Supporting Information

Restricted suitability of BODIPY for caging in biological applications based on singlet oxygen generation

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Experimental Section

Molecular Docking Studies

Molecular docking studies were performed on a Dell system including an Intel Xeon E5620 at 2.40 GHz, 24 GB RAM and a 64-bit Windows 7 Enterprise. Schrödinger Maestro 11.0, LLC, New York, USA, was used as molecular modelling software. First CDK2 Cyclin E1 structure (PDB: 4FKO[1]) was imported and processed with Protein Preparation Wizard. Missing side chains or loops were added by using Prime version 4.6. Structure was pre-processed, waters with less than three H-bonds to non-waters were removed and optimised using PROPKA at a pH of 7.0. Alternatively, MacroModel version 11.4015 was used for ligand preparation. Finally, Protein structure was minimised with OPLS3 force field. Ligands were processed with LigPrep version 4.0015. For ligand minimisation, we chose OPLS3 force field, Epik version 3.8015 generating states at a pH 7.0 ± 2.0 and at most 32 states per ligand. For receptor grid generation, the ligand was picked, and Van der Waals radius scaling was set to a factor of 1.0 with a partial charge cut-off of 0.25. Grid generation and minimised ligands were integrated in Ligand docking module that was ran in extra precision mode. For visualisation, superposition tool was used to align structure of **3** on **AZD5438**.

Synthesis

Reagents were purchased from abcr GmbH, Karlsruhe, Germany; Acros, Nidderau, Germany; Alfa Aesar by Thermo Fisher Scientific, Kandel, Germany; Fluka by Altman analytic, Munich, Germany; Merck, Darmstadt, Germany; Roth, Karlsruhe, Germany; Shangdong Shinning Pharm Co.,Ltd., China

HPLC analytics

HPLC quantification was performed with either a Agilent 1100 series instrument or a Hewlett Packard 1050 Series (Agilent Technologies, Waldbronn, Germany). Agilent 1100 series instrument was used with a Phenomenex KinetexQ C8, 5 mm (150 mm · 4.6 mm) column, 25 °C. For elution, different gradients of KH_2PO_4 buffer (0.01 M, pH 2.3)/acetonitrile (5 % CAN → 90 % ACN, 1.5 ml/min, 14 min) were used. Detection wavelength was set to 254 nm or 535 nm. For quantification with Hewlett Packard 1050 Series we used either a Kinetex® C8, 5 µm (150 mm · 4.6 mm, Phenomenex, Aschaffenburg, Germany) or a STAGROMA® C18, 5 µm (125 mm · 4 mm, Stagroma AG, Reinach, Switzerland). The mobile phase was equivalent to quantification with agilent 1100. Detection was performed at λ = 254 nm, 294 nm, 296 nm or 540 nm.

Mass spectrometry

Mass spectrometry was performed on a Bruker Esquire LC ion trap mass spectrometer (Bremen, Germany) with electron spray ionisation (dry gas 9 L min^{-1}, nebulizer 35 psi, drying temperature 350 °C, positive and negative mode). Chromatic separation was achieved with Agilent 1100 HPLC system (Waldbronn, Germany) with an RP-8 column (Agilent Eclipse XDB-C8, 150 mm · 4.6 mm, 5 mm) and a gradient of 0.1 % acetic acid/acetonitrile. Eluent flow rate was 1 ml · min^{-1}.

High-resolution mass spectrometry was performed either on a Thermo Fisher Q Exative Plus mass spectrometer (Hybrid Quadrupol Orbitrap) with electron ionisation in positive ion mode, a Joel Accu (TOF 4G) with electron impact ionisation or a BIFEXC III mass spectrometer (Bruker Daltonics) including matrix assisted laser desorption/ionisation (4-chloro-α-cyanocinnamic acid matrix, 337 nm ionisation, 19 kV acceleration) and time of flight detector.

NMR

NMR spectra were plotted on Bruker Avance III 300, tempered at 298 K. ^{1}H (300 MHz) and ^{13}C (75 MHz) spectra were referenced to the respective deuterated solvents signals of DMSO-d_6 (δ ^{1}H NMR: 2.50 ppm, δ ^{13}C NMR: 39.52 ppm) and $CDCl_3$ (δ ^{1}H NMR: 7.26 ppm, δ ^{13}C NMR: 77.16 ppm) as an internal standard. The following shortcuts have been used to classify the appropriate signals: s (singlet), d (doublet), t (triplet), q (quartet), m (complex multiplet).

All reactions have been executed under argon atmosphere and without direct incidence of light.

8-Chloromethyl-4,4-difluoro-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (1)

$C_{18}H_{24}BClF_2N_2$ (M_r 352.66)

CAS Registry Number 460719-93-1

Synthesis was performed according to literature[2]. 3-Ethyl-2,4-dimethylpyrrole (2.0 eq) was dissolved in 90 ml dry dichloromethane. Within 30 minutes, 2-chloroacetylchloride (1.0 eq) was added dropwise at room temperature. The reaction was heated to 50 °C and left stirring for 2 hours. The mixture was cooled down to 0 °C and then neutralised by addition of dry TEA (5.0 eq). After 30 minutes, boron trifluoride (7.0 eq) was added. The reaction was heated to 50 °C and left stirring for one hour.

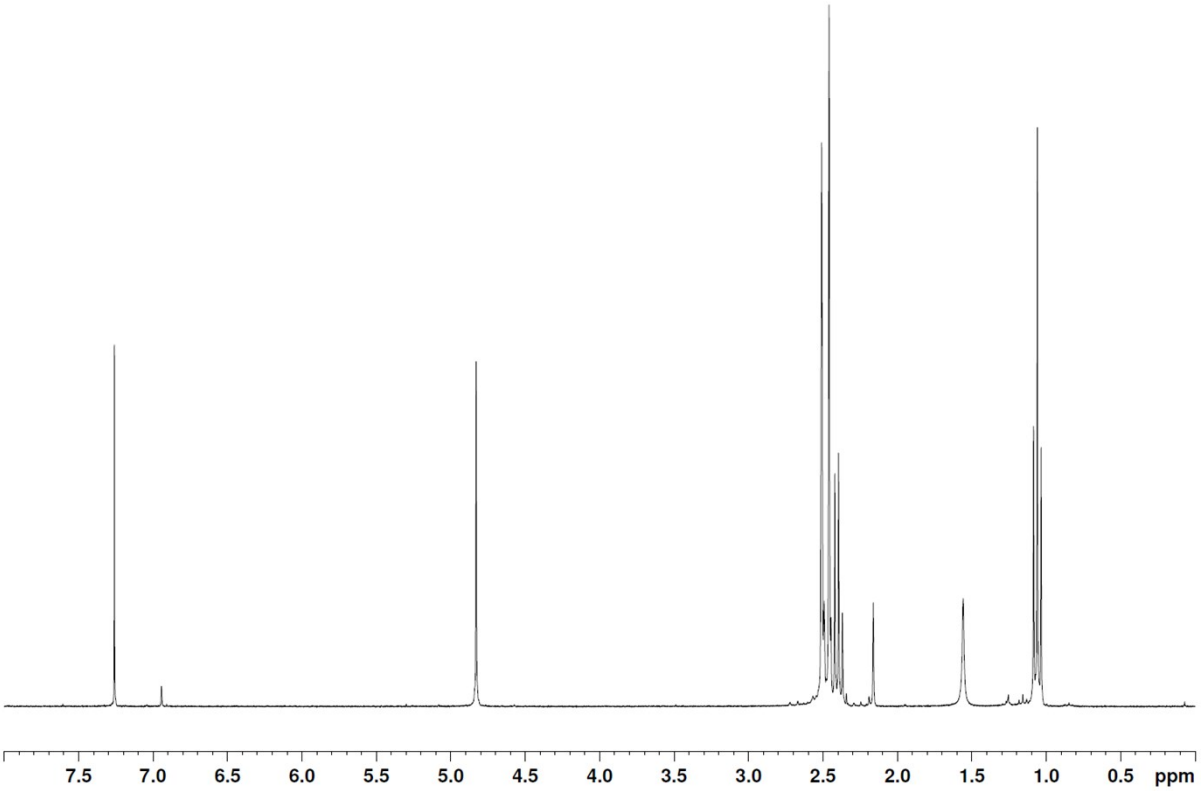
The reaction was washed with brine three times and dried over sodium sulfate. The solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography with a gradient of dichloromethane and petroleum ether (90 % HPLC purity).

Yield: 1300 mg (3.7 mmol, 52 %)

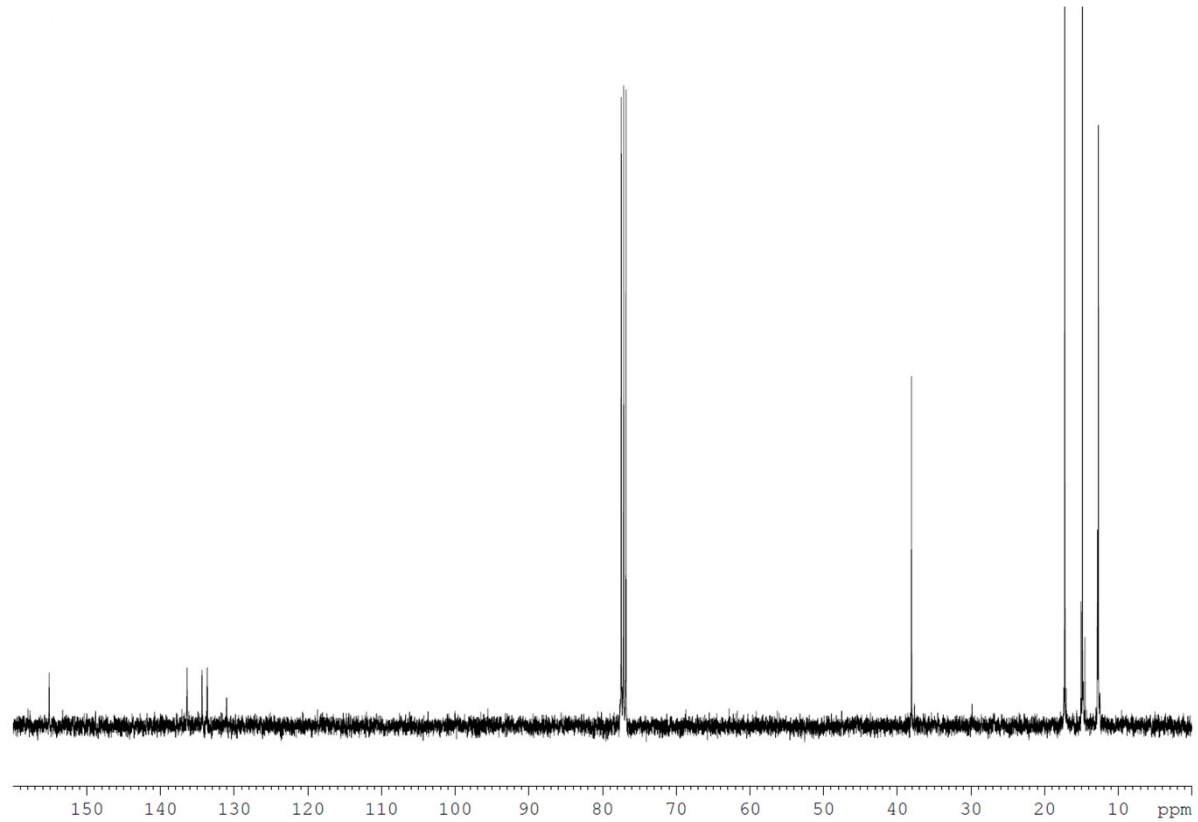
^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.06 (t, 6H), 2.41 (q, 4H), 2.46 (s, 6H), 2.51 (s, 6H), 4.83 (s, 2H) ppm.

