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Photopharmacology

Enlightening the "Spirit Molecule": Photomodulation of the 5-HT_{2A} Receptor by a Light-Controllable *N*,*N*-Dimethyltryptamine Derivative

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Abstract: Classical psychedelics are a group of hallucinogens which trigger non-ordinary states of consciousness through activation of the 5-HT_{2A} receptor (5-HT_{2A}R) in the brain. However, the exact mechanism of how 5-HT_{2A}R agonism alters perception remains elusive. When studying receptor signaling, tools which work at the same spatiotemporal resolution as the receptor are exceptionally useful. To create such a tool, we designed a set of photoswitchable ligands based on the classical psychedelic *N*,*N*-dimethyltryptamine (DMT). By incorporation of the DMT-indole ring into the photoswitchable system, we obtained red-shifted ligands which can be operated by visible light. Among these azo-DMTs, compound **2h** ("Photo-DMT") stands out as its *cis* isomer exhibits DMT like activity while the *trans* isomer acts as weak partial agonist. Such a *cis*-on "efficacy switch" substantially expands the pharmacological toolbox to investigate the complex mechanisms of 5-HT_{2A}R signaling.

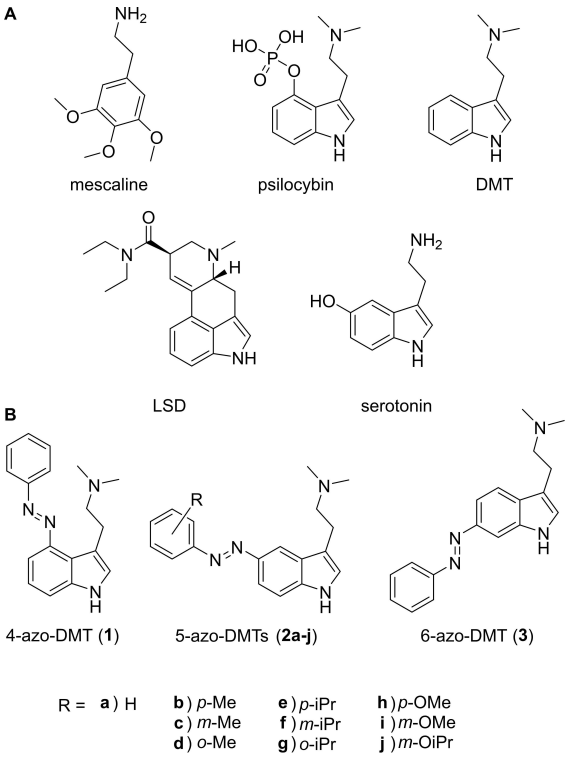

Figure 1. A) Chemical structures of 5-HT_{2A}R agonists mescaline, psilocybin, DMT, LSD and the endogenous agonist serotonin. B) First generation of photoswitchable azo-DMT derivatives ("photo-DMTs") bearing the azo group in position 4 and 6 (**1**, **2a**, **3**) and the resulting library of second generation 5-azo-DMTs (**2b–j**).

Psychedelics such as mescaline, psilocybin and *N*,*N*-dimethyltryptamine (DMT) (Figure 1A) have a long history of usage in human societies. The usage of some of these psychedelics can be dated back to prehistoric times when they were employed to trigger non-ordinary states of consciousness for ritual, spiritual or mystical purposes.[1] DMT—the "spirit molecule"—is best known for the brief and intense psychedelic effect it produces after being consumed in form of the traditional decoction "Ayahuasca".[2] Although it has mostly been used for its psychedelic effects, nowadays it has become subject of research for its very promising results as therapeutic agent and its ability to promote neural plasticity.[3]

DMT is known to bind unselectively to all receptors of the serotonin (5-hydroxytryptamin, 5-HT) receptor family[4] which is grouped into seven subfamilies comprising altogether 14 receptor subtypes. Except for the 5-HT_3 subtype, a pentameric ligand-gated ion channel, all members of this family are G protein-coupled receptors and found in nearly all tissues where they influence various functions, ranging from cognition to thermoregulation.[5] The altered state of consciousness observed after use of classical psychedelics is considered to be caused by agonism at the 5-HT_{2A} receptor in the brain, where DMT—like other classical psychedelics—acts as partial agonist.[6,7] However, the mechanisms by which these agents exert their effects are complex and remain to be under investigation.[8] To elucidate the biological function of these complex signaling cascades, tools are needed which work at the same spatiotemporal resolution as the biological system under investigation.

