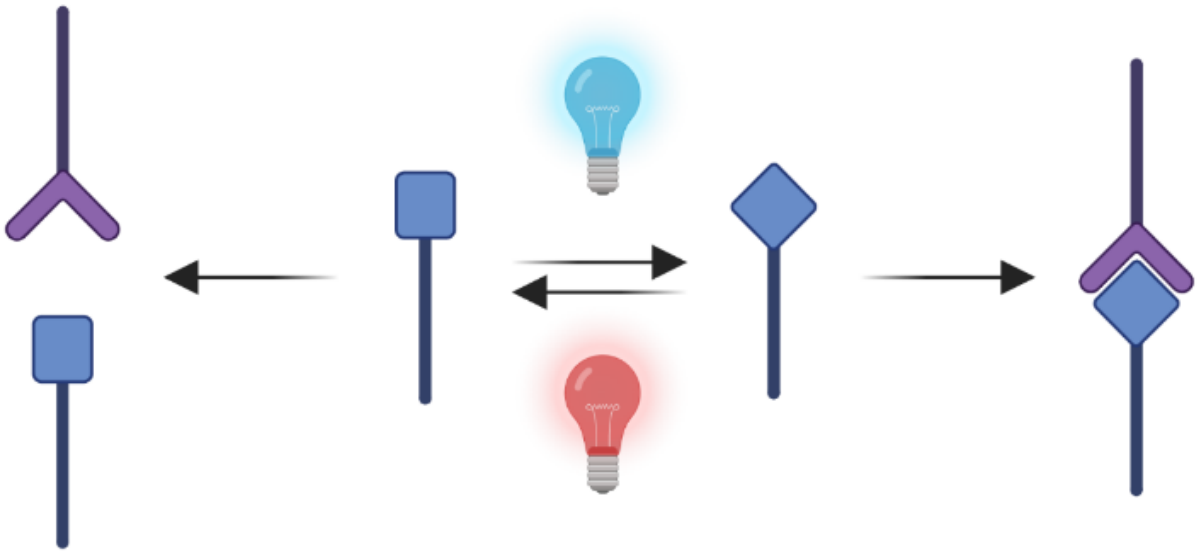
A review of molecular photoswitches and their potential applications in photopharmacology



Writing assignment | Drug Innovation

Supervised by Dr. ing. Tom Wennekes

Lin Rietveld | 5832055

April 2023

Abstract

Lack of selectivity is the main cause of failure for many experimental drugs. In order to improve selectivity, better control over its activity is necessary. Modulating the activity of drugs with light could strengthen this control as it can be applied to tissue with high precision. Photopharmacology is an upcoming field in research that utilises light in medicine. By adding a photoswitchable group in bioactive components, pharmacological processes can in theory be regulated externally with great spatiotemporal control. This review aims to introduce this emerging field with a focus on the photoswitches themselves. Many photoswitchable moieties are mentioned in literature and each has their own mechanism and advantages. Azobenzene is recognised as the most common and widely applied photoswitch, as such it is discussed in the greatest detail in this review. The review also covers other commonly used and trending photoswitches and discusses the benefits of using these switches compared to azobenzene for pharmacological functions.

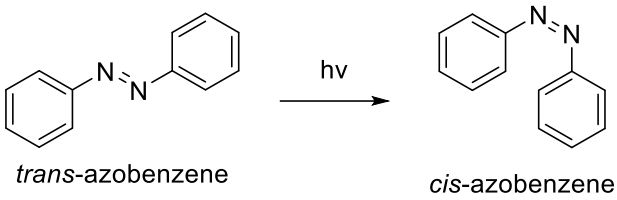
Layman's summary

Light influences many processes in the human body. It stretches from a straightforward manual reaction when a traffic light turns green, to a more complicated autonomous reaction like our sleep cycle. Light controls the sleep cycle by blocking the production of the sleep hormone melatonin which allows you to stay awake during the day. When it gets darker, melatonin levels increase, making you fall asleep. This level of control that light can have on the processes in our body, is also actively investigated in various research fields. One of these fields is photopharmacology, which is the focus of this review.

In photopharmacology, light is used to control medicine. Light is especially beneficial in this process since it can be applied non-invasively and with high precision from outside the body. In medicine, this will ideally translate into a higher efficacy of drugs and a lower amount of side effects. However, it does require an addition to the drug molecule that interacts with light. Photopharmacology uses components that are called photoswitches which change when exposed to light. This change can, for example, activate medicine to find its target, but only when it is in the area that is exposed to light. This could especially be useful in cancer therapy where the difference between healthy cells and cancerous cells are hard to distinguish for drugs. By only exposing the diseased parts of the body to light, the healthy cells will not be affected and thus lower common side effects of anti-cancer drugs. Many distinct types of photoswitches exist with each their own advantages. This review is an overview of these photoswitches and their characteristics to introduce the reader into how photoswitch work and their emerging applications in photopharmacology.

Introduction

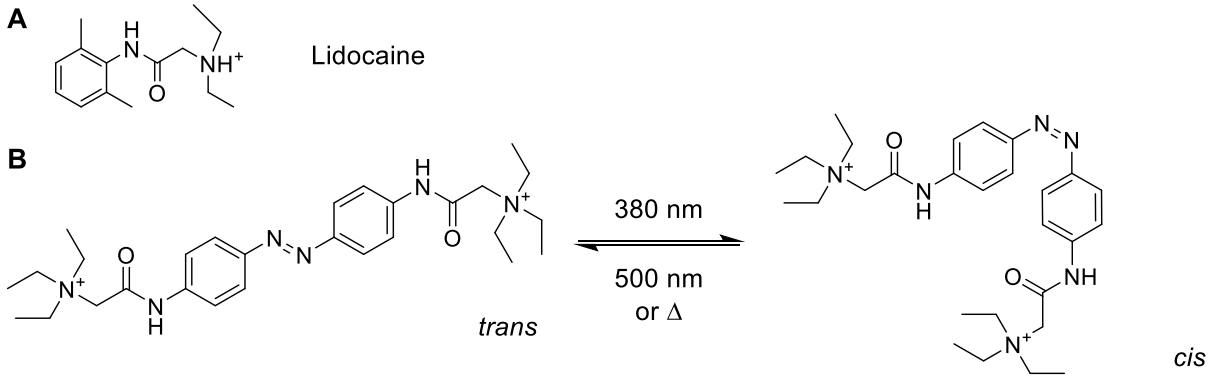
In 1937, G. Hartley reported in the journal Nature that he experienced trouble while replicating a solubility study of azobenzene using a photometric method (1). An increase in light absorption was observed after exposure to light. The absorption increase was even more rapid when the azobenzene solution was stored in a glass bottle exposed to bright daylight. After multiple tests they concluded that this observation was consistent with the reversible formation of *cis*-azobenzene when exposed to light.



Scheme 1. The conformational change of trans*-azobenzene triggered by light.*

Since then, the use of light to control molecular structures has been thoroughly investigated for use in many research subjects (2–6). The focus of this review is on the possibilities in the field of medicine. Light is an ideal tool to obtain spatiotemporal control as it is not natively present in most of the systems in the body. It can also be applied highly selectively and non-invasively. Obtaining remote control through light over chemical and biological processes could improve the precision of medicine and thereby reduce severe adverse effects (7,8). Photoswitchable compounds undergo conformational changes triggered by light at a specific wavelength. Installing said photoswitch structures at the right position of a molecular system creates the possibility to activate or inhibit a process associated with that system using specific wavelengths.

After the discovery by G. Hartley, azobenzene was defined as a photoswitch compound and has attracted a lot of attention in the research field of photopharmacology. Research has already reached *in vivo* studies in rats (9). Azobenzene was modified with a quaternary ammonium group on both sides to resemble a derivative of lidocaine, a local anaesthetic (Scheme 2). This compound enters cells selectively and silences pain-sensing neurons by blocking voltage-gated ion channels. However, the silencing goes on for many hours. The photoswitch derivative achieves the same anaesthetic effect in the *trans* conformation. However, the blockage was removed when the molecule was switched to the *cis* conformation by exposure to 380-nm light. This lidocaine derived photoswitch compound was administered to the eye of rats in combination with pain-invoking capsaicin. The rats showed a decreased response to the pain when exposed to ambient light and by exposure to 380-nm light, the decrease in pain was eliminated.



Scheme 2. A) The structure of anaesthetic Lidocaine; B) the photoswitchable derivative in its bioactive trans and inactive cis isoform.

Although the effect of the switching was very clearly observed in the *in vivo* study, the situation was not representative for all parts of a living organism. They administered the photoswitch in the eye as this is easily exposed to light. For a photoswitch to work in other parts of the body, the wavelengths necessary for switching needs to be closer to the near-infrared range (650-900 nm) for it to be safe and able to penetrate deep enough in tissue (2,10). The tissue penetration depth is dependent on the light wavelength and is visually represented in Figure 1. Other important characteristics of the photoswitch to consider for pharmacological purpose are solubility under physiological conditions, the switching mechanism, and the switching reversibility. These aspects can be optimised by modifying the main structure using several strategies.

