Supporting Information

Enlightening the “Spirit Molecule”: Photomodulation of the 5-HT$_{2A}$ Receptor by a Light-Controllable $N,N$-Dimethyltryptamine Derivative

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General Experimental Procedures

Methods and Materials

Common reagents and solvents were purchased from the following commercial suppliers: ABCR, Alfa Aesar, BLD-Pharm, Sigma-Aldrich and used without further purification unless otherwise stated. Dry Tetrahydrofuran (THF) was freshly distilled from sodium-benzophenone under an argon atmosphere. Reaction progress was monitored using analytical thin-layer chromatography (TLC) on precoated silica gel 60 GF254 plates (Macherey Nagel GmbH & Co. KG, Düren, Germany). Detection was carried out by irradiation and consequent fluorescence quenching at 254 nm or excitation at 365 nm. Compounds were purified by column chromatography using silica gel 60 (60 Å pore size, 40–63 µm; Macherey Nagel GmbH & Co. KG, Düren, Germany) as the stationary phase.

Reverse phase column chromatography was performed on an Interchim Puri Flash 430 (Ultra Performance Flash Purification) instrument connected to an Interchim Flash ELSD. FlashPure 4g, C18 columns were used. Nuclear magnetic resonance spectra were measured on a Bruker AV-400 NMR instrument (Bruker, Karlsruhe, Germany) in deuterated solvents (DMSO-d$_6$, CDCl$_3$, MeOD-d$_4$). Chemical shifts are expressed in ppm relative to DMSO-d$_6$, CDCl$_3$, MeOD-d$_4$ (2.50/7.26/3.31 for 1H; 39.52/77.16/49.00 for 13C). Measurements for verification and purity of the compounds were performed with a Shimadzu LC/MS system, comprising a DGU-20A3R controller, pump LC-20AB, degasser DGU-20A, and SPD-20A UV/Vis detector. Compounds were dissolved in MeOH and filtered through syringe filters prior to measurement. As a stationary phase, a Synergi 4U fusion RP 80 Å (150 × 4.6 mm) column was used (Phenomenex). As a mobile phase, a gradient of MeOH/water (both containing 0.1% formic acid) was used. Method: flow rate: 1.0 mL/min; detection: 254 nm; scan range: 60–1000 m/z; gradient: A: H$_2$O (0.1 % HCOOH); B: MeOH (0.1 % HCOOH) 0–8 min 5% →90 % B, 8–13 min 90 % B, 13–14 min 90 %→5 % B, 14–18 min 5 % B. The purity of all target compounds was found to be ≥95%.

ESI ionization was accomplished by a downstream Shimadzu LCMS-2020 mass spectrometer. Data are reported as mass-to-charge ratio (m/z) of the corresponding positively charged molecular ions. Target compounds were characterized by high-resolution mass spectroscopy (HRMS, ESI) on a Bruker Daltonics micrOTOF Focus.

For routine cell culture, Dulbecco’s modified Eagle Medium (DMEM) was supplemented with 10 % heat-inactivated fetal bovine serum (FBS), 100 IU/mL of penicillin, 100 µg/mL streptomycin and 0.25 µg/mL amphotericin B, all from Thermo Fisher Scientific. The analytical standard of LSD (lysergic acid diethylamide) was purchased from Chiron AS. NanoGlo Live Cell Reagent and LCS Dilution Buffer were from Promega.

Determination of Photophysical Properties

UV/Vis spectra were recorded on a Varian Cary 50 Bio UV/Vis Spectrophotometer using Hellma (Type 100-QS) cuvettes (10mm light path), connected to a water bath for temperature control. Samples were irradiated using LEDs of Seoulviosys, Cree, Lumileds and LedEngin emitting the monochromatic wavelengths (365 nm, 385 nm, 400 nm, 450 nm, 475 nm, 505 nm, 530 nm). The 50 µM solutions of the respective photoswitchable compound in DMSO or phosphate-buffered saline (PBS) were kept in the dark until thermodynamic equilibrium was achieved. The solutions were checked for decomposition and traces of cis isomer by LC/MS before spectral measurements of the dark-adapted state were performed. Irradiation of the solutions with the respective wavelengths for one minute yielded maximal conversion to the corresponding equilibrium. Thermal relaxation was measured by switching the photochromic compounds into the thermodynamically less stable cis isomer using 385 nm light. Afterwards the relaxation was determined by monitoring the change of absorption at the indicated wavelength at room temperature in PBS buffer. Half-lives were analyzed through nonlinear regression (curve fit)-plateau followed by one phase decay with Graphpad. Absence of photo-fatigue was checked for all compounds of the same generation simultaneously. Using a 96-well plate and a Spectramax 250 absorbance microplate reader (molecular devices), the compounds were irradiated alternatingly with the indicated wavelengths and absorption of the solutions was checked to be constant. The photostationary distribution (PSD) of the photostationary states (PSS) of interest were determined by HPLC. The amount of cis and trans isomer was quantified by integration of the respective peak. Absorption was measured at the respective isosbestic point.
β-arrestin2 Recruitment Assay

The activity of the photoswitchable DMT derivatives was determined by means of a previously developed HEK293T (Human Embryonic Kidney) cell line, stably expressing the 5-HT_2A and β-arrestin2 fusion constructs in the NanoBiT® system.[\textsuperscript{1}] In brief, the routinely cultured cells were seeded at a density of 50,000 cells per well in a poly-D-lysine coated 96-well plate, and incubated overnight in a humidified atmosphere, at 37°C and 5% CO_2. Subsequently, the cells were washed twice with Hank’s Balanced Salt Solution (HBSS), and 100 µL of HBSS was added to each well. To this, 25 µL of NanoGlo Live Cell Reagent (diluted 1:20 in LCS Dilution Buffer, according to the manufacturer’s protocol) was added, and the plate was transferred to an unwarmed ClarioSTAR plate reader (BMG LABTECH). The luminescent signal was monitored until stabilization, subsequently the plate was taken from the reader and 10 µL per concentration of a (irradiated) ligand dilution series was added, yielding final in-well concentrations of 10 µM – (5µM) – 2.5 µM – 1 µM – 10^{-7} M – 10^{-6} M – 10^{-5} M – 10^{-4} M – 10^{-3} M. Afterwards the plate was read again in the ClarioStar. The luminescent signal was continuously monitored for 30 min. Each substance was measured in at least three independent experiments, in which each condition was tested in duplicate. Additionally, the reference psychedelic substance LSD was included in each individual experiment.

The data were analyzed as previously described in more detail.[\textsuperscript{2}] The time-luminescence profiles were plotted and corrected for inter-well variability, and subsequently used to calculate the area under the curve (AUC), of which the AUC of the corresponding solvent control was subtracted. These values were used to generate concentration-response curves in GraphPad Prism (via the three parametric non-linear regression analysis) and normalized to the maximal response of reference agonist LSD. The values of the independent experiments were then pooled to generate the overall concentration-response curves which were fitted using built in log(agonist) vs. response equation in GraphPad Prism 5.

Investigation of dynamic photomodulation of compound 2h was conducted using the same assay. The protocol differed from the above described as follows, two concentrations of the ligand were switched into the cis/trans enriched state respectively and added to the cells after measurement of the luminescent background to give a 1 µM and a 10^{-7} M final in well concentration. Additionally, DMT was included in each individual experiment. Afterwards the plate was read in the ClarioStar for 30 min. Subsequently, the plate was removed for a 2 min irradiation with either 530 nm or 385 nm and read again in the ClarioStar for 30 min. This was repeated 2 times alternating the irradiation wavelength resulting in either a cis/trans/cis or a trans/cis/trans switching sequence. The resulting time-luminescence traces were corrected for inter-well variability.
Photophysical Characterization:

Table S1. Summary of half-lives and cis/trans ratios of the relevant photostationary states.

| Compound | $t_{1/2}$ of cis isomer in buffer @ RT [min] | PSS$_{trans}$ (%) | PSS$_{cis}$ (%) |
|----------|-------------------------------------------|-----------------|----------------|
| 1        | 53                                        | 90              | 50             |
| 3        | 2                                         | Nd.$^{[a]}$     | Nd.$^{[a]}$    |
| 2a       | 201                                       | 87              | 86             |
| 2b       | 248                                       | 87              | 87             |
| 2c       | 133                                       | 85              | 80             |
| 2d       | > 5 h                                     | 67              | 82             |
| 2e       | 204                                       | 87              | 80             |
| 2f       | 203                                       | 83              | 81             |
| 2g       | > 5 h                                     | 70              | 83             |
| 2h       | > 5 h                                     | 91              | 90             |
| 2i       | 98                                        | 85              | 81             |
| 2j       | 116                                       | 98              | 83             |

[a] Not determined due to the short half-life in aqueous medium.