^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.7, 12.8, 14.9, 17.3, 38.1, 130.8, 133.4, 134.1, 136.2, 154.9 ppm.

HRMS (EI): m/z = 352.16990 M^+ (calc. m/z = 352.16891)



Supplemental figure S1. ^{1}H-NMR spectrum of compound **1** in $CDCl_3$.



Supplemental figure S2. ^{13}C-NMR spectrum of compound **1** in $CDCl_3$.

8-Iodomethyl-4,4-difluoro-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (2)

$C_{18}H_{24}BF_2IN_2$ (M_r 444.11)

CAS Registry Number 915309-80-7

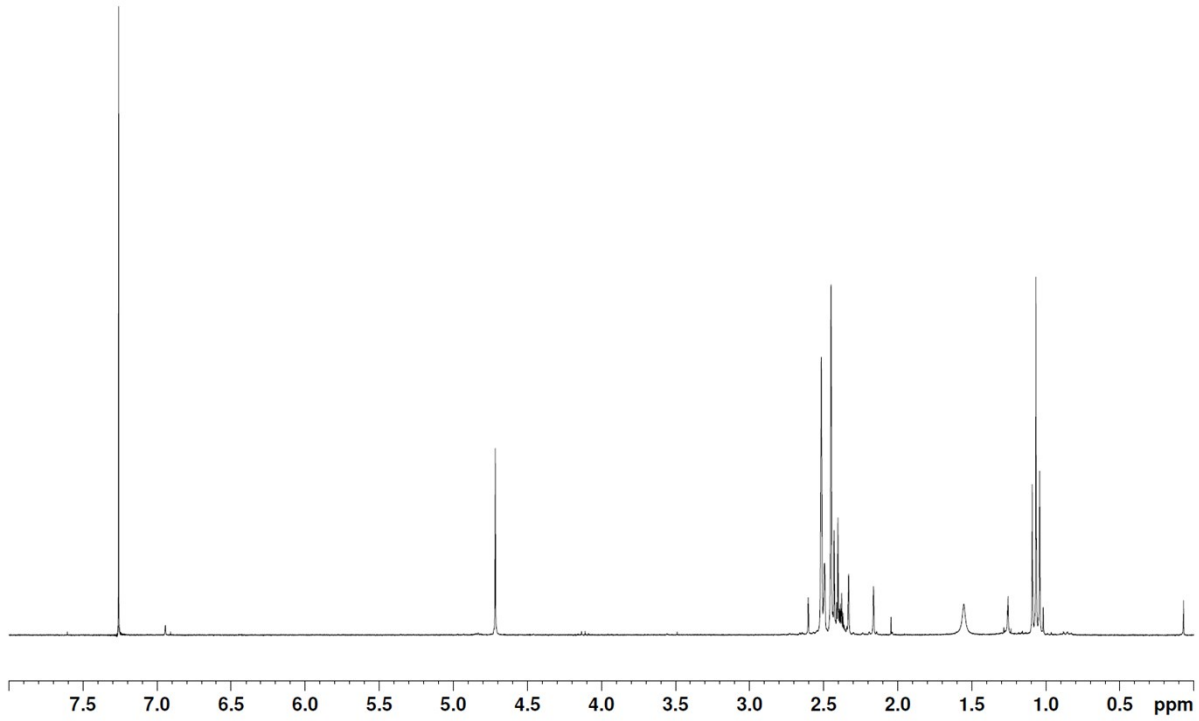
Synthesis was performed according to literature[3]. Under an argon atmosphere 8-chloromethyl-4,4-difluoro-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (500 mg, 1.4 mmol) and sodium iodide (425 mg, 2.8 mmol) were dissolved in 10 ml of dry THF. The reaction was refluxed for one hour. After cooling, the solution was diluted with Et_2O (20 ml), washed with water (20 ml) and the organic phase was dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash column chromatography (hexane/Et_2O) to give the title compound as a dark purple solid (90 % HPLC purity).

Yield: 498 mg (1.1 mmol, 80 %).

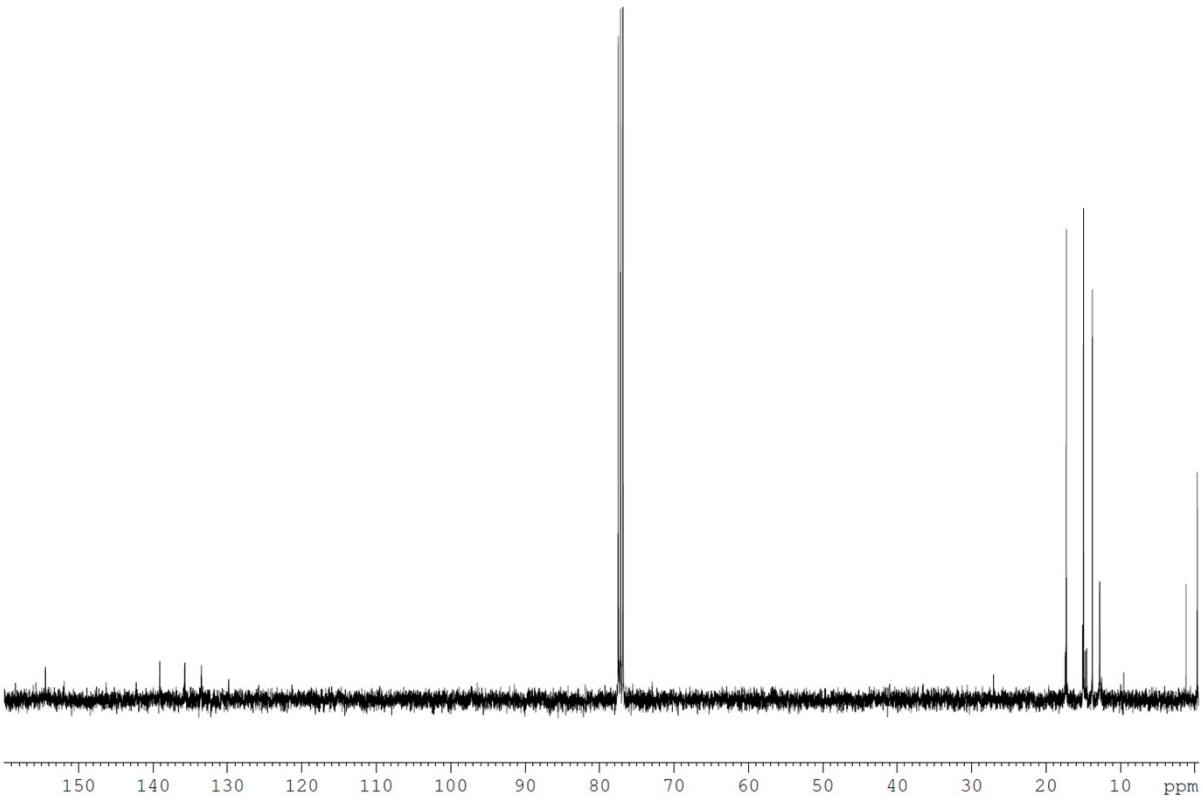
^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.07 (t, 6H), 2.38-2.45 (m, 10H), 2.52 (s, 6H), 4.72 (s, 2H) ppm.

^{13}C NMR (75 MHz, $CDCl_3$): δ = -0.3, 12.7, 13.8, 14.9, 17.3, 129.9, 133.5, 135.7, 139.1, 154.4 ppm.

HRMS (EI): m/z = 444.10421 M^+ (calc. m/z = 444.10453)



Supplemental figure S3. ^{1}H-NMR spectrum of compound **2** in $CDCl_3$.



Supplemental figure S4. ^{13}C-NMR spectrum of compound **2** in $CDCl_3$.

N-((4,4-difluoro-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-s-indacen-8-yl)methyl)-4-(1-isopropyl-2-methyl-1*H*-imidazol-5-yl)-*N*-(4-(methylsulfonyl)phenyl)pyrimidin-2-amine (3)

$C_{36}H_{44}BF_2N_7O_2S$ (M_r 687.33)

Sodium hydride (1.2 eq) was dissolved in 5 ml of dry DMF under an argon atmosphere. After cooling to 0 °C, the respective N-phenyl-pyrimidin-2-amine (1.0 eq) were dissolved in 3 ml of dry DMF and then added dropwise to the reaction. After 1 hour, the reaction was cooled to -35 °C and the respective BODIPY iodide (2.5 eq), dissolved in 10 ml of dry DMF, was added to the reaction dropwise. After stirring at -35 °C for another hour, the reaction was quenched by addition of two drops of water. The solvent was removed by reduced pressure with a rotary evaporator.

