Electronic Supporting Information

A photoswitchable CENP-E inhibitor with single blue-green light to control chromosome positioning in mitotic cells

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1. General Methods

All commercially available reagents and solvents were purchased from commercial sources (TCI chemical, Merck, Kanto Chemical, Fujifilm Wako Pure Chemical Corporation, Cytoskeleton, Inc.) and used without further purification. Thin layer chromatography was conducted on silica gel 60 F_{254}-precoated aluminium sheets (Merck) with the detection using handy UV lamp (254 nm). Purification of the synthesized compounds was carried out by Isolera One flash column chromatography (Biotage) or reverse phase high performance liquid chromatography (RP-HPLC) system (Shimadzu). All compounds were characterized with ^{1}H-nuclear magnetic resonance (NMR) and ^{13}C-NMR, and mass spectroscopy. ^{1}H NMR spectra were recorded on a JEOL JNM-ECX 400 spectrometer and all chemical shifts are cited on the δ-scale in ppm relative to the signal of solvent (DMSO-d_6) and coupling constants (*J*) are reported in Hz. Proton-decoupled ^{13}C NMR spectrum was also recorded on a JEOL JNM-ECX 400 spectrometer and all chemical shifts are reported in ppm using solvent as the internal standard (DMSO-d_6). High resolution mass spectrum (HR-MS) was recorded by electrospray ionization (ESI) method using Thermo Scientific Exactive mass spectrometer.

2. Experimental Methods

Photophysical experiments

UV-Vis absorption spectra were recorded on a Shimadzu UV-2600 spectrophotometer. Laser flash photolysis experiment for the calculation of lifetime of unstable *cis* isomer of **3** was carried out using the apparatus[1] equipped with a Q-switched Nd:YAG laser (532 nm, 7 nsec).

ATPase Assay of CENP-E

In vitro ATPase assay was carried out with ADP-Glo Max system (Promega) for the detection of ATP consumption by CENP-E according to our previous report[2]. For the experiments under the continuous light stimulus, the light source at 470 nm (50 mW/cm^2, CL-1503 with CL-H1-470-9-1, ASAHI SPECTRA) was used.

Immunofluorescence imaging experiments

We followed the methods previously described elsewhere.[2] Briefly, in the presence of **3** (6.0 μM) and MG-132 (10 μM), HeLa Kyoto cells were incubated for 1 hour with or without light illumination at 470 nm (10 mW/cm^2). To explore the phototoxicity of 405 nm (10 mW/cm^2) and 470 nm (10 mW/cm^2) lights,

in the presence of dimethyl sulfoxide (DMSO) HeLa Kyoto cells were incubated for 1 hour. After methanol fixation of cells, chromosomes were stained with 1.0 μg/mL DAPI (Wako) for overnight at 4 ºC. In addition, CENP-E and microtubules were visualized using rat monoclonal anti–α-tubulin (YOL1/34; Abcam) with Alexa Fluor 568-conjugated secondary antibodies (Abcam) and mouse monoclonal anti-CENP-E (1H12, ab5093, Abcam) with Alexa Fluor 488-conjugated secondary antibodies (Abcam), respectively. The images of cells were captured by a Ti2 microscope (Nikon) equipped with a Plan Apo VC ×60 objective lens (NA 1.40, Nikon) for high-resolution images or a Plan Apo VC ×20 objective lens (NA 0.75, Nikon) or an Axio Observer 7 microscope (Carl Zeiss) equipped with an Objective Plan-Apochromat 20x/0.8 (Carl Zeiss) for counting cells with aligned /misaligned chromosomes.

Measurement of the level of reactive oxygen species (ROS) based on phototoxicity

To explore the phototoxicity of 405 nm (10 mW/cm^2) and 470 nm (10 mW/cm^2) lights, HeLa Kyoto cells were seeded in a 96 well plate. After 24 hours, cells were incubated in the presence of 10μM photo-oxidation resistant DCFH-DA (DOJINDO) and compound **3** (6 μM) for 1 hour with or without light illumination. After washout, the fluorescence intensities (ex. 500 nm / em. 530 nm) were measured with

a plate reader (Infinite 200 Pro M Plex, Tecan).

Live cell imaging

Chromosomes in HeLa Kyoto cells (1×10^5 cells) were visualized with 1.0 μM SiR-DNA (Cytoskeleton, inc., excited with 632 nm laser in CLSM) in DMEM (supplemented with 10% FBS, w/o phenol red) and 6.0 μM **2** for 1 h. Cells were subjected to the imaging with 488 nm laser illumination. Images were captured with CLSM system (Nikon A1 and Ti2) equipped with a Plan Apo VC ×60 objective lens (NA 1.40, Nikon) and a microscope stage incubator (TOKAI HIT) with 5% CO_2 at 37 ºC.