UV/Vis Absorption Spectra, Thermal Relaxation and Switching Cycles

Figure S1. Photophysical characterization of 1 (50 μM). Absorption spectra of PSS at the dark-adapted state (thermodynamic equilibrium) and after irradiation with corresponding lights for 1 min in DMSO (A) and with lights corresponding to maximal cis/trans photoconversion in PBS buffer (pH = 7.4) (B). Half-life of cis isomer after irradiation of 400 nm LED light in DMSO at RT (C) and PBS buffer (D). Cycles of 400 nm/590 nm light irradiation performed without photodecomposition in buffer (E).
Figure S2. Photophysical characterization of 2a (50 μM). Absorption spectra of PSS at the dark-adapted state (thermodynamic equilibrium) and after irradiation with corresponding lights for 1 min in DMSO (A) and with lights corresponding to maximal cis/trans photoconversion in PBS buffer (pH = 7.4) (B). Half-life of cis isomer after irradiation of 365 nm LED light in DMSO at RT (C) and PBS buffer (D). Cycles of 365 nm/530 nm light irradiation performed without photodecomposition in buffer (E).

Figure S3. Photophysical characterization of 3 (50 μM). Absorption spectra of PSS at the dark-adapted state (thermodynamic equilibrium) and after irradiation with corresponding lights for 1 min in DMSO (A) and with lights corresponding to maximal cis/trans photoconversion in PBS buffer (pH = 7.4) (B). Half-life of cis isomer after irradiation of 385 nm LED light in DMSO at RT (C) and PBS buffer (D). Cycles of 385 nm/530 nm light irradiation performed without photodecomposition in buffer (E).
Figure S4. Photophysical characterization of 2b (50 μM). Absorption spectra of PSS at the dark-adapted state (thermodynamic equilibrium) and after irradiation with corresponding lights for 1 min in DMSO (A) and with lights corresponding to maximal cis/trans photoconversion in PBS buffer (pH = 7.4) (B). Half-life of cis isomer after irradiation of 385 nm LED light in PBS buffer at RT (C). Cycles of 385 nm/530 nm light irradiation performed without photodecomposition in buffer (D).

Figure S5. Photophysical characterization of 2c (50 μM). Absorption spectra of PSS at the dark-adapted state (thermodynamic equilibrium) and after irradiation with corresponding lights for 1 min in DMSO (A) and with lights corresponding to maximal cis/trans photoconversion in PBS buffer (pH = 7.4) (B). Half-life of cis isomer after irradiation of 385 nm LED light in PBS buffer at RT (C). Cycles of 385 nm/530 nm light irradiation performed without photodecomposition in buffer (D).
Figure S6. Photophysical characterization of 2d. (50 μM). Absorption spectra of PSS at the dark-adapted state (thermodynamic equilibrium) and after irradiation with corresponding lights for 1 min in DMSO (A) and with lights corresponding to maximal cis/trans photoconversion in PBS buffer (pH = 7.4) (B). Half-life of cis isomer after irradiation of 385 nm LED light in PBS buffer at RT (C). Cycles of 385 nm/530 nm light irradiation performed without photodecomposition in buffer (D).

Figure S7. Photophysical characterization of 2e. (50 μM). Absorption spectra of PSS at the dark-adapted state (thermodynamic equilibrium) and after irradiation with corresponding lights for 1 min in DMSO (A) and with lights corresponding to maximal cis/trans photoconversion in PBS buffer (pH = 7.4) (B). Half-life of cis isomer after irradiation of 385 nm LED light in PBS buffer at RT (C). Cycles of 385 nm/530 nm light irradiation performed without photodecomposition in buffer (D).
Figure S8. Photophysical characterization of 2f (50 μM). Absorption spectra of PSS at the dark-adapted state (thermodynamic equilibrium) and after irradiation with corresponding lights for 1 min in DMSO (A) and with lights corresponding to maximal cis/trans photoconversion in PBS buffer (pH = 7.4) (B). Half-life of cis isomer after irradiation of 385 nm LED light in PBS buffer at RT (C). Cycles of 385 nm/530 nm light irradiation performed without photodecomposition in buffer (D).

Figure S9. Photophysical characterization of 2g (50 μM). Absorption spectra of PSS at the dark-adapted state (thermodynamic equilibrium) and after irradiation with corresponding lights for 1 min in DMSO (A) and with lights corresponding to maximal cis/trans photoconversion in PBS buffer (pH = 7.4) (B). Half-life of cis isomer after irradiation of 385 nm LED light in PBS buffer at RT (C). Cycles of 385 nm/530 nm light irradiation performed without photodecomposition in buffer (D).
Figure S10. Photophysical characterization of 2h (50 μM). Absorption spectra of PSS at the dark-adapted state (thermodynamic equilibrium) and after irradiation with corresponding lights for 1 min in DMSO (A) and with lights corresponding to maximal cis/trans photoconversion in PBS buffer (pH = 7.4) (B). Half-life of cis isomer after irradiation of 385 nm LED light in PBS buffer at RT (C) and at 37°C (D). Cycles of 385 nm/530 nm light irradiation performed without photodecomposition in buffer (E).

Figure S11. Photophysical characterization of 2i (50 μM). Absorption spectra of PSS at the dark-adapted state (thermodynamic equilibrium) and after irradiation with corresponding lights for 1 min in DMSO (A) and with lights corresponding to maximal cis/trans photoconversion in PBS buffer (pH = 7.4) (B). Half-life of cis isomer after irradiation of 385 nm LED light in PBS buffer at RT (C). Cycles of 385 nm/530 nm light irradiation performed without photodecomposition in buffer (D).
Figure S12. Photophysical characterization of 2j (50 μM). Absorption spectra of PSS at the dark-adapted state (thermodynamic equilibrium) and after irradiation with corresponding lights for 1 min in DMSO (A) and with lights corresponding to maximal cis/trans photoconversion in PBS buffer (pH = 7.4) (B). Half-life of cis isomer after irradiation of 385 nm LED light in PBS buffer at RT (C). Cycles of 385 nm/530 nm light irradiation performed without photodecomposition in buffer (D).
**β-arrestin2 Recruitment Assay - Concentration-response curves**

**Figure S13.** *In vitro* characterization of all compounds in their *cis* respectively *trans* enriched states. Detection of β-arrestin2 recruitment to the 5-HT₂₅R as a measure of receptor activation. Data is given as the mean AUC ± SEM of 3 independent experiments.
β-arrestin2 Recruitment Assay - Photomodulation

Figure S14. In vitro dynamic receptor photoactivation. Traces showing photomodulation of β-arrestin2 recruitment to the receptor under trans – cis – trans (TCT) switching conditions (data corrected for inter-well variability). Irradiation with 385 nm causes decomposition of NanoLuc® substrate (Furimazine) causing a decrease in activity overcompensated by the enrichment of active cis compound.
Figure S15. *In vitro* dynamic receptor photoactivation. Traces showing photomodulation of β-arrestin2 recruitment to the receptor under cis–trans–cis (CTC) switching conditions (data corrected for inter-well variability). Irradiation with 385 nm causes decomposition of NanoLuc® substrate (Furimazine) causing a decrease in activity overcompensated by the enrichment of active cis compound.
Radioligand Binding of 2h

Table S2. Radioligand binding studies of 2h were conducted at ambient light under the National Institute of Mental Health's Psychoactive Drug Screening Program.[3] Compound can be found under PDSP# 61744.

| Receptor | LogKᵢ | Kᵢ [nM] | n |
|----------|-------|---------|---|
| 5-HT₁₆  | -7.61 ± 0.06 | 24.8 | 3 |
| 5-HT₁₇  | -7.79 ± 0.04 | 16.3 | 2 |
| 5-HT₁₀  | -8.46 ± 0.05 | 3.51 | 3 |
| 5-HT₂₆  | -6.65 ± 0.05 | 225 | 3 |
| 5-HT₂₇  | -7.81 ± 0.05 | 15.6 | 3 |
| 5-HT₁₂  | -6.46 ± 0.04 | 348 | 3 |
| 5-HT₅   | Na*     | Na*    | / |
| 5-HT₈   | Na*     | Na*    | / |
| 5-HT₃₆  | -6.02 ± 0.05 | 951 | 3 |
| 5-HT₇₆  | -6.94 ± 0.07 | 114 | 3 |
| Sigma-1  | -5.97 ± 0.08 | 1081 | 3 |
| D₂       | Na*     | Na*    | / |

*Affinity Data was not determined if inhibition in the primary binding assay employing 2h at a concentration of 10 µM was less than 50%
Synthetic Procedures

**General Method A:** The respective aniline (7b-j; 1 eq.) was dissolved in CH₂Cl₂ (10 mL). A solution of Oxone® (2 eq.) in water (30 mL) was added and the heterogeneous reaction mixture was stirred for 2 hours. Subsequently layers were separated, and the organic layer was washed with water (3x30 mL) and brine (1x30 mL). The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was filtrated over silica and used without further purification in the following Baeyer–Mills reaction.