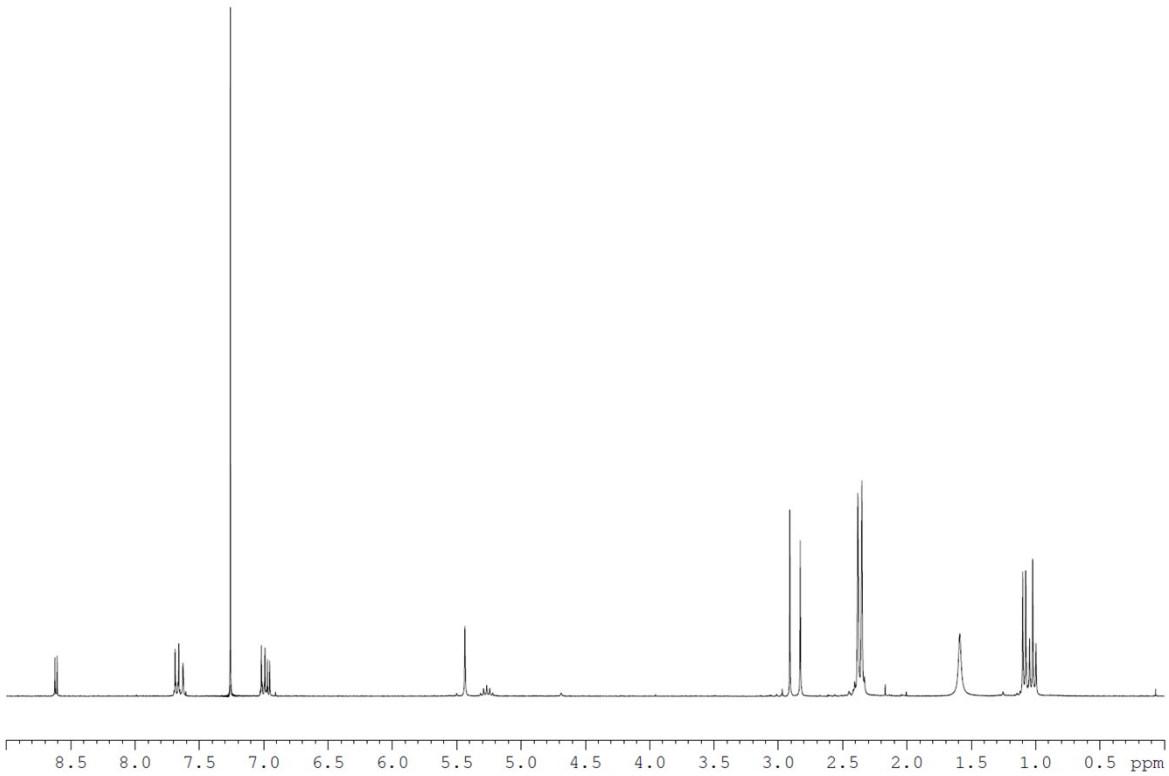
Purification by RP flash chromatography with a gradient of acetonitrile and water to get a red solid (100 % HPLC purity).

Yield: 41 mg (0.06 mmol, 15 %)

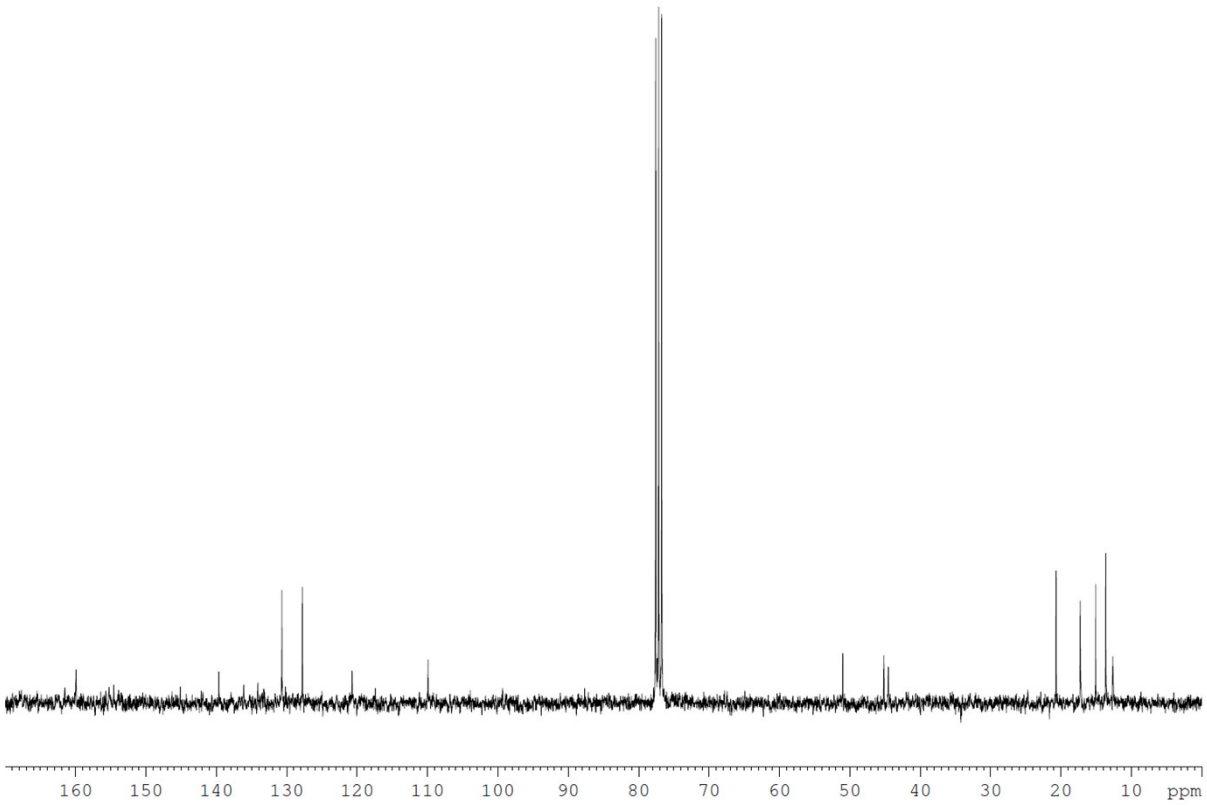
^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.02 (t, $^3J_{HH}$ = 7.5 Hz, 6H), 1.09(d, $^3J_{HH}$ = 7.1 Hz, 6H), 2.33-2.41 (m, 16H), 2.83 (s, 3H), 2.91 (s, 3H), 5.27 (sept, $^3J_{HH}$ = 7.1 Hz, 1H), 5.44 (s, 2H), 6.96 (d, $^3J_{HH}$ = 5.2 Hz, 1H), 7.00 (d, $^3J_{HH}$ = 8.5 Hz, 2H), 7.63 (s, 1H), 7.68 (d, $^3J_{HH}$ = 8.5 Hz, 2H), 8.62 (d, $^3J_{HH}$ = 5.2 Hz, 1H) ppm.

^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.6, 13.6, 15.1, 17.3, 20.7, 44.5, 45.2, 51.0, 109.9, 120.7, 127.8, 130.1, 130.7, 133.2, 133.9, 134.0, 136.1, 139.7, 145.1, 146.3, 154.4, 154.9, 159.9 ppm.

HRMS (EI): m/z = 687.33350 M^+ (calc. m/z = 687.33383)



Supplemental figure S5. ^{1}H-NMR spectrum of compound **3** in $CDCl_3$.



Supplemental figure S6. ^{13}C-NMR spectrum of compound **3** in $CDCl_3$

8-Acetoxymethyl- 4,4-difluoro-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (4)

$C_{20}H_{27}BF_2N_2O_2$ (M_r 376.25)

CAS Registry Number 137829-80-2

3-Ethyl-2,4-dimethylpyrrole (2.0 eq) was dissolved in 90 ml dry Dichloromethane. Within 30 minutes acetoxyacetyl chloride (1.0 eq) was added dropwise at room temperature. The reaction was heated to 50 °C and left stirring for 2 hours. The mixture was cooled down to 0 °C and then neutralised by addition of dry TEA (5.0 eq). After 30 min boron trifluoride (7.0 eq) was added. The reaction was heated to 50 °C and left stirring for one hour.

The reaction was washed with brine three times and two times with hydrogen carbonate and dried over sodium sulfate. The solvent was evaporated under reduced pressure.

Purification by flash chromatography isocratic with dichloromethane and petroleum ether 5 %. For 100 % HPLC purity it was recrystallised from acetone/water.

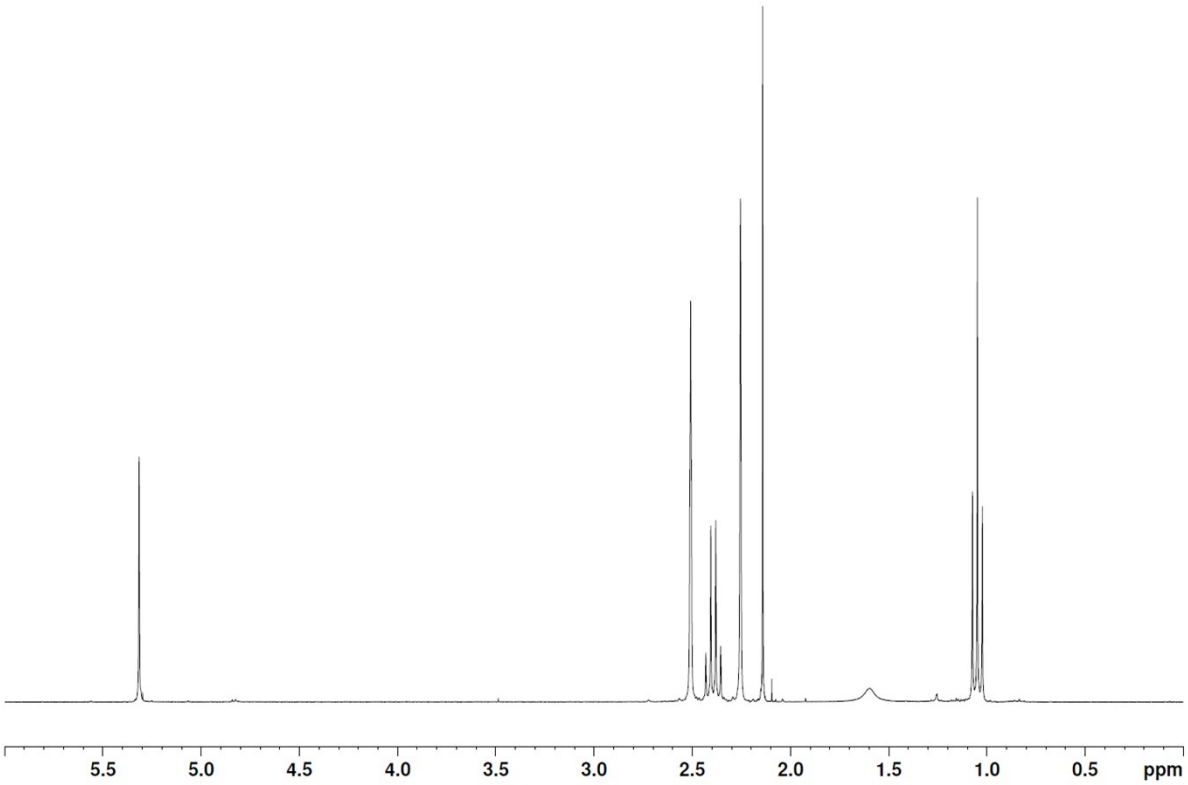
Yield: 860 mg (2.29 mmol, 21 %)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.05 (t, 6H), 2.14 (s, 3H), 2.25 (s, 6H), 2.39 (q, 4H), 2.51 (2, 6H), 5.32 (s, 2H) ppm