3. Synthetic procedures

3-1. Synthesis of 5

The solution of 4-nitrosonitrobenzene[3] (350 mg, 2.3 mmol) and the aniline derivative **4**[4] (390 mg, 1.0 mmol) in acetic acid (0.5 mL) was stirred for 2 days at 60 ºC under nitrogen atmosphere. AcOEt and sat. $NaHCO_3$aq were poured into this solution. The organic phase was extracted, washed with sat. $NaHCO_3$aq and brine, dried over $MgSO_4$, and filtered. The resulting solution was dried *in vacuo*. The crude product was purified with Isolera One flash column chromatography to afford compound **5** (389 mg, 79%) as an orange solid.

^{1}H-NMR (400 MH, DMSO-d_6) δ 8.65 (d, J = 8.4 Hz, 1H), 8.37 (d, J = 8.8 Hz, 2H), 8.07-8.03 (m, 1H), 7.98 (d, J = 8.8 Hz, 2H), 7.91 (d, J = 2.4 Hz, 1H), 7.82 (d, J = 8.0 Hz, 2H), 7.74 (dd, J = 8.4, 2.0 Hz, 1H), 7.53 (d, J = 8.0 Hz, 2H), 7.17 (d, J = 8.8 Hz, 1H), 4.75-4.67 (m, 2H), 3.22-3.17 (m, 1H), 3.09-3.03 (m, 1H), 2.60 (d, J = 4.8 Hz, 3H), 1.25 (d, J = 6.0 Hz, 6H).

^{13}C-NMR (100 MH, DMSO-d_6) δ 171.90, 165.09, 155.79, 155.71, 151.02, 148.88, 144.79, 130.90, 129.84, 128.52, 127.17, 125.60, 123.90, 123.52, 122.25, 114.79, 71.75, 55.16, 37.78, 26.22, 22.18.