**General Method B:** The respective nitrosobenzene (8b-j; 2 – 10 eq.) and the respective aniline (1 eq.) were dissolved in CH₂Cl₂ and AcOH (1 - 5 eq.) was added. The reaction mixture was stirred at room temperature until complete consumption the respective aniline (1 – 3 days). The mixture was then washed with saturated NaHCO₃ and brine. Organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. Purification by silica column chromatography (30:1 CH₂Cl₂/MeOH) yielded the product phenylazoindoles as orange to red solids.

**General Method C:** The respective oxoacetamide intermediate (9b-j, 16, 19a,b; 1 eq.) was dissolved in dry THF (2 mL) and was added dropwise (30 min) to a refluxed solution of LiAlH₄ (10 eq.) in 5 mL dry THF. The reaction mixture was then refluxed for 1 - 4 hours and subsequently allowed to cool down to room temperature. An excess of water was added dropwise to quench the remaining LiAlH₄. The resulting mixture was then extracted with EtOAc. The organic layers were combined, washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. Purification by reverse phase flash column chromatography yielded the respective phenylazo-DMTs as orange to red oils.

Scheme S1. Synthesis of 4-azo-DMT. Reagents and Conditions: (a) benzyl bromide, K₂CO₃, DMF, RT; (b) TsCl, NaH, DMF, RT; (c) 10% Pd/C, H₂, MeOH, RT; (d) nitrosobenzene, AcOH, CH₂Cl₂, RT; (e) KOH, MeOH, RT; (f) i) oxalyl chloride, Et₂O, RT; ii) 2M dimethylamine in THF, RT; (h) LiAlH₄, THF, 80 °C.
K₂CO₃ (3.14 g, 22.7 mmol) and benzyl bromide (2.59 g, 15.2 mmol) were added to a solution of 4-aminooindole (1.0 g, 7.58 mmol) in DMF (20 mL) and stirred at RT for 4 h. Excess water was added to the mixture and extracted with ethyl acetate. The organic phase was combined and washed with water and brine, dried over Na₂SO₄, then purified with column chromatography to afford 11 as blue solid (2.06 g, 6.59 mmol, 87%). ¹H NMR (400 MHz, CDCl₃): δ 8.00 (s, 1H), 7.29 – 7.11 (m, 10H), 6.99 (t, J = 2.6 Hz, 1H), 6.96 – 6.82 (m, 2H), 6.54 (s, 1H), 6.39 (d, J = 7.4 Hz, 1H), 4.52 (s, 4H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ 144.6, 139.1 (2C), 137.5, 128.4 (4C), 127.7 (4C), 126.8 (2C), 122.8, 122.2, 119.9, 107.4, 103.9, 102.0, 55.4 (2C) ppm. MS (ESI, positive): m/z calcd. for [C₂₂H₂₁N₂]⁺: 313.17 found: 313.15 ([M+H]⁺).

NaH, 60% in paraffin oil, (35 mg, 1.44 mmol) was added to a solution of intermediate 11 (0.30 g, 0.96 mmol) in DMF (3 mL) and stirred at RT for 30 min. TsCl (0.27 g, 1.44 mmol) was added to the mixture and stirred for 2 h. Excess water was added to the mixture and extracted with ethyl acetate. The organic phase was combined and washed with water and brine, dried over Na₂SO₄, then purified with column chromatography to afford 12 as a brown solid (0.45 g, 0.96 mmol, quant.). ¹H NMR (400 MHz, CDCl₃): δ 7.81 (d, J = 8.2 Hz, 2H), 7.56 (d, J = 8.3 Hz, 1H), 7.49 (d, J = 3.7 Hz, 1H), 7.32 – 7.23 (m, 14H), 7.12 (t, J = 8.1 Hz, 2H), 6.75 (d, J = 3.7 Hz, 1H), 6.62 (d, J = 7.9 Hz, 1H), 4.50 (s, 4H), 2.38 (s, 3H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ 144.8, 138.2 (2C), 136.4, 135.4, 129.9 (2C), 128.5 (4C), 128.3 (4C), 127.6, 127.01 (2C), 126.99 (2C), 125.2, 124.3, 111.5, 111.43, 107.7, 106.1, 55.6 (2C), 21.6 ppm. MS (ESI, positive): m/z calcd. for [C₂₅H₂₅N₂O₂S]⁺: 467.17 found: 467.10 ([M+H]⁺).

Pd/C (0.12 g) was added to a solution of intermediate 12 (0.25 g, 0.54 mmol) in MeOH (10 mL) and EtOAc (2 mL) and stirred at RT for 4 h under 1 atm. of hydrogen. Pd/C was filtered out over celite, the filtrate was concentrated to afford 13 as a brown oil (0.15 g, 0.54 mmol, quant.). ¹H NMR (400 MHz, CDCl₃): δ 7.67 (d, J = 8.3 Hz, 2H), 7.40 (d, J = 3.8 Hz, 1H), 7.35 (dd, J = 8.3, 0.6 Hz, 1H), 7.13 (d, J = 8.4 Hz, 2H), 7.03 (t, J = 8.0 Hz, 1H), 6.51 (d, J = 3.8 Hz, 1H), 6.44 (d, J = 7.7 Hz, 1H), 2.25 (s, 3H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ 144.8, 139.2, 135.9, 135.3, 129.8 (2C), 126.9 (2C), 125.8, 124.6, 119.3, 108.3, 105.2, 104.6, 21.6 ppm. MS (ESI, positive): m/z calcd. for [C₁₉H₁₇N₂O₂S]⁺: 287.08, found: 287.05 ([M+H]⁺).
According to general method B, AcOH (0.25 mL), intermediate 13 (0.10 g, 0.35 mmol) and nitrosobenzene (75 mg, 0.70 mmol) in CH$_2$Cl$_2$ (5 mL) were used affording 14 as an orange solid (28 mg, 74.6 µmol, 21%). $^1$H NMR (400 MHz, CDCl$_3$): δ 8.04 (d, $J=8.2$ Hz, 1H), 7.86 (d, $J=7.7$ Hz, 2H), 7.75 (d, $J=7.8$ Hz, 1H), 7.71 (d, $J=8.2$ Hz, 2H), 7.63 (d, $J=3.6$ Hz, 1H), 7.48 – 7.37 (m, 4H), 7.36 (d, $J=3.6$ Hz, 1H), 7.15 (d, $J=8.2$ Hz, 2H), 2.26 (s, 3H) ppm. $^{13}$C NMR (101 MHz, CDCl$_3$): δ 153.0, 145.2, 145.1, 136.2, 135.2, 131.0, 130.0 (2C), 129.1 (2C), 128.3, 126.9 (2C), 125.1, 124.8, 122.8 (2C), 119.5, 116.0, 108.5, 21.6 ppm. MS (ESI, positive): m/z calcd. for [C$_{21}$H$_{18}$N$_3$O$_2$S]$^+$: 376.10, found: 376.05 ([M+H]$^+$).