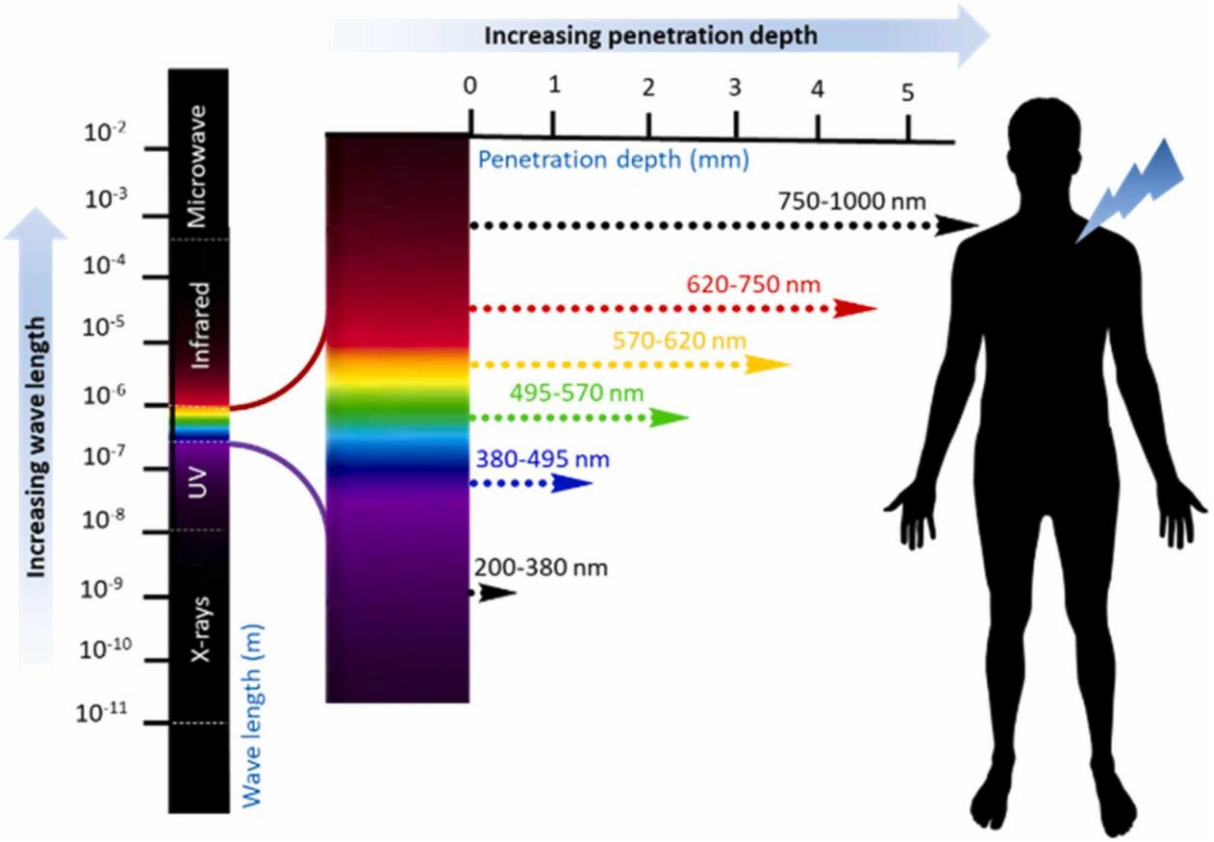


Figure 1. A schematic representation by Rad et al. on the tissue penetration depth based on light wavelength (11).

This review will first discuss the switching mechanism of azobenzene in detail. Then, several other photoswitch structures are reviewed that are being actively investigated for photopharmacological applications and the mechanisms of these photoswitches are compared to the switching mechanism of azobenzene. Additionally, the wavelength, water solubility, and switching reversibility of these photoswitches are specified and any strategies used to improve on these aspects. Finally, applications of these structures, with a focus on photopharmacology, as reported in the scientific literature of the last 10 years are discussed. The advantages of each structure are summarised and the important photoswitch features are compared, which are essential characteristics to be able to take full advantage of the photoswitch function.

The switching mechanism of azobenzene

The switching mechanism of azobenzene was first defined in 1982 by Rau and Lüddecke (12). It was already established that isomerisation of ethylene-derived compounds is enabled by weakening of the double bond. Planar *trans*-azobenzene strongly absorbs light with a wavelength of 320-380 nm (13). This amount of energy excites an electron from the pi to the pi* orbital, transitioning the molecule into the S_2 (pi,pi*) excited state. In this state, the double bond can rotate and change to the non-planar *cis*-conformation, thereby partially releasing the absorbed energy.

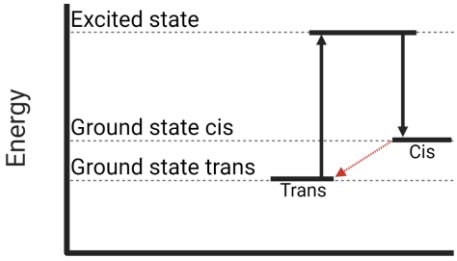


Figure 2. Simplified energy scheme representing the switching from trans- to cis-azobenzene. The red arrow represents thermal relaxation.

For azobenzene, the lone pairs of electrons from the nitrogens are also available during this process and that provides another transition state that is lower in energy. Light with a wavelength of 440 nm leads to excitation of a nitrogen lone pair electron from a non-bonding orbital to pi* and thus a S_1 (n, pi*) ← S_0 transition. Isomerisation in this pathway is assumed to be via inversion wherein the N=N-C angle increases to 180° and the nitrogen atom is temporarily sp hybridized (13,14). Relaxation from this state then either forms *cis*-azobenzene or *trans*-azobenzene which will repeatedly be excited until it forms the *cis*-isoform.

The absorption pattern of *trans*-azobenzene shows a strong absorption peak at 320 nm and a weaker absorption at 440 nm (14,15). This leads to the assumption that isomerisation mainly happens by rotation through the S_2 state with a slight contribution of switching by inversion via the S_1 state. However, Rau and Lüddecke found that excitation to the S_2 state could still lead to inversion by converting the S_2 state to S_1 state. They restricted the rotational mechanism by modifying the molecule. This lead to a relaxation pathway wherein S_2 first relaxes to S_1 via internal conversion (12). In this situation, the excited pi* electron stays in that orbital and first one of the non-bonding electrons falls back to the pi orbital. This relaxes the molecule into a S_1 (n,pi*) state and allows for isomerisation via inversion. Next, the excited pi* electron falls into the now available non-bonding orbital, returning the molecule back to the ground state.

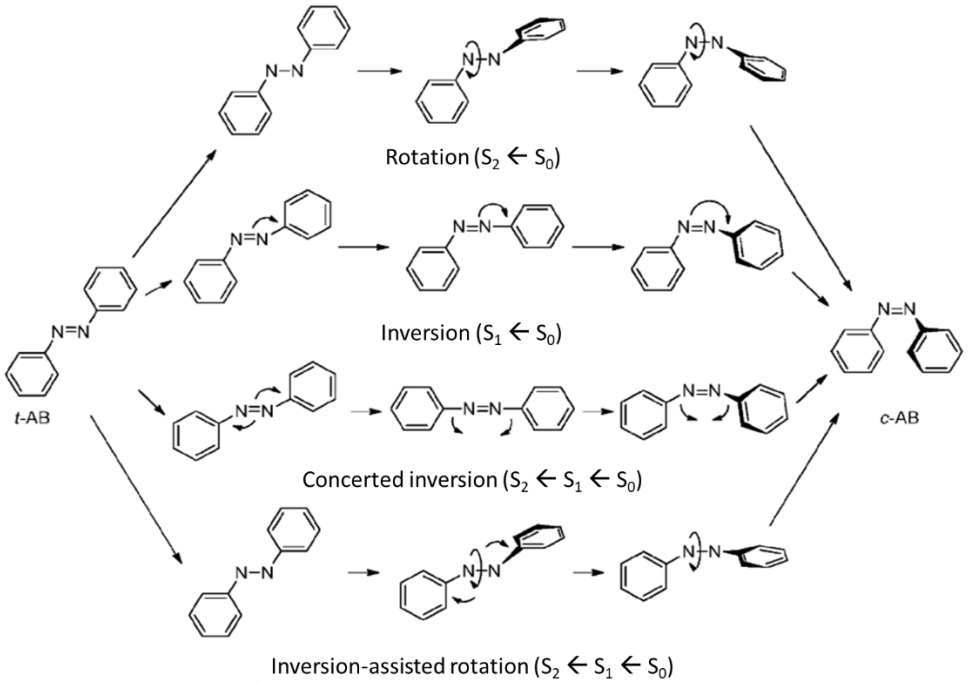


Figure 3. The four proposed isomerisation mechanisms with involved excitation states by Bandara et al. (14)

Additionally, Fujino *et al.* revealed that azobenzene in the S_2 state almost exclusively relaxes to the S_1 state (16). Thus, meaning that the rotation mechanism in S_2 is dubious. But they also reported that isomerisation via the inversion pathway according to calculations is unlikely (14). Bandara *et al.* suggested two other isomerisation routes following relaxation of S_2 to S_1 (17). One of these is the concerted inversion route in which the angles of both N=N-C bonds are straightened simultaneously. The other mechanism, inversion-assisted rotation, requires both straightening one of the N=N-C bonds and rotation of the N=N bond. All four potential isomerisation mechanisms are visualised in Figure 3.

The switching from *cis*- to *trans*-azobenzene is slightly different. The pi to pi* transition of *cis*-azobenzene requires more energy compared to *trans*-azobenzene and thus the absorption peak is blue-shifted to 280 nm (13). This is due to the electrostatically favourable formation of T-shaped benzenes in the cis-isoform as shown in Figure 4. However, the absorption for the n to pi* transition remains the same and has a stronger absorption peak. Reverse shifting should therefore be possible at a wavelength of 440 nm. This mechanism has however not yet been established in experimental studies (14,18). It is suggested that the switching mechanism is similar as for *trans*-azobenzene (19). However, switching from *cis* to *trans*-azobenzene is mainly performed via slow thermal relaxation.

