VIP Photopharmacology

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# Azo-Propofols: Photochromic Potentiators of $GABA_A$ Receptors**

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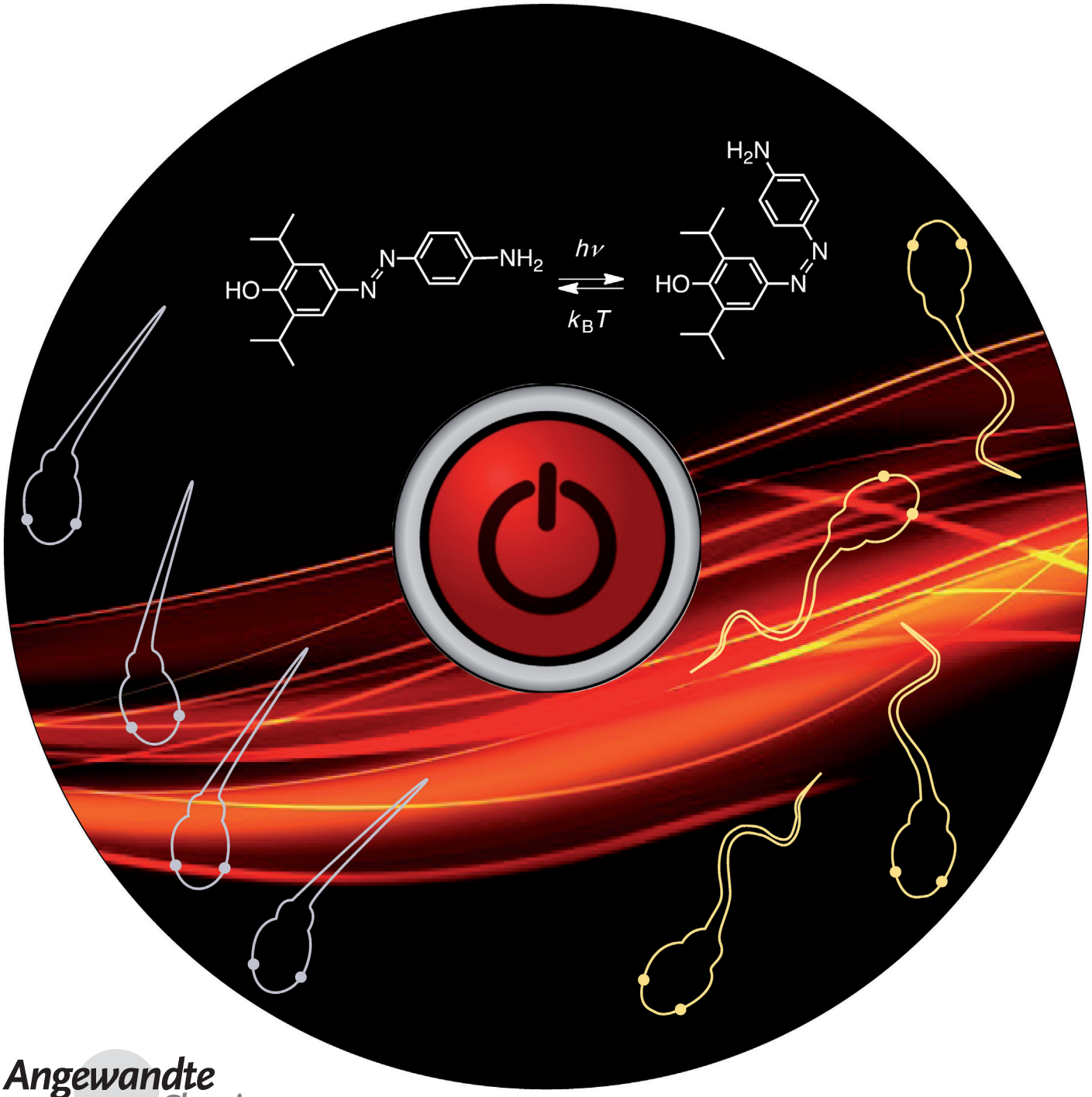
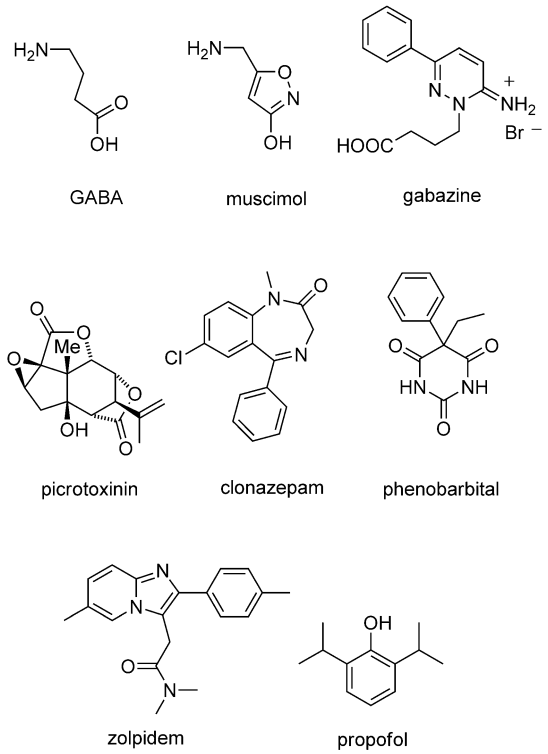


