Supplementary Information (SI) for Journal of Materials Chemistry B.

A dual-functional photosensitizer for mitochondria-targeting photodynamic therapy and synchronous polarity monitoring

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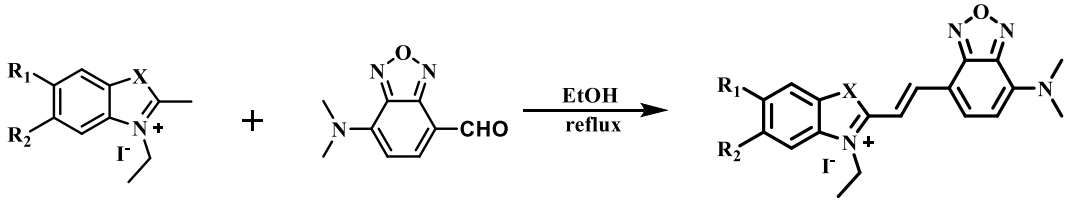
1. Experiment

1.1 General Information

All the reagents including 2,3,3-trimethyl-3H-indole, 2-methylbenzo[d]thiazole, 5-bromo-2,3,3-trimethyl-3H-indole and (4-iodophenyl)hydrazine were purchased from Bidepharm. All the solvents were of ACS grade and used without further purification. Cell culture related items, including fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), trypsin-EDTA, PBS, and penicillin/streptomycin, were purchased from Invitrogen. DCFH-DA, calcein AM and propidium iodide (PI) were purchased from Abcam. CCK-8 and JC-1 were obtained from Beyotime. 96-well of ibidi® culture plates were purchased from ibidi GmbH. Mass spectra were measured with a PC Sciex API 150 EX ESI-MS system. NMR spectra were acquired by a Bruker 400 MHz NMR spectrometer. A FiveEasy TM Fe20 pH meter was employed to measure pH values. Absorption and emission spectra were recorded on Molecular Devices SpectraMax ID5 Microplate Reader and Fluormax-4, respectively. Fluorescence imaging was conducted with a Leica TCS SP5 confocal scanning microscope.

1.2 Synthesis

Compounds **1-4** and **NBD-CHO** were synthesized according to the literatures reported[1]. The final products **BDC/S/Br/I** was obtained from Knoevenagel condensation reaction between compounds **1-5** and **BD-CHO** as illustrated in **Scheme S1**.



1 X=CMe_2, R_1=H, R_2=H
2 X=S, R_1=H, R_2=H
3 X=CMe_2, R_1=Br, R_2=H
4 X=CMe_2, R_1=I, R_2=H

BDC: X=CMe_2, R_1=H, R_2=H
BDS: X=S, R_1=H, R_2=H
BDBr: X=CMe_2, R_1=Br, R_2=H
BDI: X=CMe_2, R_1=I, R_2=H

Scheme S1. Synthetic route of compounds **BDC/S/Br/I**

General Protocol: Compounds **1-4** (1.0 equiv) and **NBD-CHO** (1.0 equiv) were dissolved in EtOH, then the mixture was refluxed for 12 h under N_2. Solvent was evaporated under vacuum and the

residue was purified with silica chromatography (DCM/MeOH=15/1) to obtain **BDC/S/Br/I**.

BDC: dark blue solid, 76.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62-8.51 (m, 1H), 8.36 (dd, J = 8.9, 1.7 Hz, 1H), 7.82 (d, J = 7.4 Hz, 1H), 7.79 (d, J = 7.9 Hz, 1H), 7.61-7.52 (m, 2H), 7.49 (t, J = 7.5 Hz, 1H), 6.67 (dd, J = 8.8, 1.7 Hz, 1H), 4.43 (q, J = 7.3 Hz, 2H), 3.68 (s, 6H), 1.81 (s, 6H), 1.49 (t, J = 7.7 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 179.1, 149.8, 149.1, 147.4, 146.5, 145.7, 143.2, 141.3, 129.4, 128.0, 123.4, 113.8, 108.5, 107.1, 106.7, 51.2, 44.2, 41.3, 26.8, 12.9.

BDS: dark blue solid, 65.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (s, 1H), 8.25 (d, J = 5.0 Hz, 1H), 8.15 (d, J = 8.4 Hz, 1H), 8.05 (d, J = 8.6 Hz, 1H), 7.77 (t, J = 7.8 Hz, 1H), 7.70-7.60 (m, 2H), 6.45 (d, J = 8.6 Hz, 1H), 4.70 (q, J = 7.2 Hz, 2H), 3.55 (s, 6H), 1.50 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.5, 148.9, 145.5, 145.4, 144.8, 144.7, 141.2, 129.6, 128.0, 127.6, 124.5, 116.1, 108.0, 107.3, 105.7, 44.2, 43.6, 13.8.

BDBr: dark blue solid, 72.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (d, J = 15.2 Hz, 1H), 8.37 (d, J = 8.9 Hz, 1H), 8.14 (d, J = 1.6 Hz, 1H), 7.79-7.70 (m, 2H), 7.50 (d, J = 15.2 Hz, 1H), 6.70 (d, J = 8.9 Hz, 1H), 4.38 (q, J = 7.2 Hz, 2H), 3.69 (s, 6H), 1.81 (s, 6H), 1.45 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 178.6, 150.2, 149.1, 147.9, 147.0, 145.7, 145.3, 140.7, 132.1, 126.7, 120.5, 115.5, 108.6, 107.1, 106.6, 51.3, 44.0, 41.3, 26.7, 12.7.

BDI: dark blue solid, 60.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (d, J = 15.2 Hz, 1H), 8.35 (d, J = 8.9 Hz, 1H), 8.24 (s, 1H), 7.89 (d, J = 8.3 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.48 (d, J = 15.2 Hz, 1H), 6.68 (d, J = 8.9 Hz, 1H), 4.36 (q, J = 6.7 Hz, 2H), 3.68 (s, 6H), 1.79 (s, 6H), 1.44 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 177.8, 149.6, 148.6, 147.3, 146.5, 145.2, 144.8, 140.7, 137.4, 131.7, 115.2, 108.1, 106.6, 106.1, 92.9, 50.6, 43.6, 40.8, 26.2, 12.3.

1.3 General Procedure for Spectra Measurement

Stock solution was prepared by dissolving **BDC/S/Br/I** in DMSO with concentration of 5 mM. Then, the probe was diluted to 5 μM of the final concentration for spectral measurement. 530 nm was set as excitation wavelength for these **BD** derivatives.

1.4 Fluorescence Imaging

Colocalization imaging: Cells were seeded in a confocal dish (35 mm) for 24 h with density of about 10^4 cells, and then co-incubated with **BDI** (1 μM) and **Mito-Tracker™ Green** (100 nM) for

20 min. The cells were washed with PBS for three times and confocal imaging was conducted. 488 nm and 543 nm were set as excitation wavelength while 500-540 nm and 620-670 nm were collected for **Mito-Tracker™ Green** and **BDI**, respectively.

Mitochondrial polarity visualization in live cells: Cells were incubated with **BDI** (5 μM) for 15 min and then washed with PBS for three times and confocal imaging was conducted. 543 nm was set as excitation wavelength while 550-600 nm and 620-670 nm were collected for green channel and red channel, respectively.

Drug-induced mitophagy[2]: Cells were incubated with **MDC** (1 μM) for 30 min and then treated with rapamycin (100 nM). Afterwards, time-lapse confocal imaging was conducted with 405 nm as excitation wavelength and 450-550 nm was collected.

Drug-induced mitochondrial polarity variation: Cells were incubated with **BDI** (5 μM) for 15 min and then treated with rapamycin (100 nM). Afterwards, time-lapse confocal imaging was conducted with 543 nm as excitation wavelength while 550-600 nm and 620-670 nm were collected for green channel and red channel, respectively.

1.5 Procedure for PDT experiment

Determination of $^1O_2$ production efficiency in DMSO[3] and H_2O[4]: With DPBF as $^1O_2$ indicator, the concentration of DPBF in DMSO was adjusted to make the absorbance at 415 nm close to 1.0. Then, 5 μM of **BDC/S/Br/I** was added, respectively. After that, the mixture was exposed to light irradiation and the absorption spectrum was recorded. With SOSG as $^1O_2$ indicator, 10 μM of SOSG and 5 μM of **BDC/S/Br/I** were mixed in deionized water. Then, the mixture solutions were exposed to light irradiation and the fluorescence spectrum was recorded with excitation at 450 nm. 590 nm and 50 mW/cm^2 LED light was selected at light source.

Phototoxicity of **BD** derivatives towards cancer cells: Cells (5×10^3 /well) were incubated on a 96-well plate for 24 h. Then, the cells were treated with different concentrations of **BDC/S/Br/I** (0, 0.625, 1.25, 2.5, 5 μM) for 15 min. After that, the old medium was replaced with fresh medium. For light irradiation, cells were irradiated with LED light (590 nm, 50 mW/cm^2) for 5 min. After an additional 12 h incubation, the cell viability was measured using CCK8 assay.

Live/dead cell co-staining[5]: HeLa cells were incubated with 5 μM of **BDI** for 15 min and irradiated with light for 5 min. HeLa cells were further cultured for 12 h and incubated with 100 nM

of Calcein AM and 10 μM of PI under dark for another 30 min. After washing with PBS, confocal imaging was conducted with Leica TCS SP5. 488 nm and 543 nm were set as excitation wavelength for Calcein AM and PI, respectively. Corresponding emission at 500-540 nm for Calcein AM, 580-650 nm for PI were collected.