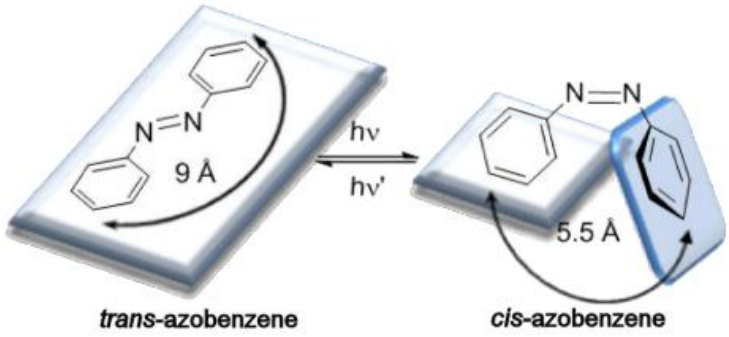


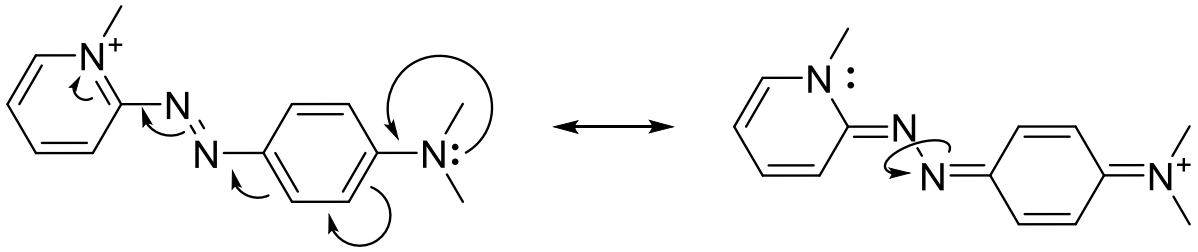
Figure 4. Visualisation of the dihedral angle between the two phenyl groups by Merino et al. (19) *These planes are in line as trans-azobenzene, but shift perpendicular when the conformation changes to cis.*

A review of the literature indicates that the correct switching pathway for both ways has not yet been conclusively proven, and many experimental observations have been explained using various differing potential pathways. However, it is quite likely that the switching mechanism is mostly dependent on steric hindrance of movement and electronic states and can thus be different for each azo-benzene structure and their substitute pattern.

Azobenzene derivatives

To improve the properties of the unsubstituted azobenzene, several alterations can be made. For better aqueous solubility, polar functional groups can be added. The switching wavelengths can be improved as well with this strategy. The addition of electron-donating and electron-withdrawing groups to the phenyl rings red shifts the absorption pattern (14,15). These so-called 'push-pull' azobenzenes have an electron-donating group on the *ortho* or *para* position of one phenyl ring and an electron-withdrawing group on the ortho or para position on the other phenyl ring. The changes this causes in the delocalisation of the conjugated electron pairs, destabilise the double bond and thereby lowers the energy necessary for excitation. Rustler *et al.* designed their photoswitch as a push-pull azobenzene to avoid using UV light (20). They installed an electron-withdrawing nitro group on the *para* position of one phenyl while an electron-donating group was already present on the other *para* position for functional reasons. Visible blue light (420 nm) was now able to penetrate guinea pig ileum tissue and switch the molecule from *trans* to *cis*.

However, this push-pull system shortens the half-life as it destabilises the photostationary *cis*-isoform as well. Especially an additional electron-donating group with a lone pair speeds up the thermal relaxation in a polar protic solvent, such as water (21). Possible tautomerism of the N=N double bond allows for free rotation of the phenyl group back to the *trans*-isoform as shown in Scheme 3. This mechanism forms an intermediate that can also act as a photoswitch but is known as a hydrazone switch which will be discussed in the next section.



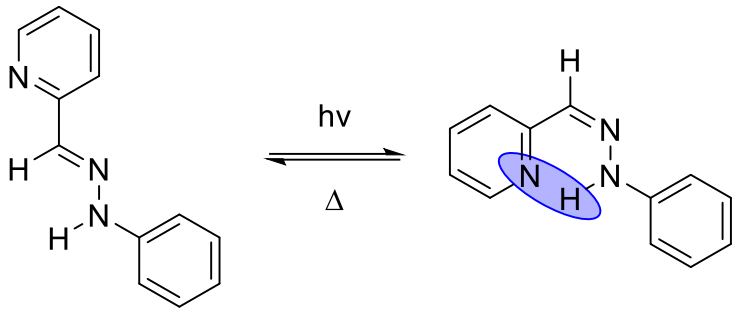
Scheme 3. Resonance structures showing the tautomerism that can take place with push-pull azobenzenes.

Another adjustment of azobenzene is replacing a phenyl ring for other heteroaryl functional groups, like pyrazoles. Most of these adjustments are also chosen to produce this 'push-pull' effect and thus have short half-lives. However, substituting one of the phenyl groups with a methyl-pyrazole surprisingly increased the thermal half-life (22). It is assumed that the *cis*-isoform is stabilised due to possible interaction of 5-membered ring with the phenyl group which are perpendicular to each other in the *cis*-conformation shown in Figure 5 (23). Adam *et al.* used arylazopyrazoles to gain photocontrol over DNA hybridisation (24). Several arylazopyrazoles were incorporated in the DNA strand via solid phase DNA synthesis. Switching the formation to *cis* showed efficient closing of the DNA hairpin where the *trans*-formation an open hairpin. Using pyrazoles instead of benzenes increased the thermal stability and thereby the half-life of the *cis*-isoform, and red-shifted the wavelengths to 365 nm (*trans* to *cis*) and 590 nm (*cis* to *trans*). A review by Crespi *et al.* extensively discusses the effects for several other heteroaryl substitutions (25).

Figure 5. Proposed mechanism by Calbo et al. for the stabilisation of the cis-conformation with a methyl-pyrazole as aromatic substitution (23).

Hydrazones

Hydrazone switches contain a C=N-NH pattern and their conformation is dependent on the imine bond. The available lone electron pair of the nitrogen is expected to provide a similar effect as was found for azobenzene. However, the advantages or disadvantages of imine bond switching over azo bond switching is not really discussed in literature. Beside the imine bond, another major difference with azobenzene is that the nitrogens of the hydrazone do not necessarily have to be directly connected to an aryl group. But the same strategies can be used to red-shift the switching wavelengths. The downside of hydrazones is that the photostationary state is quickly reversed via thermal relaxation. However, installing an H-bonding acceptor that can interact with the C=N-NH proton can greatly increase the half-life (Scheme 4). For example, this is possible by using a pyridine as aromatic group to form a hydrogen bond in the metastable state. Such a modification can even prolong the half-life up to thousands of years and forward and back switching was still possible in aqueous solutions.

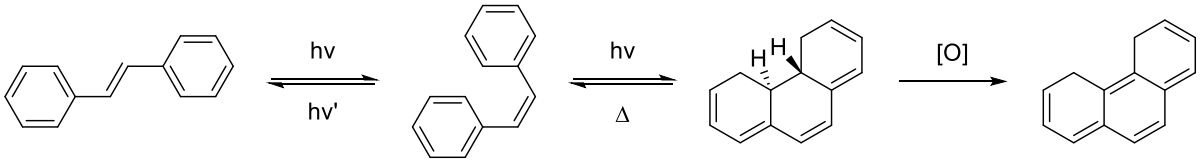


Scheme 4. The conformational change of hydrazone with a pyridine functional group. The metastable cis-isoform is stabilised by hydrogen bond formation marked in blue.

Guo *et al.* used hydrazone structures in their nanoparticle system to target tumour cells with anti-cancer medicine doxorubicin (26). They developed a hydrazone with not only a switching wavelength within the visible range (*cis* to *trans*: 450 nm) and a thermal half-life of up to 5300 years (extrapolation of an obtained Arrhenius plot). But they also modified it with the ability to be emissive, but only in the *cis*-isoform. The *cis*-isoform would exhibit fluorescence with a wavelength of 575 nm when irradiated with 430 nm light. UV light of 340 nm could switch the molecule from *trans* to *cis*, but reverse switching was not exploited for their purpose. They embedded this hydrazone in the side chains of a copolymer that the nanoparticle is composed of and loaded the particle with doxorubicin. By switching the hydrazones to the *trans*-isoform, they increased the size almost 10-fold which allowed form the drug to be released. Afterwards, the particle would disintegrate. Irradiation of the particle with 575 nm light allowed them to track cellular uptake and distribution. They were able to show that the nanoparticles were internalised in the cells and that switching to *trans* led to cell toxicity. This system showed the possibility to deliver drugs in a light-controlled manner.