To a solution of intermediate 14 (15 mg, 0.40.6 µmol) in MeOH (500 µL), KOH (4.5 mg, 0.08 mmol) was added. The mixture was stirred at 50 °C for 2 h. The mixture was concentrated under vacuum and excess water was added to the residue. The aqueous was extracted with CH$_2$Cl$_2$ and the organic phase was washed with water and brine, dried over Na$_2$SO$_4$, then concentrated to afford 15 as an orange solid (9 mg, 40.7 µmol, quant.). $^1$H NMR (400 MHz, CDCl$_3$): δ 8.34 (s, 1H), 7.92 (d, $J=7.6$ Hz, 2H), 7.77 (d, $J=7.6$ Hz, 1H), 7.49 – 7.43 (m, 3H), 7.41 – 7.36 (m, 1H), 7.34 – 7.27 (m, 3H) ppm. $^{13}$C NMR (101 MHz, CDCl$_3$): δ 153.5, 145.2, 137.5, 130.3, 129.0, 126.6, 122.6, 122.0, 120.7, 120.2, 114.4, 103.5 ppm. MS (ESI, positive): m/z calcd. for [C$_{14}$H$_{12}$N$_3$]+: 222.10, found: 222.10 ([M+H]$^+$).

N,N-Dimethyl-2-oxo-2-(4-(phenyldiazenyl)-1H-indol-3-yl)acetamide (16)

Oxalyl chloride (146 mg, 1.15 mmol) was added to a solution of intermediate 15 (50 mg, 0.23 mmol) in diethyl ether (3 mL) at 0 °C and stirred for 4 h. The mixture was concentrated under vacuum, then dimethylamine in THF (10 mL, 2 M) was added to the residue and stirred at RT over night. The mixture was concentrated and purified with column chromatography to afford 16 as an orange solid (21 mg, 65.6 µmol, 29%). $^1$H NMR (400 MHz, CDCl$_3$): δ 10.67 (s, 1H), 7.94 (d, $J=7.5$ Hz, 2H), 7.67 (d, $J=2.9$ Hz, 1H), 7.45 (t, $J=7.6$ Hz, 2H), 7.42 – 7.36 (m, 1H), 7.33 (d, $J=7.6$ Hz, 1H), 7.23 (d, $J=8.0$ Hz, 1H), 7.14 (t, $J=7.8$ Hz, 1H), 2.90 (s, 3H), 2.70 (s, 3H) ppm. $^{13}$C NMR (101 MHz, CDCl$_3$): δ 180.2, 168.7, 153.5, 147.0, 138.7, 137.7, 130.7, 129.1, 129.1 (2C), 124.1, 123.2 (2C), 115.1, 114.4, 110.8, 37.6, 34.2 ppm. MS (ESI, positive): m/z calcd for [C$_{18}$H$_{17}$N$_4$O$_2$]+: 321.13, found: 321.10 ([M+H]$^+$).
According to general method C Intermediate 16 (165 mg, 0.52 mmol) dry THF and LiAlH₄ (196 mg, 5.20 mmol) were used to afford 1 as an orange solid (26 mg, 88.9 µmol, 17%). ¹H NMR (400 MHz, MeOD-d₄): δ 7.81 – 7.76 (m, 2H), 7.51 – 7.37 (m, 5H), 7.33 (s, 1H), 7.12 (t, J = 7.9 Hz, 1H), 3.43 – 3.37 (m, 2H), 3.34 – 3.28 (m, 2H), 2.65 (s, 6H) ppm. ¹³C NMR (101 MHz, MeOD-d₄): δ 153.4, 146.0, 139.2, 130.4, 129.0 (2C), 126.2, 124.9, 122.2 (2C), 121.5, 114.5, 109.5, 105.4, 58.8, 42.4 (2C), 21.4 ppm. HRMS (ESI, positive): m/z calcd. for [C₁₈H₂₁N₄]⁺: 293.17607, found: 293.17618 ([M+H]⁺). HPLC: purity: 99.9%.

Scheme S2. Synthesis of position 5 and 6 azo-DMT. Reagents and Conditions: (a) nitrosobenzene, AcOH, CH₂Cl₂, RT; (b) i) oxalyl chloride, Et₂O, RT; ii) 2M dimethylamine in THF, RT; (c) LiAlH₄, THF, 80 °C.

5-(Phenyldiazenyl)-1H-indole (18a)

According to general method B nitrosobenzene (240 mg, 2.27 mmol), AcOH (140 mg, 2.27 mmol), 5-aminooindole (300 mg, 2.27 mmol) and CH₂Cl₂ (15 mL) were used to afford 18a as a red solid (130 mg, 0.59 mmol, 26%). ¹H NMR (400 MHz, CDCl₃): δ 8.26 (s, 1H), 8.21 (s, 1H), 7.85 (d, J = 8.0 Hz, 2H), 7.81 (dd, J = 9.0, 1.4 Hz, 1H), 7.44 (t, J = 7.7 Hz, 2H), 7.40 – 7.32 (m, 2H), 7.21 – 7.18 (m, 1H), 6.66 – 6.59 (m, 1H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ 153.0, 147.4, 137.5, 130.1, 129.0 (2C), 128.1, 125.5, 122.5 (2C), 119.2, 115.8, 111.4, 104.6 ppm. MS (ESI, positive): m/z calcd. for [C₁₄H₁₂N₃]⁺: 222.10, found: 222.10 ([M+H]⁺).

6-(Phenyldiazenyl)-1H-indole (18b)

According to general method B nitrosobenzene (240 mg, 2.27 mmol), AcOH (140 mg, 2.27 mmol), 6-aminooindole (300 mg, 2.27 mmol) and CH₂Cl₂ (15 mL) were used to afford 18b as an orange solid (75 mg, 0.34 mmol, 15%). ¹H NMR (400 MHz, CDCl₃): δ 8.38 (s, 1H), 7.97 (s, 1H), 7.86 (d, J = 7.8 Hz, 2H), 7.75 (dd, J = 8.5, 0.7 Hz, 1H), 7.64 (d, J = 8.5 Hz, 1H), 7.44 (t, J = 7.7 Hz, 2H), 7.40 – 7.34 (m, 1H), 7.27 (t, J = 2.5 Hz, 1H), 6.59 – 6.48 (m, 1H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ 152.7, 148.6, 135.9, 130.7, 130.3,
129.1 (2C), 127.5, 122.6 (2C), 120.9, 115.1, 107.8, 103.4 ppm. **MS (ESI, positive):** m/z calcd. for [C\textsubscript{14}H\textsubscript{12}N\textsubscript{3}]: 222.10, found: 222.10 ([M+H]+).

\[ \text{N,N-Dimethyl-2-oxo-2-(5-phenyldiazenyl)-1H-indol-3-yl)acetamide (19a)} \]

Oxalyl chloride (320 mg, 2.50 mmol) was added to a solution of 18a (110 mg, 0.50 mmol) in diethyl ether (10 mL) at 0 °C and stirred for 4 h. The mixture was concentrated under vacuum, then dimethylamine in THF (5 mL, 2 M) was added to the residue and stirred at RT over night. The mixture was concentrated and extracted with ethyl acetate, then washed with water and brine, dried over Na\textsubscript{2}SO\textsubscript{4} and concentrated to afford 19a as an orange solid (160 mg, 0.50 mmol, quant.). **\(^{1}H\ NMR (400 MHz, DMSO-d\textsubscript{6}):** δ 12.66 (s, 1H), 8.73 (d, \(J = 1.8\) Hz, 1H), 8.31 (s, 1H), 8.01 – 7.95 (m, 3H), 7.75 (d, \(J = 8.7\) Hz, 1H), 7.69 – 7.58 (m, 3H), 3.07 (s, 3H), 3.01 (s, 3H) ppm. **\(^{13}C\ NMR (101 MHz, DMSO-d\textsubscript{6}):** δ 187.2, 167.5, 152.6, 148.5, 139.2, 131.5, 129.9 (2C), 125.8, 122.9 (2C), 120.2, 118.4, 117.6, 114.7, 114.0, 37.3, 34.0 ppm. **MS (ESI, positive):** m/z calcd. for [C\textsubscript{18}H\textsubscript{17}N\textsubscript{4}O\textsubscript{2}]: 321.13, found: 321.10 ([M+H]+).

\[ \text{N,N-Dimethyl-2-oxo-2-(6-phenyldiazenyl)-1H-indol-3-yl)acetamide (19b)} \]