Photoswitchable ligands i.e., molecules that can reversibly change their chemical structure (Figure 2A) and concomitantly their biological activity upon irradiation with light, enable researchers to make use of light with its unmatched spatiotemporal precision as an external stimulus.

For a variety of targets, azobenzene-based photoswitchable ligands have shown to be powerful tools for the investigation of elusive problems.[9–23] Surprisingly, there has been a lack of such tool compounds for the 5-HT receptors, only most recently a set of photoswitchable serotonins acting as "potency switches" has been reported.[24] Complementary to this work, we herein report the design and synthesis of an "efficacy switch" based on the classical psychedelic DMT. Modulating the efficacy of this ligand with light allows precise control over the activity of the 5-HT_{2A}R, effectively turning it into an artificial photoreceptor.

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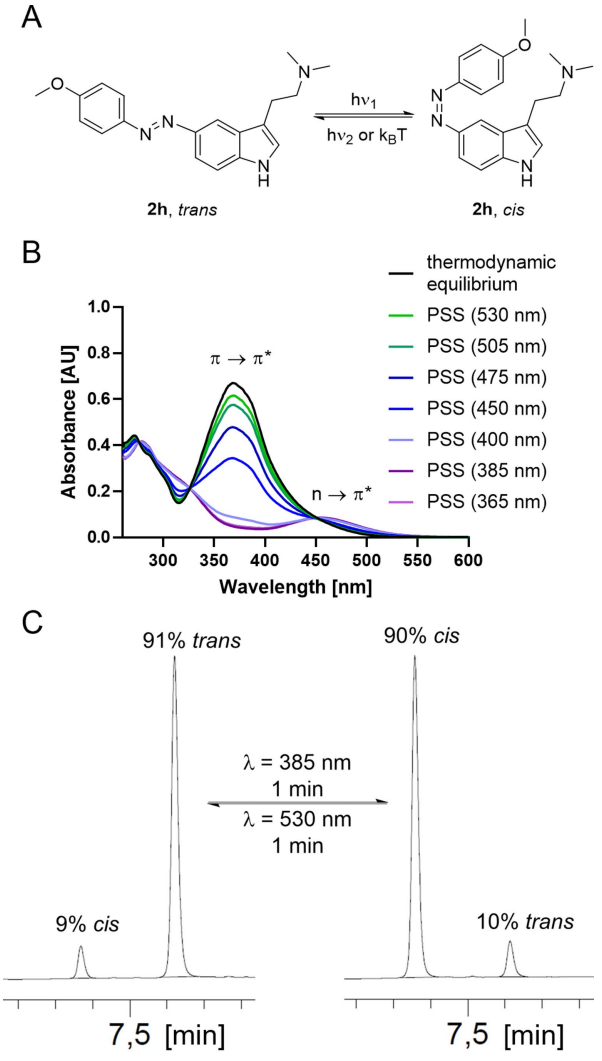


Figure 2. A) Photo-DMT (**2h**) shown in its *cis* and *trans* configuration. B) UV/Vis spectra of compound **2h** at photostationary states (PSSs) after irradiation with given wavelengths in DMSO. C) Ratios of *trans* and *cis* isomers after switching into the respective PSSs in MeOH/H_2O.