Stilbenes

Stilbenes are structurally very similar to azobenzenes but contain an alkene group instead of an azo group. This still allows for switching via double bond isomerisation by weakening the double bond between the carbons. However, isomerisation via n-pi* excitation is not available and thus, switching can only occur via rotation. This increases the half-life of the photostationary state as well since a higher energy transition is necessary for thermal relaxation. It is stated that thermal relaxation of the *cis* isomer does not typically happen at room temperature as it does with azobenzene. An advantage is that it allows for more control on reverse switching (21,27). However, an important disadvantage of this photoswitch moiety is that it switches at low wavelengths that damage living organisms; 300 nm to switch from *trans* to *cis* and 280 nm from *cis* to *trans* (27). Another problem is that the *cis*-isoform of stilbene can undergo electrocyclic ring-closure. The formed dihydrophenanthrene can then easily be oxidised and form a fused ring structure as shown in Scheme 5 (28). While the ring closing mechanism is reversible and known as a diarylethene switch (discussed in a following section), the oxidation process is irreversible and thus restricts the switching mechanism.



Scheme 5. Photoisomerisation and photocyclisation of stilbene and the possible oxidation of the closed isoform forming phenanthrene.

Nevertheless, modifications to stilbene can prevent the cyclisation. For example, a stilbene derivative fused with another ring is unable to undergo this cyclisation process due to steric hindrance (28). This class of molecules is called stiff-stilbene or overcrowded alkene and has mostly become famous as Feringa's molecular motor (29). These structures have a part that rotates 360 °C in one direction when stimulated by light. Directional control of the rotation is gained by installing another stereogenic centre which pushes the rotational isomerisation in a certain direction via steric interaction shown in Figure 6. This mechanism has been applied in various fields; from supramolecular assembly (27), to catalysis control (28), and the creation of artificial muscles (29). The artificial muscle is even applicable in aqueous solutions due to several added modifications like phosphate groups. However, this moiety still needs UV light stimulation. Structural modifications that enable significant red-shifting are thus still in a goal that researchers hope to achieve in order to allow for applications in photopharmacology.

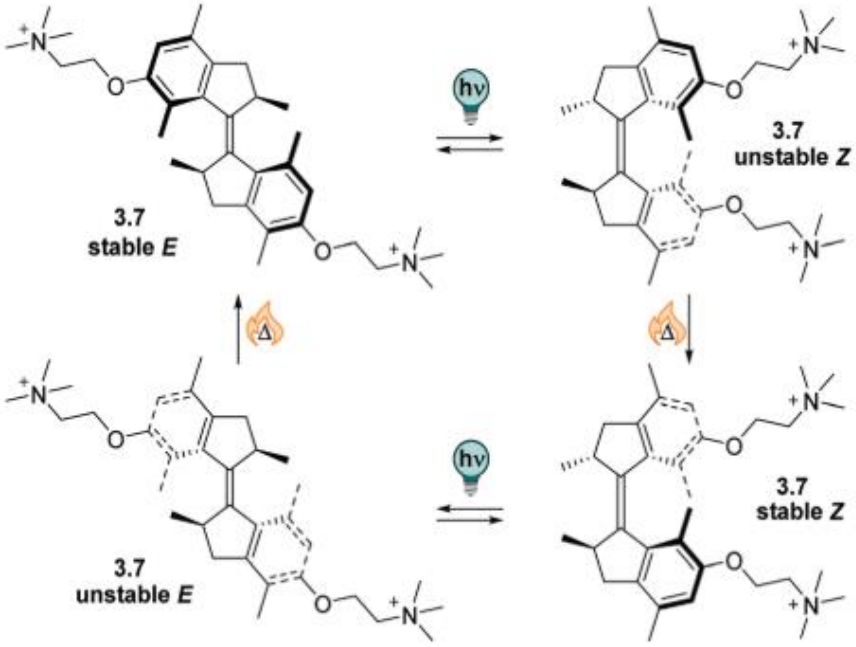
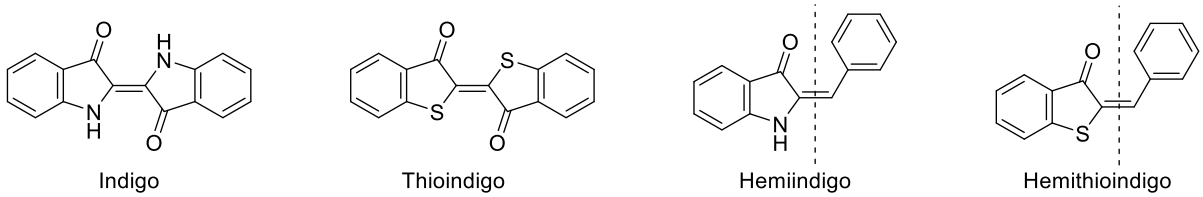


Figure 6. A figure from Volaric et al. displaying a molecular motor (21)*. The rotation is directed with steric interaction created by the methyl groups.*

Indigoids

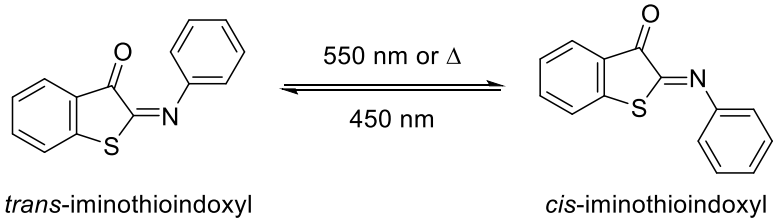
Another extensively researched class of photoswitches are the indigoids. This class consists of many derivatives based on the indigo compound which itself is well known as a chromophore and for its use as a blue dye (30). Indigo has a similar structure as the stiff stilbene in Figure 6 but contains a pyrrolidine with carbonyl as 5-membered ring (Scheme 6). Also the position of the double bond differs compared to the stiff-stillbene. Indigo itself is not applicable as photoswitch moiety as the excited state relaxes very efficiently via proton transfer of the pyrrolidine-nitrogens to a carbonyl (21). Alkylation or acetylation of these nitrogens does allow for switching from stable *trans* to metastable *cis* using light. An interesting feature of this switch is that the light necessary to switch is already within the visible light range (500-670 nm) without major substitutions. However, it is not yet within the therapeutic window mentioned in the introduction. Petermayer *et al.* discovered that arylation of only one of the nitrogens already allows for photoisomerization, even though a proton is present for the deexcitation pathway (30). However, the photostationary state half-life of these substitutions is incredibly low.



Scheme 6. Indigo and its variations. Hemiindigo and hemithioindigo are split with a dashed line to show the stilbene part from the indigo part.

The indigoid photoswitch class contains many variations of the original indigo. One variation is called thioindigo, shown in Scheme 6, which has the nitrogens substituted for sulfurs. Beside that thioindigo can switch without substitutions on the sulfurs, no other significant differences are mentioned in literature (31). The hemi-variations of indigo are a lot more present in literature with many examples of it being used as photoswitch. The two most mentioned hemi-variations are hemiindigo and hemithioindigo. These structures are also shown in Scheme 6 and contain on one side a stilbene fragment and on the other side an indigo or thioindigo fragment, respectively. As they are structurally a mix of stilbene and indigo, the switching wavelength is also in within values. Fortunately, the switching wavelength was found to be around 450 nm for both versions which is still within the visible region (21,32). And just like stilbene, the photostationary state is thermally stable at room temperature and can be switched back with light, which is for hemi(thio)indigo possible with visible light of 480 nm (4). While the difference between hemiindigo and hemithioindigo is minimal, hemithioindigo is used a lot more in research (4,21,27). For example, this moiety has been used to control the folding of a supramolecular receptor (30). This receptor consisted of two hemithioindigo moieties of which only one switched with blue light stimulation (420 nm). The switching formed a helical structure which created a binding pocket with great binding affinity for electron-poor aromatic structures. This could again be reversed with heat which would also release the captured molecule. Why one would assess only the thio-variant is not mentioned in literature.

Another novel derivative of indigoid is iminothioindoxyl which has been presented by Hoorens et al in 2012 (33). This compound is on one side thioindigo and on the other side azobenzene, which makes the imine the switchable bond (Scheme 7). Also in this structure, the switching mechanism of imine is not entirely explained but the absorbance pattern does predict a significant n to pi* contribution. It was also found that the *trans*-isomer undergoes inversion during de-excitation as was observed with azobenzenes which also supports the n to pi* contribution (33). Switching *trans* to *cis* and back are both activated with visible light with an encouraging band gap between wavelengths of over 100 nm when tested in several solvents. The *cis*-isoform absorbs light with a wavelength of 400-500 nm while the *trans*-isoform absorbs wavelengths of 500-600 nm. In comparison, the band gap found for hemithioindigoids is generally between 10-50 nm which complicates orthogonal switching either way (30). Another exciting part is that this the switching ability is operatable in aqueous solutions as the N=C double bond also makes the molecule more polar than its other indigoid switches, enabling it to be soluble in phosphate buffered saline (PBS) (33). They also confirmed that the molecule was still able to switch both ways using visible light in PBS. With these characteristics, it could be presumed that this photoswitch is highly applicable in photopharmacology.