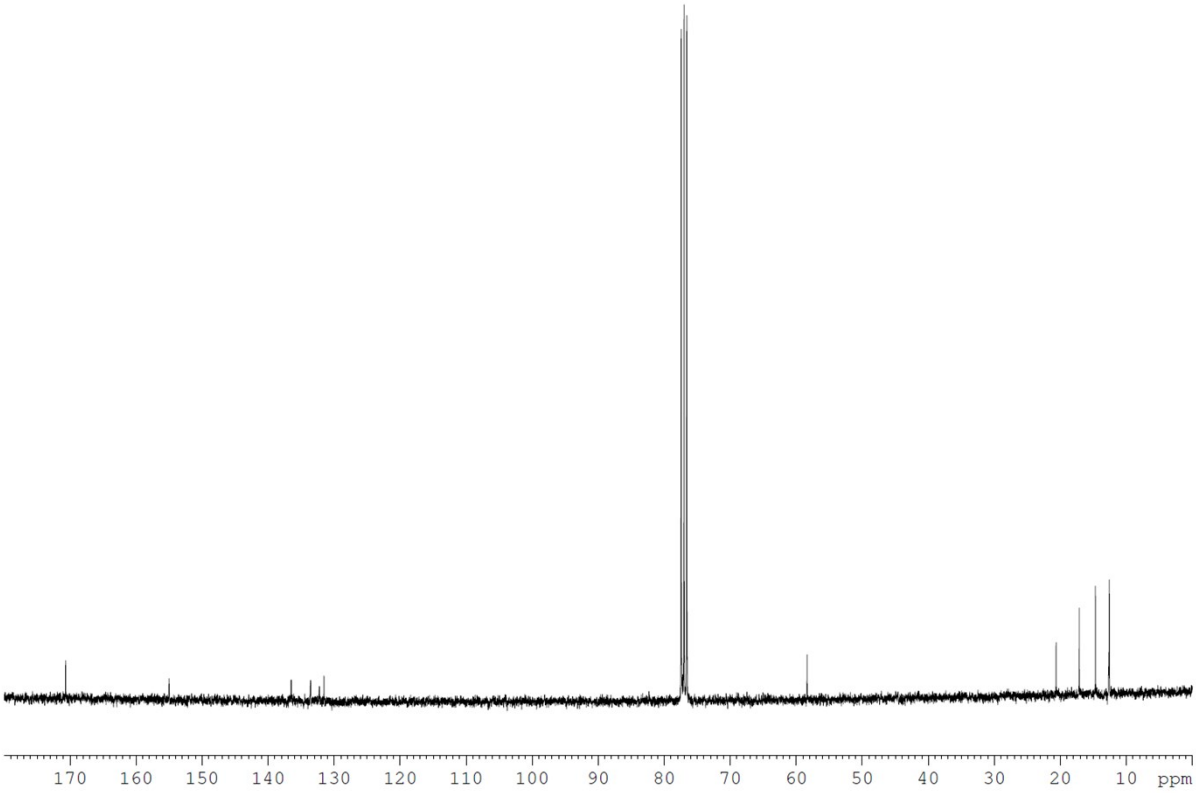
^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.6, 12.7, 14.7, 17.1, 20.7, 58.4, 131.6, 132.3, 133.6, 136.5, 155.0, 170.7 ppm.

HRMS (EI): m/z = 376.21340 M^+ (calc. m/z = 376.21282)

Synthesis and analytical data have been described by Amat-Guerri et al, 2003.[2]



Supplemental Figure S7. ^{1}H-NMR spectrum of compound **4** in $CDCl_3$.



Supplemental Figure S8. ^{13}C-NMR spectrum of compound **4** in $CDCl_3$.

8-Hydroxymethyl- 4,4-difluoro-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (5)

$C_{18}H_{25}BF_2N_2O$ (M_r 334.21)

CAS Registry Number 615574-26-0

1 was dissolved in tetrahydrofuran/water (1:1) and solid lithium hydroxide (5.0 eq) was added. The reaction was left stirring at room temperature for three hours.

Purification by column chromatography isocratic with 20 % ethyl acetate in cyclohexane.

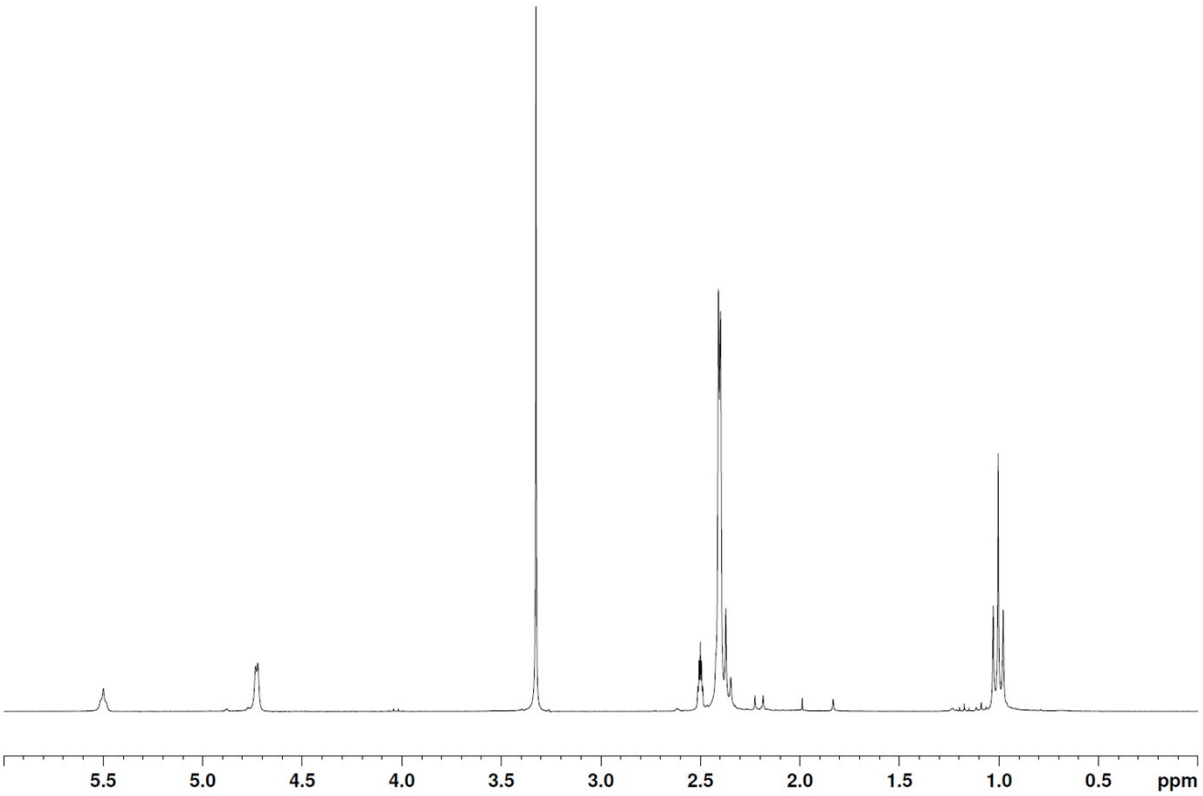
Yield: 704 mg (2.11 mmol, 92 %)

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.00 (t, 6H), 2.34-2.43 (m, 16H), 4.73 (s, 2H), 5.50 (s, 1H) ppm

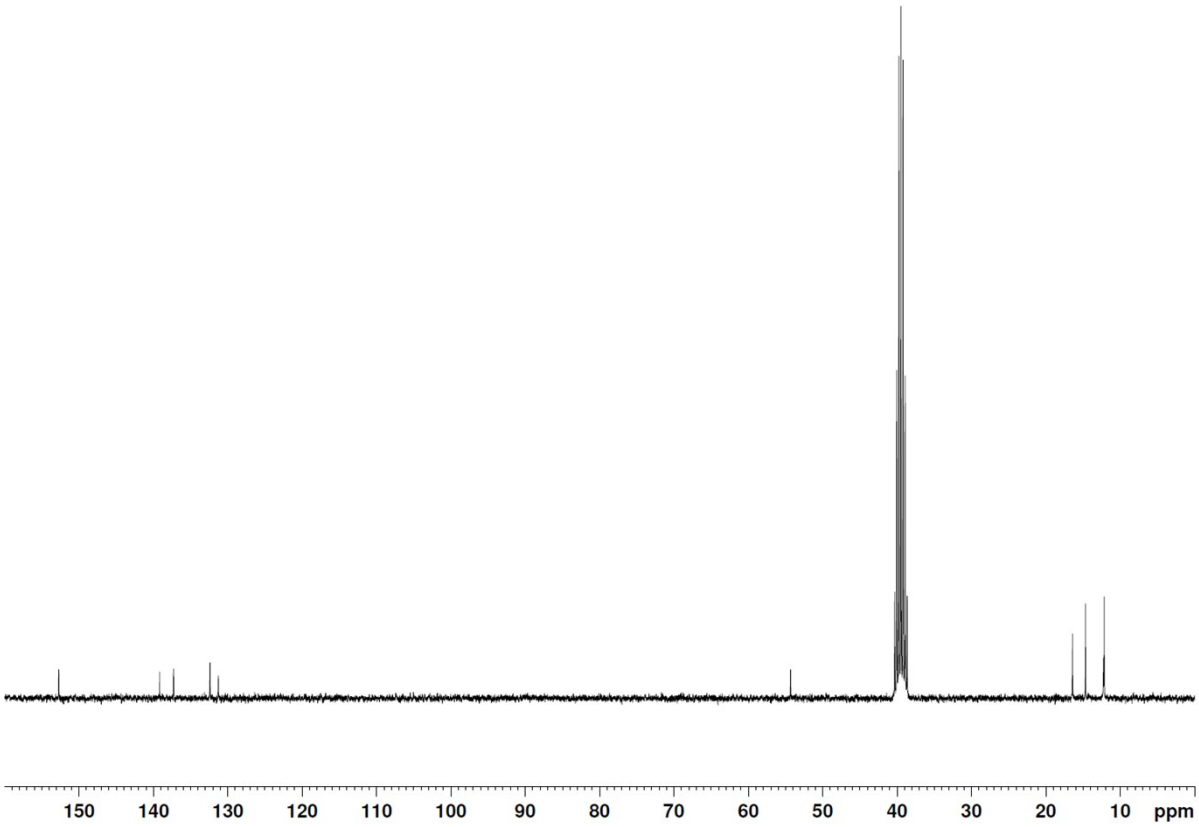
^{13}C NMR (75 MHz, DMSO- d_6): δ = 12.1, 12.2, 14.7, 16.4, 54.3, 131.3, 132.4, 137.3, 139.2, 152.7 ppm.