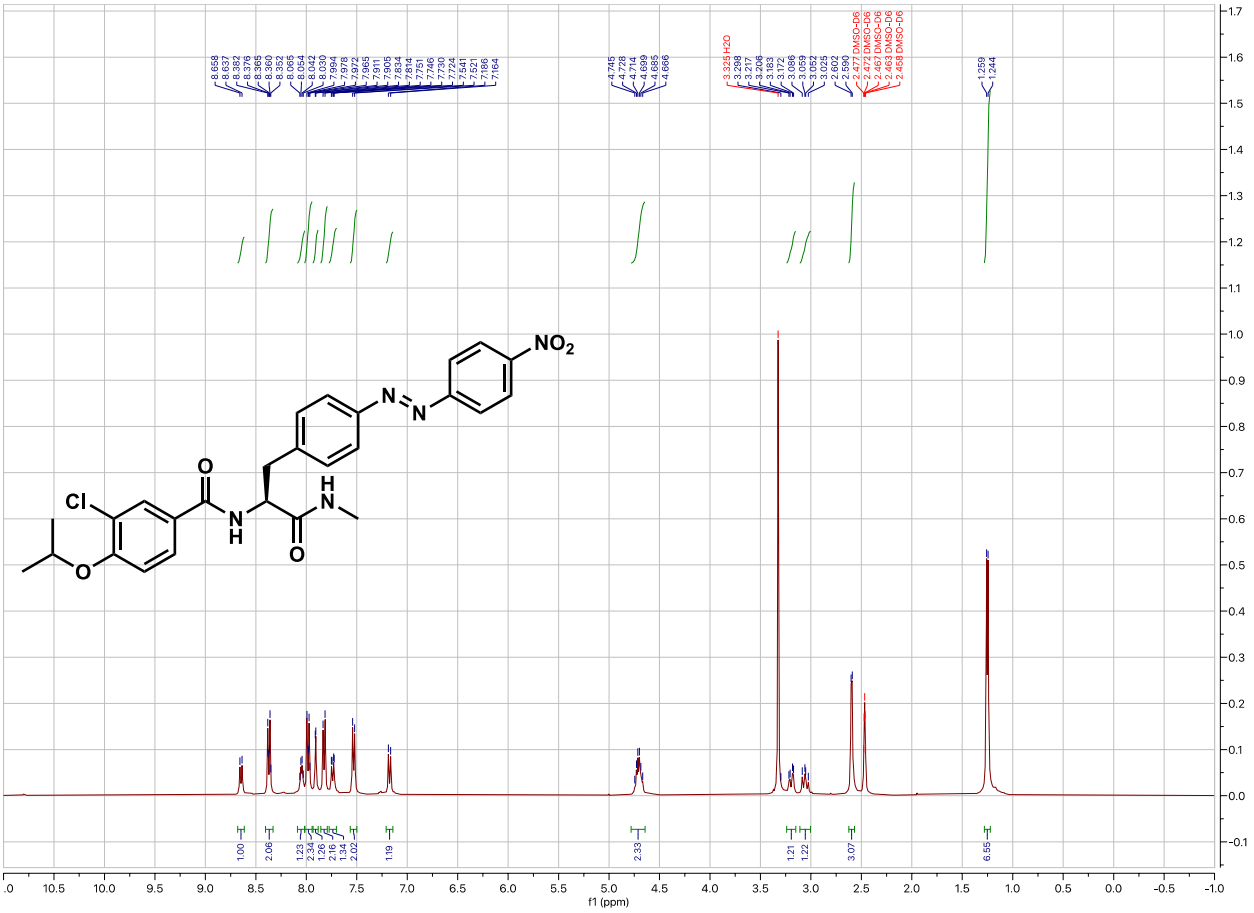

Fig. S1. ^{1}H-NMR of **5**.

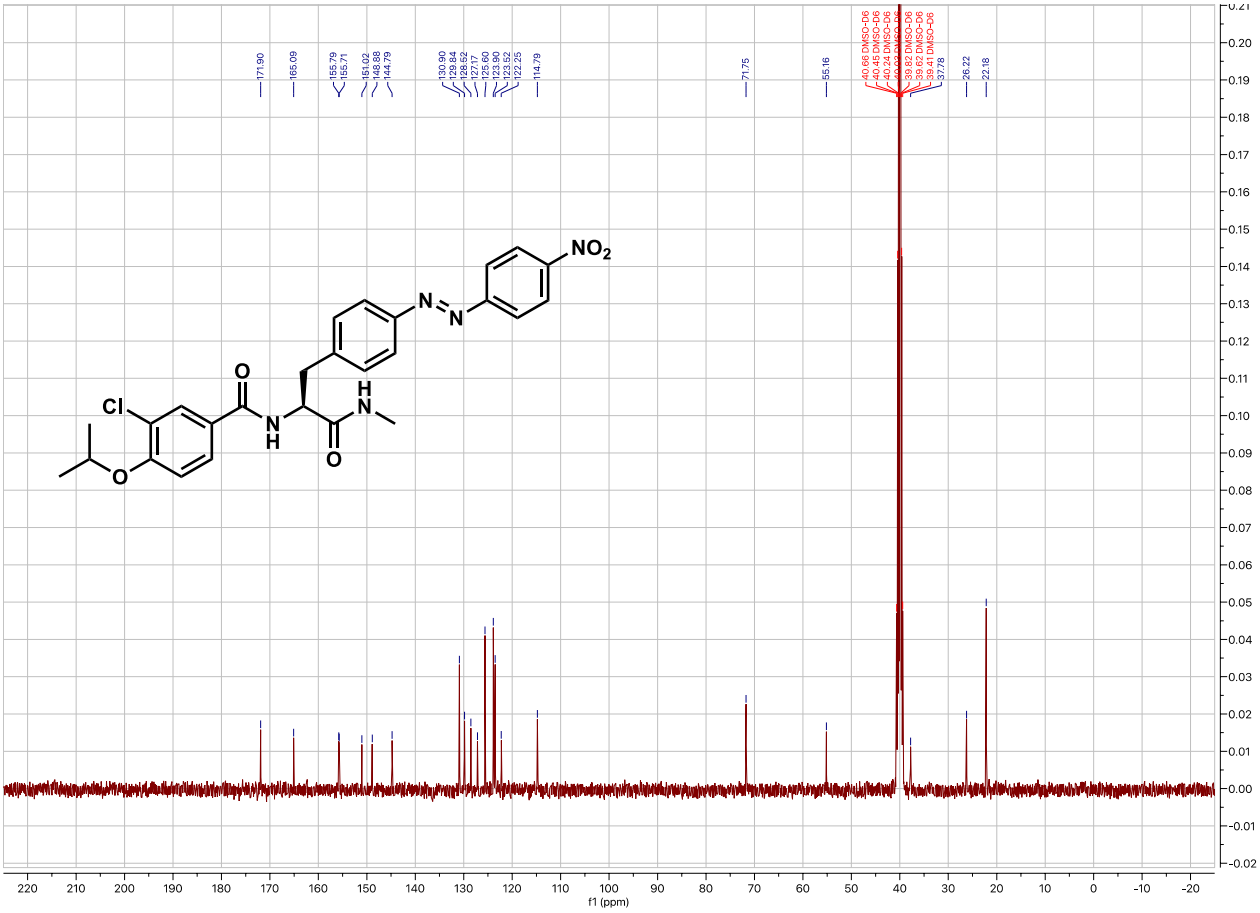

Fig. S2. ^{13}C-NMR of **5**.

3-2. Synthesis of 6

The solution of **5** (152 mg, 0.29 mmol) and sodium sulfide (120 mg, 1.5 mmol) in THF (15 mL) and H_2O (5 mL) was refluxed for 8 h. The reaction mixture was evaporated *in vacuo*. The crude product was purified with reverse phase HPLC system to afford compound **6** (86 mg, 60%) as a yellow solid.