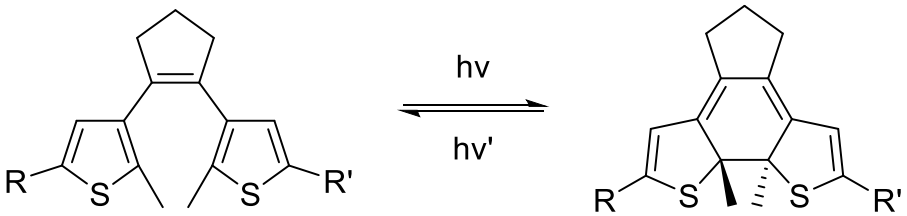


Scheme 7. The switching of iminothioindoxyl

Diarylethenes

All photoswitches that have been discussed until now, have been based on *cis*/*trans* isomerisation that cause big changes in the structure of the molecule. Another photoswitch mechanism has already briefly been mentioned stilbenes section. This mechanism is based ring formation and create rigidness in a free moving structure. Diarylethenes are the most well-defined photoswitches that operate via this mechanism. These structures consist of two aryl groups with an ethene connectivity, just like stilbene, which creates a hexatriene system shown in Scheme 9. By exciting the molecule with UV light, a sigma-bond can be formed to create a 6-membered ring (27). For this bond to form the two aryl groups need to rotate in such a way that the necessary orbitals overlap. These rotations occur in the same direction (conrotatory) which determines the stereochemistry of the closed-ring isomer as is shown in Scheme 8. Rotation in opposite direction (disrotatory) could be activated by ring closing using heat. The other pi electrons are moved further through the ring making this mechanism also known as 6pi electrocyclisation. Ring opening can occur via the reverse pathway by stimulation with visible light (27). However, opening the structure via thermal relaxation is forbidden according to the Woodward-Hoffman rules (21,34). An overview of the quite complex specific excited states involved can be found in the following articles (35–37), but is beyond the scope of this review.

Scheme 8. The stereochemical effect of disrotatory or conrotatory ring closure. The rotation in conrotatory ring closure is in the same direction and thus places the methyl groups in opposite directions. The rotation in disrotatory ring closure is in opposite direction and places the methyl groups in the same direction.



Scheme 9. The conrotatory ring closure of diarylethenes

As mentioned before with stilbenes, this structure is also able to undergo *trans*/*cis* isomerisation with light stimulation. But for diarylethenes this movement is hindered by bridging the ethene moiety with a 5-membered ring. Next to this bridging structure, it also consists of thiophenes as aryl groups and methyl groups at the ring-closing carbons to inhibit irreversible oxidation as was observed with stilbene. This basic structure is shown in Scheme 9. However, a disadvantage of this molecule is that thiophenes are prone to lose their switching performance. Every time when the molecule is in the excited state, a by-product can be formed which is the cause of this so-called switching fatigue (21). But this is easily hindered by substituting the thiophenes for other aromatic groups like 6-membered aromatic rings. In this particular situation, the gain in aromaticity when the photoswitch is changed from the closed to the open isoform, is so large that reverse switching via thermal relaxation is enabled. However, this requires more energy to form the closed isoform and thus blue-shifts the switching wavelength. Common strategies are also available for this photoswitch to red-shift the ring-closing wavelength, like extending the pi-conjugation of the molecule. However, a significant disadvantage is that installing all these modifications complicates the already difficult synthesis and makes it less soluble in aqueous solutions (27,38).

Babii *et al.* benefited from the stable photostationary state of diarylethene in the creation of a peptide analogue (39). They used the basic diarylethene structure and connected a proline and a carboxylic acid group at the ends. This structure was integrated in gramicidin S, a natural antimicrobial cyclic peptide that is highly cytotoxic in both prokaryotic and eukaryotic cells. The cyclic peptide can inhibit tumour growth but does this with low selectivity which induces high systemic toxicity as adverse effect. The photoswitch replaced a phenylalanine and proline group of gramicidin S (Figure 7) and switched to the open-ring isomer by irradiation with blue light (570 nm). The ring closing wavelength is probably still within the UV region, but they did not examine this characteristic. In its open form, the photoswitch peptide retained cytotoxicity with almost the same intensity as the original peptide. However, by switching the structure to the closed form, an 8-fold decrease in effect was observed. They tested their therapeutic peptide on mice with developed tumours. The mice that received treatment were exposed to locally irradiated visible light and showed significantly improved animal survival. Post-mortem analysis showed necrotic tumour tissue and shrinkage and the tumour of one animal was completely gone. Even though the required wavelength did not yet reach the therapeutic window, the light was able to penetrate tissue deep enough as a clear effect was observed.

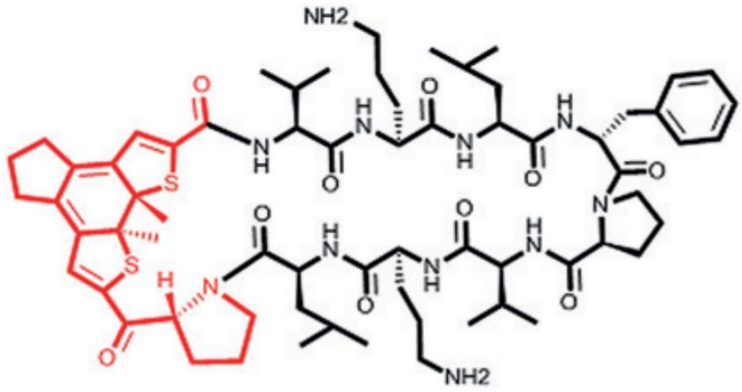
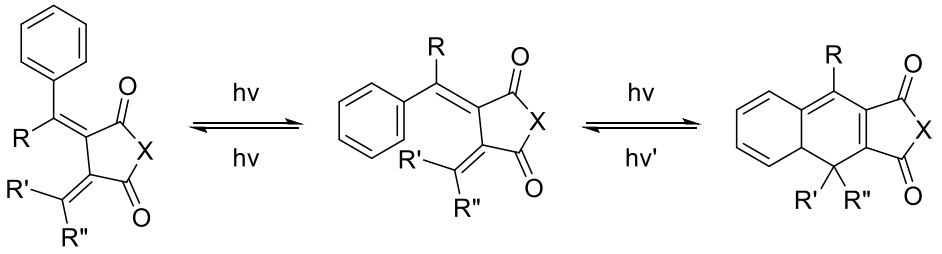


Figure 7. The photo controllable peptide from Babii et al. containing a diarylethene (39)*. A phenylalanine and proline were substituted for the diarylethene switch which is marked in red.*

Fulgides

The switching mechanisms of remaining photoswitches that will be discussed, consist of both *trans*/*cis* isomerisation and cyclisation and the first class that will be discussed are the fulgide switches. Its structure consists of an aromatic group and either a succinic anhydride (fulgide) or a succinimide (fulgimide) connected with an ethene bond as seen in Scheme 10. The ethene bond allows for *trans*/*cis* isomerisation and can be utilised by adding a substituent on the aromatic site of the ethene. However, the *trans*-isoform can also switch to the closed form instead of the *cis*-isoform without this substituent. Since the closed-ring version absorbs light at a different wavelength, it makes the switching irreversible when still exposed to light. But the absorption spectrum of the *cis*-isoform overlaps with the spectrum of the *trans*-isoform and thus the *cis*-isoform could switch back when irradiated with the same wavelength. This allows again for the possibility to from the closed isomer as final product. The back switching of the closed isoform is performed by using visible light and is also returned to the open *trans*-isomer. So, the fulgide switch is therefore mostly used as cyclisation switch.



Scheme 10. Photoisomerisation and photocyclisation of fulgides (X=O) and fulgimides (X=N). The R substituent regulates the trans/cis photoisomerisation ability.

Substituting any part of the basic fulgide structure has a slight impact on the switching characteristics. For example, changing the aromatic group could significantly improve the quantum yield of the cyclic photoswitching. However, crucial differences are found between fulgide and fulgimide. The closed isoform of fulgides generally absorbs light around 450-550 nm which is already within the visible region. However, the fulgimide version absorbs light around 600 nm which is nearly in the therapeutic window (21). Additionally, fulgimides are able to switch in aqueous solutions due to the succinyl group whereas fulgides are easily hydrolysed and therefore rendered inoperative (4).

Incorporation of fulgide or fulgimide is commonly surpassed by the mechanism-alike counterpart, diarylethene, in literature. However, Simeth *et al.* chose to replace a diarylethene core with a fulgimide instead (40). They wanted to improve on a previously reported inhibitor for sirtuins, a class of histone deacetylases, shown in Figure 8. They first investigated a diarylethene switch version but found it to be limited by the activation conditions (41). Ring closure had to be triggered by UV light and was only able to switch in organic solvents which is not tolerated in many enzyme assays. By using a fulgimide core, they found that switching was possible both ways with visible light (400 nm and 590 nm) and that it could be performed in aqueous solutions. Unfortunately, they did lose some affinity (5-fold) for sirtuins with the fulgimide version. But they suspected that structural optimisation could recover this loss.