Determination of cellular ROS generation in cells: Cells were seeded in a confocal dish (35 mm) for 24 h with density of about 5×10^4 cells. Then, the cells were treated with 5 μM of **BDI** for 15 min. Afterwards, 10 μM DCFH-DA was added and incubated for another 30 min. The cells were then washed with PBS and irradiated with LED light for 5 min. Confocal imaging was conducted with Leica TCS SP5 at 488 nm excitation and the fluorescence channel at 500-550 nm was collected.

Mitochondrial membrane potential detection[6]: HeLa cells were incubated with 5 μM of **BDI** for 15 min, followed by irradiation at 600 nm for 5min. Then, cells were stained with JC-1 (5μg/mL) in the dark for 20 min. Afterwards, cells were washed with PBS and confocal imaging was conducted with Leica TCS SP5. 488/543 nm was set as excitation wavelength and corresponding emission at 500-540/550-600 nm was collected for JC-1 monomers and aggregates, respectively.

1.6 Mitochondrial polarity visualization during PDT process

HeLa/HepG2 cells were incubated with 5 μM of **BDI** for 15 min and then irradiated for 30 s. Then, time-lapse CLSM images were obtained at different time intervals. 543 nm was set as excitation wavelength while 550-600 nm and 620-670 nm were collected for the green channel and red channel, respectively.

2. Supplementary Figures

Table S1. Photophysical properties of **BDC** in various solvents

Comp	Solvents	$\lambda_{abs,\ max}$ (nm)	ε (M^{-1} cm^{-1})	λ_{em} (nm)	Φ_F	Stokes shift (nm)
BDC	1,4-dioxane	605	58300	626	0.564	21
	DCM	608	75780	625	0.156	17
	Acetone	602	63234	625	0.174	23
	EtOH	601	78464	622	0.173	21
	MeOH	600	68082	621	0.152	21
	ACN	600	74874	624	0.142	24
	DMF	607	72790	631	0.203	24
	DMSO	609	80098	635	0.209	26
	H_2O	599	69072	628	0.018	29

Table S2. Photophysical properties of **BDS** in various solvents

Comp	Solvents	$\lambda_{abs,\ max}$ (nm)	ε (M^{-1} cm^{-1})	λ_{em} (nm)	Φ_F	Stokes shift (nm)
BDS	1,4-dioxane	570	48908	627	0.482	59
	DCM	567	61382	619	0.457	52
	Acetone	565	51736	622	0.558	57
	EtOH	562	56046	618	0.491	56
	MeOH	561	59346	618	0.338	57
	ACN	561	57766	622	0.409	61
	DMF	570	49802	629	0.449	59
	DMSO	573	60798	630	0.340	57
	H_2O	563	56724	631	0.010	68

Table S3. Photophysical properties of **BDBr** in various solvents

Comp	Solvents	$\lambda_{abs,\ max}$ (nm)	ε (M^{-1} cm^{-1})	λ_{em} (nm)	Φ_F	Stokes shift (nm)
BDBr	1,4-dioxane	611	63182	628	0.505	17
	DCM	614	107156	630	0.195	16
	Acetone	607	86778	628	0.242	21
	EtOH	608	94502	626	0.256	18
	MeOH	605	87406	624	0.185	19
	ACN	606	94288	627	0.199	21
	DMF	611	81868	633	0.312	22
	DMSO	614	87714	637	0.379	23
	H_2O	603	81828	630	0.029	27

Table S4. Photophysical properties of **BDI** in various solvents

Comp	Solvents	$\lambda_{abs,\ max}$ (nm)	ε (M^{-1} cm^{-1})	λ_{em} (nm)	Φ_F	Stokes shift (nm)
BDI	1,4-dioxane	614	65830	630	0.541	16
	DCM	615	84084	633	0.251	18
	Acetone	608	76802	629	0.229	21
	EtOH	609	95472	628	0.248	19
	MeOH	610	83536	627	0.188	17
	ACN	607	98428	629	0.186	22
	DMF	614	89860	636	0.270	22
	DMSO	616	90232	641	0.305	25
	H_2O	607	65506	631	0.033	24

Note: For fluorescence quantum yield test of **BD** compounds, Nile Blue was chosen as reference with Φ_F=0.30 in DMSO[7].

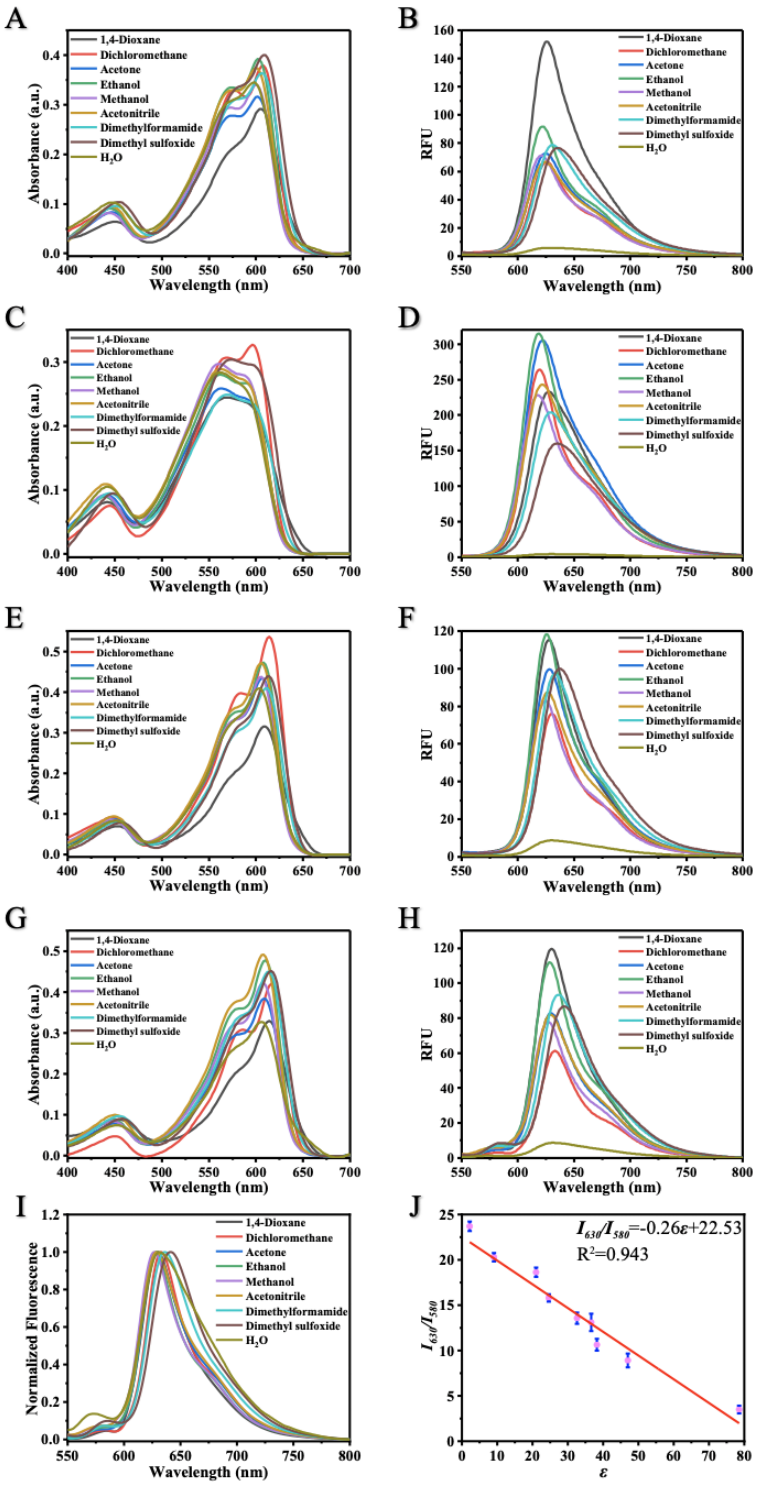


Figure S1. Absorption and emission spectra of **BD** derivatives in different solvents. (A) Absorption and (B) emission of **BDC**. (C) Absorption and (D) emission of **BDS**. (E) Absorption and (F) emission of **BDBr**. (G) Absorption and (H) emission of **BDI**. (I) Normalized emission and (J) I_{630}/I_{580} ratio of **BDI** in various solvents. ε denotes dielectric constant that characterizes polarity of the solvent. Ex = 530 nm.