Oxalyl chloride (172 mg, 1.36 mmol) was added to a solution of 18b (0.60 mg, 0.27 mmol) in diethyl ether (5 mL) at 0 °C and stirred for 4 h. The mixture was concentrated under vacuum, then dimethylamine in THF (2.5 mL, 2 M) was added to the residue and stirred at RT over night. The mixture was concentrated and extracted with ethyl acetate, then washed with water and brine, dried over Na\textsubscript{2}SO\textsubscript{4} and concentrated to afford 19b as an orange solid (86 mg, 0.27 mmol, quant.). **\(^{1}H\ NMR (400 MHz, DMSO-d\textsubscript{6}):** δ 12.68 (s, 1H), 8.36 (s, 1H), 8.30 (d, \(J = 8.5\) Hz, 1H), 8.13 (d, \(J = 1.6\) Hz, 1H), 7.99 – 7.90 (m, 3H), 7.69 – 7.56 (m, 3H), 3.06 (s, 3H), 2.99 (s, 3H) ppm. **\(^{13}C\ NMR (101 MHz, DMSO-d\textsubscript{6}):** δ 187.1, 167.5, 152.5, 149.1, 140.1, 137.6, 131.6, 129.9 (2C), 128.0, 122.9 (2C), 121.8, 117.5, 113.8, 109.0, 37.3, 34.0 ppm. **MS (ESI, positive):** m/z calcd. for [C\textsubscript{18}H\textsubscript{17}N\textsubscript{4}O\textsubscript{2}]: 321.13, found: 321.10 ([M+H]+).

\[ \text{N,N-dimethyl-2-(5-phenyldiazenyl)-1H-indol-3-yl)ethan-1-amine (2a)} \]

According to general method C intermediate 19a (150 mg, 0.47 mmol), dry THF and LiAlH\textsubscript{4} (179 mg, 4.70 mmol) were used to afford product 2a as an orange solid (59 mg, 0.20 mmol, 43%). **\(^{1}H\ NMR (400 MHz, MeOD-d\textsubscript{4}):** δ 8.45 (s, 1H), 8.30 (d, \(J = 1.7\) Hz, 1H), 7.93 – 7.88 (m, 2H), 7.86 (dd, \(J = 8.8, 1.7\) Hz, 1H), 7.57 – 7.45 (m, 4H), 7.35 (s, 1H), 3.55 – 3.49 (m, 2H), 3.34 – 3.23 (m, 2H), 2.99 (s, 6H). **\(^{13}C\ NMR (101 MHz, MeOD-d\textsubscript{4}):** δ 152.9, 146.6, 138.8, 129.9, 128.8 (2C), 126.9, 124.8, 122.0 (2C), 116.4, 115.0, 111.8, 110.7, 57.7, 42.2 (2C), 20.5 ppm. **HRMS (ESI, positive):** m/z calcd. for [C\textsubscript{18}H\textsubscript{21}N\textsubscript{4}]: 293.17607, found: 293.17602 ([M+H]+). **HPLC:** purity: 97.4%.
According to general method C intermediate 19b (70 mg, 0.22 mmol), dry THF and LiAlH₄ (84 mg, 2.20 mmol) were used to afford product 3 as an orange solid (23 mg, 79 µmol, 36%). ^1H NMR (400 MHz, MeOD-d₄): δ 7.99 (d, J = 1.4 Hz, 1H), 7.92 – 7.87 (m, 2H), 7.75 (d, J = 1.7 Hz, 1H), 7.68 (s, 1H), 7.57 – 7.50 (m, 2H), 7.49 – 7.44 (m, 1H), 7.33 (s, 1H), 3.10 – 3.04 (m, 2H), 2.97 – 2.91 (m, 2H), 2.56 (s, 6H) ppm. ^13C NMR (101 MHz, MeOD-d₄): δ 152.9, 148.3, 136.7, 129.9, 128.8 (2C), 126.0, 122.0 (2C), 118.1, 112.7, 112.1, 108.5, 59.3, 43.4 (2C), 22.0 ppm. HRMS (ESI, positive): m/z calcd. for [C₁₈H₂₁N₄]: 293.17607, found: 293.17689 ([M+H]+). HPLC: purity: 97.3%.

Scheme S3. Synthesis of substituted 5-azo-DMTs. Reagents and conditions: (a) i) oxalyl chloride, Et₂O, 0 °C to RT; ii) 2M dimethylamine in THF, RT, 92%; (b) 10 wt% Pd/C, 1 atm. H₂, MeOH, RT, quant.; (c) Oxone®, H₂O, CH₂Cl₂, RT; (d) AcOH, CH₂Cl₂, RT, 15 - 98%; (e) LiAlH₄, THF, reflux, 16 - 88%.
Oxalyl chloride (2.64 mL, 3.91 g, 30.84 mmol) was added to a solution of 5-nitro-1H-indole (1.00 g, 6.17 mmol) in diethyl ether (100 mL) at 0 °C and stirred for 2 hours. Subsequently the reaction was warmed up to room temperature and stirring was continued overnight. The mixture was concentrated under vacuum and dimethylamine in THF (31 mL, 2 M) was added to the residue and stirred at room temperature for 2 hours. The mixture was concentrated and extracted with ethyl acetate, then washed with water and brine, dried over Na₂SO₄ and concentrated. Purification by column chromatography (40:1 CH₂Cl₂/MeOH) afforded 5 as a brown solid (1.47 g, 5.63 mmol, 92%). ¹H NMR (400 MHz, DMSO-d₆): δ 12.85 (s, 1H), 8.97 (d, J = 2.3 Hz, 1H), 8.41 (s, 1H), 8.17 (dd, J = 9.0, 2.4 Hz, 1H), 7.73 (d, J = 9.0 Hz, 1H), 3.01 (s, 3H), 2.95 (s, 3H). ¹³C NMR (101 MHz, DMSO-d₆): δ 186.60, 166.52, 143.14, 140.35, 140.08, 124.41, 118.88, 117.14, 114.19, 113.48, 36.78, 33.55. MS (ESI, positive): m/z calcd. for C₁₂H₁₂N₃O₄⁺: 262.08, found: 262.10 ([M+H]⁺).

Intermediate 5 (200 mg, 0.77 mmol) was dissolved in MeOH (5 mL) and Pd/C (20 mg, 10 wt%) was added. The mixture was stirred under an atmosphere of hydrogen for 1.5 hours and subsequently filtered. The filter was washed extensively with MeOH and the filtrate was concentrated under reduced pressure to afford 6 as a beige solid (176 mg, 0.77 mmol, quant.). ¹H NMR (400 MHz, DMSO-d₆): δ 11.87 (s, 1H), 7.80 (d, J = 3.3 Hz, 1H), 7.32 (s, 1H), 7.18 (d, J = 8.5 Hz, 1H), 6.60 (dd, J = 8.5, 2.2 Hz, 1H), 4.90 (s, 2H), 2.96 (s, 3H), 2.89 (s, 3H). ¹³C NMR (101 MHz, DMSO-d₆): δ 186.18, 167.70, 144.83, 135.60, 129.75, 126.33, 113.01, 112.64, 112.14, 104.02, 36.76, 33.33. MS (ESI, positive): m/z calcd. for C₁₂H₁₄N₂O₂⁺: 232.11, found: 232.10 ([M+H]⁺).
According to general method B the respective nitrosobenzene (78 mg, 0.65 mmol), AcOH (156 mg, 2.60 mmol), intermediate 6 (75 mg, 0.32 mmol) and CH₂Cl₂ (2.5 mL) were used to afford 9b as an orange solid (106 mg, 0.32 mmol, 98%).

**1H NMR (400 MHz, DMSO-d₆):** δ 8.64 (d, J = 1.9 Hz, 1H), 8.24 (s, 1H), 7.89 (dd, J = 8.7, 2.0 Hz, 1H), 7.87 – 7.81 (m, 2H), 7.68 (d, J = 8.7 Hz, 1H), 7.43 – 7.37 (m, 2H), 3.02 (s, 3H), 2.95 (s, 3H), 2.41 (s, 3H).

**13C NMR (101 MHz, DMSO-d₆):** δ 186.66, 167.01, 148.06, 141.09, 138.65, 129.92, 125.29, 122.38, 117.90, 116.84, 114.13, 113.43, 36.80, 33.49, 21.00.

**MS (ESI, positive):** m/z calcd. for C₁₉H₁₉N₄O₂⁺: 335.15, found: 335.15 ([M+H]+).

According to general method B the respective nitrosobenzene (78 mg, 0.65 mmol), AcOH (156 mg, 2.60 mmol), intermediate 6 (75 mg, 0.32 mmol) and CH₂Cl₂ (2.5 mL) were used to afford 9c as an orange solid (61 mg, 0.18 mmol, 56%).

