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Azogabazine; a photochromic antagonist of the $GABA_A$ receptor†

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The design and synthesis of azogabazine is described, which represents a highly potent (IC_{50} = 23 nM) photoswitchable antagonist of the $GABA_A$ receptor. An azologization strategy is adopted, in which a benzyl phenyl ether in a high affinity gabazine analogue is replaced by an azobenzene, with resultant retention of antagonist potency. We show that cycling from blue to UV light, switching between *trans* and *cis* isomeric forms, leads to photochemically controlled antagonism of the GABA ion channel.

$GABA_A$ receptors are ligand-gated chloride ion channels,[1] which are activated by γ-aminobutyric acid (GABA); the major inhibitory neurotransmitter present in the central nervous system.[2] $GABA_A$ receptors are also modulated by an array of compounds including neurosteroids, benzodiazepines and barbiturates.[3] A number of small molecule antagonists are known for the $GABA_A$ receptor,[4] of which gabazine (Fig. 1, also called SR-95531)[4a,5] is one of the most widely used.[4e] Gabazine is a competitive antagonist (IC_{50} = 300 nM), and we recently described the development of enhanced potency analogues including Gz-i1 (IC_{50} = 13 nM).[6] Furthermore, by incorporation of a benzophenone, we constructed a photoaffinity labelled version (GZ-B1, IC_{50} = 153 nM) which upon irradiation could be employed to irreversibly block populations of native neuronal $GABA_A$ receptors, facilitating the study of receptor trafficking.[7] We envisaged that reversible light mediated control of activity would offer further opportunities to probe this important class of ion channels.

Chemical photoswitches are photochromic compounds which can be activated and deactivated in cycles.[8] These can be used to reversibly control the function of a biological system with light.[9] By far the most well-known and widely-used

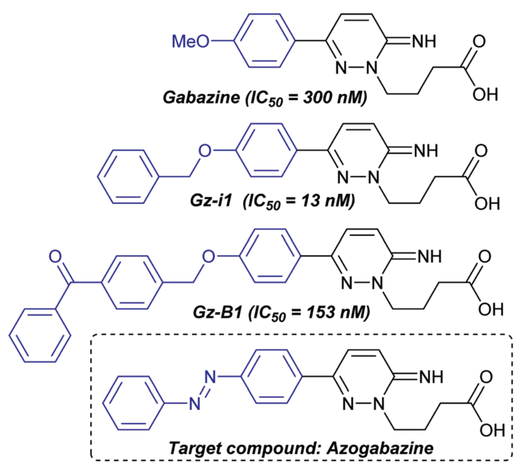

Fig. 1 Gabazine, potent analogues and the targeted azogabazine as a photochromic antagonist of the $GABA_A$ receptor.

photoswitches are azobenzenes, which upon irradiation with UV light form a *cis*-enriched photostationary state, which converts to the more stable *trans* isomer upon visible light irradiation or thermal relaxation. Two main strategies have been developed to exploit the use of photoswitches to infer photochemical control over ion channel activity. The first involves covalent attachment of ligands onto the channel, such that the ligand can bind in its active isomeric form, but upon irradiation is expelled from the binding site. This approach is known as photoswitchable tethered ligands (PTL) and essentially serves to provide a constant high local concentration of the ligand.[9] The second strategy involves the use of freely diffusible ligands, which are only able to bind effectively in one of the isomeric forms. Such compounds are known as photochromic ligands (PCL), and preclude the requirement for mutagenesis to incorporate reactive handles for covalent attachment.

Examples of PTLs and PCLs for $GABA_A$ receptors have been described recently in the literature. Propofol analogues have been designed as photochromic potentiators of $GABA_A$ recep-

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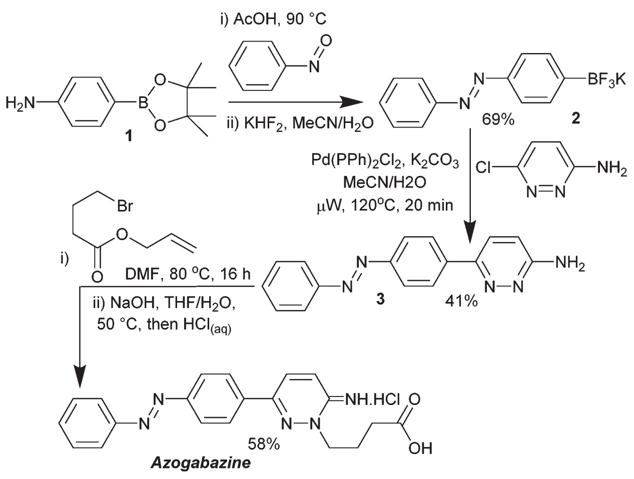
tors.[10] Lin *et al.* have also described the use of an engineered $GABA_A$ receptor to enable the tethering of the agonist muscimol. Rather than the expected agonism they observed antagonism using this PTL, which was also observed using guanidinium analogues of GABA.[10c,d]

Having previously established that elaboration of the gabazine molecule to incorporate benzyl substituents leads to enhanced binding to the $GABA_A$ receptor (*i.e.* Gz-i1), we envisaged that this strategy could be harnessed for the design of potent antagonists as PCLs. Following the 'azologization' approach pioneered by Trauner and co-workers[11] we identified the benzyl ether in Gz-i1 (Fig. 1) as being an ideal structural motif to be replaced by an azobenzene photoswitch. Thus we targeted 'azogabazine' as a prospective new tool for this important class of ion channels.