^{1}H-NMR (400 MH, DMSO-d_6) δ 8.59 (d, J = 8.4 Hz, 1H), 8.01 (q, J = 4.8 Hz, 1H), 7.90 (d, J = 2.4 Hz, 1H), 7.72 (dd, J = 8.8, 2.0 Hz, 1H), 7.60-7.56 (m, 4H), 7.39 (d, J = 8.4 Hz, 2H), 7.18 (d, J = 8.8 Hz, 1H), 6.62 (d, J = 8.4 Hz, 2H), 4.73 (septet, J = 6.0 Hz, 1H), 4.66-4.60 (m, 1H), 3.14-3.09 (m, 1H), 3.01-2.95 (m, 1H), 2.57 (d, J = 4.8 Hz, 3H), 1.26 (d, J = 6.0 Hz, 6H).

^{13}C-NMR (100 MH, DMSO-d_6) δ 172.06, 165.09, 155.76, 153.01, 151.48, 143.42, 140.79, 130.42, 129.84, 128.51, 127.26, 125.53, 122.23, 122.02, 114.81, 114.01, 71.76, 55.38, 37.60, 26.20, 22.21.

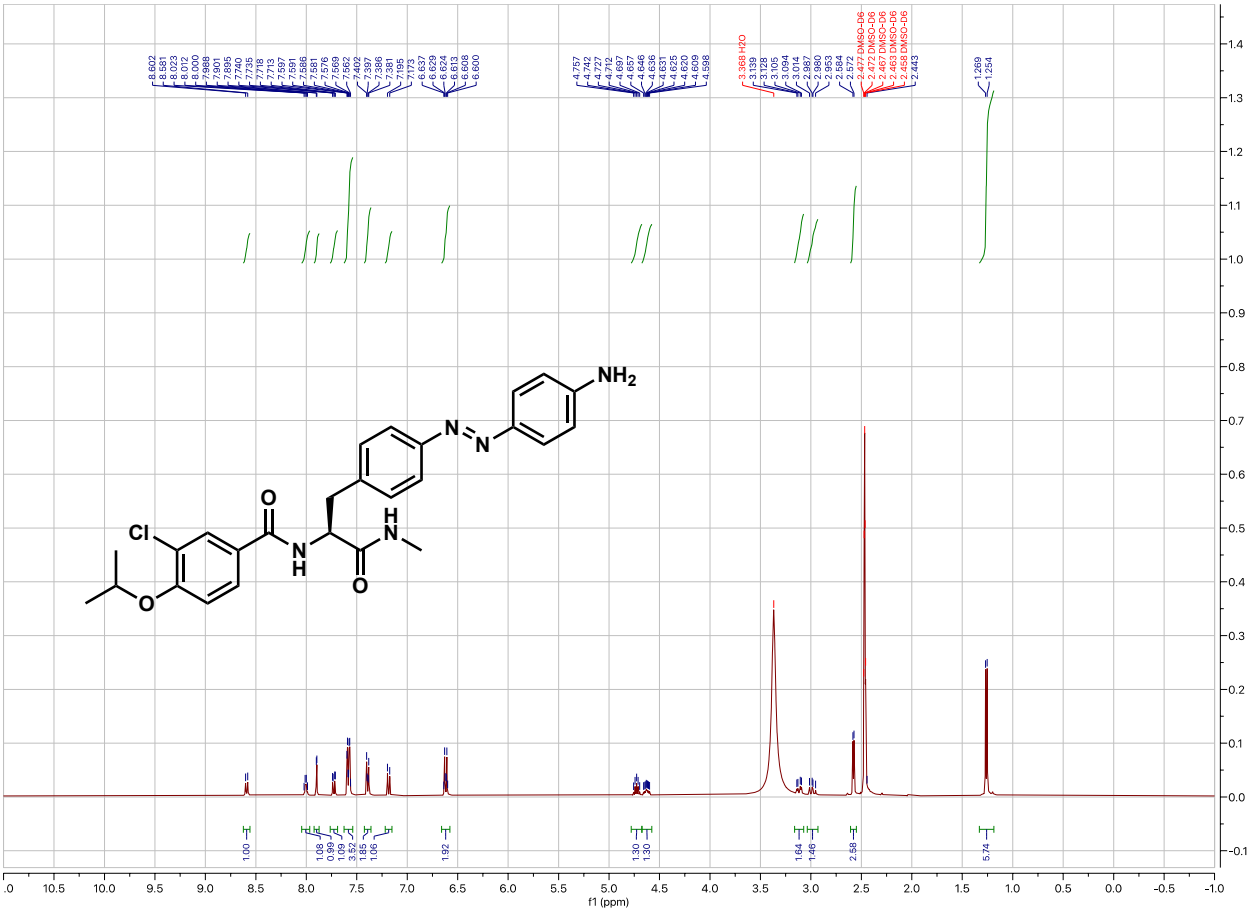

Fig. S3. ^{1}H-NMR of **6**.