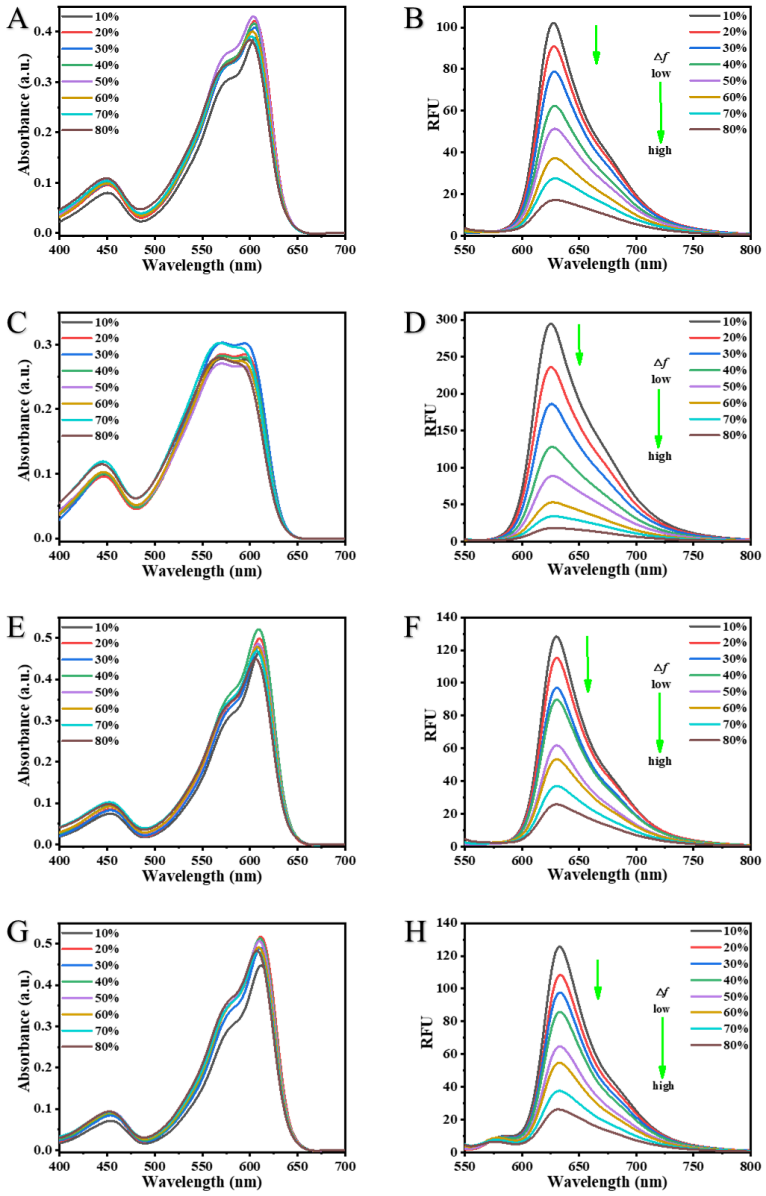


Figure S2. Absorption and emission spectra of **BD** derivatives in a H_2O/1,4-dioxane mixture with water fraction from 10% to 80%. (A) Absorption and (B) emission of **BDC**. (C) Absorption and (D) emission of **BDS**. (E) Absorption and (F) emission of **BDBr**. (G) Absorption and (H) emission of **BDI**. Δf denotes the parameter characterizes solvent polarity. Ex = 530 nm.

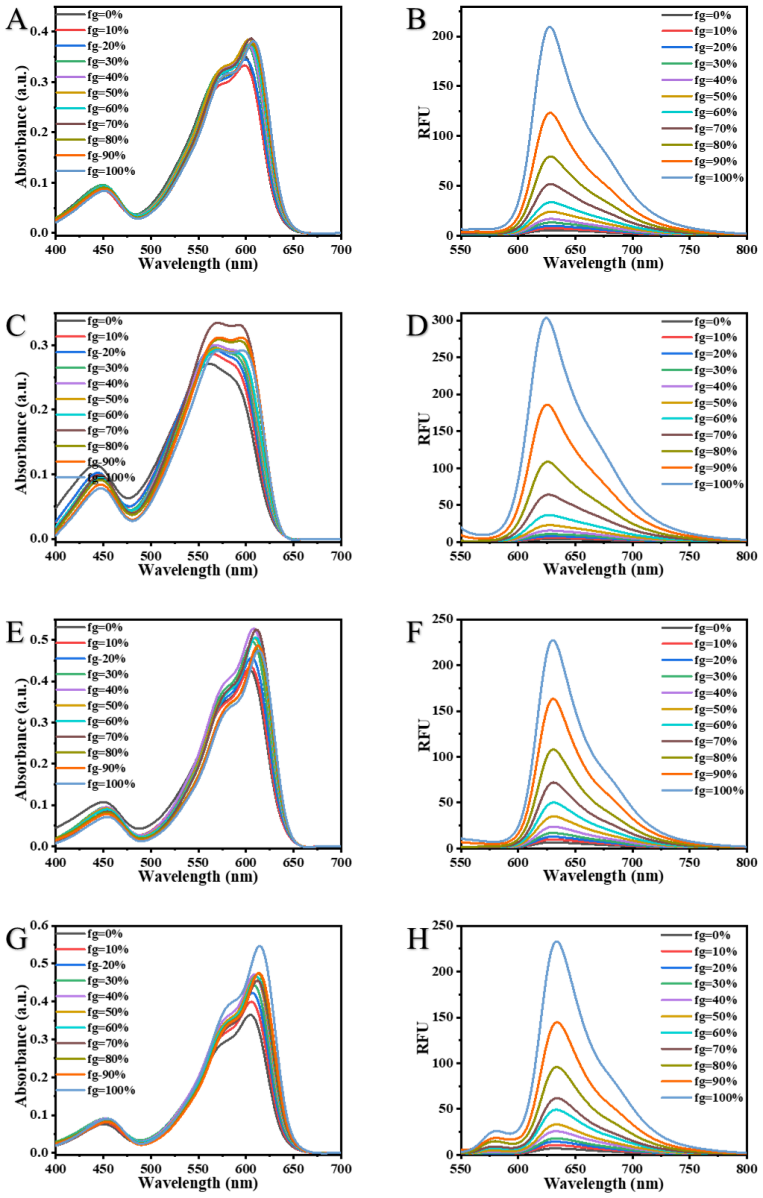


Figure S3. Absorption and emission spectra of **BD** derivatives in a glycerol/H_2O mixture with glycerol fraction from 0% to 100%. (A) Absorption and (B) emission of **BDC**. (C) Absorption and (D) emission of **BDS**. (E) Absorption and (F) emission of **BDBr**. (G) Absorption and (H) emission of **BDI**. Ex = 530 nm.

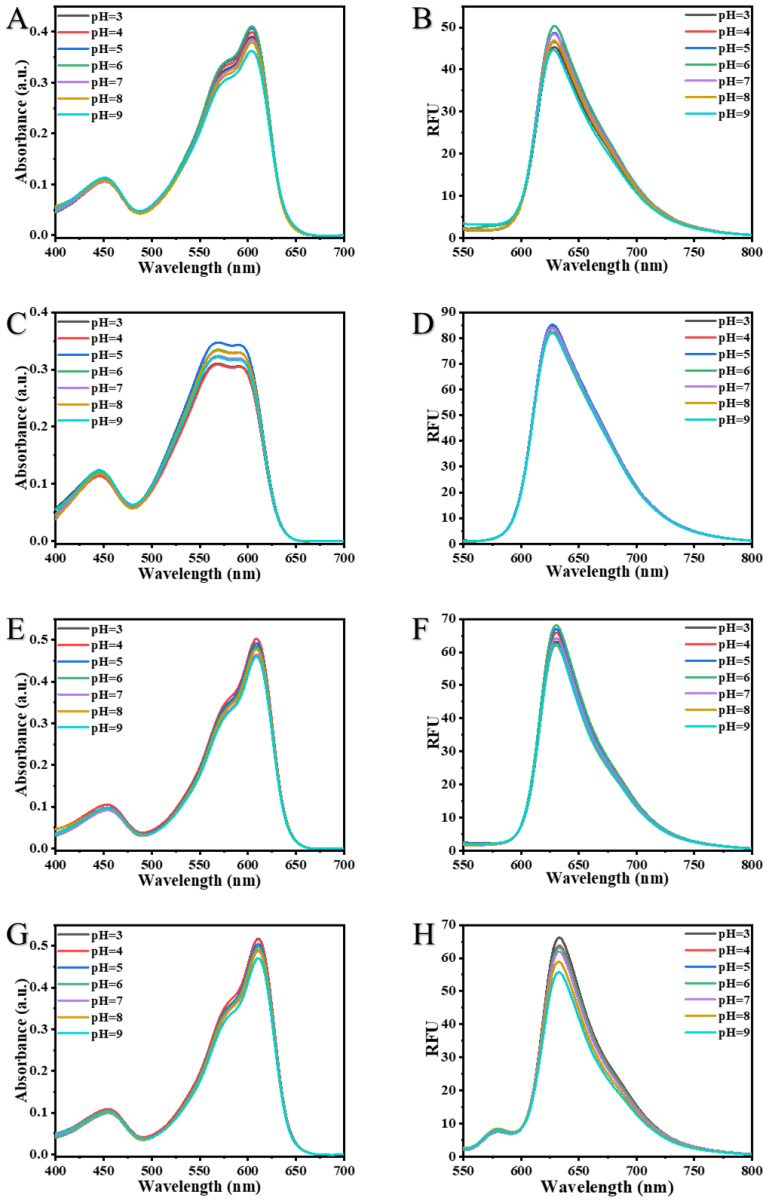


Figure S4. Absorption and emission spectra of **BD** derivatives in various *p*H solutions. (A) Absorption and (B) emission of **BDC**. (C) Absorption and (D) emission of **BDS**. (E) Absorption and (F) emission of **BDBr**. (G) Absorption and (H) emission of **BDI**. The experiment was conducted in a 50/50 dioxane/B-R buffer mixture. Ex = 530 nm.