$GABA_A$ receptors are pentameric ligand-gated ion channels that are activated by the major inhibitory neurotransmitter in the mammalian brain, γ-aminobutyric acid (GABA).[1] Binding of GABA results in the opening of a chloride ion selective pore, thus hyperpolarizing the postsynaptic neuron and decreasing the likelihood of action-potential firing. As such, $GABA_A$ receptors are a prominent target for anesthetic, hypnotic, and anticonvulsant drugs (Scheme 1).[2,3]

While agonists, antagonists, and blockers of $GABA_A$ receptors, such as muscimol, gabazine, or picrotoxinin, respectively, have proven to be valuable research tools, their impact on human medicine has been limited. Drugs that target these receptors are dominated by allosteric modulators that potentiate, that is, increase, chloride currents elicited by the neurotransmitter. Well-established potentiators include benzodiazepines (e.g. clonazepam), barbiturates (e.g. phenobarbital), the imidazopyridine zolpidem, and the simple phenol propofol.[2] These drugs bind to distinct allosteric sites on $GABA_A$ receptors, thereby increasing the mean open time or the opening frequency of the channel. However, the analysis of their exact binding sites at a molecular level has been complicated by a lack of detailed structural data.

After its discovery in 1980, propofol has become the most widely used intravenous general anesthetic.[4] Although its mode of action has not been fully elucidated, it is commonly accepted that the anesthesia induced by this unusually lipophilic drug mostly results from potentiation of GABA-induced currents, as well as a direct activation of the chloride ion channel at high concentrations. Propofol has a rapid onset and offset of action and shows only minimal accumulation upon prolonged use. The intravenous administration of propofol is also associated with reduced postoperative nausea and vomiting.[5]

While $GABA_A$ receptors respond to a variety of ligands, they are normally not sensitive toward light. It would be fascinating to confer light sensitivity to these ion channels, since light is unsurpassed in terms of the temporal and spatial precision it provides. This light sensitivity could be indirectly achieved by using ligands that act on the receptors but can be optically switched between an active and an inactive form. Photochromic ligands of $GABA_A$ receptors could be agonists, antagonists, or allosteric modulators. In principle, these ligands could be covalently attached as photoswitched tethered ligands (PTLs) or act as soluble photochromic ligands (PCLs).[6] Indeed, both approaches have been used to convert neuronal[7] and neuromuscular[8] nicotinic acetylcholine receptors, another type of pentameric ligand-gated ion channels, as well as ionotropic glutamate receptors[9] into artificial photoreceptors. Tethered and soluble photochromic blockers of $K^+$, $Na^+$, and $Ca^{2+}$ ion channels have been described as well and have been used to control heartbeat,[10] pain sensation[11] and visual responses[12] in different animals with light.