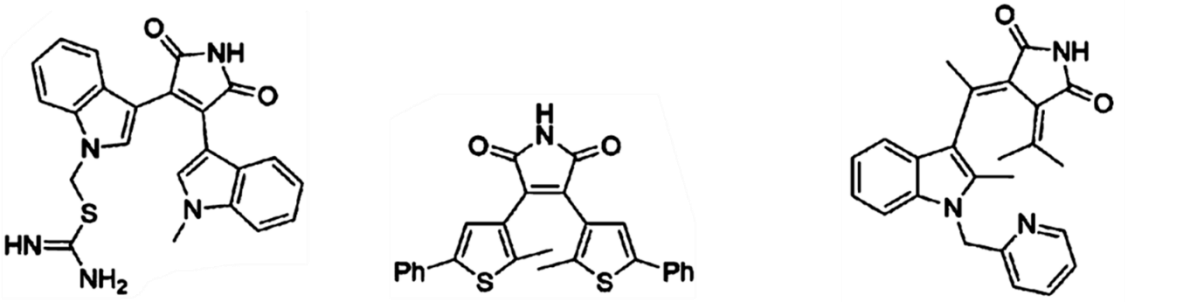
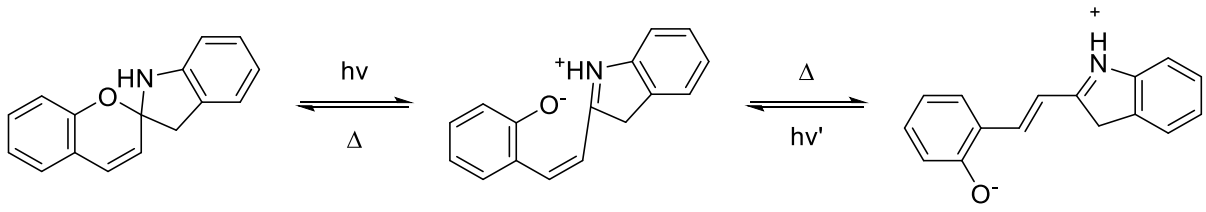


Figure 8. The sirtuin inhibitors compared by Simeth et al. (40)

Spiropyran

Another class of photoswitches that employ both mechanisms are the spiropyrans. The basic structure consists of a chromene and indoline structure connected at the alpha carbons. Irradiation with UV light breaks the bond between the oxygen and alpha carbon which opens the chromene structure to a merocyanine. This creates a linear structure with the still intact indoline structure and a phenolic structure that are linked with an ethene in *cis*-formation as shown in Scheme 11. The formation then switches quickly to the *trans*-isoform via thermal relaxation. Ring closing can then be induced again by light of 500-600 nm or heat and follows the mechanism in reverse order (38). Also for this moiety, the *trans*/*cis* isomerisation cannot be controlled individually. It would be possible when the phenolic oxygen is alkylated, but photocyclisation is then inaccessible (21).



Scheme 11. The photoisomerisation and photocyclisation of spiropyran

Another feature of spiropyran is that it can act as an acid generator operated by light as well. The formed molecule after ring opening has a deprotonated phenolic oxygen. When protonated, it has a pK_a of around 4 which can be tweaked by placing specific substituents at the para position (21,42). For example, an electron withdrawing group at the para position could lower the pK_a. By forcing ring closure of the protonated open form with light, it forms a protonated spiropyran with a very acidic hydrogen (pK_a around 1). This mechanism is fully visualised in Figure 9. By keeping spiropyran in the right environment, an acidic compound is created when switched with visible light.

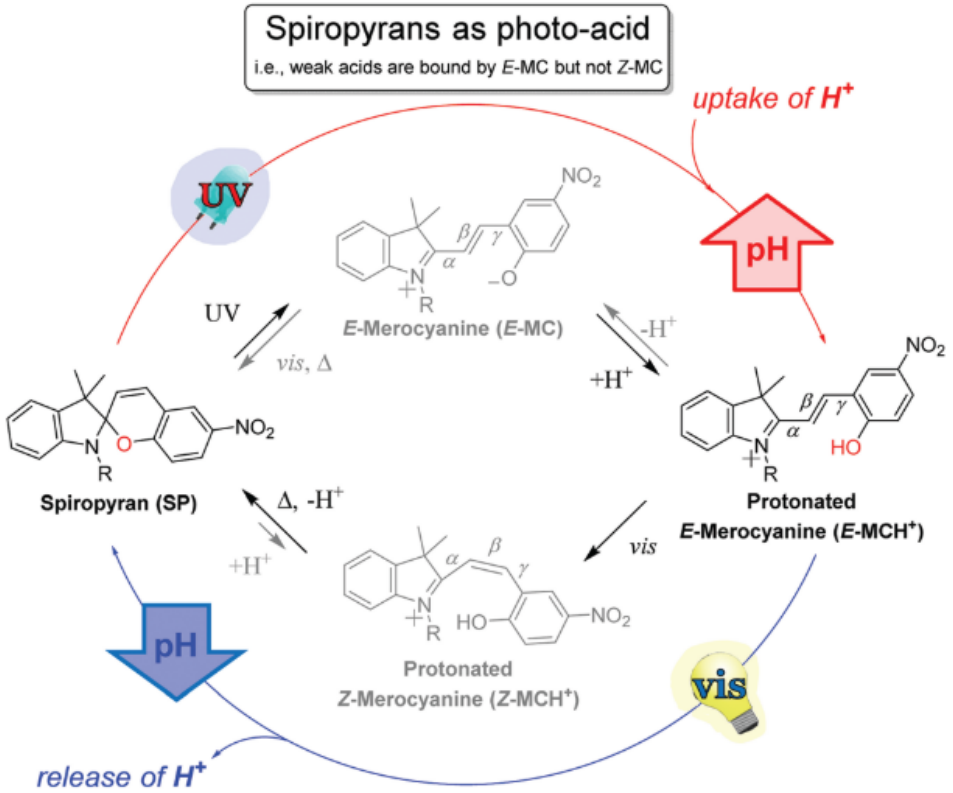
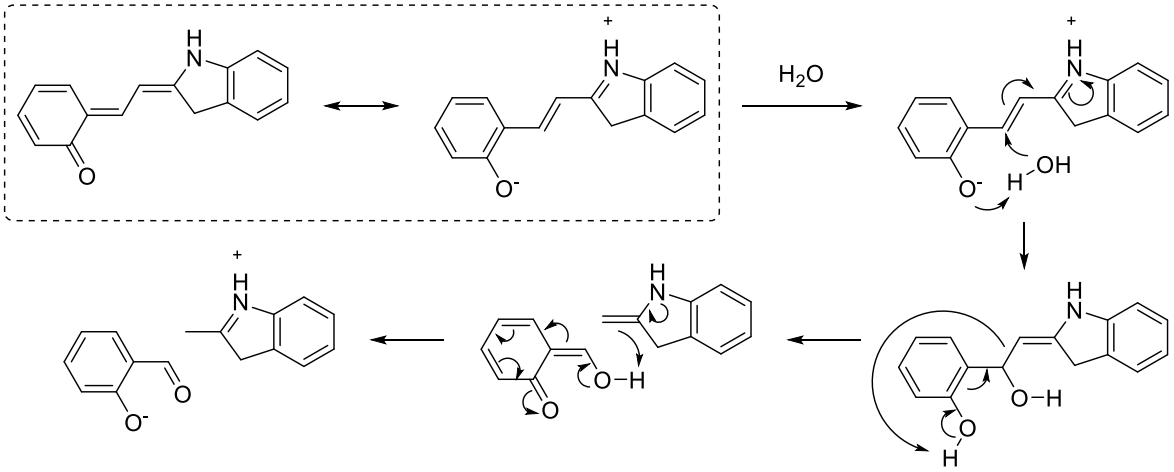


Figure 9. A mechanistical overview by Bonefacino et al. showing how spiropyran can function as a photo-acid (43). *Irradiating spiropyran with UV creates the linear form that can take up a proton and raise the pH value. By stimulating this form with visible light it releases the proton again creating an acidic environment.*

Spiropyran has frequently been applied in aqueous environments. In this environment, the switching equilibrium shifts more to the open form in aqueous solutions due to stabilisation of the zwitterionic form (shown in Scheme 12). However, the open form can be hydrolysed at the double bond to form an aldehyde and Fischer's Base. This is only possible when the phenolic oxygen is deprotonated as it directs nucleophilic water to the double bond (44). To support this mechanism, it confirmed that the protonated form was more resistant towards degradation (44). But other methods to resist hydrolytic degradation are available as well. For example, by substituting the phenyl group for pyridine which also enhances the water solubility of the spiropyran as switch (21). Another interesting modification is exchanging the carbon double bond for a carbon-nitrogen double bond, making it a spirooxazine switch. This modification not only enhances water solubility, but also redshifts the switching wavelengths. However, thermal relaxation of this compound is very fast.

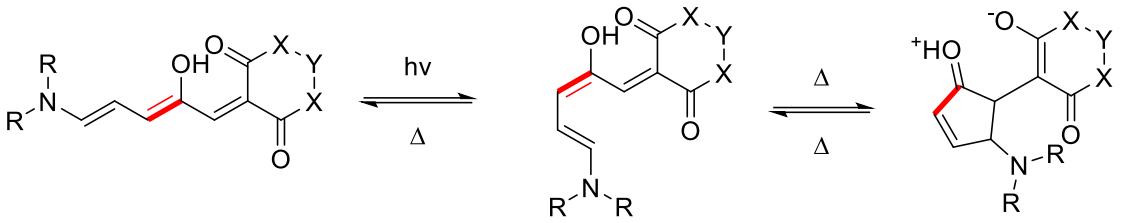


Scheme 12. The isomerisation between the neutral and zwitterionic form of the open spiropyran is shown in the marked area. The zwitterionic form can be hydrolysed via the mechanism visualised in the rest this figure.

Spiropyran has been used as photoswitch in similar ways as the other compounds discussed before. For example, Zhang *et al.* incorporated spiropyran in a protease inhibitor to control the activity (45). However, as this function has been revised in other photoswitches, an article using the photo-acid functionality will be presented instead. Tatum *et al.* employed a spiropyran with a propyl sulfonate attached to the indoline nitrogen (46). Their goal was to switch a hydrazone using acidity as activator. Acid-base powered switching commonly creates a lot of waste. By using spiropyran as an acid generator, no large amounts of waste products are formed. They used violet light (430 nm) to close the ring and release the proton, which in turn switched the hydrazone from *trans* to *cis*. In the dark, this process was reversed and could be activated again. The use of spiropyran to activate the hydrazone seems inconvenient since hydrazone could also switch with light. However, hydrazones need UV light to switch. Thus, using a spiropyran as intermediate indirectly redshifts the wavelength.