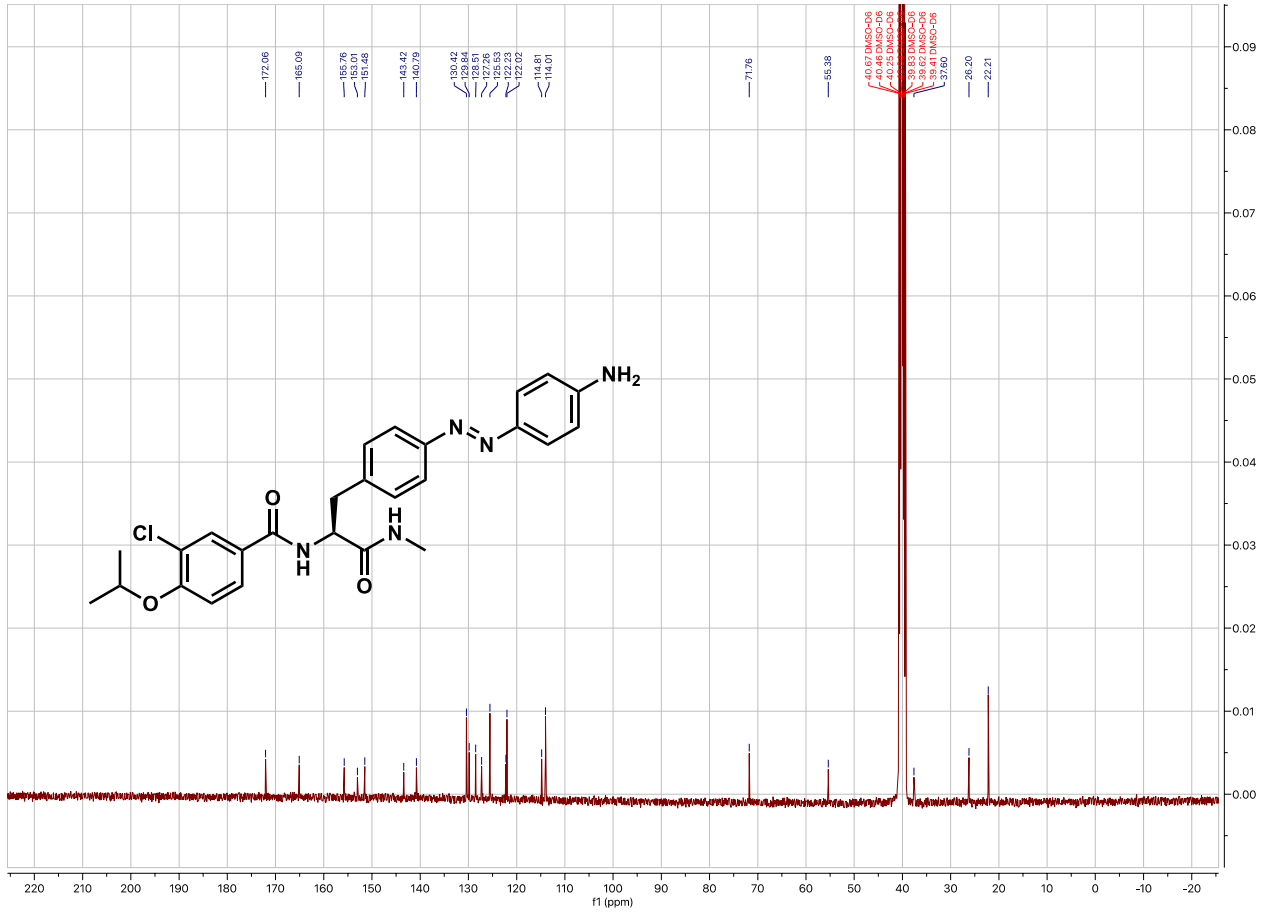

Fig. S4. ^{13}C-NMR of **6**.

3-3. Synthesis of 3

Sodium methoxide (300 mg, 5.6 mmol) was added to the solution of **6** (40 mg, 81 μmol) in MeOH (10 mL). The reaction mixture was stirred for 10 min. Paraformaldehyde (150 mg) was added to the mixture. After stirring for 18 hours at 60 ºC, sodium borohydride (5.0 mg, 132 μmol) was added. It was stirred for 5 hours at 60 ºC. After cooling, AcOEt and sat. $NaHCO_3$aq was added to this solution. The organic phase was extracted, washed with sat. $NaHCO_3$aq and brine, dried over $MgSO_4$, and filtered. The resulting solution was dried *in vacuo*. The crude product was purified with reverse phase HPLC system to afford compound **3** (31 mg, 75%) as a yellow solid.