***Scheme 1.*** Agonists (GABA, muscimol), antagonists (gabazine), blockers (picrotoxinin), or potentiators (clonazepam, phenobarbital, zolpidem, propofol) of $GABA_A$ receptors.

We now report photochromic potentiators of GABA currents that change the strength of GABA-induced currents in a light-dependent fashion. Our program was prompted by a recent report on a photoaffinity probe based on propofol, *p*-4-aziC5-propofol that underscored that a relatively large substituent in the *para*-position of the phenol would be tolerated and that the propofol pharmacophor would be compatible with photochemistry (Scheme 2a).[13] Accordingly, we designed a series of azobenzene derivatives of propofol; in these derivatives an aryldiazene unit is directly coupled to the pharmacophor. These molecules, termed azopropofols 1–16 (**AP1**–**16**) are shown in Scheme 2a. Their

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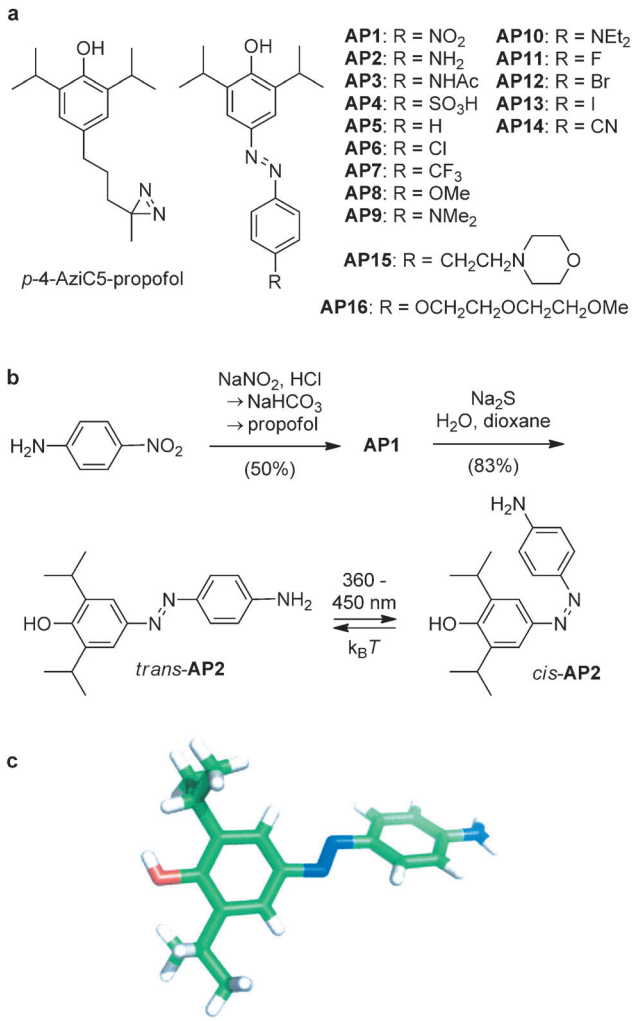


***Scheme 2.*** a) *p*-4-aziC5-propofol, a photoreactive derivative of propofol, and **AP1**–**16**, photoswitchable derivatives of propofol. b) Synthesis of **AP1** and **AP2**, which is shown in its *trans* and *cis* configuration. c) X-ray structure of *trans*-**AP2**; C green, O red, H white, N blue.

varying substituent in the 4′-position of the azobenzene core determines their pharmacodynamic as well as spectral properties.

Compounds **AP1**–**16** were synthesized using classical diazo-coupling chemistry, as shown in the representative synthesis of **AP1** and **AP2** (Scheme 2b, also see the Supporting Information).[14] The X-ray structure of **AP2** is displayed in Scheme 2c.[15] Owing to crystal packing effects, the *trans*-azobenzene is not fully planar in this solid-state structure (see the Supporting Information for a more detailed discussion of these effects).