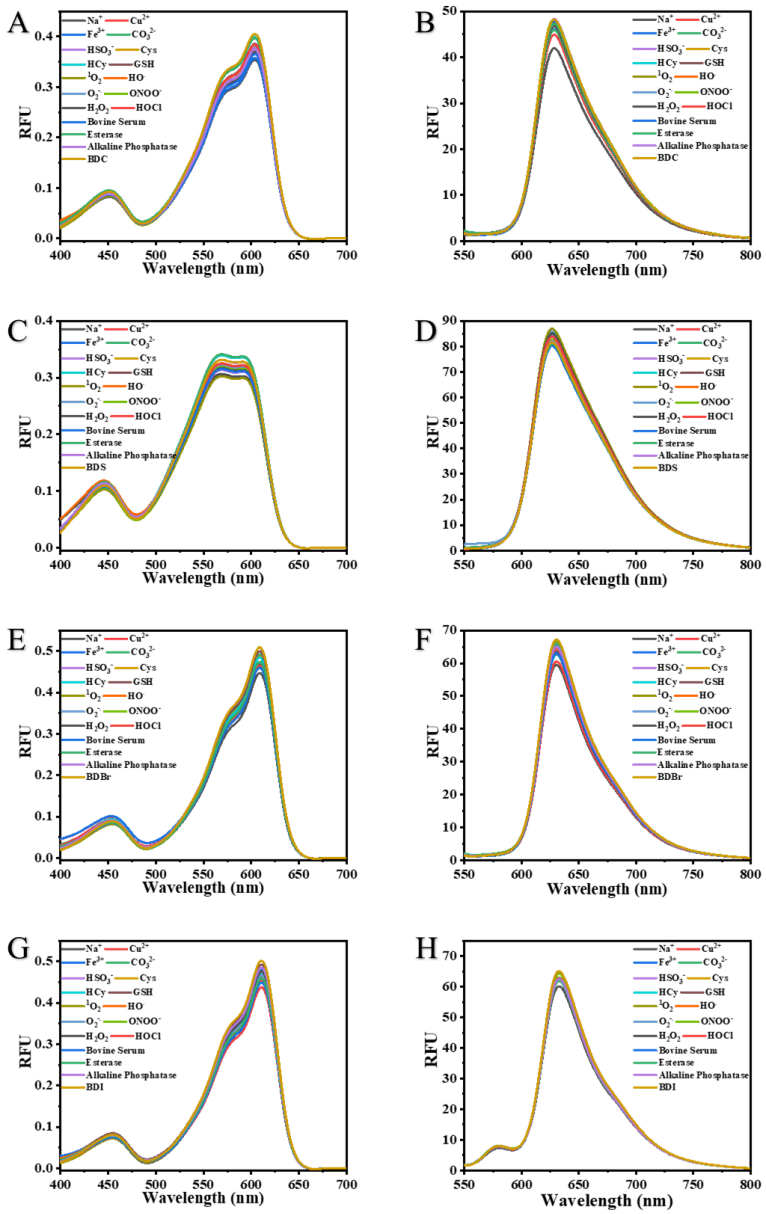


Figure S5. Absorption and emission spectra of **BD** derivatives in the presence of various bio-analytes. (A) Absorption and (B) emission of **BDC**. (C) Absorption and (D) emission of **BDS**. (E) Absorption and (F) emission of **BDBr**. (G) Absorption and (H) emission of **BDI**. The experiment was conducted in a 50/50 dioxane/water mixture. Ex = 530 nm.

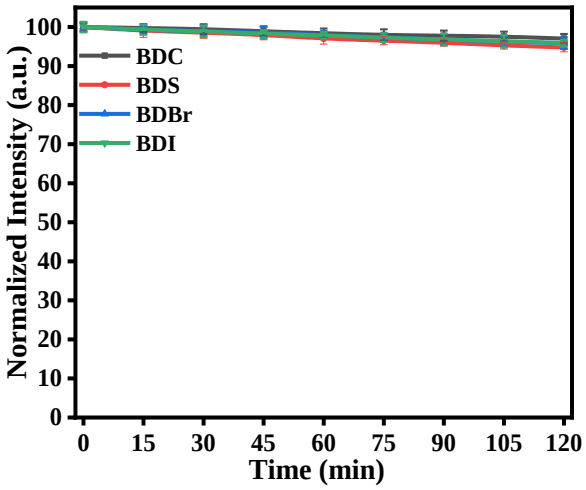


Figure S6. Photodegradation of **BD** derivatives under continuous light irradiation (600 nm, 50 mW/cm^2).

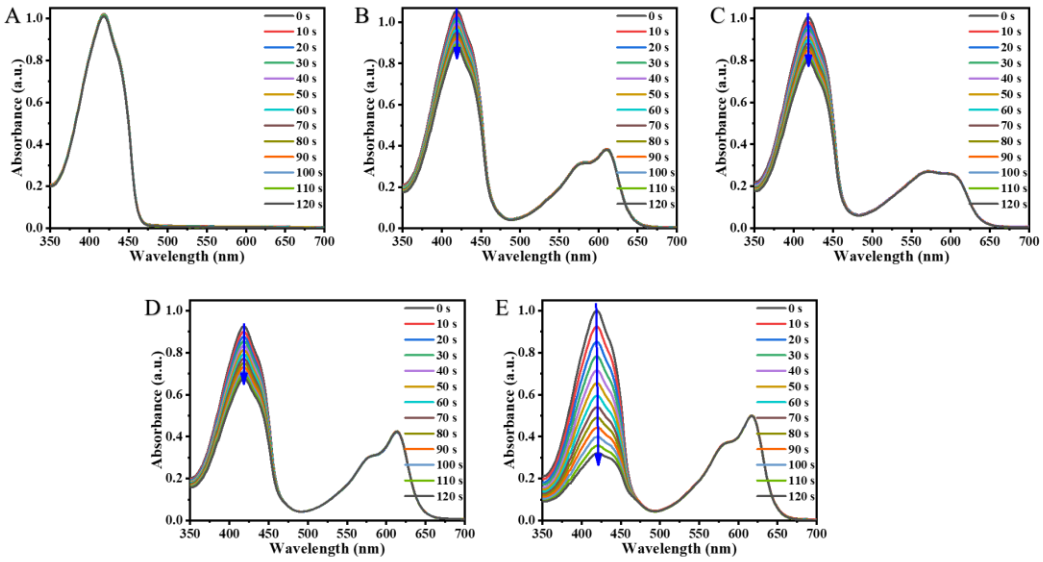


Figure S7. Photodegradation curve of DPBF in the presence of 5 μM of **BD** derivatives in DMSO under light irradiation (600 nm, 50 mW/cm^2). (A) DPBF alone. (B) **BDC**. (C) **BDS**. (D) **BDBr**. (E) **BDI**.

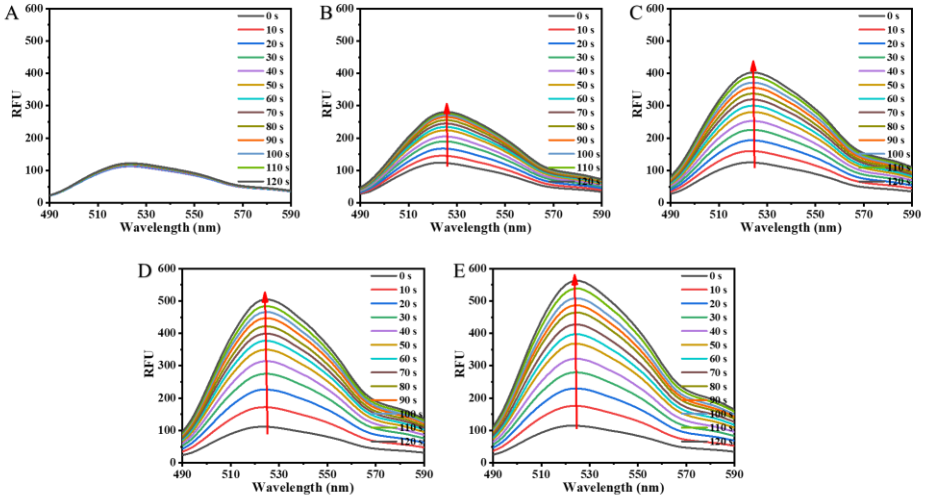


Figure S8. Fluorescence variation of SOSG in the presence of 5 μM of **BD** derivatives in water under light irradiation (600 nm, 50 mW/cm^2). (A) SOSG alone. (B) **BDC**. (C) **BDS**. (D) **BDBr**. (E) **BDI**. Ex = 450 nm.

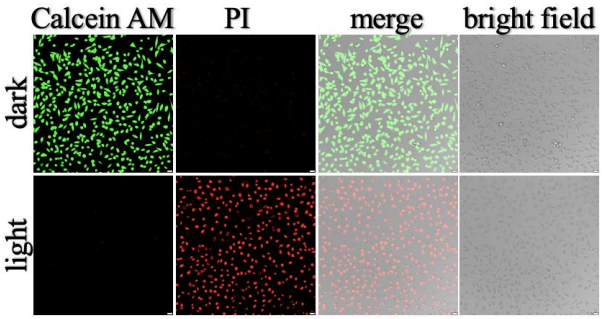


Figure S9. Live/dead cell staining images of HeLa cells pretreated with **BDI**. Scale bar = 20 μm.