HRMS (EI): m/z = 334.20280 M^+ (calc. m/z = 334.20225)

Synthesis and analytical data have been described by Amat-Guerri et al, 2003.[2]



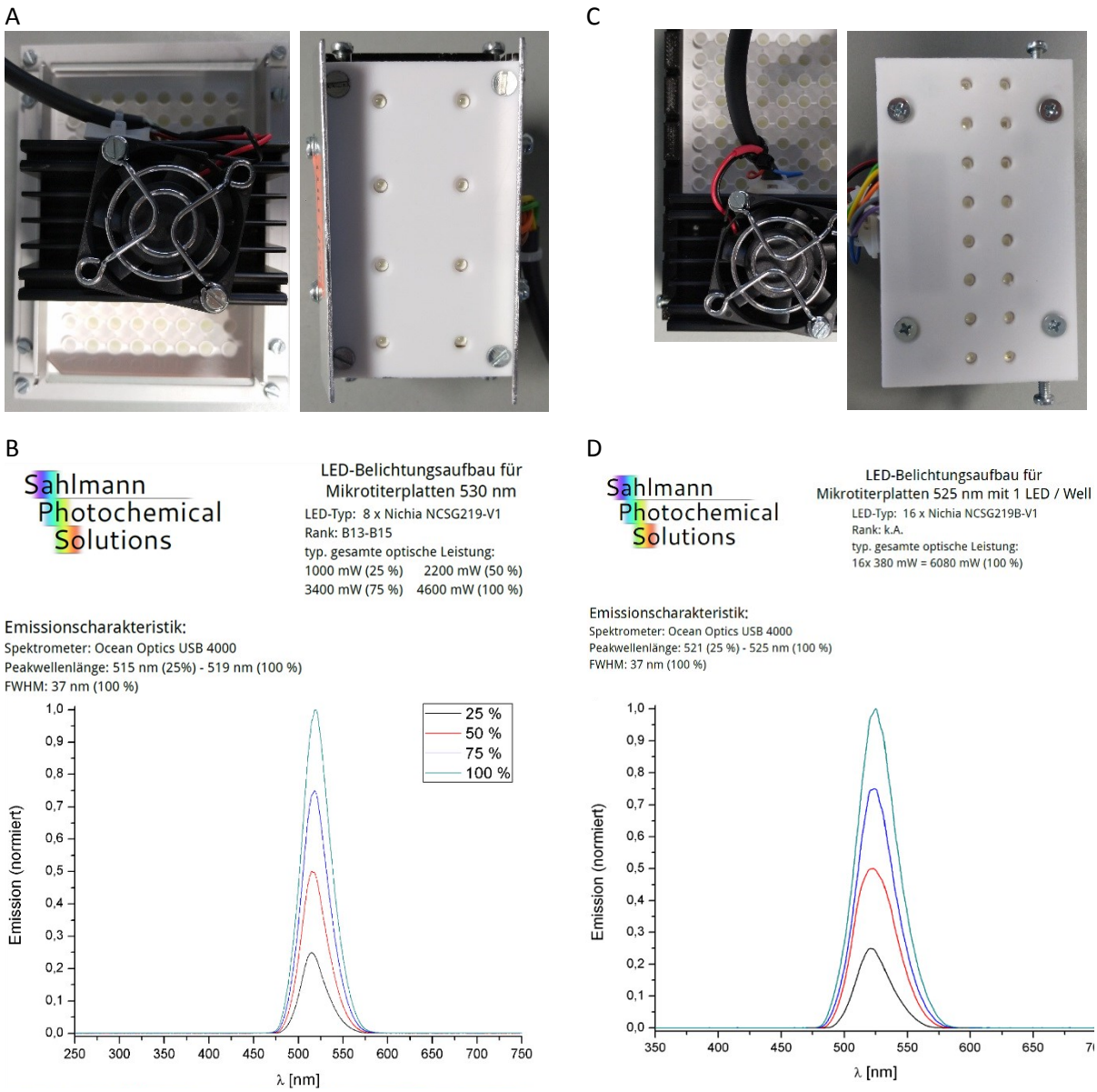
Supplemental figure S9. ^{1}H-NMR spectrum of compound **5** in DMSO-d_6.



Supplemental figure S10. ^{13}C-NMR spectrum of compound **5** in DMSO-d_6.

Light sources

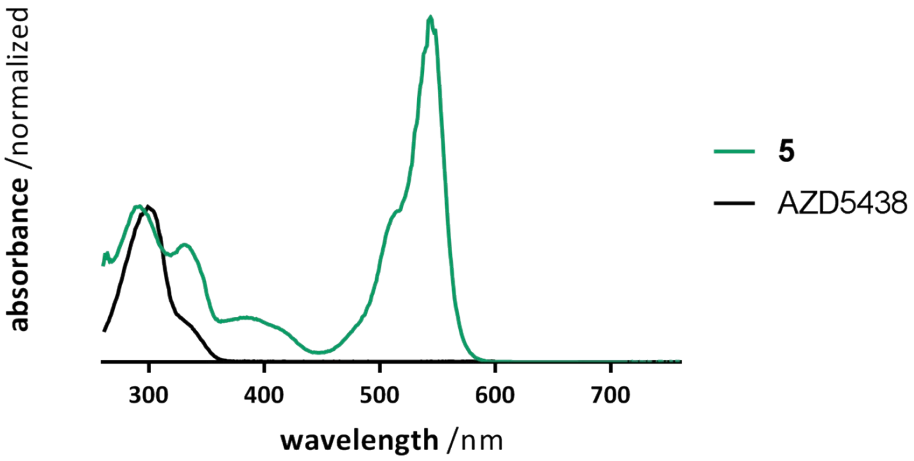
For compound irradiation several LEDs with well plate attachment were used: A green eight LED lamp for well plate irradiation, λ = 519 nm, Nichia NCSG219-V1, 4.6 W, estimated to 6.5 mW/cm² per well (100 % power) and a green 16 LED lamp for well plate irradiation, λ = 525 nm, Nichia NCSG219B-V1, 6.08 W, estimated to 90 mW/cm² per well (100 % power) or 4.5 mW/cm² per well (5 % power). Sahlmann Photochemical Solutions, Germany, manufactured mentioned light sources. During irradiation, ambient light was excluded, and only a weak red light was used for lighting. 10 minutes of irradiation with 519 nm LED correlates to one minute of irradiation via 525 LED (S21).



Supplemental Figure S11. Light sources. (A) 519 nm 8 LED lamp (1 LED per 4 wells) in top-down (left) and bottom-up view (right). (B) Technical data sheet of 519 nm light source. (C) 525 nm 16 LED lamp (1 LED per well) in top-down (left) and bottom-up view (right). (D) Technical data sheet of 525 nm light source.

UV/Vis spectroscopy

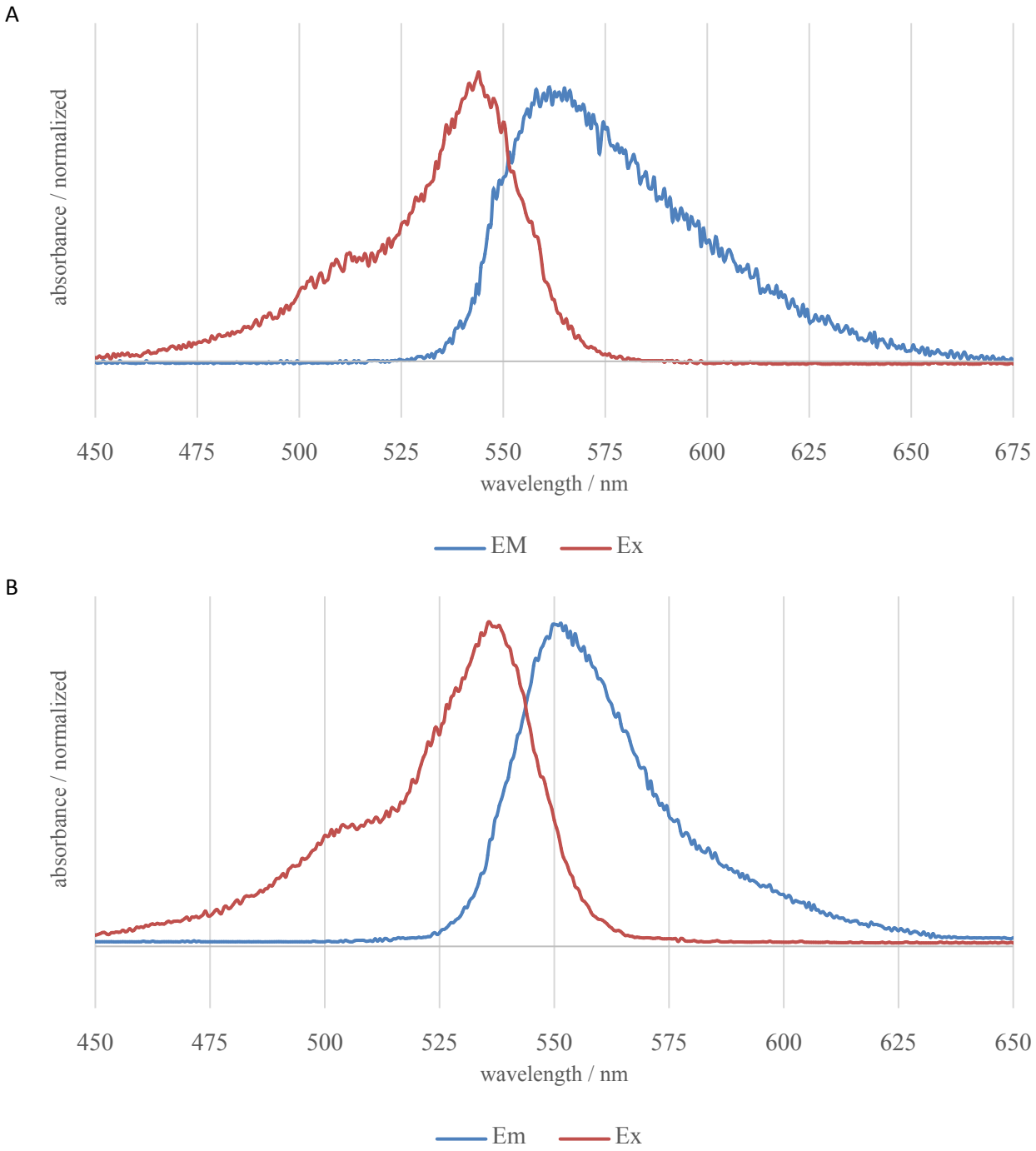
UV/VIS spectra were acquired with either a Varian Cary® 50 Scan, Agilent Technologies, in DMSO or DMSO/DPBS solution, or a Fluostar OMEGA, BMG Labtech, in a transparent 96 well plate, in DMSO or 20 % DMSO in double distilled water. Final compound concentration was 0.1 mM.