With a molecular weight of just 188 g mol^{-1} and only one functional group, the chemically most interesting characteristic of the serotonin analogue DMT is its simplicity. The goal of our design approach was to maintain this simplicity while aiming for a maximal effect on efficacy upon photoisomerization. Therefore, we tried to incorporate the indole ring of DMT into the photoswitchable system, so that only one additional aromatic ring had to be added, keeping the molecular weight of the compound around 300 g mol^{-1}. By design, this requires an "azo-extension" strategy at one of the indole *C*-atoms.[25]

Several structure–activity relationships (SARs) have been investigated concerning *N*-alkylation and side chain alkylation of tryptamines, but only few SARs are described for substitution at the aromatic system, most of which show that sterically small substitutions at the C4 or the C5 position of the indole are tolerated.[26–28] But none of the previously investigated substituents was comparable in size to a benzene ring, therefore, in the absence of conclusive SARs we based our design approach on the photophysical properties of phenylazoindoles. Although phenylazoindoles have not yet found application in photopharmacology, these promising scaffolds have already been thoroughly characterized for their photophysical properties.[29,30]

Knowing that "azo-extension" in C2 and C3 position would lead to undesirably fast relaxing *cis*-isomers, we performed (and therefore synthesized) an azo-walk from the C4 to the C6 position instead (Figure 1B). This provided us with three photoswitchable DMT derivatives (**1**, **2a**, **3**) which were tested for their ability to activate the 5-HT_{2A}R in vitro. A recently developed β-arrestin2 recruitment NanoLuc® Binary Technology (NanoBiT®) assay was used to detect β-arrestin2 recruitment to the receptor as a quantifiable parameter of its activation.[31,32] Besides the primary signaling cascade of the 5-HT_{2A}R, mediated by the $G_{\alpha q}$ family of G proteins, also β-arrestin2 is recruited to the receptor upon activation. This results in desensitization, internalization, inactivation, and induction of separate signaling pathways, the latter seem to be pivotal for the mediation of psychedelic effects.[33]

Due to the substantial likelihood that addition of a phenylazo group might result in complete abrogation of activity at the receptor, we were pleasantly surprised that two out of three derivatives maintained DMT-like activity. Only the C6 derivative (**3**) was inactive while the C4 and C5 derivatives (**1**, **2a**) maintained activity and even showed tendencies of light-dependent activity differences /nl:(Figure 3A, B). Interestingly, photopharmacological properties of the latter two compounds were inverted. Compound **1** was found to be slightly more potent in its *trans* isoform (*trans*-on), while **2a** showed a higher potency in its *cis* isoform (*cis*-on).

We decided to focus on the optimization of the *cis*-on derivative, since in contrast to a *trans*-on compound it cannot spontaneously activate itself, which would reduce spatiotemporal resolution. Therefore, a library of 5-azo-DMT derivatives (**2b–j**) was synthesized, derived from a methyl-, isopropyl- and methoxy-walk on the phenylazo substituent (Figure 1B). The methyl- and isopropyl-walk strategies were employed as these had previously proven effective in maximizing the activity differences between *cis* and *trans* isoforms.[34] Methoxy and isopropoxy derivatives were synthesized as the position of methoxy substituents was reported to have a pronounced effect on the binding affinity of DMT.[35] Therefore, we speculated that relocation of a methoxy substituent due to photoisomerization would have a significant effect on receptor activity. To check if such an effect is additive with a sterical hindrance, we also synthesized one isopropoxy derivative.

Starting from commercially available 5-nitro-1*H*-indole (**4**) the 3 position was acylated with oxalyl chloride in ether (Scheme 1). The resulting acyl chloride was subsequently reacted with dimethylamine in THF to produce amide (**5**) in 92 % yield over two steps. Hydrogenation of the nitro moiety in MeOH, using 10 wt % palladium on carbon under hydrogen atmosphere, afforded indole-amine (**6**) in a quantitative manner. Derivatization had to be carried out in