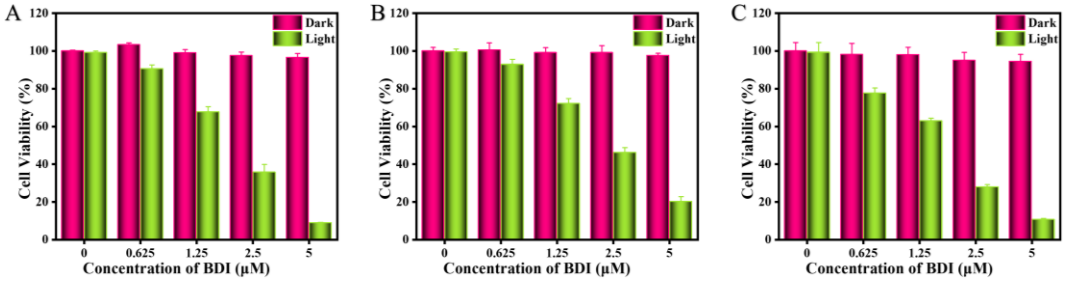


Figure S10. Cytotoxicity of **BDI** toward (A) HepG2; (B) MCF-7; (C) A549 cells w/o light irradiation.

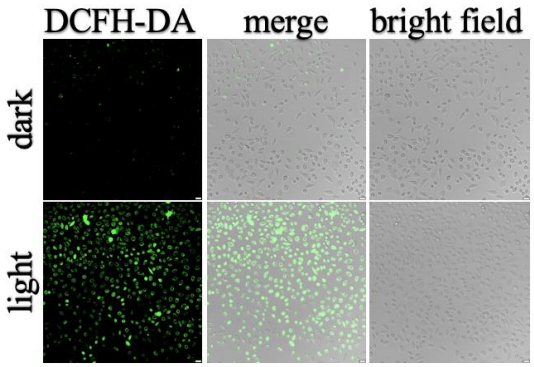


Figure S11. Intracellular ROS detection of HeLa cells pretreated with **BDI**. Scale bar = 20 μm.

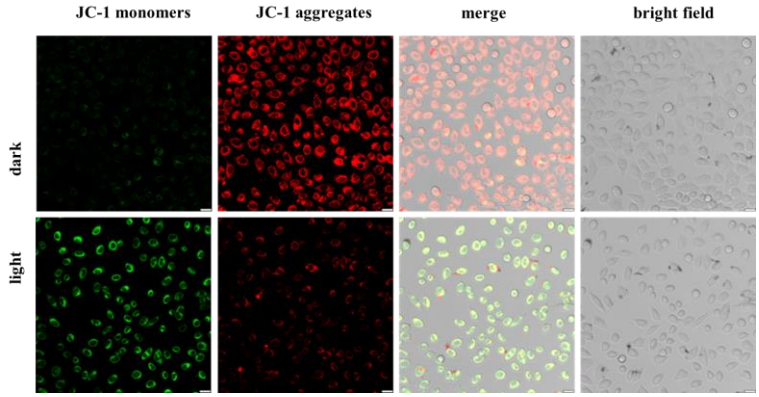


Figure S12. Mitochondrial membrane potential detection of HeLa cells incubated with **BDI** w/o light irradiation. Scale bar = 20 μm.

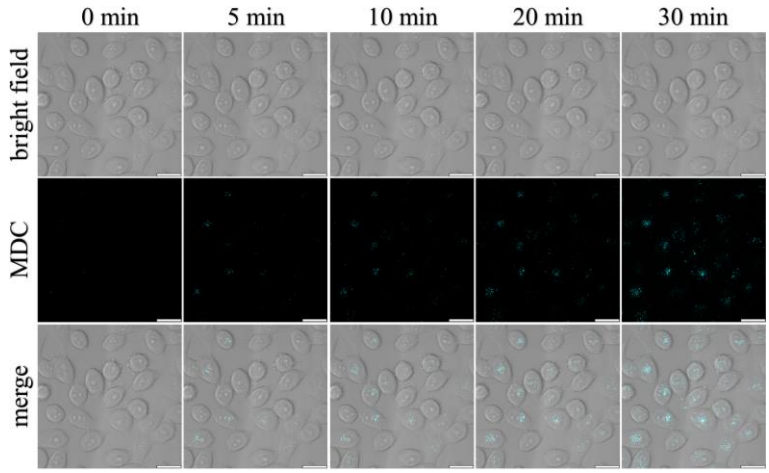


Figure S13. (A) Time-lapse CLSM images of HeLa cells incubated with **MDC** (1 μM) after treated with rapamycin. 405 nm was set as excitation wavelength and 450-550 nm was collected. Scale bar

= 20 μm.

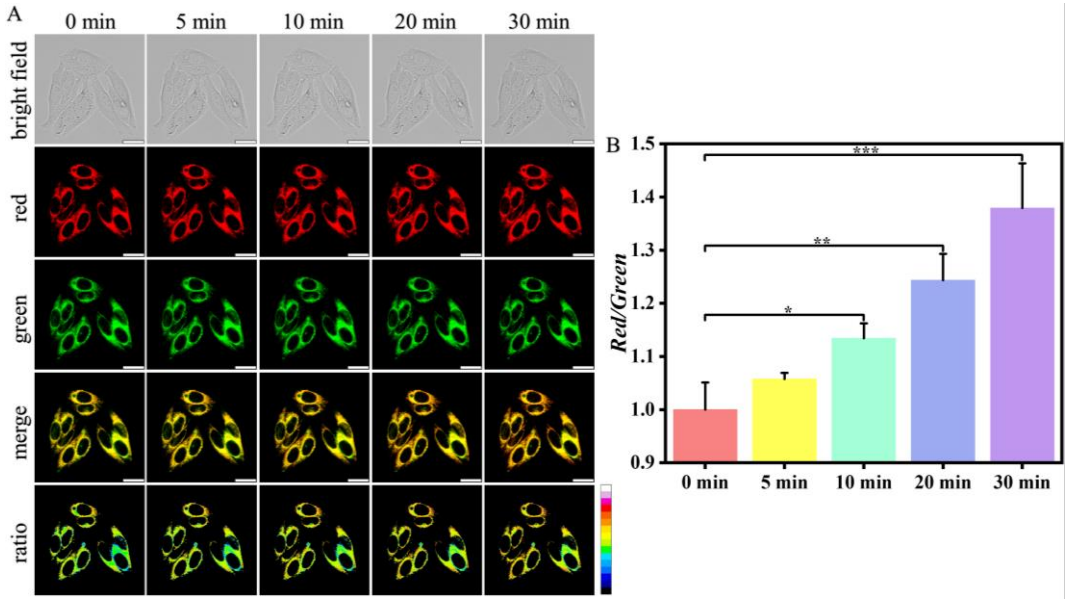


Figure S14. (A) Time-lapse CLSM images of HepG2 cells incubated with **BDI** (5 μM) after treated with rapamycin. Ex = 543 nm, 550-600 nm was collected for green channel and 620-670 nm was collected for red channel, respectively. Scale bar = 20 μm. (B) Fluorescence intensity ratio as a function of mitochondrial polarity changes. The ratio at 0 min is defined as 1.0. Significant differences (*p < 0.05, **p < 0.01, ***p < 0.001) are obtained by Student's t-test.

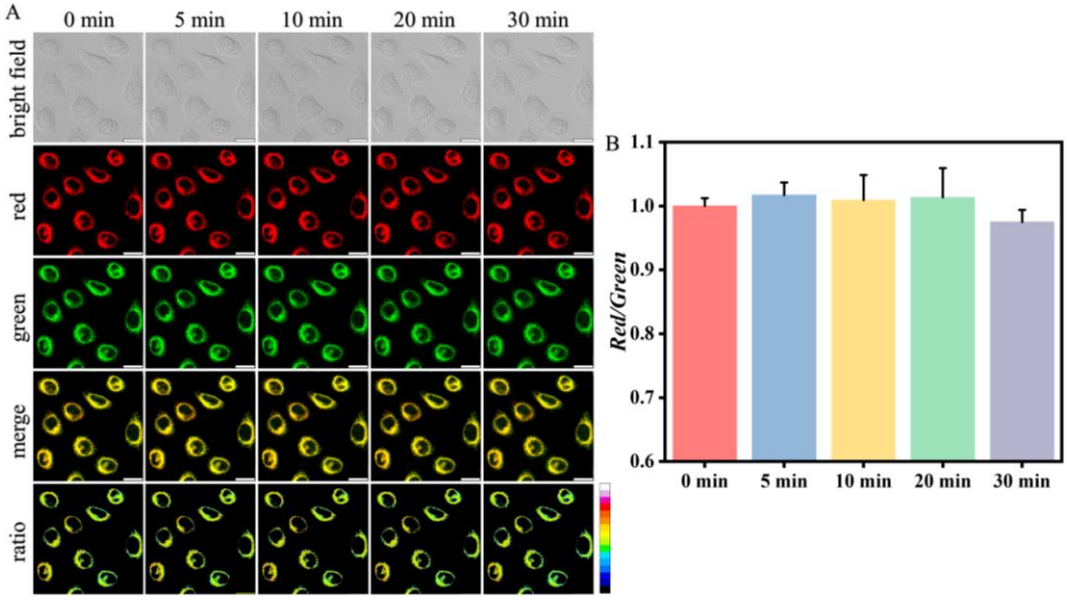


Figure S15. (A) Time-lapse CLSM images of HeLa cells incubated with **BDI** (5 μM) without

treatment of rapamycin. Ex = 543 nm, 550-600 nm was collected for green channel and 620-670 nm was collected for red channel, respectively. Scale bar = 20 μm. (B) Fluorescence intensity ratio as a function of mitochondrial polarity changes. The ratio at 0 min is defined as 1.0.

