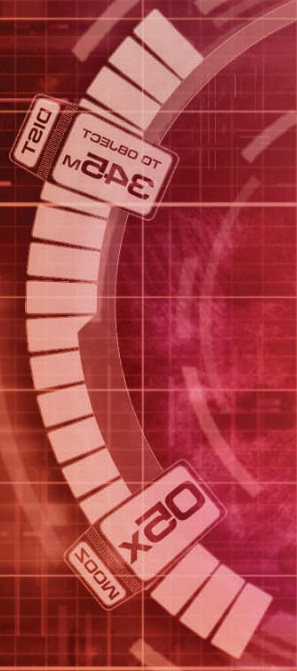
drugs on demand



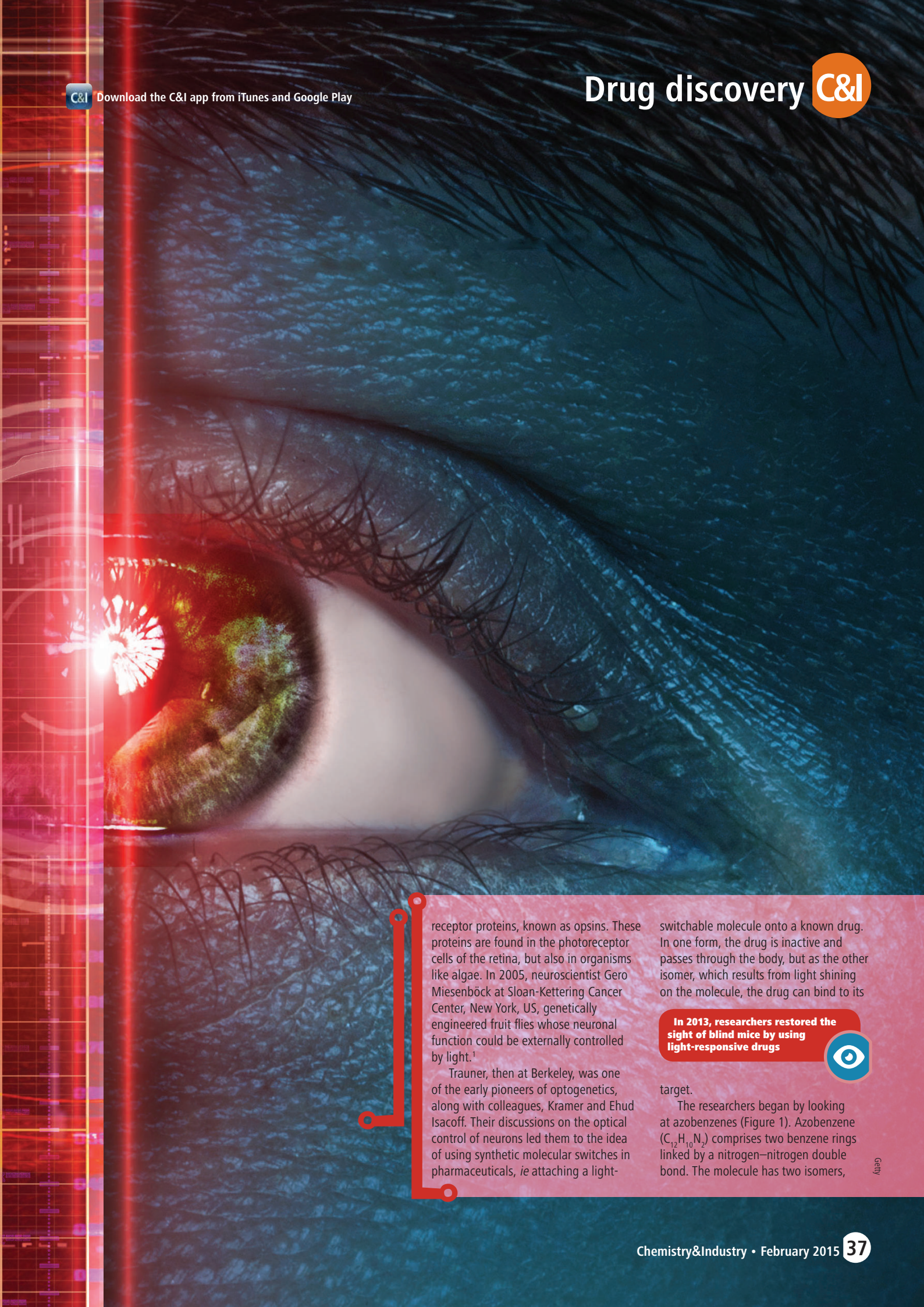
By designing photoswitchable groups into drug molecules, they can be turned on and off – at the flick of a light switch. Rachel Brazil reports

The ideal drug works only where it's needed in the body – cutting down on side effects and toxicity. Creating drugs that act as 'magic bullets' and target specific cells has become the main strategy for achieving this result. But a different approach – which uses light to activate drugs – could provide the same outcome.