Supplemental figure S12. Adsorption spectra of AZD5438 and 5. Absorbance was recorded in pure DMSO. **5** revealed an absorbance maximum at 544 nm. **AZD5438** indicated an absorbance maximum at 299 nm.

Fluorescence spectroscopy

For fluorescence spectra, 0.5 µM compounds were dissolved in DMSO, half micro cuvette. Spectra were plotted using a Fluorescence spectrometer LS55 (Slit width: 2.5 nm), Perkin Elmer, Waltham, USA.



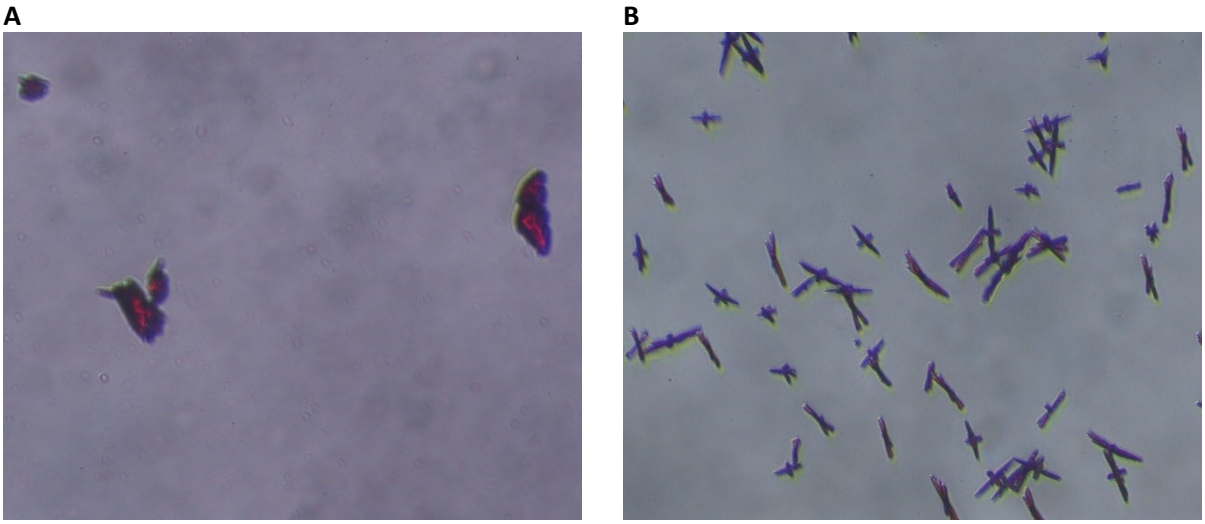
Supplemental figure S13. Fluorescence spectra of 3 and 5. (A) 0.5 µM of compound **3** dissolved in DMSO indicated maxima at 544 nm (excitation) and 558 (emission). (B) 0.5 µM of compound **5** dissolved in DMSO indicated maxima at 536 nm (excitation) and 551.5 (emission).

Uncaging experiments

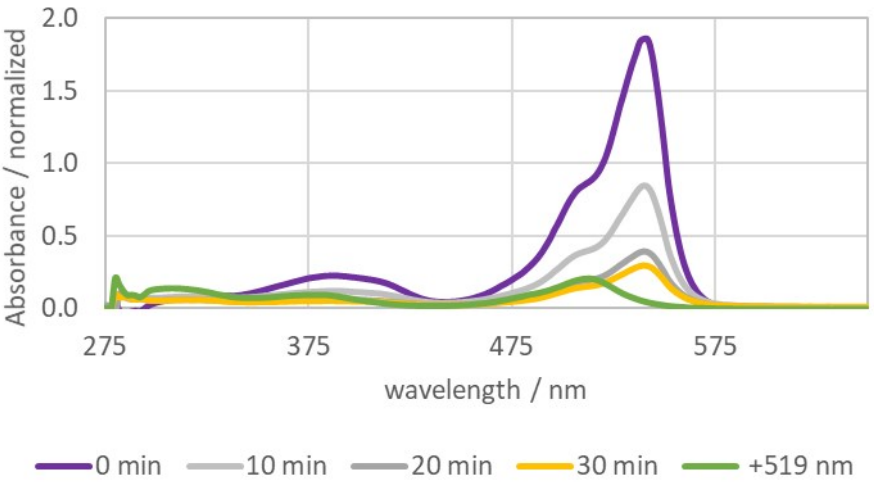
For uncaging experiments, 25 µM compounds were dissolved in DMSO, DMSO/PBS solution or 20 % DMSO in double distilled water. Compounds have been irradiated in a white 96 well plate, Perkin Elmer, Rodgau, Germany, with 519 nm at 6.5 mW/cm² per well (1 – 30 min). Quantification were completed by high-performance liquid chromatography (HPLC).

Microscopy

Precipitation was photographed with a Canon EOS 80D attached to an Olympus CK2 microscope. Compounds were dissolved in DMSO or 20 % DMSO in double distilled water.

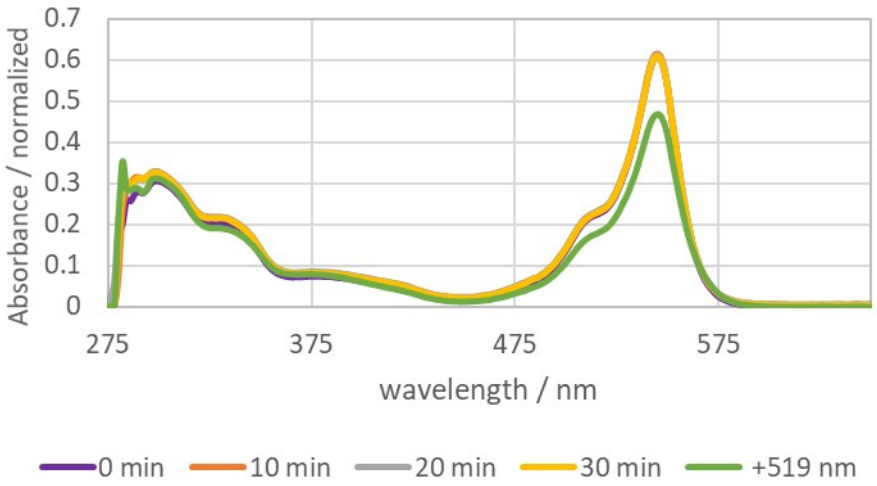


Supplemental figure S14. Precipitation of *meso*-methyl-BODIPY derivatives in aqueous solution. (A) 0.1 mM **3** in 20 % DMSO in double distilled water with crystals of **3**. (B) 0.1 mM **5** in 20 % DMSO in double distilled water with crystals of **5** Compounds dissolved in pure DMSO did not revealed any precipitation.

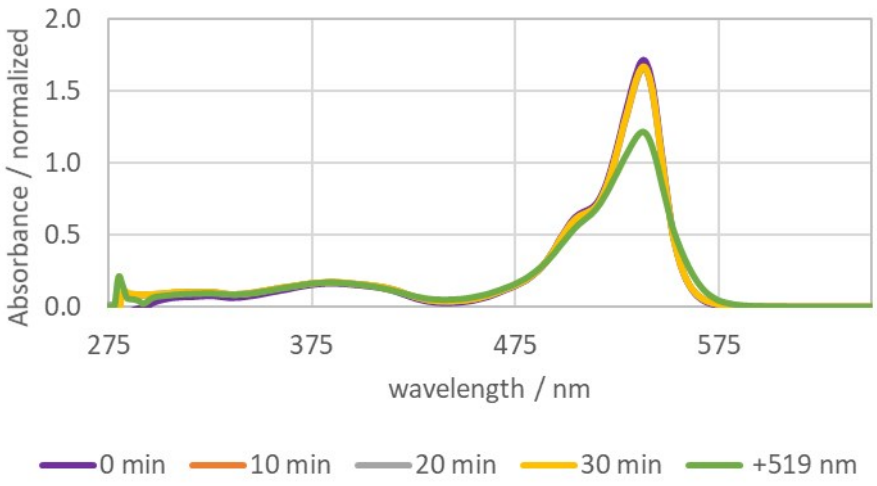


Supplemental figure S15. Photodegradation of 5 in aqueous solution. A solution of 0.1 mM **5** dissolved in 20 % DMSO in double distilled water was observed (0 – 30 min) or irradiated with 519 nm for 10 min. Adsorption decreased over time in aqueous solution. By irradiation, compound can be quantitatively degraded.