The synthesis of azogabazine was accomplished in just five synthetic steps (Scheme 1). Condensation of aniline **1** with nitrosobenzene was followed by conversion of the boronate ester into the trifluoroborate salt **2**. Suzuki reaction with 3-amino-6-chloropyridazine[12] afforded the triaryl intermediate **3**, which underwent *N*(2) alkylation and subsequent deallylation to afford azogabazine. The overall yield for the sequence was 16%.

Azogabazine was tested for potency on $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors expressed in HEK293 cells, using whole-cell patch-clamp recording, and proved to be one of the most potent GABA antagonists known (Fig. 2). An IC_{50} of 23 nM was determined, representing a 13-fold improvement over gabazine. This enhanced potency is similar to that observed with Gz-i1, suggesting that the benzyl phenyl ether and azobenzene represented ideal bioisosteres in this application.

UV/Vis absorbance spectra were taken to confirm the retention of azobenzene absorbance characteristics. The initial spectrum was consistent with the thermodynamically favoured *trans*-azogabazine, showing a characteristic large absorbance with λ_{max} of 342 nm representing a $\pi \rightarrow \pi^*$ transition. After 30 s of irradiation with a hand-held torch containing LEDs of



Scheme 1 Synthesis of azogabazine.

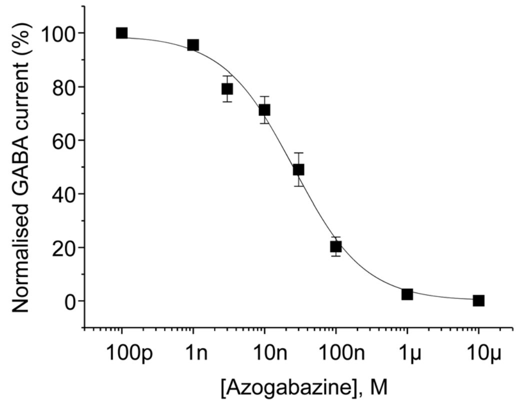


Fig. 2 Concentration inhibition curve for azogabazine. Azogabazine was applied in combination with 10 μM GABA. pIC50: 7.646 ± 0.141, *n* = 6 (IC_{50}: 22.6 nM).

365 nm a further UV spectrum was taken (Fig. 3). This was now characteristic of a *cis* azobenzene-containing compound, with an increase in absorbance at 435 nm and a concomitant decrease at lower wavelengths.

^{1}H-NMR studies confirmed this photoswitching behaviour and allowed quantification of the ratios at photostationary states. Irradiation with a UV light (a 365 nm LED) afforded the *cis*-enriched photostationary state in a 5 : 1 ratio. By contrast irradiation with blue light (a 470 nm LED) afforded predominantly the *trans* product in a 3 : 1 ratio. No further changes were seen after 60 s irradiation in each case, showing the interconversions to form the photostationary states to be rapid. Once formed, the *cis* isomer was found to show slow thermal conversion to the more stable *trans* isomer, requiring 20 days in the dark to achieve full conversion; thus representing a high bistability system.

Azogabazine was then constantly perfused onto $GABA_A$ receptor expressing HEK cells, along with 10 μM GABA, followed by alternating application of blue (a 470 nm LED) and UV light (a 365 nm LED; Fig. 4). Each cycle of blue light led to reduced currents, confirming that the predominating *trans* isomer was serving as the most potent antagonist. In contrast,

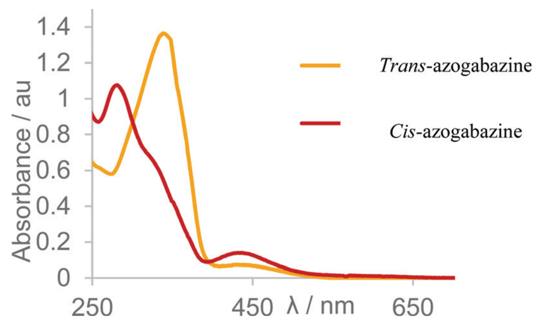


Fig. 3 Overlaid UV/Vis absorbance spectra for (i) sample of azogabazine (orange) representing the *trans* isomer and (ii) sample of UV irradiated azogabazine (red) representing the *cis* isomer.

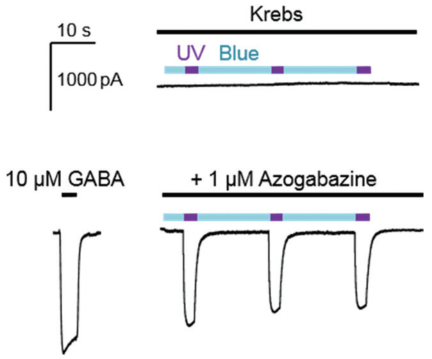


Fig. 4 Whole-cell GABA currents recorded from α1β2γ2 $GABA_A$ receptors expressed in a HEK cell. Upper panel: Cell exposed to Krebs (bar) and alternating periods of blue and UV light which do not induce current responses. Lower panel: The control 10 μM GABA current (left) is reduced in the presence of co-applied azobenzene, and GABA currents are only revealed by exposure to UV light (right).

UV light produced an inward current, as the *cis* azogabazine was generated and no longer served as an effective antagonist. Thus after presumed ejection from the GABA binding sites GABA-mediated activation was taking place.

Conclusions

We have described the design and convenient synthesis of azogabazine, a potent photochromic antagonist of the $GABA_A$ receptor. This study represents a powerful demonstration of the 'azologization' strategy for the design of photochromic ligands. The identification of a benzyl phenyl ether as an 'azostere' guided our strategy for the introduction of the azobenzene motif, and resulted in an antagonist which was 13 times more potent than gabazine itself. Repeated cycling from blue-to-UV light showed robust photo-control of $GABA_A$ receptor channel activity making azogabazine a useful new research tool for studying $GABA_A$ receptors.

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