In 2013, chemist Dirk Trauner at Ludwig-Maximilians University in Munich, Germany, along with neuroscientist Richard Kramer at University of California, Berkeley, US, succeeded in restoring the sight of blind mice using light-responsive drugs. While it is still early days, researchers worldwide are already designing a range of photoswitchable pharmaceuticals, including antibiotics, painkillers, anticancer and diabetes drugs.

Optogenetics

Photo- or opto-pharmacology grew out of optogenetics. Highlighted in 2010 by the journal *Science* as one of the breakthroughs of the decade, optogenetics uses light to control neurons in genetically engineered animals to study brain function. The neurons are engineered to include light-sensitive



receptor proteins, known as opsins. These proteins are found in the photoreceptor cells of the retina, but also in organisms like algae. In 2005, neuroscientist Gero Miesenböck at Sloan-Kettering Cancer Center, New York, US, genetically engineered fruit flies whose neuronal function could be externally controlled by light.[1]

Trauner, then at Berkeley, was one of the early pioneers of optogenetics, along with colleagues, Kramer and Ehud Isacoff. Their discussions on the optical control of neurons led them to the idea of using synthetic molecular switches in pharmaceuticals, *ie* attaching a light-switchable molecule onto a known drug. In one form, the drug is inactive and passes through the body, but as the other isomer, which results from light shining on the molecule, the drug can bind to its target.

In 2013, researchers restored the sight of blind mice by using light-responsive drugs

The researchers began by looking at azobenzenes (Figure 1). Azobenzene ($C_{12}H_{10}N_2$) comprises two benzene rings linked by a nitrogen–nitrogen double bond. The molecule has two isomers,

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'The retinal ganglion cells are made "naturally blind"; they don't respond to light, but with our synthetic photoswitches they suddenly become light sensitive.'

Dirk Trauner *Ludwig-Maximilians University, Germany*

a *trans* and a *cis* form. On irradiating with UV light, the more stable *trans* form converts to the *cis*-isomer, which reverts to the *trans*-isomer in time or under blue light. What makes azobenzenes work so well is the large difference in geometry between the two forms. Moreover, azobenzene is small enough to be incorporated into drugs and doesn't generate any further photochemical species.

Therapeutic success

One of the first types of pharmaceuticals Trauner designed was a photoswitchable version of the painkiller fentanyl (*N*-(1-(2-phenylethyl)-4-piperidinyl)-*N*-phenylpropanamide).[2] Fentanyl binds to opioid receptors, which decreases neuronal activity, diminishing pain. The photoswitch gave the researchers a way of localising its action. This work led Trauner to hypothesise that it may also be possible to restore vision, given that the light-sensitive receptor proteins in human eyes are in-built photoswitches. When these stop working or are damaged, vision irreversibly fails. This happens, for example, in age-related macular degeneration, the leading cause of blindness in the Western world, with more than 20m elderly sufferers and no present treatments.

Trauner and his colleagues at Berkeley experimented to see if they could use photoswitching molecules to replace the damaged receptors. Their most successful molecule is diethylamine-azobenzene-quaternary ammonium (DENAQ), a *para*-substituted azobenzene that can target ion channels in retinal ganglion cells,

A monthly injectable slow release formulation for treating blindness in humans could be available for clinical trials within the next few years

a type of neuron on the inner surface of the retina. These cells transmit signals from the eyes' photoreceptors to the brain. Trauner explains: 'The retinal ganglion cells are made "naturally blind"; they don't respond to light, but with our synthetic photoswitches they suddenly become light-sensitive.'

DENAQ was ideal in that the *trans* to *cis* change occurs in the visible region (Figure 2).[3] This means the molecule could activate the ion channel at similar wavelengths and intensities to daylight and in the dark would rapidly return to the *trans* form, turning off any signalling. Trauner tested the approach in mice genetically engineered to be blind. He injected DENAQ into the vitreous cavity of their eyes, and did experiments looking at various movements and learning patterns in light and dark environments. His results confirmed the mice clearly had some level of vision restored. Trauner also showed that DENAQ only worked with diseased retinas and did not interfere with healthy cells. Without photoreceptors, somehow the retinal ganglion cells become electropysiologically hyperactive, allowing them to interact with DENAQ.

Trauner and his colleagues have since set up a company, Photoswitch Biosciences, in California, US, to continue preclinical development of DENAQ. 'We have already been able to show it works in rodents and we are very hopeful it will work in humans,' says Trauner. He is confident that a monthly injectable slow release formulation could be available to test on humans within the next few years as long as the toxicity evaluation tests go well.