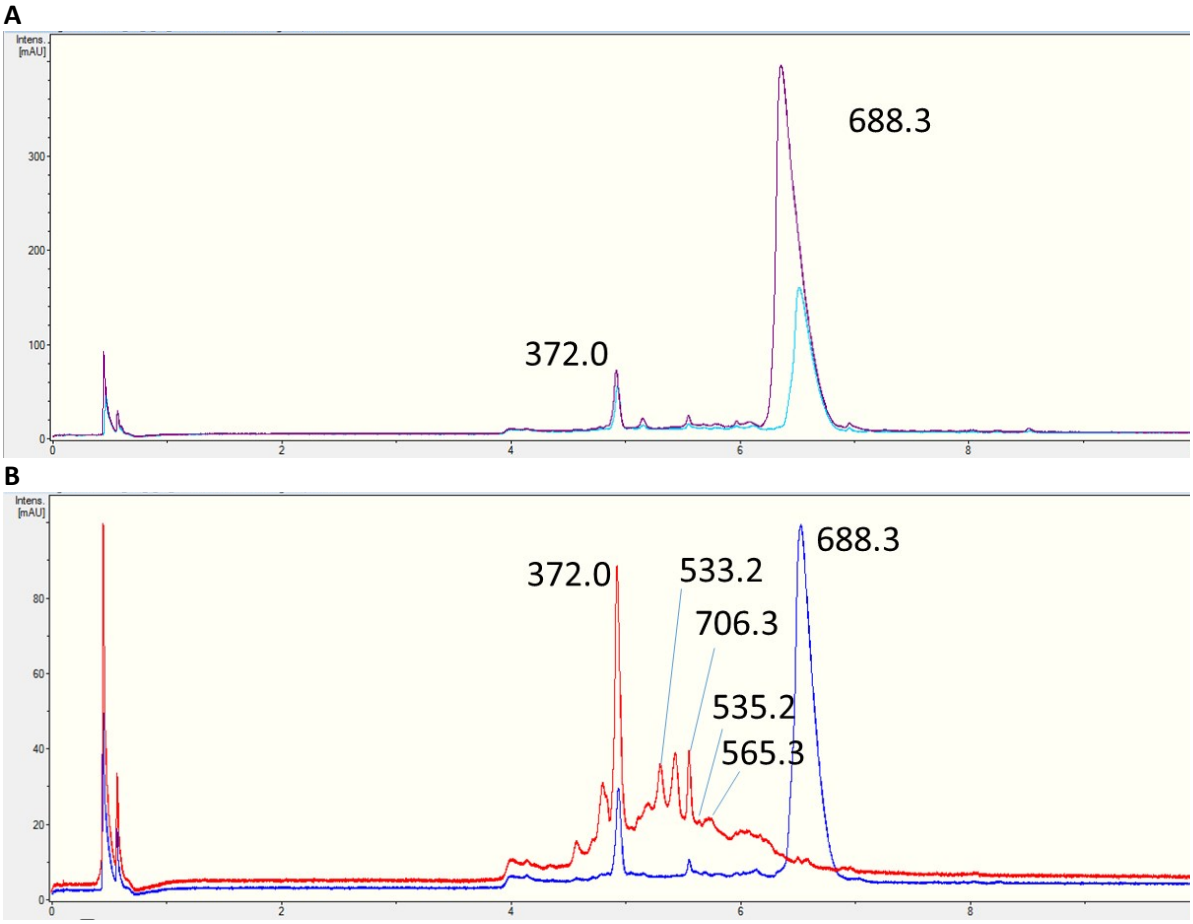
A



B

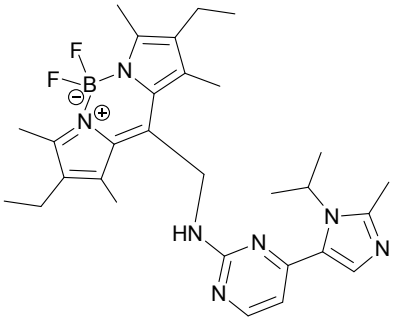
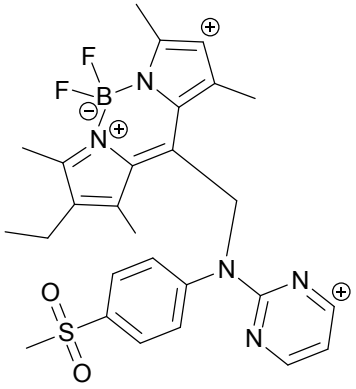
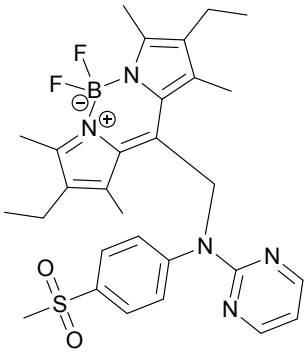


Supplemental figure S16. Photodegradation of BODIPY derivatives in DMSO. (A) Adsorption spectrum of 0.1 mM **3** in pure DMSO. Irradiation with 525 nm shows a decent decrease in adsorption at 535 nm. (B) Adsorption spectrum of 0.1 mM **5** in pure DMSO. Irradiation with 525 nm shows a decrease in adsorption at 535 nm.



Supplemental figure S17. Mass spectrometry of irradiation of compound 3 in DMSO or aqueous solution. (A) LC-MS chromatogram of 0.1 mM **3** in DMSO without 525 nm (purple) or with one minute irradiation (cyan). Mass of 372.0 represents **AZD5438** while mass of 688.3 represents mass of **3**. A BODIPY-PPG cleaved from **AZD5438** has not been detected. (B) LC-MS chromatogram of 0.1 mM **5** in 20 % DMSO/80 % water without 525 nm (blue) or with one minute irradiation (red). Choosing the appropriate solvent has a critical influence in uncaging experiments. Irradiation in aqueous solutions leads mostly into fragmentation. We observed fragments with higher masses than **3** or **5**, e.g. 706.3. Therefore, we presume that the irradiation facilitates aggregation formation, which leads into precipitation.[4,5] Detection wavelength was set to 254 nm. Instability of several BODIPY derivatives are already described.[6,7]

Supplemental table S1. Selected potential fragments of supplemental figure S13.

Mass	Potential fragment
533.2	
535.2	
565.3	

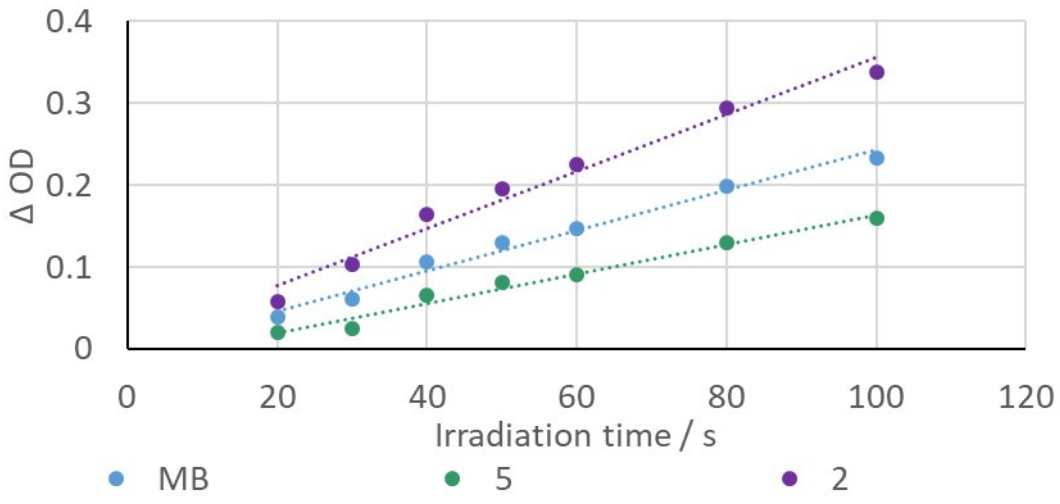
Electron spin resonance spectroscopy (ESR) experiments

The test substances **AZD5438**, compound **3** and **5** dissolved in DMSO (25 µM) were mixed with the spin probe 2,3,7,8-Tetramethoxythianthrene (TMTH) in water (2.5 mM) in an white 96 well. The water contained EDTA (1 mM) to complex metal ions to avoid interference with ROS. As positive control, the photosensitiser methylene blue (MB) in DMSO was mixed with the spin probe TMTH to get a concentration of 16.7 µM and 2.5 mM of the latter. As negative controls the substances were measured either without the use of the spin probe or without prior irradiation. The samples were irradiated with a 16 LED lamp for a duration of 30 s and intensity of 90 mW/cm² per well.

Spin probing experiments were carried out using an ELEXSYS E 500 (Bruker Biospin, Rheinstetten, Germany). For the acquisition of the ESR spectra, samples were taken from the 96 well plate using 25 µl ringcaps® (Hirschmann, Eberstadt, Germany). The ringcap® was then placed in an ESR tube and put into the cavity of the spectrometer. The measurements were done in duplicate of two distinct samples using the X-band and the following parameters: centre field: 3511.35 GHz; resonance frequency 9.85 GHz; microwave power 20 mW; modulation amplitude 1.0 G; modulation frequency 100 kHz; conversion time 40 ms; time constant 40.96 ms.

Singlet oxygen assays

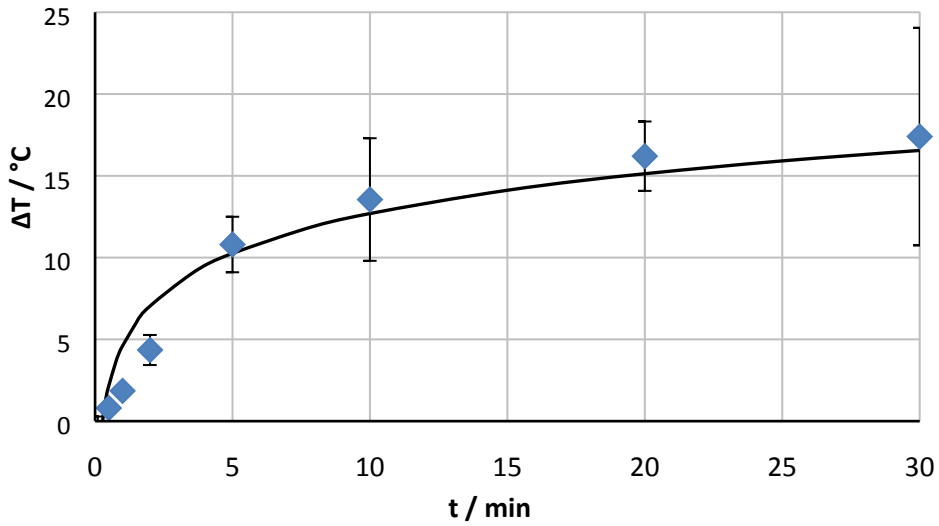
Released singlet oxygen degenerates 1,3-diphenylisobenzofuran (DPBF) into a colourless 1,2-dibenzoylbenzene.[8] Compounds like **3** and **5** are generating singlet oxygen that leads into an absorption decay of DPBF at 410 nm. Five µM compounds were dissolved in DMSO, 90 µM DPBF and irradiated with 525 nm (4.5 mW/cm², 10 s – 300 s). Absorption was determined with a Fluostar Omega, BMG Labtech, at 410 nm.[9] Singlet oxygen quantum yield was determined and calculated as described by Usui.[10] Methylene blue was used as internal standard with a quantum yield of 0.52.



Supplemental figure S18. Singlet oxygen quantum yield of 3 and 5. Graphical illustration of absorption decrease of DPBF (λ = 410 nm) in DMSO. Methylene blue (MB) was applied as internal standard.