Although several of the azo-propofols shown in Scheme 2 function as photochromic potentiators, **AP2** emerged early on as our most promising candidate owing to its favorable pharmacological and photochemical features. By virtue of its amino substituent, the photoswitch has a red-shifted absorption spectrum, which means that a maximum *cis* content is achieved at irradiation with 404 nm light. However, owing to the broad absorption spectrum, slightly longer or shorter wavelengths could be used effectively (see the Supporting Information). In addition to this, the substitution of the azobenzene core with electron-donating substituents greatly decreases the thermal stability of the *cis* isomer. Therefore, **AP2** quickly reverts to its *trans* form once the light is switched off. Since the absorption spectra of the *cis* and *trans* isomers are very similar (see the Supporting Information), this process cannot be accelerated by irradiation with a different wavelength. Other APs studied have less favorable photophysical properties, show decreased potency (e.g. **AP3**, **AP9**, **AP10**), no activity at all (e.g. **AP4**),[14c] or unfavorable solubility and distribution (e.g. **AP6**).

The effect of **AP2** on $Cl^-$ currents was investigated with electrophysiology using $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors expressed in *Xenopus* oocytes (Figure 1).[16] This receptor subtype represents the most prevalent form in the human brain.[17] First, the heterologously expressed $GABA_A$ receptors were exposed to GABA at a concentration eliciting 0.3% of the maximal current amplitude in combination with increasing concentrations of propofol or **AP2** in the dark to compare the relative effect of the compounds. From the resulting dose–response curves, we extracted an $EC_{50}$ value of $(17.1 \pm 2.9)$ μM for propofol and of $(6.1 \pm 0.4)$ μM for **AP2** (mean ± standard error of the mean (SEM), $n = 4$; Figure 1a). Thus, **AP2** in its dark-adapted *trans* form has a significantly higher affinity than propofol itself, albeit its efficacy is reduced by about twofold when compared with its parent compound.

Having established that **AP2**, in its dark-adapted form, has an effect on $GABA_A$ receptors, we investigated the light dependency of the current potentiation. UV/Vis light from an Ultrafire 1 Watt UV LED pocket lamp (YonC Trading, Zürich; emission wavelength 390–450 nm) had no effect on the GABA response or the combined GABA/propofol response (data not shown). Figure 1b illustrates the effect of UV/Vis light on currents elicited by the combined application of GABA and **AP2**. Stimulation of GABA currents by **AP2** (1.5 μM) was $(159 \pm 25)$% (mean ± SEM, $n = 6$). Exposure to light decreased the residual stimulation to $(18 \pm 3)$% (mean ± SEM, $n = 6$). Similar observations were made using a UV high power LED pocket lamp, 5 Watt (Uveco GmbH, Bruckmühl, Germany), emission wavelength 355-380 nm equipped with a CHROMA bandpass filter D365/10×, to limit light emission to 360-370 nm. The possibility to use these different light sources reflects the broad absorption spectrum of **AP2**. Owing to redistribution of the hydrophobic compound **AP2** into egg yolk, the rate of photoswitching could not be determined in *Xenopus* oocytes. For this purpose, we expressed $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors in HEK cells and performed experiments using the whole-cell patch-clamp technique. GABA was co-applied with **AP2**. Subsequently, the perfusion was stopped to prevent arrival of new *trans*-**AP2** during the measurement, and the cells were exposed to the light. The current amplitude decreased rapidly and increased again upon turning off the light source. Current traces were fitted with a mono-exponential function. The time constant τ amounted to $(1.1 \pm 0.4)$ s (mean ± SD, $n = 7$) for the *trans*-to-*cis* transition and $(2.0 \pm 0.7)$ s (mean ± SD, $n = 6$) for the *cis*-to-*trans* transition.