**1H NMR (400 MHz, DMSO-d₆):** δ 12.59 (s, 1H), 8.66 (d, J = 1.9 Hz, 1H), 8.25 (s, 1H), 7.90 (dd, J = 8.7, 2.0 Hz, 1H), 7.78 – 7.62 (m, 3H), 7.48 (t, J = 7.6 Hz, 1H), 7.37 (d, J = 7.5 Hz, 1H), 3.02 (s, 2H), 2.96 (s, 3H), 2.44 (s, 2H).

**13C NMR (101 MHz, DMSO-d₆):** δ 186.67, 166.97, 152.12, 148.06, 138.89, 138.67, 138.62, 131.58, 129.19, 125.27, 122.43, 119.98, 117.91, 117.03, 114.15, 113.44, 36.79, 33.48, 20.90.

**MS (ESI, positive):** m/z calcd. for C₁₉H₁₉N₄O₂⁺: 335.15, found: 335.15 ([M+H]+).

According to general method B the respective nitrosobenzene (78 mg, 0.65 mmol), AcOH (156 mg, 2.60 mmol), intermediate 6 (75 mg, 0.32 mmol) and CH₂Cl₂ (2.5 mL) were used to afford 9d as an orange solid (93 mg, 0.28 mmol, 86%).

**1H NMR (400 MHz, DMSO-d₆):** δ 12.59 (s, 1H), 8.66 (d, J = 1.9 Hz, 1H), 8.25 (s, 1H), 7.90 (dd, J = 8.8, 2.0 Hz, 1H), 7.69 (d, J = 8.8 Hz, 1H), 7.64 – 7.59 (m, 1H), 7.44 – 7.42 (m, 2H), 7.37 – 7.30 (m, 1H), 3.02 (s, 3H), 2.96 (s, 3H), 2.70 (s, 2H).

**13C NMR (101 MHz, DMSO-d₆):** δ 186.65, 167.02, 150.05, 148.52, 138.54, 137.07, 131.35, 130.80, 126.61, 125.26, 117.87, 117.31, 115.16, 114.16, 113.48, 36.80, 33.47, 17.14.

**MS (ESI, positive):** m/z calcd. for C₁₉H₁₉N₄O₂⁺: 335.15, found: 335.15 ([M+H]+).
According to general method **B** the respective nitrosobenzene (97 mg, 0.65 mmol), AcOH (156 mg, 2.60 mmol), intermediate **6** (75 mg, 0.32 mmol) and CH$_2$Cl$_2$ (2.5 mL) were used to afford **9e** as an orange solid (88 mg, 0.24 mmol, 75%). **$^1$H NMR (400 MHz, DMSO-de$_6$):** δ 12.58 (s, 1H), 8.65 (d, $J = 1.9$ Hz, 1H), 8.25 (s, 1H), 7.92 – 7.84 (m, 3H), 7.68 (d, $J = 8.7$ Hz, 1H), 7.50 – 7.45 (m, 2H), 3.04 – 2.99 (m, 4H), 2.95 (s, 3H), 1.27 (s, 3H), 1.25 (s, 3H). **$^{13}$C NMR (101 MHz, DMSO-de$_6$):** δ 186.66, 166.98, 151.73, 150.44, 148.08, 138.65, 138.53, 127.26, 125.27, 122.46, 117.88, 116.88, 114.12, 113.41, 36.79, 33.47, 33.36, 23.67. **MS (ESI, positive):** m/z calcd. for C$_{21}$H$_{23}$N$_4$O$_2$: 363.18, found: 363.20 ([M+H]+).

According to general method **B** the respective nitrosobenzene (97 mg, 0.65 mmol), AcOH (156 mg, 2.60 mmol), intermediate **6** (75 mg, 0.32 mmol) and CH$_2$Cl$_2$ (2.5 mL) were used to afford **9f** as an orange solid (91 mg, 0.25 mmol, 77%). **$^1$H NMR (400 MHz, DMSO-de$_6$):** δ 12.59 (s, 1H), 8.67 (d, $J = 1.8$ Hz, 1H), 8.25 (s, 1H), 7.91 (dd, $J = 8.7$, 1.9 Hz, 1H), 7.81 (t, $J = 1.8$ Hz, 1H), 7.77 – 7.70 (m, 1H), 7.69 (d, $J = 8.7$ Hz, 1H), 7.51 (t, $J = 7.7$ Hz, 1H), 7.44 (dt, $J = 7.6$, 1.5 Hz, 1H), 3.08 – 3.00 (m, 3H), 2.96 (s, 3H), 1.30 (s, 2H), 1.28 (s, 2H). **$^{13}$C NMR (101 MHz, DMSO-de$_6$):** δ 186.68, 166.96, 152.23, 149.83, 148.07, 138.69, 138.61, 129.31, 129.31, 125.27, 120.32, 119.81, 117.95, 117.01, 114.15, 113.43, 36.79, 33.48, 33.35, 23.77. **MS (ESI, positive):** m/z calcd. for C$_{21}$H$_{23}$N$_4$O$_2$: 363.18, found: 363.20 ([M+H]+).

According to general method **B** the respective nitrosobenzene (97 mg, 0.65 mmol), AcOH (156 mg, 2.60 mmol), intermediate **6** (75 mg, 0.32 mmol) and CH$_2$Cl$_2$ (2.5 mL) were used to afford **9g** as an orange solid (95 mg, 0.26 mmol, 81%). **$^1$H NMR (400 MHz, DMSO-de$_6$):** δ 12.59 (s, 1H), 8.65 (d, $J = 1.9$ Hz, 1H), 8.25 (s, 1H), 7.91 (dd, $J = 8.7$, 1.9 Hz, 1H), 7.81 (t, $J = 1.8$ Hz, 1H), 7.77 – 7.70 (m, 1H), 7.69 (d, $J = 8.7$ Hz, 1H), 7.51 (t, $J = 7.7$ Hz, 1H), 7.44 (dt, $J = 7.6$, 1.5 Hz, 1H), 3.08 – 3.00 (m, 3H), 2.96 (s, 3H), 1.30 (s, 2H), 1.28 (s, 2H). **$^{13}$C NMR (101 MHz, DMSO-de$_6$):** δ 186.65, 167.02, 148.99, 148.58, 146.88, 138.54, 131.12, 126.51, 126.46, 125.28, 118.06.
2-(5-((4-methoxyphenyl)diazenyl)-1H-indol-3-yl)-N,N-dimethyl-2-oxoacetamide (9h)

According to general method B the respective nitrosobenzene (1179 mg, 8.60 mmol), AcOH (387 mg, 6.45 mmol), intermediate 6 (200 mg, 0.86 mmol) and CH2Cl2 (2.5 mL) were used to afford 9h as an orange solid (138 mg, 0.39 mmol, 46%). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 12.56 (s, 1H), 8.61 (d, \(J = 1.9\) Hz, 1H), 8.23 (s, 1H), 7.96 – 7.90 (m, 2H), 7.87 (dd, \(J = 8.7, 2.0\) Hz, 1H), 7.67 (d, \(J = 8.7\) Hz, 1H), 7.22 – 7.09 (m, 2H), 3.87 (s, 2H), 3.01 (s, 2H), 2.95 (s, 2H). \(^{13}\)C NMR (101 MHz, DMSO-\(d_6\)): \(\delta\) 187.14, 167.51, 162.06, 148.60, 146.76, 139.03, 138.78, 125.77, 124.75, 118.33, 116.92, 115.05, 114.56, 113.84, 56.09, 37.28, 33.96. MS (ESI, positive); m/z calcd. for C\(_{19}\)H\(_{19}\)N\(_4\)O\(_3\): 351.15, found: 351.15 ([M+H]+).