Kinase assays

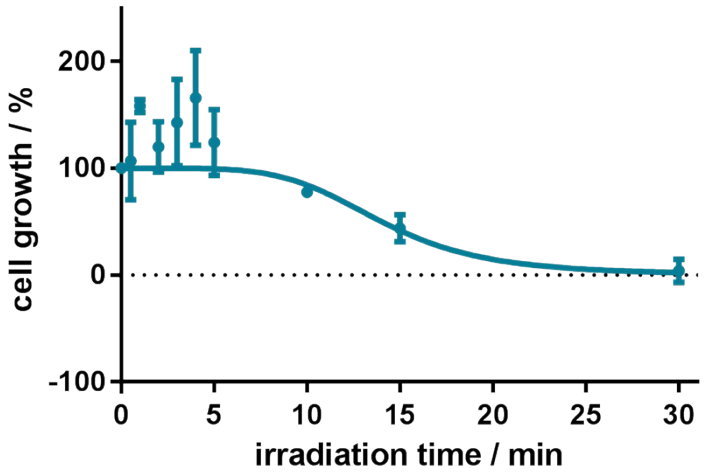
To evaluate IC_{50}-values on the CDK2/cyclin E1, ATP conversion were detected via bioluminescence. ADP-Glo Kinase Assay were used to manufacturer's manual. ADP-Glo Kinase Assay and CDK2/CyclinE1 Kinase Enzym System (V6280) were purchased from Promega, Mannheim, Germany. Assay was performed in a white 96 well plate (Perkin Elmer). Inhibitors were dissolved in DMSO and Reaction Buffer A to a final concentration of 10 µM and diluted in 1:3 dilution steps. Then compounds were irradiated with 519 nm LED, 10 minutes, 6.5 mW/cm² . To prevent denaturation, compounds have to be irradiated before kinase were added due to strong heat generation in wells (S19). After pre-incubation with kinase (0.4 ng/µl) for 10 minutes, ATP and substrate histone H1 (10 µM and 0.1 µg/µl, respectively) were added and incubated for one hour at 30 °C. Afterwards, ADP-Glo Reagent was appended; solutions were incubated for additional 30 minutes. Lastly, Detection Reagent was added for 30 minutes. Signals were detected via bio luminescence on a Fluostar Omega, BMG Labtech, Germany, using luminescence measurement, interval time of 0.2 and gain of 3600. Kinase assays were performed with a DMSO concentration of 1 % and a final DTT concentration of 0.05 mM. IC_{50}-values were determined with GraphPad Prism version 7, Graphpad Software, LLC, San Diego, USA, using the function Y=Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*HillSlope)). Outliers were detected via D'Agostino-Pearson omnibus normality test. Kinase incubated with 1 % DMSO was set as 100 % control and wells without kinase as 0 %. Assay was performed with three technical replicates.



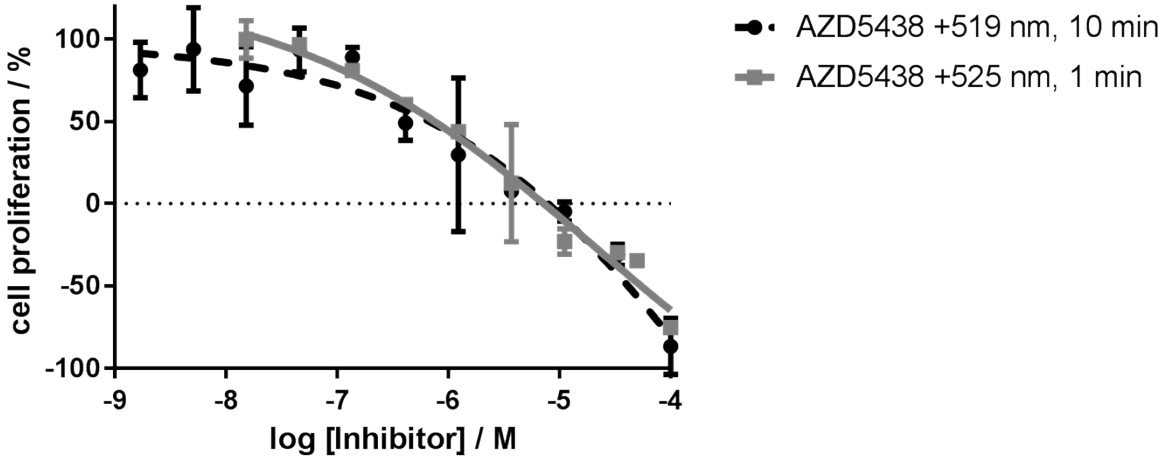
Supplemental figure S19. Temperature increase during irradiation with 519 nm in kinase assay. Compounds have to be irradiated separately due to high temperature increase in white 96 well plate. Compounds for kinase assays were irradiated with 519 nm before kinase was added to prevent kinase from denaturation. Temperatures were determined with an IR thermometer, TFA Dostermann, Wertheim, Germany.

Cell proliferation assays

Panc89, also known as T3M4 were obtained from Christian Röder, Institute for Experimental Cancer Research, CAU Kiel, Germany. Cells were cultivated at 37 °C, 5 % CO_2, in a humidified atmosphere. Panc89 were nourished with Dulbecco's MEM (Lifetech) concentrated with 10 % fetal calf serum (Lifetech) and 1 mM pyruvate (Lifetech). For cell proliferation assay, cells were counted with Cell Scepter (Merck Millipore) and seeded to a final number of 5,000 – 15,000 cells/ml in white 96 well plates (PerkinElmer). After 24 hours incubation at cultivation parameters compounds were added and irradiated with 525 nm LED, 0.5 to one minute, 6.5 mW/cm. For dose-response curves, compounds were dissolved in DMSO to a final concentration of 10 mM in assay and diluted in 1:3 steps. A day zero plate was incubated with resazurin to determine cell proliferation at compound treatment. After additional 48 hours incubation and medium removal cells were incubated with two mg/ml resazurin for two hours.[11] Mitochondria in eukaryote reducing resazurin (blue) to resorufin (violet) that can be detected via fluorescence measurement. Read-out was performed with Fluostar Omega (Fluorescence measurement, excitation of 544 nm, emission of 590 nm, 10 flashes per well). For IC_{50}-value determination, GraphPad Prism 7 was used. Inhibitor curves were plotted with Y=Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*HillSlope)), DMSO treatment as high control and medium only as negative control. The background was subtracted from measured values. Outliers were detected via D'Agostino-Pearson omnibus normality test as mentioned in kinase assay description. Assay was performed with three technical replicates.



Supplemental figure S20. Light titration of Panc89 with 519 nm. Low-dose green light irradiation stimulates cell growth.[12] An irradiation of 10 minutes, 519 nm impacts cell confluence insignificantly.



Supplemental figure S21. Cell proliferation of Panc89 under different light treatments. 10 minutes irradiation of 519 nm 8 LED lamp (6.5 mW/cm²) is bio-equivalent to one minute irradiation of 525 nm 16 LED lamp (90 mW/cm²).

Apoptosis assays

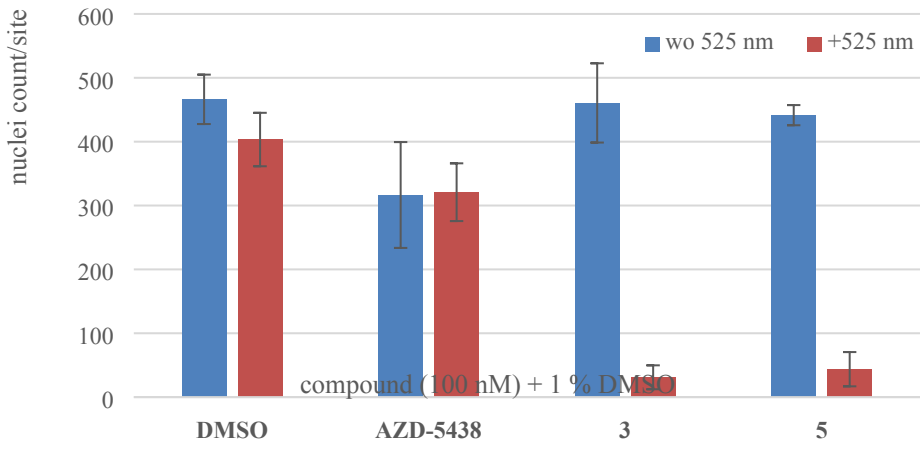
For apoptosis assay, APOlive-GLO assay (Promega) was used to manufacturer's protocol. First, 5,000 – 15,000 cells/ml of Panc89 were seeded and incubated for 24 hours. Second, dissolved compounds in DMSO were added and irradiated with 525 nm (90 mW/cm² per well, 60 sec). After incubation for 48 h, the medium was changed against detection viability reagent and incubated for 30 minutes at RT. Subsequently, caspase reagent was added and recently luminescence was recorded with a Tecan Spark (Tecan, Switzerland) using fluorescence top reading. Curves were plotted with GraphPad Prism 7. Assay was run in duplicates.

Fluorescence microscopy

Panc89 were seeded to a final number of 10,000 – 20,000 cells per ml in black 96 well microplate (half area, Greiner Bio One) and incubated for 24 hours (37 °C, 5 % CO_2, saturated humidity). After pre-incubation, compounds dissolved in DMSO were added, irradiated with 525 nm (90 mW/cm² per well, 30 s), and incubated for additional 48 hours. After medium removal and washing with DPBS, cells were fixed with 4 % PFA and stained with 5 µg/ml Hoechst 33342 (life technologies) for 15 minutes. Images were taken with ImageXpress Micro XL (Molecular Devices, Sunnyvale CA, USA) and Meta Xpress using 10x or 60x magnification. Following filters were used: DAPI (377 nm excitation, 543 nm emission) and TRITC (447 nm excitation, 593 nm emission).

Supplemental table S2. Nuclei count per well site.

	+525 nm				wo 525 nm			
	DMSO	**AZD5438**	**3**	**5**	DMSO	**AZD5438**	**3**	**5**
1	368.0	271.7	16.3	41.3		423.5	391.0	420.5
2	399.0	308.5	27.2	18.2	430.5	335.7	468.0	455.2
3	383.2	380.5	22.3	71.7	507.3	233.0	540.3	452.0
4	463.2	322.8	58.3		461.0	273.8	443.3	438.3
mean	403.3	320.9	31.0	43.7	466.3	316.5	460.7	441.5
SD	41.8	45.2	18.7	26.8	38.7	82.9	62.1	15.8
dev %	-13.5	-31.2	-93.3	-90.6	0.0	-32.1	-1.2	-5.3
SD %	10.4	11.2	4.6	6.7	8.3	17.8	13.3	3.4



Supplemental figure S22. Nuclei count in fluorescence microscopy. Panc89 were treated with different compounds (100 nM) and either without irradiation (blue bars) or with 525 nm irradiation (red bars).

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