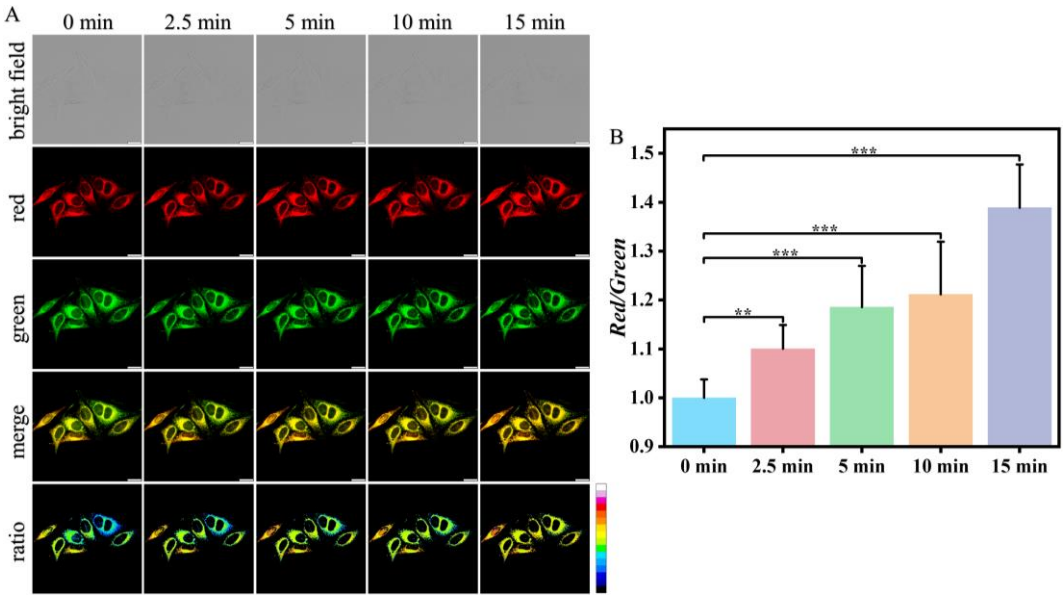
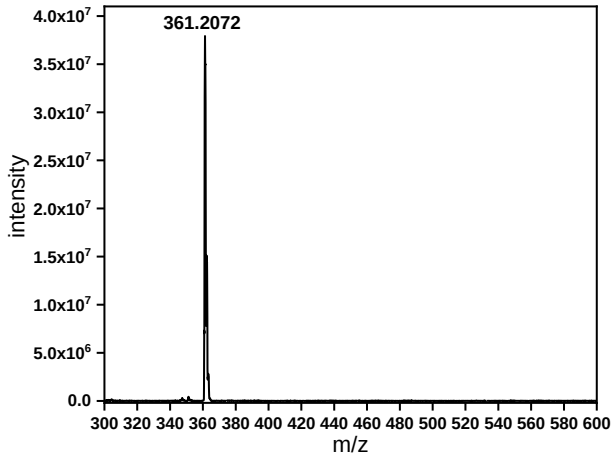
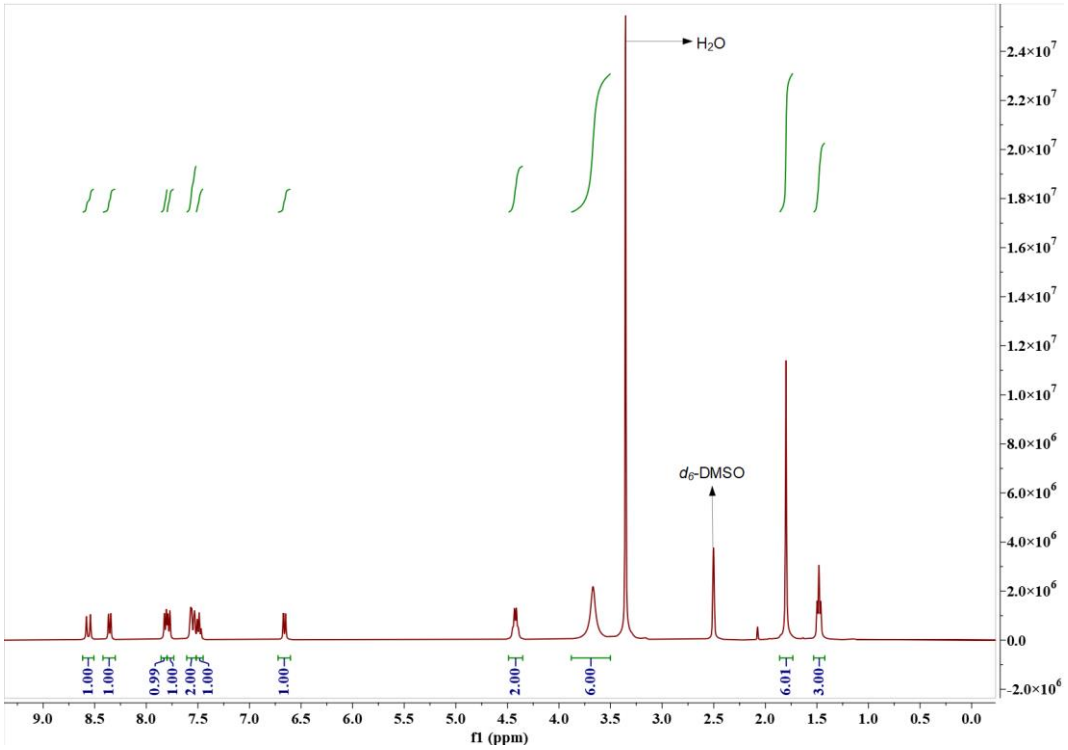


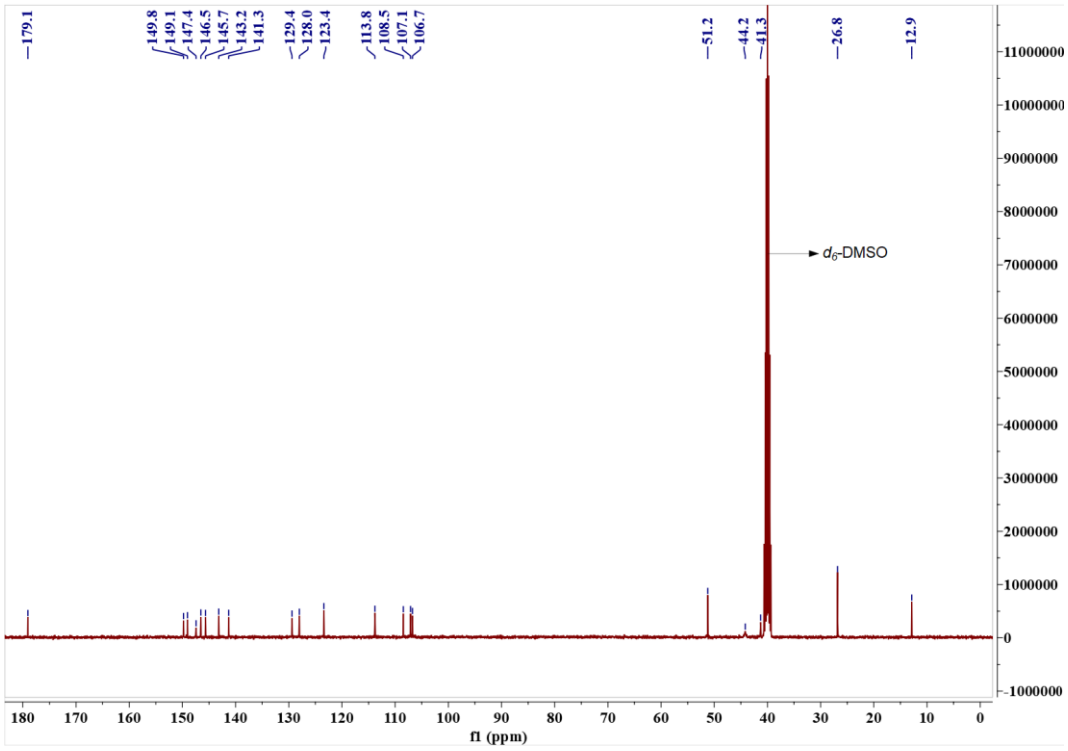
Figure S16. (A) Time-lapse CLSM images of HepG2 cells incubated with **BDI** (5 μM) after light irradiation. Ex = 543 nm, 550-600 nm was collected for green channel and 620-670 nm was collected for red channel, respectively. Scale bar = 20 μm. (B) Fluorescence intensity ratio as a function of mitochondrial polarity changes. The ratio at 0 min is defined as 1.0. Significant differences (**p < 0.01, ***p < 0.001) are obtained by Student's t-test.



