without the occurrence of photodecomposition.

Photoswitchable kinases that exhibit OFF/ON catalytic activity that mimics that of the parent enzyme have been achieved by either the inclusion of an allosteric LOV2 photosensory domain or the insertion of a photodissociable dimeric domain into the kinase of interest. It is also worth noting that all examples employ longer wavelengths (400–500 nm) for isomerisation compared to the UV light used for the decaging reactions.

Due to the challenges regarding the design and synthesis of photoswitchable kinase inhibitors in which significant differ-

ences in the affinity for the target between the active and inactive form is achieved, only a handful of examples have been reported. In order to achieve distinct OFF/ON switching of kinase activity, the difference in the affinity for the target between the active and inactive form should be significant (> 100-fold). To date, only a modest difference in kinase activity between the two forms has been observed.

As the field of photopharmacology continues to rapidly evolve, so does the possibilty to move past the simple '*Proof-of-Concept*' phase into the next stage in which these light-responsive tools can be applied in advanced pre-clinical biological systems. The ability to selectively probe the relationship between kinase activity and cellular behaviour, with high spatiotemporal resolution, will further our understanding of the role that kinases play in cell signalling pathways, and may even give rise to the identification of new therapeutic targets.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: chemical biology · kinase inhibition · photocaging · photopharmacology · photoswitching

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