^{1}H-NMR (400 MH, DMSO-d_6) δ 8.59 (d, J = 8.4 Hz, 1H), 8.01 (q, J = 4.8 Hz, 1H), 7.90 (d, J = 2.4 Hz, 1H), 7.72 (dd, J = 8.8, 2.4 Hz, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.59 (d, J = 8.0 Hz, 2H), 7.39 (d, J = 8.4 Hz, 2H), 7.18 (d, J = 8.8 Hz, 1H), 6.61 (d, J = 8.8 Hz, 2H), 4.73 (septet, J = 6.0 Hz, 1H), 4.66-4.60 (m, 1H), 3.14-3.10 (m, 1H), 3.02-2.95 (m, 1H), 2.74 (s, 3H), 2.57 (d, J = 4.4 Hz, 3H), 1.26 (d, J = 6.0 Hz, 6H).

^{13}C-NMR (100 MH, DMSO-d_6) δ 172.06, 165.09, 155.76, 153.39, 151.48, 143.28, 140.75, 130.42, 129.84, 128.51, 127.27, 125.54, 122.23, 122.00, 114.81, 111.86, 71.75, 55.38, 37.62, 29.91, 26.20, 22.21.

HR-MS (ESI) for $[M + H]^+$ calcd. 508.2110, found 508.2111.

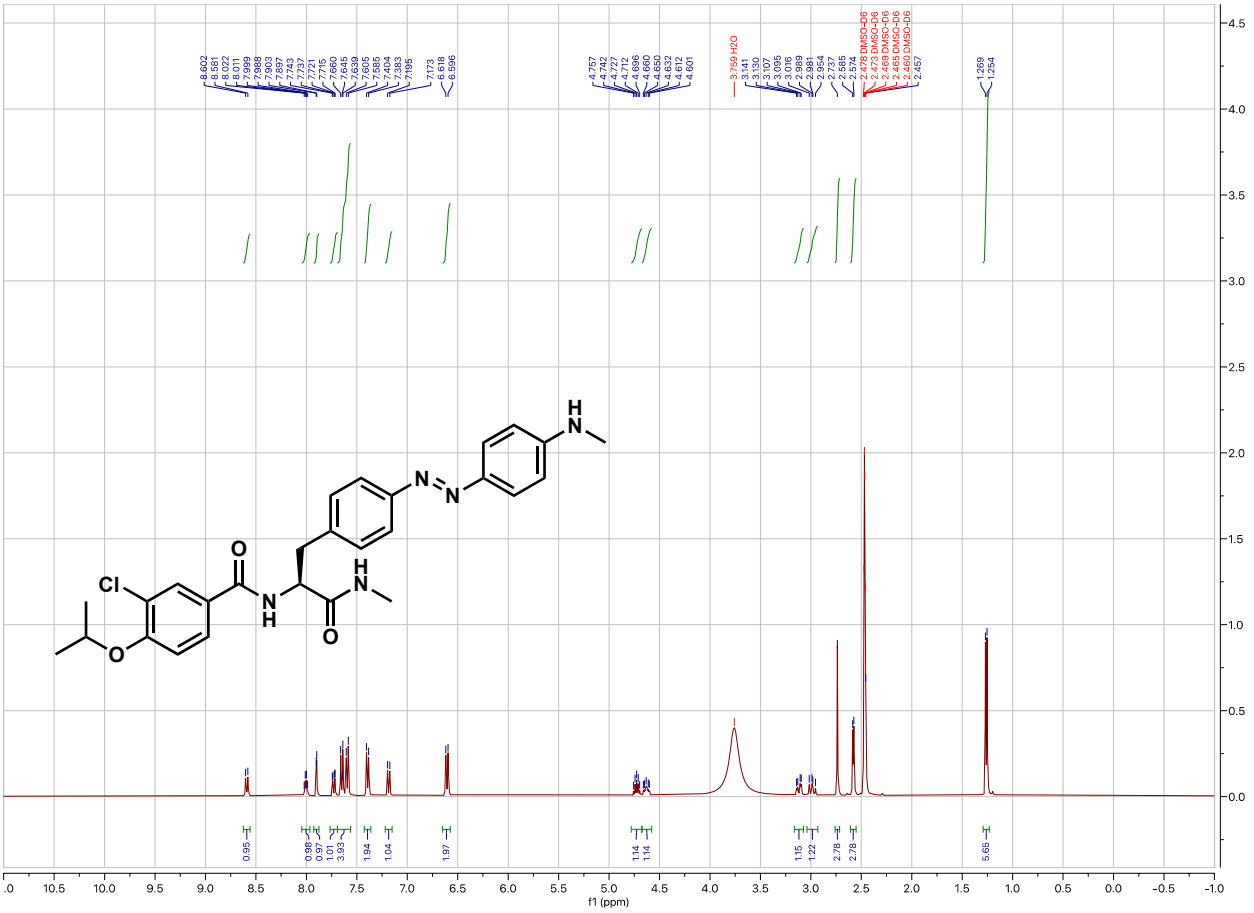

Fig. S5. ^{1}H-NMR of **3**.