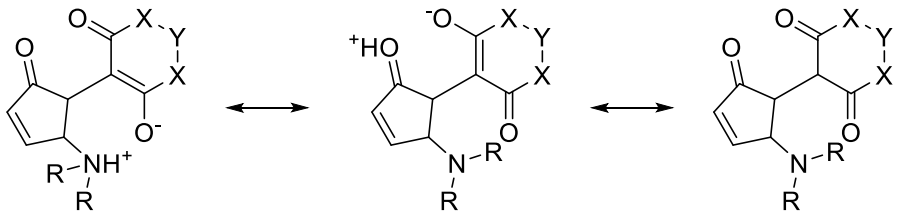
Donor-Acceptor Stenhouse Adducts

The last photoswitch moiety that will be discussed has only been featured in literature since 2014 (47). The Donor-Acceptor Stenhouse Adduct (DASA) switches via *trans*/*cis* isomerisation and conratory 4pi cyclisation. This structure consists of a conjugated triene with a hydroxyl group on C_4, a tertiary amine on the C_1 end and a carbonyl conjugated to the triene on the C_6 end. Irradiating the molecule with visible light triggers the C_3-C_4 double bond to switch from *trans* to *cis* mediated by the hydroxyl group. Afterwards the C_4-C_5 bond rotates and a 5-membered ring can be formed. The switching is then concluded by pushing the electrons to the carbonyl moiety. This process is reversed to the linear form via thermal relaxation. The DASA switching mechanism is shown in Scheme 13.



Scheme 13. The photoswitching of DASA triggered by light and fully reversible by thermal relaxation. The double bond undergoing photoisomerisation is marked in red. The acceptor part can be Meldrum's acid (X=O, Y=dimethyl), barbituric acid (X=N, Y=carbonyl), or other electron withdrawing carbon acids.

The first DASA switch contained on one side a tertiary amine as donor and on the other side either Meldrum's acid or barbituric acid as acceptor. Irradiation of these structures with 545 nm and 570 nm light respectively activates the structures to switch to the closed form. However, back-switching was only possible in nonpolar solvents. Thermal relaxation in polar solvents was not observed when the molecule was in its cyclic form as this form was stabilised due to its zwitterionic nature highlighted in Scheme 14. This inspired others to develop other versions of this photoswitch with arylamines as donor and more electron withdrawing carbon acids as acceptor (48,49). These modifications did lead to applicability in more polar solvents as acetonitrile and ethyl acetate, but not yet in aqueous solutions. But the new modifications did lead to a bathochromic shift to far-red light as activator with wavelengths of around 700 nm mentioned in literature(49,50).



Scheme 14. A few cyclic DASA isomers obtained via electron and proton transfer. The structure in the middle is created after photoswitching.

However, reverse switching in water has once been reported when they incorporated DASAs in nanocarriers (51). Yap *et al.* constructed a copolymer with DASAs conjugated on PEG to create a photocontrolled micelle. The DASA structure was similar to the first generation of DASAs with a tertiary amine as donor and Meldrum's acid as acceptor. The micelle is formed with the open and linear DASA form and loaded them with anticancer drug ellipticine. It releases its content when the DASA is switched to the cyclic form. They were able to track the conformational changes by measuring the absorbance at 518 nm which is consistent with the linear open form of DASA. They irradiated the micelles in water with green light (530 nm) and were able to observe a decrease in absorbance. Afterwards, they left the micelles to recover in the dark during which they observed an increase in absorbance again, indicative for the back switching of the DASAs. They also observed this effect in phosphate buffered saline. However, only 70-85% of the original signal was observed which showed that not all DASAs were switched back. This percentage decreased to 25-45% when loaded with ellipticine. They suspect that this effect was due to a preferred interaction of the zwitterionic form with the drug. Thus, control over drug release was not optimal for this nanocarrier. However, the reversibility DASA switching in aqueous solutions was very promising for the future studies of DASA.

Discussion and Conclusion

In this review, many photoswitchable structures have been discussed with different switching mechanisms and characteristics. The older switches like azobenzene, stilbene and fulgides are extensively researched and have already been applied in many situations. To solve any issues regarding these switches, either adjustments have been made to the main structure or novel photoswitches like DASA or iminothioindoxyl were engineered to obtain new opportunities. All main structures and important derivatives are summarised in Table 1, including key features.

Table 1. Summary of all discussed photoswitch classes. The data is based on the basic photoswitch structure or the most important derivative. The activating wavelength switches the respective molecule from stable to metastable.

	Switching mechanism	**Activating wavelength**	**Switching reversibility**	**Water solubility**	**Characteristic**
Azobenzene	*Trans*/*cis* isomerisation	320 nm	Thermal relaxation or 440 nm	Aided by polar additions	Most commonly used in literature
Hydrazone	*Trans*/*cis* isomerisation	320 nm	Thermal relaxation or 440 nm	Aided by polar additions	Short half-life
Stilbene	*Trans*/*cis* isomerisation	300 nm	280 nm	Aided by polar additions	Stiff-stilbene motor
(Thio)Indigo	*Trans*/*cis* isomerisation	500-670 nm	Thermal relaxation	Moderate	Activated with visible light
Hemi(thio)indigo	*Trans*/*cis* isomerisation	450 nm	480 nm	Moderate	Activated with visible light
Iminothioindoxyl	*Trans*/*cis* isomerisation	400-500 nm	500-600 nm	Good	Clear band gap
Diarylethene	Cyclisation	100-400 nm	380-700 nm	Aided by polar additions	Switching in small volume
Fulgide	*Trans*/*cis* isomerisation and cyclisation	450-550 nm	380-700 nm	Hydrolysis	
Fulgimide	*Trans*/*cis* isomerisation and cyclisation	600 nm	380-700 nm	Good	Operative in aqueous solutions
Spiropyran	*Trans*/*cis* isomerisation and cyclisation	100-400 nm	500-600 nm	Good	Photo-acid generator
DASA	*Trans*/*cis* isomerisation and cyclisation	545-700 nm	Thermal relaxation	Excellent	Longest activating wavelength

With the many differing photoswitch structures available, a wide variety of functions could be fulfilled. The applications ranged from nanoparticles, to folding agents, and photoswitchable ligands and it could also be integrated in various constructs as small molecules, DNA, lipids, and peptides. Far more functions can be found in literature for each structure. Another fascinating example that has not been discussed is the photoswitchable antibiotic from Ben Feringa (52). By letting light control its activity, the drug activity can be turned off once it has been eliminated from the body. This prevents the development of resistant bacterial strains in nature, which is becoming a major problem nowadays.

However, the biggest limitation of photoswitches is still the switching wavelength. The wavelengths of the most commonly used photoswitches ranges from UV light, which is harmful for living tissue, to visible green light, which is not able to penetrate tissue deeply. Photoswitches used in living organisms were either applied just below the skin or in the eye, allowing light to easily reach the intended areas. A novel solution for this problem is the optical fibre that has been published in 2021, allowing light to be delivered deep into tissue (7). But more traditional solutions, like red-shifting strategies and engineering new photoswitch moieties, are still being developed as well.

In conclusion, photopharmacology is well on its way to become a major field in medicine. This field opens up many new ways of tackling current obstacles in medicine. This review highlights the many photoswitchable structures that are available to us and the distinctiveness of each. By discussing each structure and comparing them to each other, a clear overview is generated that highlights the benefits of each structure. Moreover, it also shows what is already possible within this field and where improvement is still necessary. Overall, photopharmacology has opened up lots of opportunities and started many investigations which in turn lead to more control in medicine.

References

1. Hartley GS (1937). The Cis-form of Azobenzene. Nature. 140(281):281–281
2. Welleman IM, Hoorens MWH, Feringa BL, Boersma HH, Szymański W (2020). Photoresponsive molecular tools for emerging applications of light in medicine. Chem Sci. 11(43):11672–91
3. Göstl R, Senf A, Hecht S (2014). Remote-controlling chemical reactions by light: Towards chemistry with high spatio-temporal resolution. Chem Soc Rev. 43(6):1982–96
4. Brieke C, Rohrbach F, Gottschalk A, Mayer G, Heckel A (2012). Light-Controlled Tools. Angew Chemie Int Ed. 51(34):8446–76
5. Polosukhina A, Litt J, Tochitsky I, Nemargut J, Sychev Y, De Kouchkovsky I, et al. (2012). Photochemical restoration of visual responses in blind mice. Neuron. 75(2):271–82
6. Andresen M, Wahl MC, Stiel AC, Gräter F, Schäfer L V., Trowitzsch S, et al. (2005). Structure and mechanism of the reversible photoswitch of a fluorescent protein. Proc Natl Acad Sci U S A. 102(37):13070–4
7. Palasis KA, Lokman NA, Quirk BC, Adwal A, Scolaro L, Huang W, et al. (2021). Optical fibre-enabled photoswitching for localised activation of an anti-cancer therapeutic drug. Int J Mol Sci. 22(19)
8. Velema WA, Szymanski W, Feringa BL (2014). Photopharmacology: Beyond Proof of Principle. J Am Chem Soc. 136(6):2178–91
9. Mourot A, Fehrentz T, Le Feuvre Y, Smith CM, Herold C, Dalkara D, et al. (2012). Rapid optical control of nociception with an ion-channel photoswitch. Nat Methods. 9(4):396–402
10. Alachouzos G, Schulte AM, Mondal A, Szymanski W, Feringa BL (2022). Computational Design, Synthesis, and Photochemistry of Cy7-PPG, an Efficient NIR-Activated Photolabile Protecting Group for Therapeutic Applications. Angew Chemie. 134(27)
11. Rad JK, Balzade Z, Mahdavian AR (2022). Spiropyran-based advanced photoswitchable materials: A fascinating pathway to the future stimuli-responsive devices. J Photochem Photobiol C Photochem Rev. 51:100487
12. Rau H, Lüddecke E (1982). On the Rotation-Inversion Controversy on Photoisomerization of Azobenzenes. Experimental Proof of Inversion. J Am Chem Soc. 104(6):1616–20
13. Zhu M, Zhou H (2018). Azobenzene-based small molecular photoswitches for protein modulation. Org Biomol Chem. 16(44):8434–45
14. Bandara HMD, Burdette SC (2012). Photoisomerization in different classes of azobenzene. Chem Soc Rev. 41(5):1809–25
15. Zhu M, Zhou H (2018). Azobenzene-based small molecular photoswitches for protein modulation. Org Biomol Chem. 16(44):8434–45
16. Fujino T, Arzhantsev SY, Tahara T (2001). Femtosecond Time-Resolved Fluorescence Study of Photoisomerization of trans-Azobenzene. J Phys Chem A. 105(35):8123–9
17. Bandara HMD, Friss TR, Enriquez MM, Isley W, Incarvito C, Frank HA, et al. (2010). Proof for the Concerted Inversion Mechanism in the trans → cis Isomerization of Azobenzene Using Hydrogen Bonding To Induce Isomer Locking. J Org Chem. 75(14):4817–27
18. Giles LW, Faul CFJ, Tabor RF (2021). Azobenzene isomerization in condensed matter: Lessons for the design of efficient light-responsive soft-matter systems. Mater Adv. 2(13):4152–64