Meanwhile, Ben Feringa, professor of organic chemistry at the University of Groningen in the Netherlands, has spent many years studying molecular switches for electronic devices and molecular machines and motors. Recently, his group has been working on how to incorporate photoswitches into various drug molecules. According to Feringa: 'We have found several cases where it is quite easy to replace a small piece of the molecule without affecting the binding….we typically look at structural mimics [to see] if we can then replace them with a photoswitchable unit….'

Feringa's group has so far made light-switchable antibiotics, the advantage of which is not only to localise the effects of the drugs, but also to switch off biological activity when they are not in the body. Feringa explains: 'The major problem is that when you release the antibiotic into the environment in an active form, you build a resistance. A switchable drug could help to stem this serious problem.'

The research chemists designed a switchable version of the antibiotic quinolone, which is made up of a benzene ring fused to a carboxypyridone. Quinolone is thought to work by targeting enzymes needed for bacterial DNA replication. Typically, quinolone antibiotics have a piperazine moiety substituted on the benzene ring (R^3) as well as fluorine substituents. By exchanging the piperazine unit with an aryldiazo group, Feringa and his team created similar structures containing azobenzene photoswitches (Figure 3).[4] Several of these structures could be switched on by UV light with the *cis* form being an active antibiotic. After only several hours, they reverted to the *trans* form, losing their antibiotic activity and thus safe if released into the environment. The most successful example contained a 4-methyoxy-3-methyl substituted aryldiazo.

A photoswitchable treatment for diabetes may not seem immediately useful but one of the first line drug classes prescribed, sulfonylureas, have problematic side effects that switchable

Figure 1 ***Trans*** **to** ***cis*** **isomer conversion occurs in the UV-visible; blue light will convert the molecule back to the** ***trans*** **form**

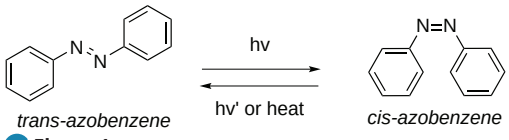


Figure 2 DENAQ, ***trans*** **and** ***cis*** **forms**

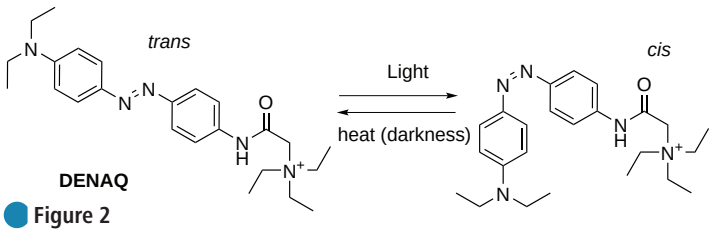
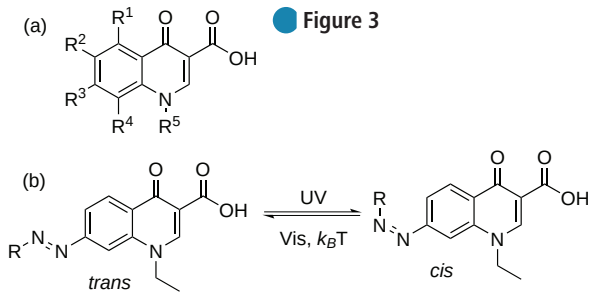


Figure 3
(a) Quinoline antibiotics;
(b) modified version with azobenzene



drugs might control. Sulfonylureas work by binding to receptors on the membranes of pancreas β-cells, which increases insulin secretion. But these drugs can cause over-production of insulin, leading to blood sugar crashes and increases in cardiovascular disease. The idea of being able to control their release to the pancreas is therefore attractive.

David Hodson, a diabetes researcher at Imperial College London, UK, has recently teamed up with Trauner to see if together they can create a photoswitachable sulfonylurea drug. Using the drug glimepiride as a template, they synthesised the molecule JB253 containing a light-switchable azobenzene group.[5] Hodson explains: 'The only difference is one molecule has a diazene unit, otherwise they are pretty much identical.' The drug molecule they created could be switched from the inactive *trans* form to the active *cis* form with blue LED light (Figure 4).

Hodson envisages that patients with type 2 diabetes would be able to shine light on their abdomens after eating, activating the drug only when needed. Hodson comments: 'Because the drug switches on very quickly in milliseconds and because it switches off within about 2s, fine control is possible.' Trauner and Hodson are now keen to find industrial partners to further develop their diabetes drug.

Switching cells on and off

Bionanotechnologist, Pau Gorostiza at the Institute for Bioengineering of Catalonia, Barcelona, Spain, is focusing his research on stapled peptides. These molecules can adopt a protein-like α-helical structure by forming a hydrocarbon bridge between amino acids across the peptide chain. They can penetrate cells and interact with previously inaccessible disease targets.