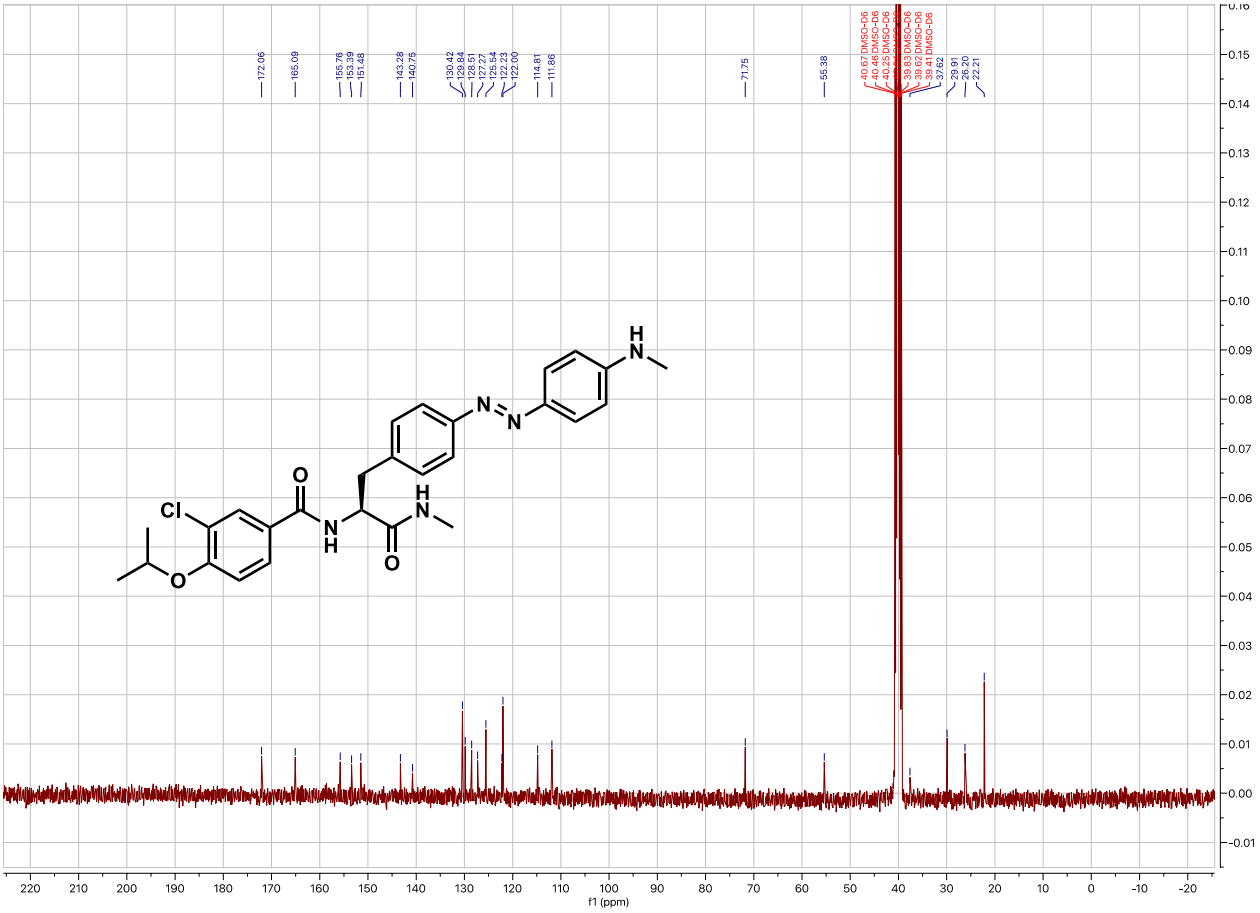

Fig. S6. ^{13}C-NMR of **3**.