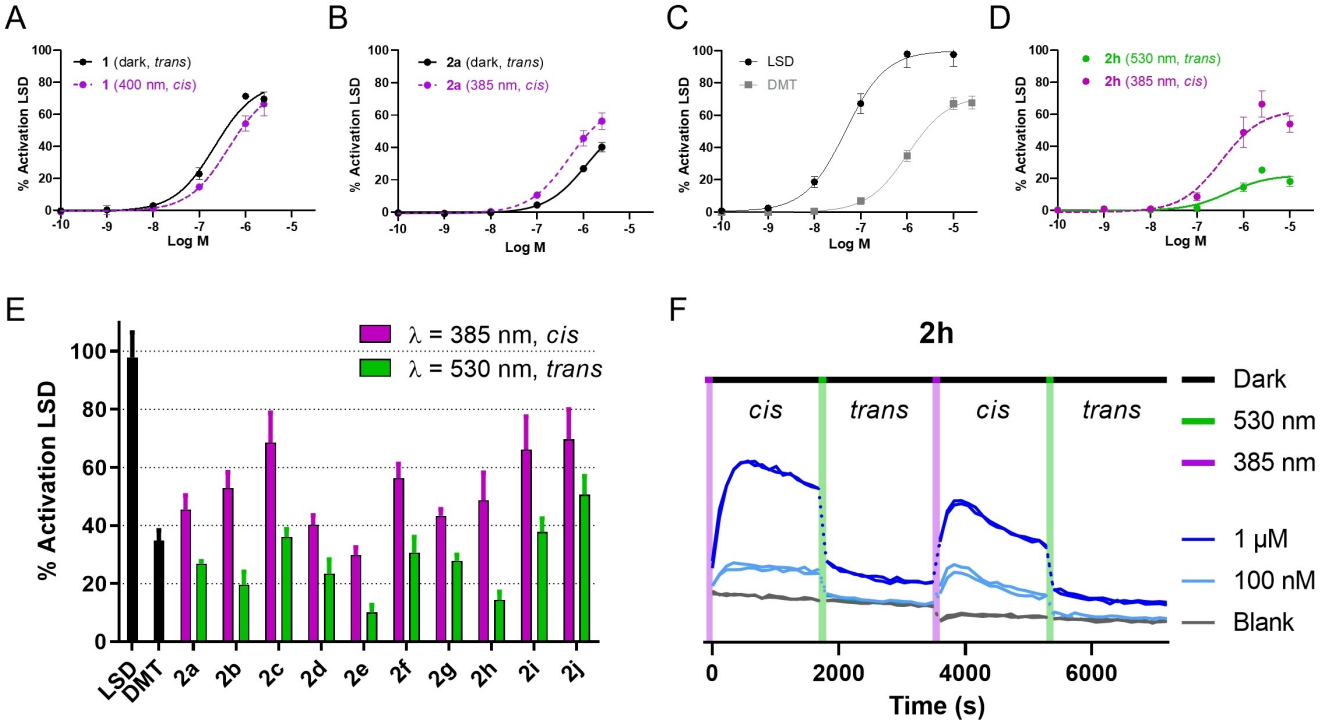
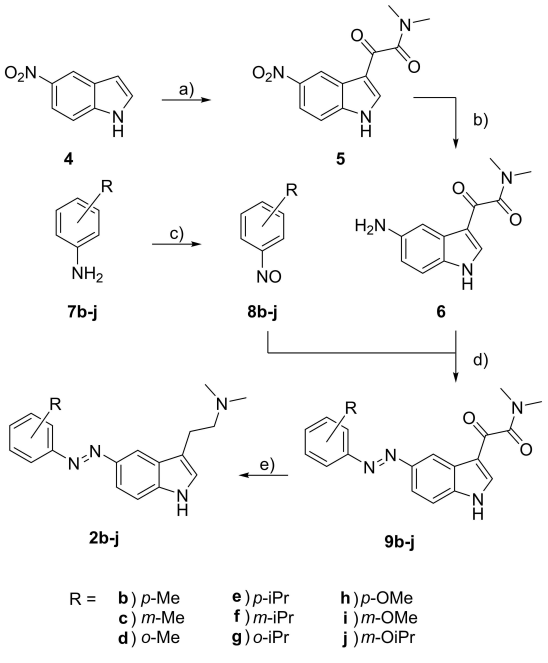