Next, we investigated anesthetic activity and photoreversibility of both propofol and **AP2** in a small animal model,

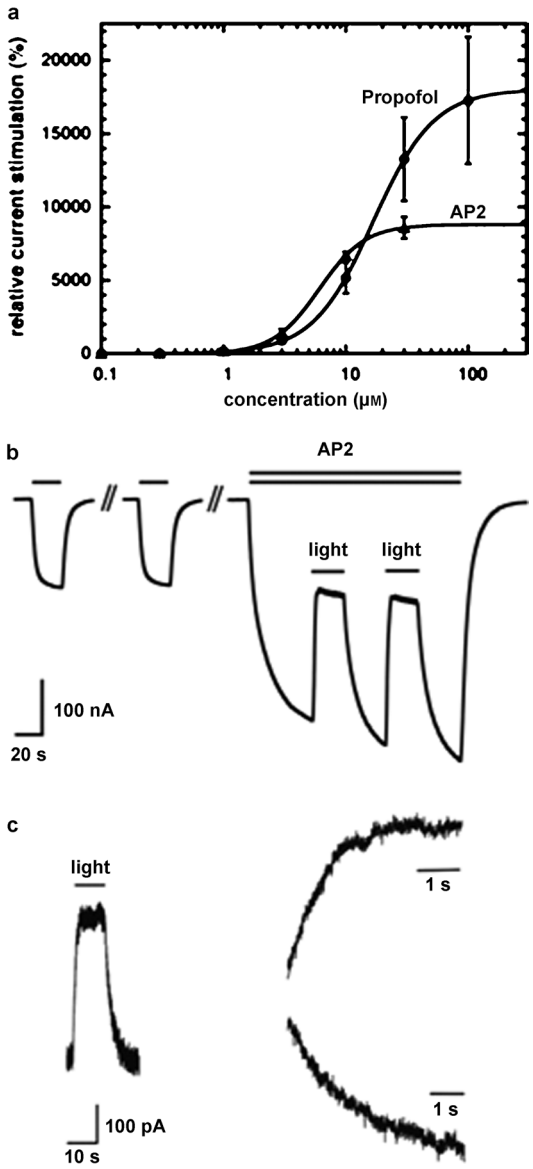


***Figure 1.*** a) $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors were expressed in *Xenopus* oocytes. Currents were activated with a concentration of GABA eliciting 0.3% of the maximal current amplitude ($EC_{0.3}$) with increasing amounts of either propofol or **AP2**. Mean ± SEM of four experiments is shown. b) GABA (1 μM) was applied repetitively until a stable current response was observed. Co-application of **AP2** (1.5 μM) with GABA resulted in current potentiation. During co-application, the oocyte was exposed to a light source emanating 390–450 nm light. As a consequence, current stimulation rapidly decreased until it reached a steady level. When the light-source was turned off, the amplitude increased again. This procedure was repeated. This experiment was repeated independently six times using different oocytes. c) $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors were expressed in HEK cells. GABA (0.5 μM) was co-applied with **AP2** (5 μM). Subsequently, the perfusion was stopped and the cells were exposed to the light source. The inward current amplitude decreased rapidly and increased again upon turning off the light source (trace left). Current decrease (trace top right) and increase (trace bottom right) were each fitted with a mono-exponential function.

albino *Xenopus laevis* tadpoles. Groups of animals were placed in aqueous solutions containing either propofol or **AP2** and tested every five minutes for loss of righting reflexes (LORR), a standard assay for anesthesia.[13] Steady-state LORR results were observed at 10 min for propofol and 25 min for **AP2**. After 30 min in drug solution, each animal was exposed for five to ten seconds to 360–370 nm bandpass filtered UV light (details in the Supporting Information) while retesting for LORR. Propofol alone produced LORR with an $EC_{50}$ of 1.3 μM (Figure 2a). Illumination induced

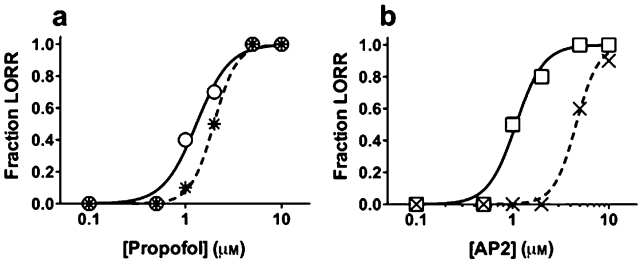


***Figure 2.*** Light-dependent anesthesia in tadpoles. Loss of righting reflexes is plotted against aqueous anesthetic concentration, overlayed with logistic fits. Each point represents data from ten animals. a) 360–370 nm light, an apparently noxious stimulus in *Xenopus* tadpoles, produces a small rightward shift in propofol-dependent loss of righting reflexes (LORR) from (1.1 ± 0.1) μM (circles) to (2.0 ± 0.1) μM (stars). b) 360–370 nm light shifts the **AP2**-dependent LORR curve to the right from (1.1 ± 0.1) μM (squares) to 4.6 ± 0.2 μM (crosses). This larger shift is due to photoisomerization of **AP2**.