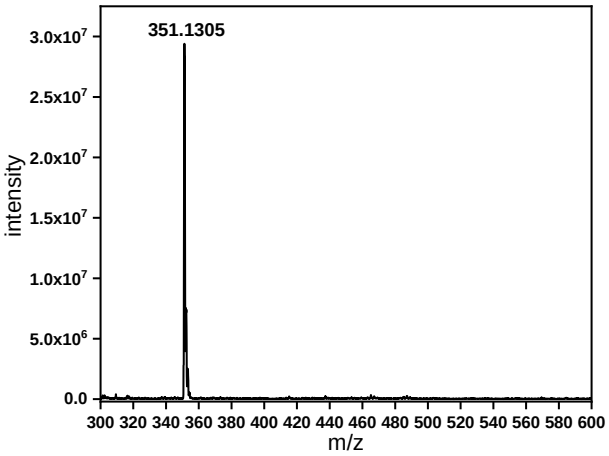
MS spectrum of **BDC** compound



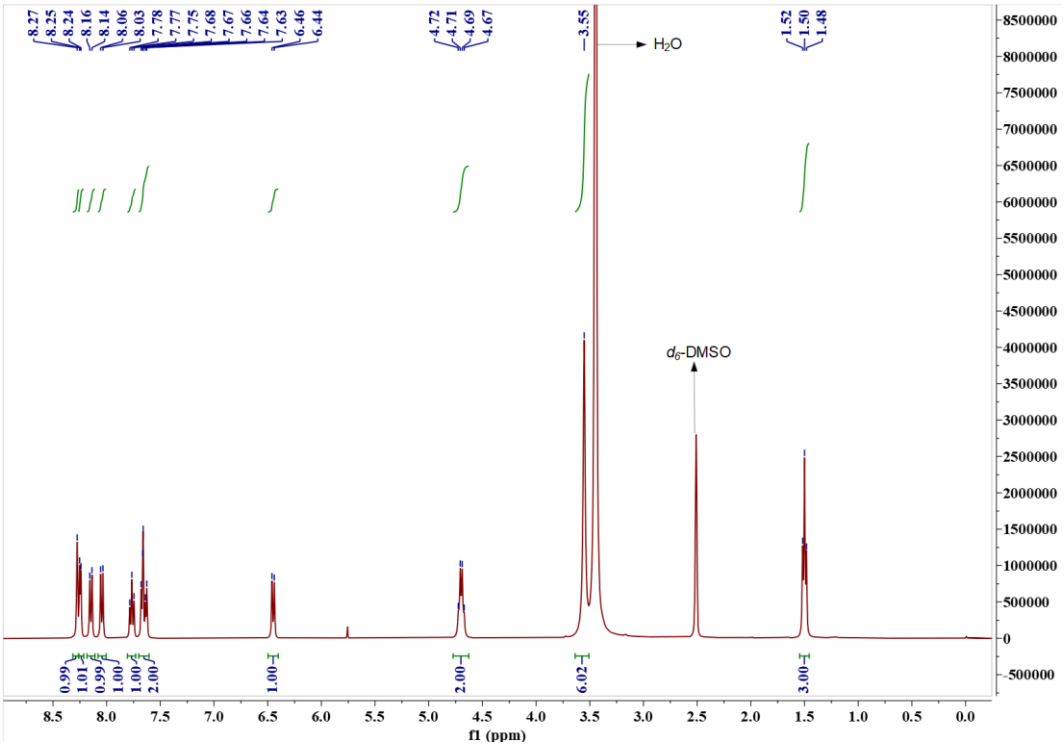
^{1}H NMR spectrum of compound **BDC** in DMSO-d_6



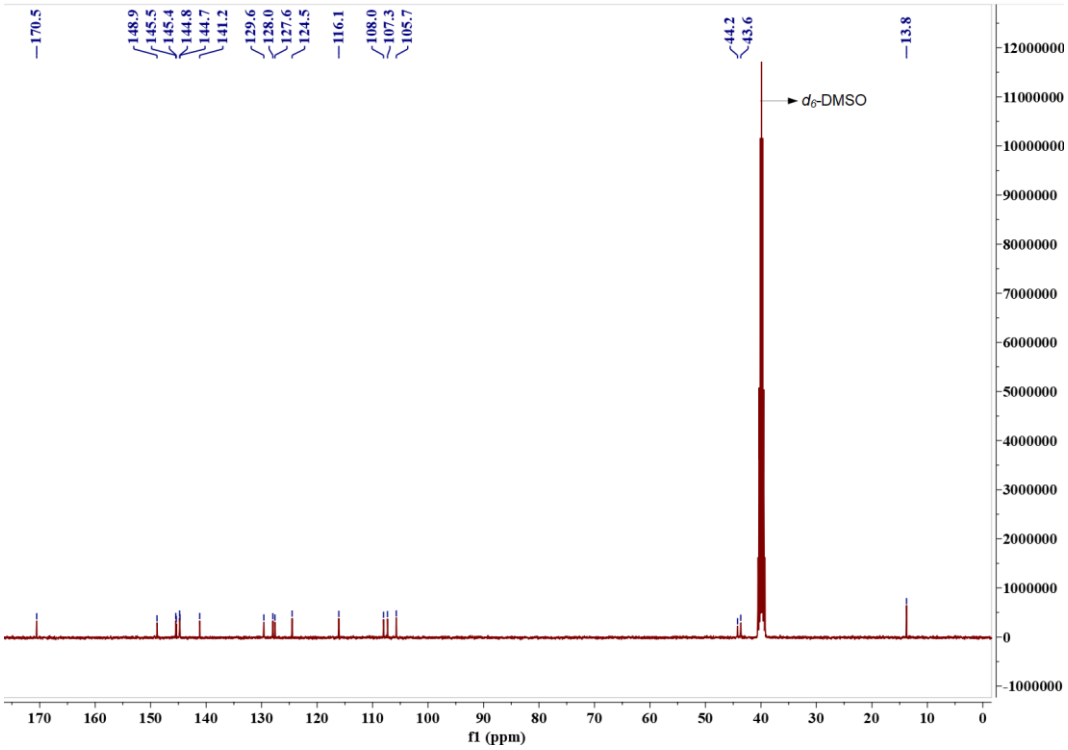
^{13}C NMR spectrum of compound **BDC** in DMSO-d_6



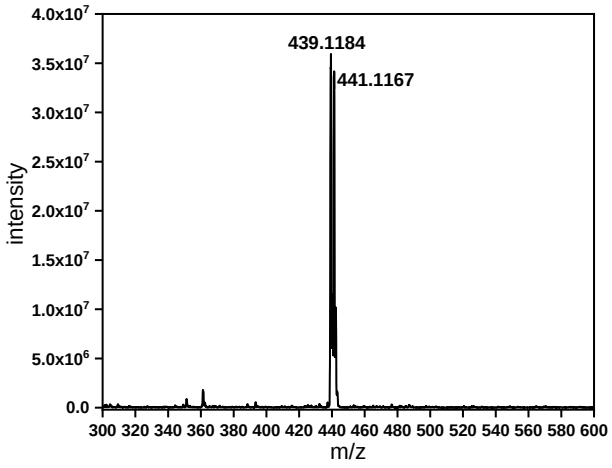
MS spectrum of **BDS** compound



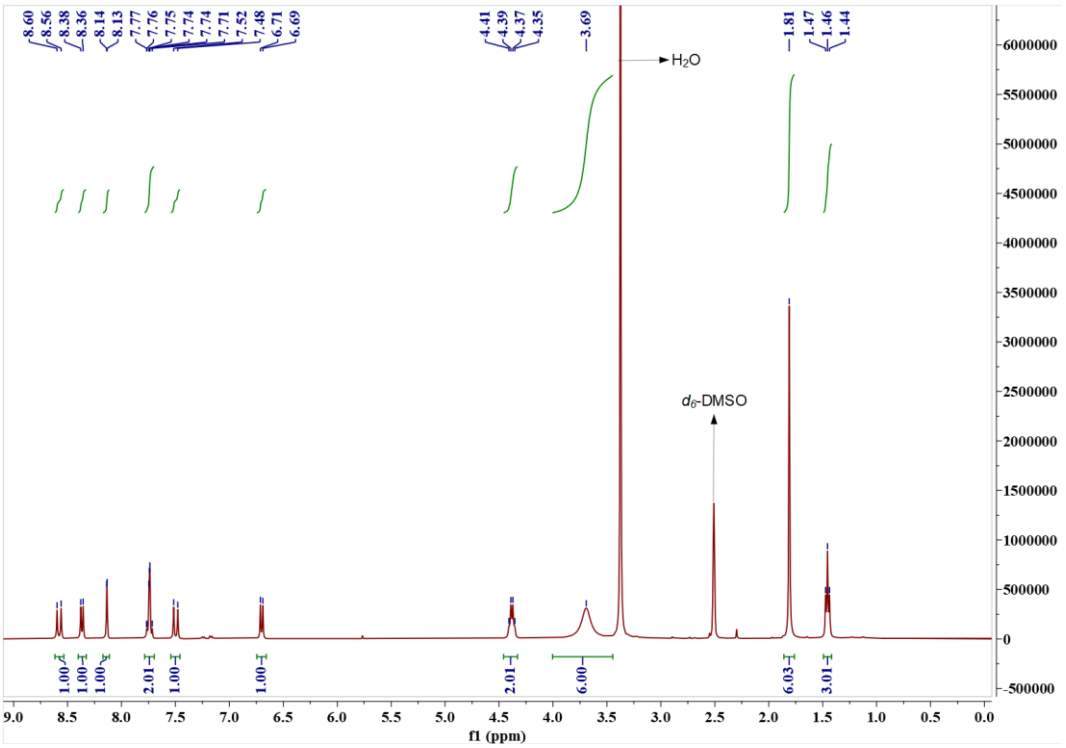
^{1}H NMR spectrum of compound **BDS** in DMSO-d_6