19. Merino E, Ribagorda M (2012). Control over molecular motion using the cis–trans photoisomerization of the azo group. Beilstein J Org Chem. 8:1090

20. Rustler K, Pockes S, König B (2019). Light-Switchable Antagonists for the Histamine H1 Receptor at the Isolated Guinea Pig Ileum. ChemMedChem. 14(6):636–44

21. Volarić J, Szymanski W, Simeth NA, Feringa BL (2021). Molecular photoswitches in aqueous environments. Chem Soc Rev. 50(22):12377–449

22. Weston CE, Richardson RD, Haycock PR, White AJP, Fuchter MJ (2014). Arylazopyrazoles: Azoheteroarene photoswitches offering quantitative isomerization and long thermal half-lives. J Am Chem Soc. 136(34):11878–81

23. Calbo J, Weston CE, White AJP, Rzepa HS, Contreras-García J, Fuchter MJ (2017). Tuning azoheteroarene photoswitch performance through heteroaryl design. J Am Chem Soc. 139(3):1261–74

24. Adam V, Prusty DK, Centola M, Škugor M, Hannam JS, Valero J, et al. (2018). Expanding the Toolbox of Photoswitches for DNA Nanotechnology Using Arylazopyrazoles. Chem – A Eur J. 24(5):1062–6

25. Crespi S, Simeth NA, König B (2019). Heteroaryl azo dyes as molecular photoswitches. Nat Rev Chem. 3(3):133–46

26. Guo X, Shao B, Zhou S, Aprahamian I, Chen Z (2020). Visualizing intracellular particles and precise control of drug release using an emissive hydrazone photochrome. Chem Sci. 11(11):3016–21

27. Peddie V, Abell AD (2019). Photocontrol of peptide secondary structure through non-azobenzene photoswitches. J Photochem Photobiol C Photochem Rev. 40:1–20

28. Villarón D, Wezenberg SJ (2020). Stiff-Stilbene Photoswitches: From Fundamental Studies to Emergent Applications. Angew Chemie Int Ed. 59(32):13192–202

29. Chen S, Yang L, Leung FKC, Kajitani T, Stuart MCA, Fukushima T, et al. (2022). Photoactuating Artificial Muscles of Motor Amphiphiles as an Extracellular Matrix Mimetic Scaffold for Mesenchymal Stem Cells. J Am Chem Soc. 144(8):3543–53

30. Petermayer C, Dube H (2018). Indigoid Photoswitches: Visible Light Responsive Molecular Tools. Acc Chem Res. 51(5):1153–63

31. Pereira RC, Pineiro M, Galvão AM, Seixas de Melo JS (2018). Thioindigo, and sulfonated thioindigo derivatives as solvent polarity dependent fluorescent on-off systems. Dye Pigment. 158:259–66

32. Gonzalez A, Kengmana ES, Fonseca M V., Han GGD (2020). Solid-state photoswitching molecules: structural design for isomerization in condensed phase. Mater Today Adv. 6

33. Hoorens MWH, Medved' M, Laurent AD, Di Donato M, Fanetti S, Slappendel L, et al. (2019). Iminothioindoxyl as a molecular photoswitch with 100 nm band separation in the visible range. Nat Commun. 10(1):1–11

34. Albert L, Vázquez O (2019). Photoswitchable peptides for spatiotemporal control of biological functions. Chem Commun. 55(69):10192–213

35. Celani P, Bernardi F, Olivucci M, Robb MA (1995). Excited-state reaction pathways for s-cis buta-1,3-diene. J Chem Phys. 102(14):5742

36. Sotome H, Nagasaka T, Une K, Morikawa S, Katayama T, Kobatake S, et al. (2017).

Cycloreversion Reaction of a Diarylethene Derivative at Higher Excited States Attained by Two-Color, Two-Photon Femtosecond Pulsed Excitation. J Am Chem Soc. 139(47):17159–67

37. Irie M (2021). Reaction Mechanism. Diarylethene Mol Photoswitches. :15–30

38. Leistner AL, Pianowski ZL (2022). Smart Photochromic Materials Triggered with Visible Light. European J Org Chem. 2022(19)

39. Babii O, Afonin S, Garmanchuk L V., Nikulina V V., Nikolaienko T V., Storozhuk O V., et al. (2016). Direct Photocontrol of Peptidomimetics: An Alternative to Oxygen-Dependent Photodynamic Cancer Therapy. Angew Chemie. 128(18):5583–6

40. Simeth NA, Altmann LM, Wössner N, Bauer E, Jung M, König B (2018). Photochromic Indolyl Fulgimides as Chromo-pharmacophores Targeting Sirtuins. J Org Chem. 83(15):7919–27

41. Falenczyk C, Schiedel M, Karaman B, Rumpf T, Kuzmanovic N, Grøtli M, et al. (2014). Chromo-pharmacophores: photochromic diarylmaleimide inhibitors for sirtuins. Chem Sci. 5(12):4794–9

42. Kortekaas L, Browne WR (2019). The evolution of spiropyran: fundamentals and progress of an extraordinarily versatile photochrome. Chem Soc Rev. 48(12):3406–24

43. Bonefacino J, Vincent Tse M-L, Jeff Pun C-F, Cheng X, Kin Edward Chan W, Boersma A, et al. (2013). Characterization of Spirooxazine and Spiropyran Hosted in Poly(Methyl Methacrylate) for Germicidal UV Source Indicator Application. Opt Photonics J. 3:11–6

44. Hammarson M, Nilsson JR, Li S, Beke-Somfai T, Andréasson J (2013). Characterization of the thermal and photoinduced reactions of photochromic spiropyrans in aqueous solution. J Phys Chem B. 117(43):13561–71

45. Zhang X, Heng S, Abell AD (2015). Photoregulation of α-Chymotrypsin Activity by Spiropyran-Based Inhibitors in Solution and Attached to an Optical Fiber. Chem – A Eur J. 21(30):10703–13

46. Tatum LA, Foy JT, Aprahamian I (2014). Waste management of chemically activated switches: Using a photoacid to eliminate accumulation of side products. J Am Chem Soc. 136(50):17438–41

47. Helmy S, Leibfarth FA, Oh S, Poelma JE, Hawker CJ, Read de Alaniz J (2014). Photoswitching Using Visible Light: A New Class of Organic Photochromic Molecules. J Am Chem Soc. 136(23):8169–72

48. Mallo N, Brown PT, Iranmanesh H, MacDonald TSC, Teusner MJ, Harper JB, et al. (2016). Photochromic switching behaviour of donor–acceptor Stenhouse adducts in organic solvents. Chem Commun. 52(93):13576–9

49. Hemmer JR, Page ZA, Clark KD, Stricker F, Dolinski ND, Hawker CJ, et al. (2018). Controlling Dark Equilibria and Enhancing Donor-Acceptor Stenhouse Adduct Photoswitching Properties through Carbon Acid Design. J Am Chem Soc. 140(33):10425–9

50. Berraud-Pache R, Santamaría-Aranda E, de Souza B, Bistoni G, Neese F, Sampedro D, et al. (2021). Redesigning donor–acceptor Stenhouse adduct photoswitches through a joint experimental and computational study. Chem Sci. 12(8):2916–24

51. Yap JE, Zhang L, Lovegrove JT, Beves JE, Stenzel MH (2020). Visible Light—Responsive Drug Delivery Nanoparticle via Donor–Acceptor Stenhouse Adducts (DASA). Macromol Rapid Commun. 41(21):2000236

52. Velema WA, Van Der Berg JP, Hansen MJ, Szymanski W, Driessen AJM, Feringa BL (2013). Optical control of antibacterial activity. Nat Chem 2013 511. 5(11):924–8