2-(5-((3-methoxyphenyl)diazenyl)-1H-indol-3-yl)-N,N-dimethyl-2-oxoacetamide (9i)

According to general method B the respective nitrosobenzene (110 mg, 0.8 mmol), AcOH (156 mg, 2.60 mmol), intermediate 6 (90 mg,0.39 mmol) and CH2Cl2 (2.5 mL) were used to afford 9i as an orange solid (28 mg, 80 \(\mu\)mmol, 15%). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 12.60 (s, 1H), 8.68 (d, \(J = 1.9\) Hz, 1H), 8.26 (s, 1H), 7.91 (dd, \(J = 8.7, 1.9\) Hz, 1H), 7.69 (d, \(J = 8.7\) Hz, 1H), 7.58 – 7.48 (m, 2H), 7.48 – 7.44 (m, 1H), 7.13 (ddd, \(J = 7.6, 2.6, 1.5\) Hz, 1H), 3.88 (s, 2H), 3.02 (s, 2H), 2.96 (s, 2H). \(^{13}\)C NMR (101 MHz, DMSO-\(d_6\)): \(\delta\) 186.67, 166.95, 160.09, 153.32, 147.93, 138.77, 138.74, 130.17, 125.28, 117.88, 117.32, 117.31, 116.08, 114.16, 113.48, 105.75, 55.37, 36.79, 33.48. MS (ESI, positive); m/z calcd. for C\(_{19}\)H\(_{19}\)N\(_4\)O\(_3\): 351.15, found 351.15 ([M+H]+).
According to general method B the respective nitrosobenzene (345 mg, 1.3 mmol), AcOH (26 mg, 0.43 mmol), intermediate 6 (100 mg, 0.43 mmol) and CH₂Cl₂ (5 mL) were used to afford 9j as an orange solid (48 mg, 126.8 mmol, 29%). ¹H NMR (400 MHz, Chloroform-d): δ 10.31 (s, 1H), 8.93 – 8.87 (m, 1H), 7.88 (dd, J = 8.8, 1.9 Hz, 1H), 7.81 (d, J = 2.6 Hz, 1H), 7.56 – 7.52 (m, 1H), 7.49 – 7.46 (m, 1H), 7.43 – 7.37 (m, 2H), 7.03 – 6.97 (m, 1H), 4.68 (hept, J = 6.1 Hz, 1H), 3.10 (s, 3H), 3.07 (s, 3H), 1.40 (s, 3H), 1.38 (s, 3H). ¹³C NMR (101 MHz, Chloroform-d): δ 185.87, 167.86, 158.78, 154.13, 149.38, 138.31, 136.82, 129.91, 125.77, 119.51, 119.37, 118.15, 116.88, 115.61, 112.70, 107.79, 70.27, 37.74, 34.72, 22.21. MS (ESI, positive) m/z calcd. for C₂₁H₂₃N₄O₃+: 379.18, found 379.20 ([M+H]+).

N,N-dimethyl-2-(5-(p-tolyl diazenyl)-1H-indol-3-yl)ethan-1-amine (2b)

According to general method C intermediate 9b (148 mg, 0.44 mmol), dry THF and LiAlH₄ (168 mg, 4.43 mmol) were used to afford 2b as an orange solid (31 mg, 0.10 mmol, 23%). ¹H NMR (400 MHz, Methanol-d₄): δ 8.16 (d, 1H), 7.79 – 7.74 (m, 3H), 7.41 (d, J = 8.8 Hz, 1H), 7.29 (d, J = 8.1 Hz, 2H), 7.12 (s, 1H), 3.02 – 2.94 (m, 2H), 2.72 – 2.64 (m, 2H), 2.38 (s, 3H), 2.32 (s, 6H). ¹³C NMR (101 MHz, Methanol-d₄): δ 152.30, 147.65, 141.55, 139.85, 130.65, 128.81, 124.89, 123.31, 117.94, 115.87, 115.81, 112.77, 61.27, 45.36, 24.15, 21.38. HRMS (ESI, positive): m/z calcd. for C₁₉H₂₃N₄+: 307.19172, found 307.19241 ([M+H]+). HPLC: purity: 98.1%.

N,N-dimethyl-2-(5-(m-tolyl diazenyl)-1H-indol-3-yl)ethan-1-amine (2c)

According to general method C intermediate 9c (60 mg, 0.18 mmol), dry THF and LiAlH₄ (68 mg, 1.79 mmol) were used to afford 2c as an orange solid (20 mg, 65.3 µmol, 36%). ¹H NMR (400 MHz, Methanol-d₄): δ 8.23 – 8.19 (m, 1H), 7.85 – 7.78 (m, 1H), 7.75 – 7.67 (m, 2H), 7.49 – 7.40 (m, 2H), 7.34 – 7.28 (m, 1H), 7.21 (s, 1H), 3.12 – 3.01 (m, 1H), 2.82 – 2.73 (m, 2H), 2.49 (s, 3H), 2.42 (s, 6H). ¹³C NMR (101 MHz, Methanol-d₄): δ 154.42, 147.79, 140.16, 140.03, 131.79, 129.95, 128.70, 125.30, 123.65, 120.70, 118.06, 115.95, 114.97, 112.90, 60.85, 44.99, 23.62, 21.42. HRMS (ESI, positive): m/z calcd. for C₁₉H₂₃N₄+: 307.19172, found 307.19208 ([M+H]+). HPLC: purity 99.6%.
According to general method C intermediate 9d (100 mg, 0.30 mmol), dry THF and LiAlH₄ (113 mg, 2.99 mmol) were used to afford 2d as an orange solid (42 mg, 0.14 mmol, 46%). ¹H NMR (400 MHz, Methanol-d₄): δ 8.23 – 8.19 (m, 1H), 7.83 – 7.78 (m, 1H), 7.66 – 7.59 (m, 1H), 7.48 – 7.41 (m, 1H), 7.37 – 7.24 (m, 3H), 7.17 (s, 1H), 3.08 – 2.99 (m, 2H), 2.72 (s, 5H), 2.38 (s, 6H). ¹³C NMR (101 MHz, Methanol-d₄): δ 150.88, 146.81, 138.51, 136.74, 130.73, 129.53, 127.41, 126.04, 123.54, 117.24, 114.95, 114.52, 114.16, 111.43, 59.97, 44.00, 22.78, 16.25. HRMS (ESI, positive): m/z calc. for C₁₉H₂₃N₄⁺: 307.19172, found 307.19312 ([M+H]⁺). HPLC: purity 99.4%.

According to general method C intermediate 9e (80 mg, 0.22 mmol), dry THF and LiAlH₄ (84 mg, 2.21 mmol) were used to afford 2e as an orange solid (42 mg, 0.13 mmol, 57%). ¹H NMR (400 MHz, Methanol-d₄): δ 8.21 – 8.15 (m, 1H), 7.85 – 7.77 (m, 3H), 7.46 – 7.41 (m, 1H), 7.40 – 7.34 (m, 2H), 7.15 (s, 1H), 3.04 – 2.91 (m, 3H), 2.74 – 2.66 (m, 2H), 2.35 (s, 6H), 1.30 (s, 3H), 1.28 (s, 3H). ¹³C NMR (101 MHz, Methanol-d₄): δ 152.59, 152.48, 147.69, 139.88, 128.83, 128.05, 124.91, 123.43, 117.98, 115.91, 115.81, 112.78, 61.32, 45.39, 35.28, 24.32, 24.18. HRMS (ESI, positive): m/z calc. for C₂₁H₂₇N₄⁺: 335.22302, found 335.22380 ([M+H]⁺). HPLC: purity: 99.6%.

According to general method C intermediate 9f (80 mg, 0.22 mmol), dry THF and LiAlH₄ (84 mg, 2.21 mmol) were used to afford 2f as an orange solid (12 mg, 35.9 µmol, 16%). ¹H NMR (400 MHz, Methanol-d₄): δ 8.25 – 8.20 (m, 1H), 7.85 – 7.80 (m, 1H), 7.80 – 7.78 (m, 1H), 7.74 – 7.69 (m, 1H), 7.48 – 7.42 (m, 2H), 7.38 – 7.31 (m, 1H), 7.18 (s, 1H), 3.09 – 2.98 (m, 3H), 2.79 – 2.69 (m, 2H), 2.38 (s, 6H), 1.35 (s, 3H), 1.33 (s, 3H). ¹³C NMR (101 MHz, Methanol-d₄): δ 153.10, 152.48, 147.69, 139.88, 128.83, 128.05, 124.91, 123.43, 117.98, 115.91, 115.81, 112.78, 61.32, 45.39, 35.28, 24.32, 24.18. HRMS (ESI, positive): m/z calc. for C₂₁H₂₇N₄⁺: 335.22302, found 335.22380 ([M+H]⁺). HPLC: purity: 99.8%.
According to general method C intermediate 9g (95 mg, 0.26 mmol), dry THF and LiAlH₄ (100 mg, 2.62 mmol) were used to afford 2g as an orange solid (22 mg, 65.8 µmol, 25%). ¹H NMR (400 MHz, Methanol-d₄): δ 8.23 – 8.18 (m, 1H), 7.83 – 7.77 (m, 1H), 7.64 – 7.58 (m, 1H), 7.49 – 7.36 (m, 3H), 7.30 – 7.23 (m, 1H), 7.17 (s, 1H), 4.11 (hept, J = 7.0 Hz, 1H), 3.06 – 2.99 (m, 2H), 2.76 – 2.68 (m, 2H), 2.37 (s, 6H), 1.38 (s, 3H), 1.36 (s, 3H). ¹³C NMR (101 MHz, Methanol-d₄): δ 151.22, 148.25, 148.07, 139.88, 131.25, 128.82, 127.29, 127.26, 124.96, 118.50, 116.28, 115.91, 115.68, 112.86, 61.37, 45.40, 29.10, 24.20, 24.18. HRMS (ESI, positive): m/z calcd. for C₂₁H₂₇N₄⁺: 335.22302, found 335.22528 ([M+H]⁺). HPLC: purity: 99.1%.