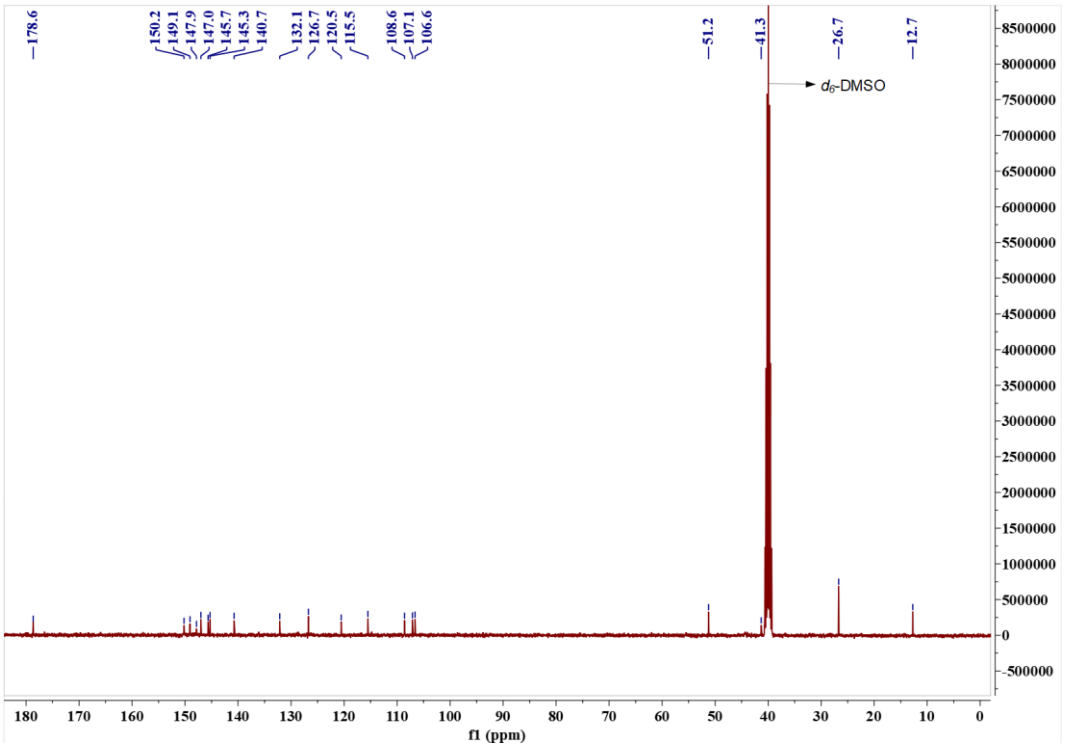
^{13}C NMR spectrum of compound **BDS** in DMSO-d_6



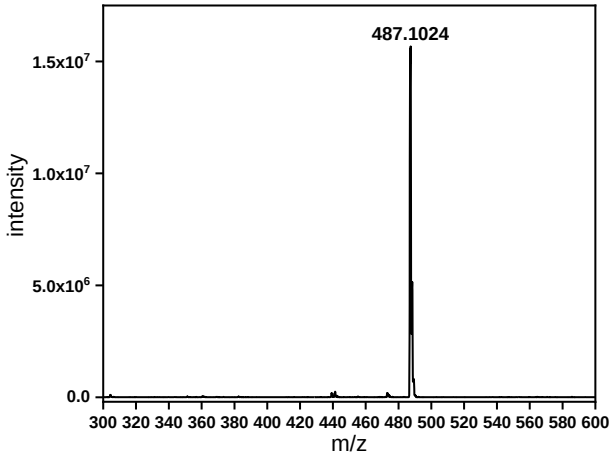
MS spectrum of **BDBr** compound



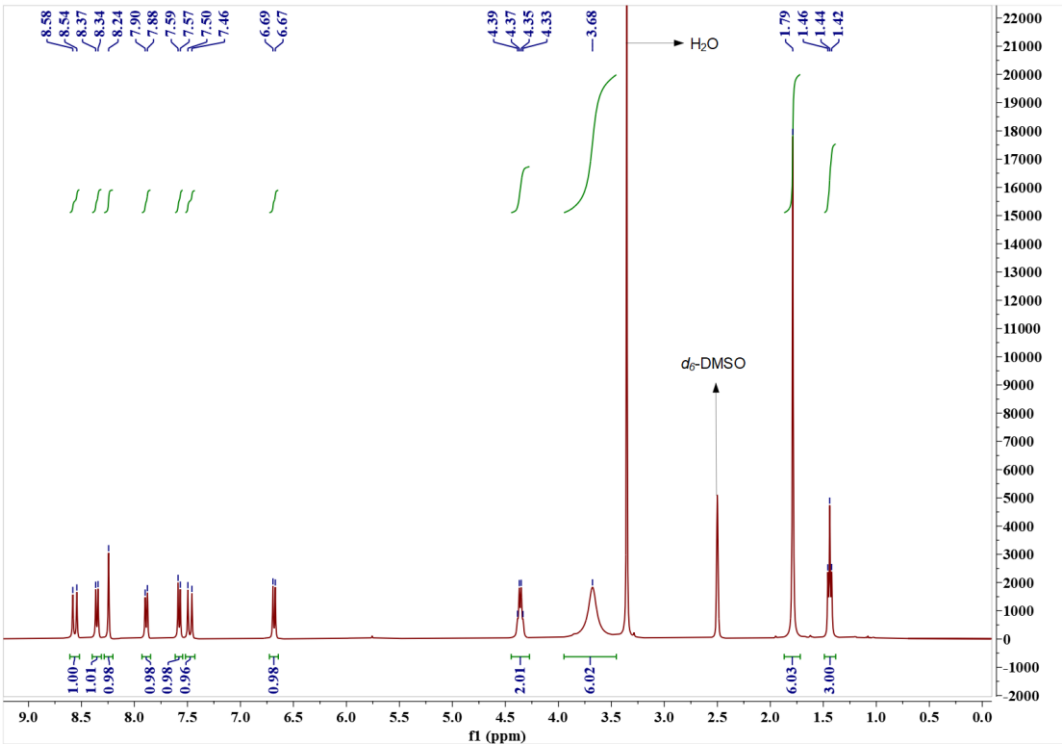
^{1}H NMR spectrum of compound **BDBr** in DMSO-d_6



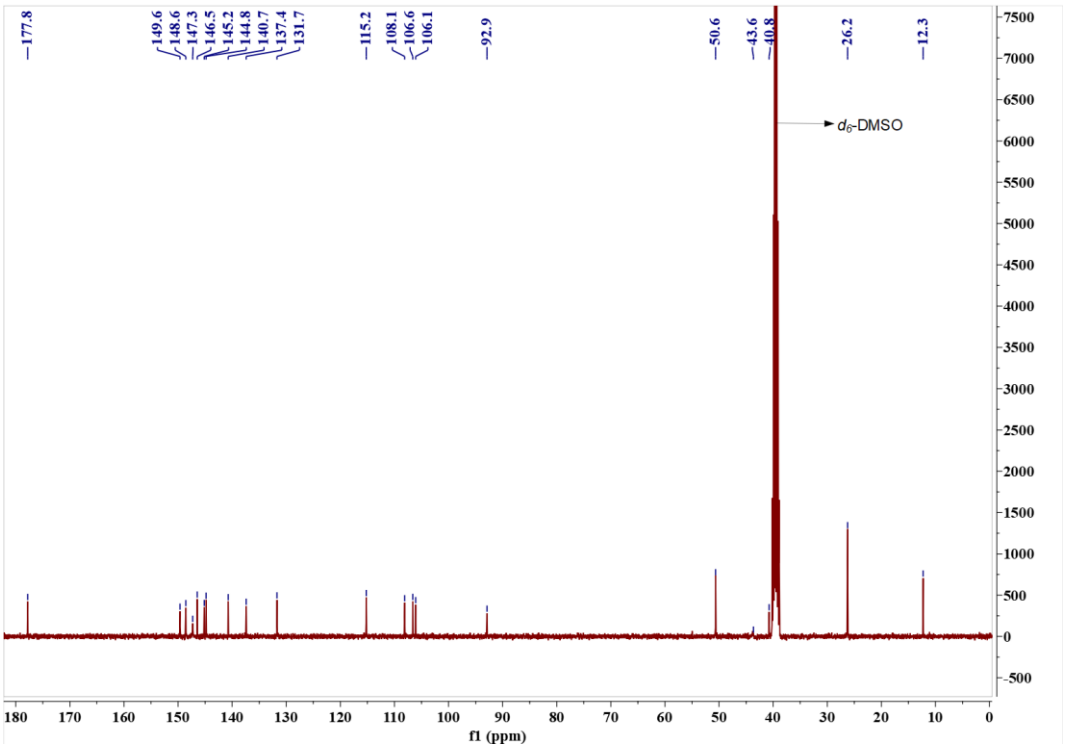
^{13}C NMR spectrum of compound **BDBr** in DMSO-d_6



MS spectrum of **BDI** compound



^{1}H NMR spectrum of compound **BDI** in DMSO-d_6



^{13}C NMR spectrum of compound **BDI** in DMSO-d_6

3. References

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