4. Self-assembling properties of 3 in aqueous buffer and cell culture medium

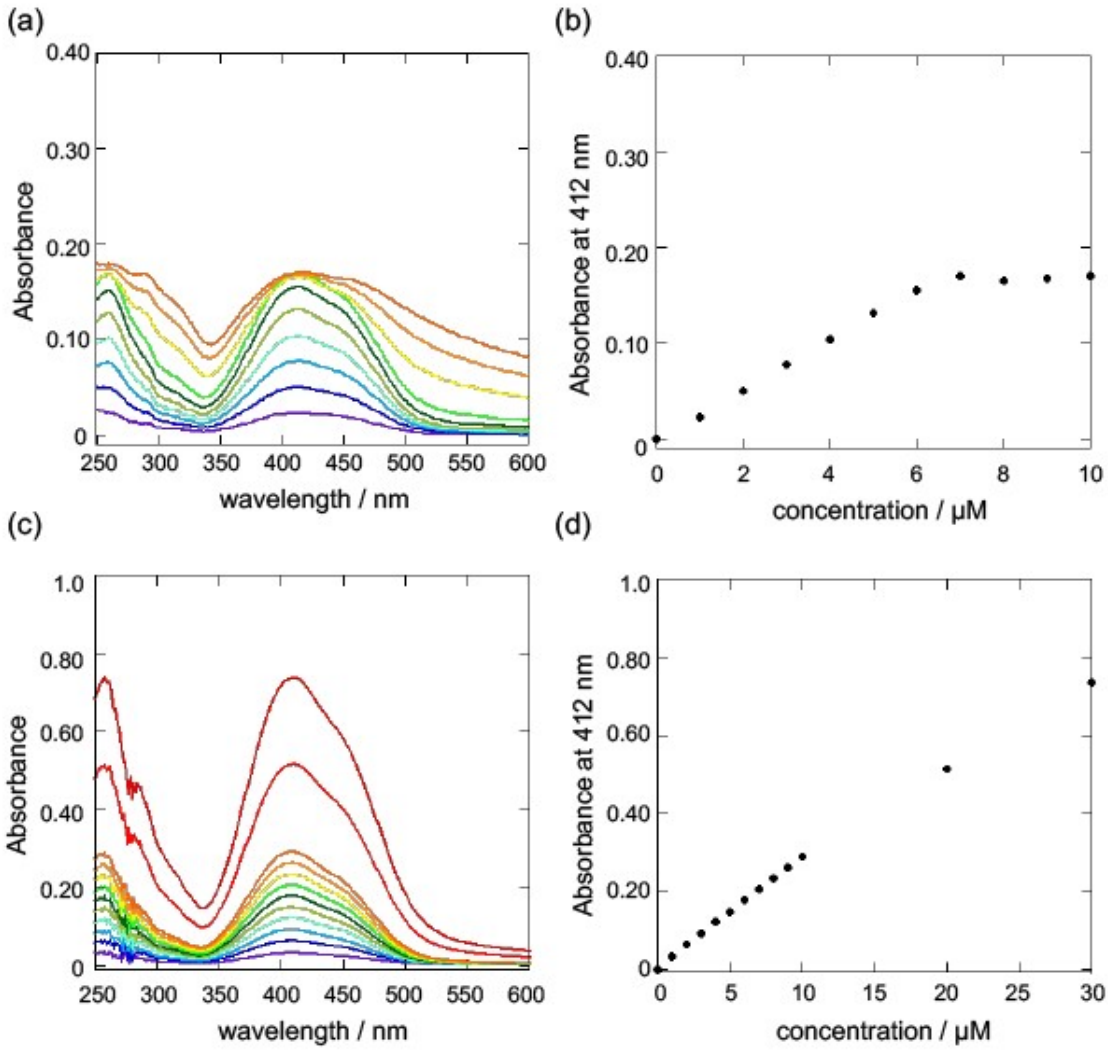

Fig. S7. (a) Absorption spectra of **3** in BRB80 buffer depending on the concentration (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 μM). (b) Plots of the absorbance of **3** at 412 nm in BRB80 buffer. (c) Absorption spectra of **3** in DMEM supplemented with 10% FBS depending on the concentration (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 μM). (d) Plots of the absorbance of **3** at 412 nm in DMEM with 10% FBS.

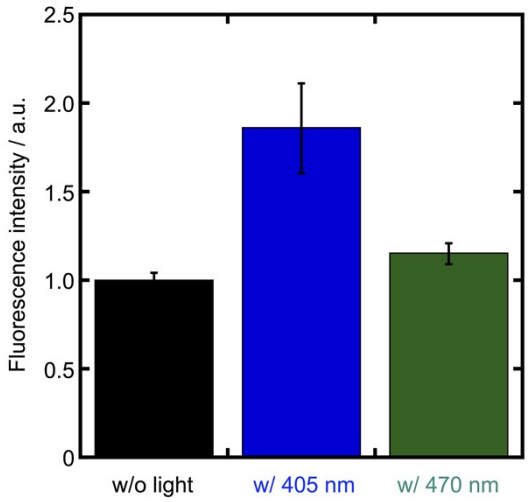

Fig. S8. Relative fluorescence intensity of photo-oxidation resistant DCFH-DA in cells with compound **3** (6.0 μM) with or without light illumination at 405 nm (10 mW/cm^2) or 470 nm (10 mW/cm^2) for 1 hour. w/o light : without light illumination. w/ 405 nm : with light illumination at 405 nm. w/ 470 nm : with light illumination at 470 nm.

References

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