Figure 3. In vitro characterization of photoswitchable 5-HT_{2A}R ligands. Detection of β-arrestin2 recruitment to the 5-HT_{2A}R as a measure of receptor activation. A)–D) Data is given as the mean area under the curve (AUC) ± standard error of mean (SEM) of n independent experiments. Concentration-response curves of compounds **1** (A) and **2** (B) in the dark adapted as well as in the *cis* enriched state; ($n=3$). C) Reference compounds LSD and DMT; ($n=6$). Data is normalized to maximal LSD response while DMT was measured to function as benchmark for all Photo-DMTs. D) Efficacy *cis*-on switch (**2h**) in its 385 nm and 530 nm irradiated PSSs; ($n=3$). E) Overview of light-dependent 5-HT_{2A}R activation at 1 µM for all 5-azo-DMTs; ($n=3$). F) Dynamic receptor photoactivation employing compound **2h** (Photo-DMT) demonstrated by photomodulation of β-arrestin2 recruitment to the receptor (representative traces; data corrected for inter-well variability).



Scheme 1. Synthesis of position 5-azo-DMTs (**2b–j**). Reagents and conditions: a) i) oxalyl chloride, Et_2O, 0 °C to RT; ii) 2 M dimethylamine in THF, RT, 92%; b) 10 wt% Pd/C, 1 atm H_2, MeOH, RT, quant.; c) Oxone®, H_2O, CH_2Cl_2, RT; d) AcOH, CH_2Cl_2, RT, 15–98%; e) $LiAlH_4$, THF, reflux, 16–88%.

the next, penultimate step to mask the amino function which was interfering with reduction of the oxoacetamide. Therefore, the nitroso compounds (**8b–j**) were synthesized by partial oxidation of the corresponding anilines (**7b–j**) with Oxone® in a heterogeneous H_2O/CH_2Cl_2 system. The nitroso compounds were directly reacted in a Baeyer-Mills reaction with compound **6** and a stoichiometric amount of AcOH in CH_2Cl_2, yielding the photoswitchable oxoacetamides (**9b–j**). The subsequent reduction of the oxoacetamide moiety with $LiAlH_4$ in THF under reflux afforded the target 5-azo-DMTs (**2b–j**).

The photophysical properties of compounds **2a–j** were determined both in DMSO and phosphate buffered saline (PBS, pH 7.4) by UV/Vis spectroscopy (cf. Supporting Information). This provided the spectra of the respective photostationary states (PSS), i.e. the *cis*/*trans* equilibrium as a function of the wavelength used for irradiation (Figure 2B). By virtue of the electronic properties of the indole, absorption spectra of the phenylazoindoles are slightly red-shifted in comparison to a regular, unsubstituted azobenzene. This allows operating the photoswitchable ligands in the visible spectrum, using 385 nm to achieve maximal conversion to *cis* and 530 nm for the reversed isomerization to *trans* (Figure 2C). In comparison to an unsubstituted azobenzene other hallmarks stayed mostly unaffected (Supporting Information Table 1).

Afterwards, concentration-response curves in *cis* and *trans* enriched PSSs were determined. As expected, the *cis*-on behavior of the parent compound was maintained in the whole set and for some compounds even increased. In general, the following qualitative trends were observed when comparing at a concentration of 1 μM (Figure 3E):

Substitution in *ortho* position was observed to be detrimental for activity in general as well as for the activity differences of the isomers. *Ortho* substituted DMTs (**2d**) and (**2g**) scored among the lowest in both categories. The small *cis*/*trans* efficacy quotient at 1 μM (**2d**, $cis_{Eff}/trans_{Eff}$ = 1.72; **2g**, $cis_{Eff}/trans_{Eff}$ = 1.55) is potentially caused—among other effects—by the poor *cis*/*trans* ratios in the 530 nm PSS (**2c**, 67 % *trans*; **2f**, 70 % *trans*) which were likewise the lowest of the set of compounds tested.