vigorous swimming activity in unanesthetized tadpoles (thus suggesting that illumination represents a noxious stimulus) and a small rightward shift in the corresponding plot of LORR versus propofol concentration was observed ($EC_{50}$ ca. 2.0 μM; Figure 2a). **AP2** alone produced LORR with an $EC_{50}$ value similar to that of propofol (1.1 μM; Figure 2b). However, illumination produced a large rightward shift in the **AP2** $EC_{50}$ value to 4.6 μM (Figure 2b). All animals recovered from anesthesia when returned to water alone. In an independent set of experiments (see video in the Supporting Information), propofol (3 μM) produced LORR in all tadpoles with or without light, whereas in **AP2** (3 μM), all animals showed LORR without light and all spontaneously righted themselves and swam during illumination with UV light.

The photoreversibility of both **AP2**-induced $GABA_A$ receptor modulation and its anesthetic action in animals supports the hypothesis that anesthesia caused by **AP2** and propofol is largely mediated by $GABA_A$ receptors. However, evidence also implicates other targets, including HCN1 channels (hyperpolarization-activated cation channels),[18] in propofol's anesthetic actions. The examination of the effects of **AP2** on these other targets and the investigation of the photoreversibility of the modulation of these targets might help to further elucidate their roles in the pharmacology of general anesthesia.

In summary, we have developed photoswitchable versions of propofol that allow the indirect optical control of $GABA_A$ receptors. Functionally, our compounds differ from previously introduced PCLs, because they act as photochromic

  

potentiators rather than photochromic agonists, antagonists, or channel blockers. Application of our lead compound, **AP2**, in the dark potentiates GABA-induced $Cl^-$ currents, which can be reversed upon irradiation with violet light. The ability of azo-propofols to control neural systems has been demonstrated, since **AP2** functions as a light-dependent anesthetic in translucent tadpoles. Future work will address the usefulness of azo-propofols in other systems, such as brain slices and retinas lacking innate photoreceptors, wherein photochromic potentiators could restore visual responses through their action on neurons expressing $GABA_A$ receptors.

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[15] **AP2**: $C_{18}H_{23}N_3O$, $M_r = 297.395\ g\,mol^{-1}$, red block, $0.14 \times 0.25 \times 0.30$ mm, monoclinic, $P21$, $a = 10.5454(3)$, $b = 9.5435(3)$, $c = 17.0651(4)$ Å, $\alpha = 90$, $\beta = 90.0955(16)$, $\gamma = 90°$, $V = 1704.28(8)$ Å$^3$, $Z = 4$, $\rho = 1.159\ g\,cm^{-3}$, $\mu(MoK\alpha) = 0.073\ mm^{-1}$, MoKα radiation ($\lambda = 0.71073$ Å), $T = 200$ K, $2\theta_{max}$ 55.02°, 13750 refls., 4143 independent, 3271 with $I \geq 2\sigma(I)$, $R_{int} = 0.042$, mean $\sigma(I)/I = 0.0392$, 429 parameters, $R(F_{obs}) = 0.0432$, $R_w(F^2) = 0.1097$, $S = 1.014$, min. and max. residual electron density: $-0.21$, $0.15\ e\,Å^{-3}$; data collection by means of a Nonius KappaCCD diffractometer equipped with a rotating anode generator (ω-scans), structure solution by direct methods with SIR97, structure refinement with SHELXL-97, O- and N-bonded H atoms have been refined freely, C-bound H atoms have been added geometrically treated as riding on their parent atoms. CCDC 890176 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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