Working with Ernest Giralt, professor of organic chemistry at the University of Barcelona, Spain, Gorostiza wanted to create switchable versions of stapled proteins that could control endocytosis, the process by which molecules enter cells. Such peptides would need to stop the formation of the protein complex crucial to the process. So the researchers designed their peptide to mimic the α-helical end of β-arrestin, part of the protein complex needed for endocytosis.

Using an azobenzene containing the crosslinker molecule 3,3′-bis(sulfonato)-4,4′-bis (chloroacetamido)azobenzene (BSBCA), the Spanish researchers experimented to find how far apart they needed to attach each end of the crosslinker molecule on the peptide chain to get it to form a helix that would bind to its target. They found that by varying the number of helical turns across which the linker was attached, they could change whether the binding helix was formed in the *cis* or *trans* configurations.[6] In this way, they were able to create two oppositely switching peptides, one that switched off endocytosis in the dark, the other by uv light.

While in time this may have therapeutic applications, for now Gorostiza says its importance is as a tool to study cellular processes that involve endocytosis, such as cell signalling, and the defective endocytosis process that seems to occur in tumour cells.

Light-switchable antibiotics have the advantage they can be switched off when not in the body, impeding resistance problems

Challenges

Ultimately, however, azobenzene is not the ideal photoswitch because it changes conformation at 340nm in the UV region, which is damaging to cells. So researchers are now looking for molecules that will switch at wavelengths in the red region. This has the additional advantage that red light can penetrate further into the body. According to Trauner: 'The redder the better…if you are able to operate in the 700–750nm range, the peak red, almost near infrared, you can go deep into tissues.'

There has already been some success in designing such photoswitchable molecules. By substituting all four ortho-positions on both aromatic rings of azobenzene with electron-rich substituents, such as chlorine, chemist Andrew Woolley at the University of Toronto, Canada, has created a stapled peptide that can be switched to its helical-active form in the red region.[7]

While still in its infancy, photopharmacology is now becoming more than a mere novelty. An important area where light switchable drugs could have a big impact is cancer chemotherapy. Targeting specific areas could prevent the wide spread off-target damage that causes severe side effects like hair loss and nausea. Feringa, for example, has already started to design photoswitchable proteasome inhibitors, drugs that block the breakdown of tumour-suppressing proteins for treating myelomas and lymphomas.

Photopharmacology is an expanding area of research and it may not be long before the pharmacist gives you an LED along with your prescription drugs.

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Photoswitchable diabetic drugs could avoid over-production of insulin, which results in blood sugar crashes and increases in cardiovascular disease

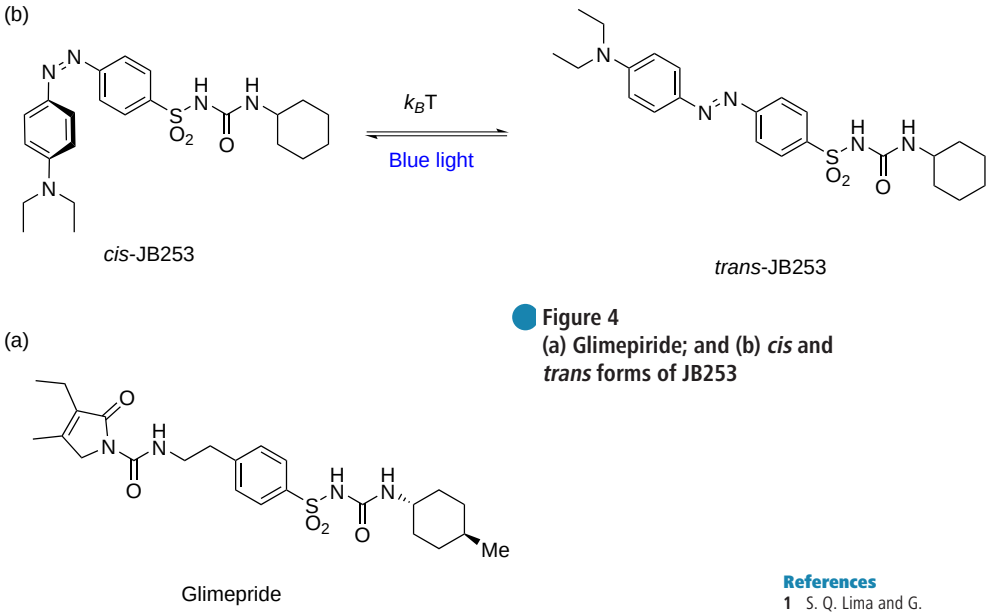

Figure 4
(a) Glimepiride; and (b) *cis* and *trans* forms of JB253

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