Substitution in *meta* position is beneficial for overall activity but does not result in significant differences between the activities of the photoisomers. The *meta* substituted ligands (**2c**, **2f**, **2i**, **2j**) showed even higher activity than DMT and the highest activity within our set of compounds, but the *cis*/*trans* efficacy quotient was moderate only (**2c**, $cis_{Eff}/trans_{Eff}$ = 1.91). Interestingly, introduction of the isopropoxy substituent removed the *cis*/*trans* activity differences which were observed for *meta* methoxy and *meta* isopropyl substitution.

Substitution in *para* position proved to be optimal for induction of high activity differences of the respective photoisomers. In the order methyl < isopropyl < methoxy the *para* substituted ligands exhibited the highest *cis*/*trans* efficacy quotients at 1 μM ($cis_{Eff}/trans_{Eff}$ = 2.69 < 2.96 < 3.39). The *para* methoxy compound **2h** showed submicromolar potency (EC_{50} = 334 nM) and a DMT like efficacy (E_{max} = 63 %; *cf.* DMT: EC_{50} = 1.03 μM, E_{max} = 72 %) in its *cis* enriched PSS, while the *trans* enriched PSS showed substantially reduced activity (EC_{50} = 464 nM, E_{max} = 22 %) (Figure 3C, D).

Next, we tested if compound **2h** allowed reversible dynamic photocontrol over the receptor. Dilutions were switched to *cis* and *trans* enriched states, respectively, and added to the cells of the NanoLuc® assay described above. After 30 min of measurement the plate was taken out of the reader for two-minute irradiation with 530 nm and again measured for another 30 min. This was done two more times alternating the irradiation wavelengths. Starting from the *cis* enriched state, receptor activation could be turned off by irradiation with 530 nm, this inactivated state was dynamically reactivated by irradiation with 385 nm light and *vice versa* (Figure 3F). Starting from the *trans* enriched state, a corresponding light-dependent activity was observed while the reference showed no such behavior (cf. Supporting Information).

Interestingly, switching of compound **2h** at a concentration of 1 μM from *cis* to *trans* reduces receptor activation to a level which is comparable to the response caused by a 100 nM *cis*-enriched solution. This correlates with the *cis*/*trans* ratio of the *trans*-enriched state (cf. Figure 2C), indicating that residual activity of the *trans* enriched state might be caused by remaining amount of *cis*-photoisomer.

In radioligand binding studies (performed at ambient light) conducted under the National Institute of Mental Health's Psychoactive Drug Screening Program (PDSP)[36] the *trans* isomer of compound **2h** was found to bind with submicromolar affinity to the 5-HT_{2A}R (K_i = 225 nM) verifying that compound **2h** acts as a true "efficacy switch". Binding data at the entire 5-HT receptor family showed that compound **2h** kept the unselective binding properties of its parent compound DMT (cf. Supporting Information; PDSP# 61744).

In summary, we have developed and characterized a set of photoswitchable versions of DMT (Photo-DMTs). Notably, our approach largely retains the simplicity of DMT using the innovative phenylazoindole moiety as photoswitchable unit. In doing so, we extended the SARs of the tryptamine class of psychedelics, showing that "azo-extension" in positions 4 and 5 results in highly active compounds, comparable to DMT. Out of these compounds, Photo-DMT (**2h**) stands out as *cis*-on "efficacy switch" that allows reversible optical control of the 5-HT_{2A}R. As such, we think that this will be an interesting tool compound in the ongoing efforts to understand the effects of psychedelics in the mammalian brain.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

Keywords: 5-HT_{2A} Receptor · Phenylazoindoles · Photopharmacology · Psychedelics · Tryptamines

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