According to general method C intermediate 9h (200 mg, 0.57 mmol), dry THF and LiAlH₄ (216 mg, 5.71 mmol) were used to afford 2h as an orange solid (102 mg, 0.32 mmol, 55%). ¹H NMR (400 MHz, Methanol-d₄): δ 8.17 – 8.13 (m, 1H), 7.92 – 7.85 (m, 2H), 7.81 – 7.74 (m, 1H), 7.46 – 7.40 (m, 1H), 7.16 (s, 1H), 7.10 – 7.03 (m, 2H), 3.88 (s, 3H), 3.06 – 2.97 (m, 2H), 2.76 – 2.69 (m, 2H), 2.37 (s, 6H). ¹³C NMR (101 MHz, Methanol-d₄): δ 162.89, 148.45, 147.68, 139.68, 128.83, 125.02, 124.85, 117.43, 115.80, 115.79, 115.23, 112.74, 61.34, 56.03, 45.39, 24.20. HRMS (ESI, positive): m/z calcd. for C₁₉H₂₃N₄O⁺: 323.18664, found 323.18731 ([M+H]⁺). HPLC: purity: 98.8%.

According to general method C intermediate 9i (87 mg, 0.25 mmol), dry THF and LiAlH₄ (95 mg, 2.48 mmol) were used to afford 2i as an orange solid (38 mg, 0.12 mmol, 46%). ¹H NMR (400 MHz, Methanol-d₄): δ 8.24 – 8.21 (m, 1H), 7.82 (dd, J = 8.8, 1.8 Hz, 1H), 7.55 – 7.51 (m, 1H), 7.49 – 7.43 (m, 3H), 7.20 (s, 1H), 7.09 – 7.03 (m, 1H), 3.92 (s, 3H), 3.12 – 3.01 (m, 2H), 2.83 – 2.72 (m, 2H), 2.41 (s, 6H). ¹³C NMR (101 MHz, Methanol-d₄): δ 161.94, 147.60, 140.08, 130.81, 128.85, 125.06, 118.43, 117.39, 117.38, 115.95, 115.78, 112.85, 106.61, 61.32, 55.88, 45.37, 24.14. HRMS (ESI, positive) m/z calcd. for C₁₉H₂₃N₄O⁺: 323.18664, found 323.18798 ([M+H]⁺). HPLC: purity: 99.0%.
According to general method C intermediate 9j (45 mg, 0.12 mmol), dry THF and LiAlH₄ (46 mg, 1.19 mmol) were used to afford 2j as an orange solid (37 mg, 0.11 mmol, 88%).

**1H NMR (400 MHz, Methanol-d₄):** δ 8.24 (d, J = 1.8 Hz, 1H), 7.82 (dd, J = 8.8, 1.8 Hz, 1H), 7.52 – 7.39 (m, 4H), 7.23 (s, 1H), 7.05 – 6.98 (m, 1H), 4.70 (hept, J = 6.0 Hz, 1H), 3.15 – 3.08 (m, 2H), 2.99 – 2.93 (m, 2H), 2.55 (s, 6H), 1.37 (d, J = 6.0 Hz, 6H).

**13C NMR (101 MHz, Methanol-d₄):** δ 158.69, 154.19, 146.30, 138.70, 129.48, 127.30, 123.99, 117.79, 116.87, 115.67, 114.59, 113.45, 111.57, 107.56, 69.83, 59.32, 43.48, 22.13, 20.95.

**HRMS (ESI, positive):** m/z calcd. for C₂₁H₂₇N₄O⁺: 351.21794, found 351.21856 ([M+H]+).

**HPLC:** purity: 95.8%. 
**1H-, 13C-NMR Spectra**

*N,N*-Dibenzy1-1H-indol-4-amine (11)
N,N-Dibenzyl-1-tosyl-1H-indol-4-amine (12)
1-tosyl-1-indol-4-amine (13)
4-(Phenyldiazenyl)-1-tosyl-1H-indole (14)
N,N-Dimethyl-2-oxo-2-(4-phenyldiazenyl)-1H-indol-3-yl]acetamide (16)
N,N-Dimethyl-2-(4-(phenyldiazenyl)-1H-indol-3-yl)ethan-1-amine (1)
6-(Phenyldiazenyl)-1H-indole (18b)
N,N-Dimethyl-2-oxo-2-(5-(phenyldiazenyl)-1H-indol-3-yl)acetamide (19a)
N,N-Dimethyl-2-oxo-2-(6-(phenyldiazenyl)-1H-indol-3-yl)acetamide (19b)
$N,N$-dimethyl-2-[(5-phenyldiazenyl)-1H-indol-3-yl]ethan-1-amine (2a)
$N,N$-dimethyl-2-(6-(phenyldiazenyl)-1H-indol-3-yl)ethan-1-amine (3)
$N,N$-dimethyl-2-(5-nitro-1H-indol-3-yl)-2-oxoacetamide (5)
2-(5-amino-1H-indol-3-yl)-N,N-dimethyl-2-oxoacetamide (6)
$N,N$-dimethyl-2-oxo-2-(5-(p-tolyldiazenyl)-1H-indol-3-yl)acetamide (9b)
N,N-dimethyl-2-oxo-2-(5-(m-tolyldiazenyl)-1H-indol-3-yl)acetamide (9c)
Supplemental Information

*N,N*-dimethyl-2-oxo-2-(5-(o-tolyldiazenyl)-1H-indol-3-yl)acetamide (9d)
2-(5-((4-isopropylphenyl)diazene)-1H-indol-3-yl)-N,N-dimethyl-2-oxoacetamide (9e)
2-(5-((3-isopropylphenyl)diazenyl)-1H-indol-3-yl)-N,N-dimethyl-2-oxoacetamide (9f)
2-(5-((4-methoxyphenyl)diazenyl)-1H-indol-3-yl)-N,N-dimethyl-2-oxoacetamide (9h)
Supporting Information

- (5-methoxyphenyldiazenyl-1H-indol-3-yl-N,N-dimethyl-2-oxoacetamide (9i)
2-(5-((3-isopropoxyphenyl)diazenyl)-1H-indol-3-yl)-N,N-dimethyl-2-oxoacetamide (9j)
$N,N$-dimethyl-2-(5-(p-tolyldiazenyl)-1H-indol-3-yl)ethan-1-amine (2b)
N,N-dimethyl-2-(m-tolyldiazenyl)-1H-indol-3-yl)ethan-1-amine (2c)
N,N-dimethyl-2-[5-(o-toluidinyl)-1H-indol-3-yl]ethan-1-amine (2d)
2-(5-((4-isopropylphenyl)diazenyl)-1H-indol-3-yl)-N,N-dimethylethan-1-amine (2e)
2-(5-[(3-isopropylphenyl)diazenyl]-1H-indol-3-yl)-N,N-dimethylethan-1-amine (2f)
2-((2-isopropylphenyl)diazenyl)-1H-indol-3-yl)-N,N-dimethylethan-1-amine (2g)
2-(5-((4-methoxyphenyl)diazenyl)-1H-indol-3-yl)-N,N-dimethylethan-1-amine (2h)
2-(5-((3-methoxyphenyl)diazenyl)-1H-indol-3-yl)-N,N-dimethylethan-1-amine (2i)
2-(5-(3-isopropoxyphenyl)diazenyl)-1H-indol-3-yl-N,N-dimethylethan-1-amine (2j)
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